APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. LOAELs for serious health effects (such as irreparable damage to the liver or kidneys, or serious birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

APPENDIX A

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Office of Innovation and Analytics, Toxicology Section, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Office of Innovation and Analytics, Toxicology Section, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S106-5, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Inhalation
Duration: Acute
MRL: 0.001 ppm (0.005 mg/m^3)
Critical Effect: Nasal lesions
Reference: Larson et al. 1996; Templin et al. 1996b
Point of Departure: NOAEL of 2 ppm ($NOAEL_{HEC}$ of 0.04 ppm)
Uncertainty Factor: 30
LSE Graph Key: 9, 24
Species: Rat, Mouse

MRL Summary: An acute-duration inhalation MRL of 0.001 ppm was derived for chloroform based on nasal lesions in rats and mice exposed to concentrations ≥10 ppm for 4 days (6 hours/day); a NOAEL of 2 ppm was identified (Larson et al. 1996; Templin et al. 1996b). The MRL is based on the NOAEL of 2 ppm, which was adjusted to continuous duration exposure and converted to a $NOAEL_{HEC}$ of 0.04 ppm and divided by a total uncertainty factor of 30 (3 for extrapolation from animals to humans after dosimetric adjustment and 10 for human variability). The $LOAEL_{HEC}$ value was 0.19 ppm.

Selection of the Critical Effect: No acute-duration human studies with reliable exposure estimates were identified. The most sensitive effects following acute-duration inhalation exposure were hepatic and respiratory effects (Table A-1). Changes to nasal bones and olfactory neuron loss were also observed at similar concentrations as nasal epithelial changes.

Table A-1. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Inhalation Exposure to Chloroform

		Effect level (ppm)			
Species	Duration	NOAEL	LOAEL	Effect	Reference
Hepatic effects					
B6C3F1 mouse	7 days 6 hours/day	1.2	3	18% increase in relative liver weight	Larson et al. 1994c
B6C3F1 mouse	4 days 6 hours/day	2	10	Diffuse lipid hepatocytic vacuolation, scattered hepatocyte necrosis	Larson et al. 1996
Respiratory effects					
C57BL/6 mouse	5 days 1 hour/day (20 minutes 3 times/day)	ND	7	Increased white blood cells in BALF, increased alveolar area, and decreased density of alveolar septa; decreased relative lung weight in females	de Oliveira et al. 2015
Fischer 344 rat	4 days 6 hours/day	2	10	Loss of olfactory glands; periosteal hypercellularity and proliferation; mineralization of the basal lamina; new nasal bone growth	Templin et al. 1996b

Table A-1. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Inhalation Exposure to Chloroform

		Effect level (ppm)			
Species	Duration	NOAEL	LOAEL	Effect	Reference
B6C3F1 mouse	4 days 6 hours/day	2	10	Connective tissue proliferation in the nasal lamina propria; periosteal cell proliferation in nasal cavity	Larson et al. 1996
B6C3F1 mouse	7 days 6 hours/day	3	10	Nasal periosteal cell proliferation	Mery et al. 1994
Fischer 344 rat	7 days 6 hours/day	3.1	10.4	Goblet cell hyperplasia in nasal respiratory epithelium; olfactory gland degeneration in lamina propria; periosteal proliferation and new bone formation	Larson et al. 1994c; Mery et al. 1994
Nervous system effects					
Fischer 344 rat	7 days 6 hours/day	3.1	10.4	Olfactory neuron loss	Larson et al. 1994c; Mery et al. 1994

BALF = bronchoalveolar lavage fluid; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

In order to identify the most sensitive POD, benchmark dose (BMD) modeling was attempted for endpoints in Table A-1 when data were amenable to modeling. The data were fit to all available continuous models in EPA's Benchmark Dose Software (BMDS; version 3.3) using a benchmark response (BMR) of 1 SD for liver weight, nasal lesion severity score, periosteal labeling index (proliferation), and nasal turbinate width. Adequate model fit was judged by four criteria: goodness-of-fit statistics (p-value >0.1), visual inspection of the dose-response curve, a 95% lower confidence limit on the BMD (BMCL) that is not 10 times lower than the lowest non-zero dose, and scaled residual within ±2 units at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest 95% lower confidence limit concentration (BMCL) was selected as the POD when the difference between the BMCLs estimated from these models was >3-fold; otherwise, the BMCL from the model with the lowest Akaike information criterion (AIC) was chosen.

The datasets used for BMD modeling are presented in Table A-2 for elevated liver weight in mice reported by Larson et al. (1994c), Table A-3 for nasal lesion severity score and periosteal proliferation in mice reported by Larson et al. (1996), Table A-4 for periosteal proliferation and width of central nasal turbinate in rats reported by Mery et al. (1994), Table A-5 for periosteal proliferation in mice reported by Mery et al. (1994), and Table A-6 for periosteal proliferation in rats reported by Templin et al. (1996b). Data for increased severity of hepatic lesions in mice were not suitable for modeling because mean severity scores were reported without a measure of variance (Larson et al. 1996). Data for pulmonary effects reported by de Oliveira et al. (2015) were not suitable for modeling because only one exposure group was included. Data for nasal epithelial lesions in rats were not suitable for modeling because incidence data were not provided and/or mean severity scores were reported without a measure of variance (Larson et al. 1994c; Mery et al. 1994; Templin et al. 1996b). ATSDR used the NOAEL/LOAEL approach for endpoints with data unsuitable for BMD modeling.

Table A-2. Relative Liver Weights in Female Mice Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day)

	Analytical concentration (ppm)						
Endpoint[a]	0	1.2	3	10	29.5	101	288
Relative liver weight (% body weight)	5.7±0.6 (5)	6.3±0.5 (5)	6.7±0.7[b] (5)	7.0±1.1[b] (5)	7.3±0.6[b] (5)	9.5±1.7[b] (5)	10.1±1.1[b] (5)

[a]Mean±SD (number of animals).
[b]p<0.05.

BW = body weight; SD = standard deviation

Source: Larson et al. 1994c

Table A-3. Nasal Lesions and Periosteal Proliferation in Female Mice Following Inhalation Exposure to Chloroform for 4 Days (6 Hours/Day)

	Analytical concentration (ppm)					
Endpoint[a]	0	0.3	2	10	30	88
Severity score[b]	0±0 (5)	0±0 (5)	0.5±0.5 (5)	1.6±0.5[c] (5)	1.8±1.0[c] (5)	2.4±0.5[c] (5)
Nasal turbinate lamina propria labeling index	15±8 (5)	9±3 (5)	16±5 (5)	164±49[d] (5)	281±158[d] (5)	397±27[d] (5)

[a]Mean±SD (number of animals).
[b]Nasal lesions were scored according to a 1–4 score: 0 = within normal limits; 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
[c]p<0.05, as calculated for this review.
[d]p<0.05, as reported by the study authors.

SD = standard deviation

Source: Larson et al. 1996

Table A-4. Periosteal Proliferation and Endoturbinate Width in Male Rats Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day)

	Analytical concentration (ppm)						
Endpoint[a]	0	1.5	3.1	10.4	29.3	100	271
Labelled cells in nasal turbinate							
Proximal	55±30 (5)	52±41 (5)	140±130 (5)	270±54[b] (5)	330±100[b] (5)	250±95[b] (5)	450±110[b] (5)
Central	26±15 (5)	19±13 (5)	90±13 (5)	220±80[b] (5)	200±60[b] (5)	230±110[b] (5)	340±140[b] (5)
Distal	36±19 (5)	34±19 (5)	96±19 (5)	150±69[b] (5)	120±52[b] (5)	130±47[b] (5)	220±93[b] (5)

Table A-4. Periosteal Proliferation and Endoturbinate Width in Male Rats Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day)

	Analytical concentration (ppm)						
Endpoint[a]	0	1.5	3.1	10.4	29.3	100	271
Width of central turbinate (µm)	41±12 (5)	45±17 (5)	40±9 (5)	61±17[b] (5)	51±16[b] (5)	66±8[b] (5)	68±10[b] (5)

[a]Mean±SD (number of animals).
[b]p<0.05, as reported by the study authors.

SD = standard deviation

Source: Mery et al. 1994

Table A-5. Periosteal Proliferation in Female Mice Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day)

	Analytical concentration (ppm)						
Endpoint[a]	0	1.2	3	10	29.5	101	288
Labelled cells in nasal turbinate							
Proximal	19±11 (5)	31±32 (5)	63±34 (5)	360±94[b] (5)	190±130[b] (5)	190±100[b] (5)	330±70[b] (5)
Distal	14±11 (5)	21±12 (5)	15±10 (5)	82±42[b] (5)	54±48[b] (5)	77±24[b] (5)	100±30[b] (5)
Ventral	31±23 (5)	95±130 (5)	110±140 (5)	310±49[b] (5)	230±140[b] (5)	260±160[b] (5)	370±130[b] (5)
Dorsal	21±13 (5)	36±69 (5)	27±14 (5)	200±11[b] (5)	120±74[b] (5)	110±140[b] (5)	220±140[b] (5)

[a]Mean±SD (number of animals).
[b]p<0.05, as reported by the study authors.

SD = standard deviation

Source: Mery et al. 1994

Table A-6. Periosteal Proliferation in Male Rats Following Inhalation Exposure to Chloroform for 4 Days (6 Hours/Day)

	Concentration (ppm)					
Endpoint[a]	0	2	10	30	90	300
Proximal turbinate labeling index	30±15 (5)	24±11 (5)	490±99[b] (5)	566±155[b] (5)	752±74[b] (5)	809±48[b] (5)

[a]Mean±SD, estimated from graphically presented data using *GrabIt!* software (number of animals).
[b]p<0.05, as reported by the study authors.

SD = standard deviation

Source: Templin et al. 1996b

Details of the modeling results for the model predictions for relative liver weight in female mice reported by Larson et al. (1994c) are in Table A-7. The frequentist, restricted, Exponential 5 model was selected based on the selection criteria outlined above. No adequate models were identified for connective tissue or periosteal cell proliferation in mice following exposure for 4 days (Larson et al. 1996) or periosteal cell proliferation in rats following exposure for 4 days (Templin et al. 1996b) or 7 days (Mery et al. 1994) because they failed to meet conventional goodness-of-fit criteria using constant or nonconstant variance. While statistical model fits were identified for increased width of the central turbinate in rats exposed for 7 days and distal turbinate labeling index in mice exposed for 7 days, inspection of the recommended and alternate models showed poor visual fit, particularly in the low-exposure region of the curves.

Table A-7. Results from Benchmark Dose (BMD) Analysis (Constant Variance) of Relative Liver Weight in Female Mice Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day) (Larson et al. 1994c)

					Scaled residuals[c]	
Model	BMC_{1SD}[a] (ppm)	$BMCL_{1SD}$[a] (ppm)	p-Value[b]	AIC	Dose below BMC	Dose above BMC
Exponential (model 3)[d]			0.001	116.36	3.07	-0.84
Exponential (model 5)[d,e]	**16.72**	**9.89**	**0.49**	**101.74**	0.64	-0.82
Hill[f]	13.97	6.94	0.43	102.16	0.46	-1.03
Polynomial (3-degree)[f]			0.004	113.93	0.46	2.86
Polynomial (2-degree)[f]			0.004	113.93	0.46	2.86
Power			0.004	113.93	0.46	2.86
Linear			0.004	113.93	0.46	2.86

[a]BMC and BMCLs values for models that do not provide adequate fit are not included in this table.
[b]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMC.
[d]Power restricted to ≥1.
[e]Selected model. The constant variance model provided an adequate fit. Only the Exponential 5 and Hill models provided an adequate fit to the means. BMCLs were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected (Exponential 5).
[f]Coefficients restricted to be positive.

AIC = Akaike Information Criterion; BMC = benchmark concentration (maximum likelihood estimate of the concentration associated with the selected benchmark response); BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{1SD}$ = exposure dose associated with a change of 1 standard deviation from the control); NA = not applicable

In order to accurately compare candidate PODs across different species and target tissues, POD values were converted into human equivalent concentrations (HECs). For systemic (hepatic) effects, exposure was not adjusted for continuous exposure because data provided by the PBPK model by Corley et al. (1990) demonstrate that the arterial blood concentration (CA) of chloroform in the mouse exposed to chloroform for 6 hours reached "periodicity" (the pattern of repeated increases and decreases in arterial blood concentration that occurs when steady state is achieved during repeated intermittent exposures) within 15 minutes following exposure (Table A-8). Therefore, adjustment from 6 hours to 24 hours is not required.

Table A-8. Corley PBPK Model for Chloroform to Simulate 6-Hour Inhalation Exposure in Mice

Time (hours)	Blood concentration (CA) (mg/L)
0.00	0.014
0.25	0.040
0.50	0.041
0.75	0.041
1.25	0.042
1.50	0.042
1.75	0.042
2.00	0.042
2.25	0.042
2.50	0.042
3.375	0.042
4.5	0.042
5.625	0.042
6.75 (post-exposure)	0.0006

Source: Corley et al. (1990) in the Scop version (courtesy of Dr. Nancy Chiu, EPA)

The NOAELs for hepatic effects in mice reported by Larson et al. (1994c, 1996) were converted into $NOAEL_{HEC}$ values using guidance from EPA (1994) on dosimetric adjustments for systemic effects using the ratio of animal:human blood gas partition coefficients. In the case of chloroform, using reported blood:air partition coefficients of 21.3 for the mouse and 7.34 for the human (Corley et al. 1990) provides a ratio of mouse: human partition coefficients >1; therefore, a default value of 1 is used to derive the $NOAEL_{HEC}$.

Larson et al. (1994c), 7-day mouse study (increased relative liver weight):

$$BMCL_{HEC} = BMCL \times \frac{mouse\ partition\ coefficient}{human\ partition\ coefficent} = 9.9\ ppm \times 1 = 9.9\ ppm$$

Larson et al. (1996), 4-day mouse study (hepatic lesions):

$$NOAEL_{HEC} = NOAEL \times \frac{mouse\ partition\ coefficient}{human\ partition\ coefficent} = 2\ ppm \times 1 = 2\ ppm$$

The candidate POD values for nasal effects were adjusted to continuous exposure because kinetic data reported by Sarangapani et al. (2002) indicate that the periodicity reported by Corley et al. (1990) is not applicable to nasal tissue exposures. Using a PBPK model, Sarangapani et al. (2002) showed a steeper external exposure-internal dose relationship for the nasal compartment compared to the hepatic compartment. This steeper dose relationship is driven by the tissue:air partition coefficient and is relatively insensitive to the blood perfusion rate or other systemic parameters. Additionally, longer-duration studies indicate increased severity of nasal lesions with increased duration of exposure, which further supports duration-adjustment for nasal effects. Since the nasal bone effects and olfactory neuron loss are presumably due to portal-of-entry effects, extrathoracic HEC calculations were applied for these endpoints as well.

For each study evaluating nasal endpoints, POD_{ADJ} values were converted to POD_{HEC} values using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the regional gas dose ratio (RGDR) for extrathoracic effects ($RGDR_{ET}$). This $RGDR_{ET}$ is calculated using the following equation as defined by EPA (1994):

$$RGDR_{ET} = \frac{V_{E\,a}}{SA_a} \div \frac{V_{E\,h}}{SA_h}$$

where:
$V_{E\,a}$ = ventilation rate for animals: male F344 rats = 0.137 L/minute; female B6C3F1 mice = 0.028 L/minute
SA_a = surface area of the extrathoracic region in rats = 15 cm^2
$V_{E\,h}$ = ventilation rate for humans = 13.8 L/minute
SA_h = surface area of the extrathoracic region in humans = 200 cm^2

Note, below, that rat and mouse have different extrathoracic RGDR values and these will be critical in calculating $NOAEL_{HEC}$ values for each endpoint.

$$Rat\text{:}\ RGDR_{ET} = \frac{V_{E\,a}}{SA_a} \div \frac{V_{E\,h}}{SA_h} = \frac{0.137}{15} \div \frac{13.8}{200} = 0.132$$

$$Mouse\text{:}\ RGDR_{ET} = \frac{V_{E\,a}}{SA_a} \div \frac{V_{E\,h}}{SA_h} = \frac{0.028}{3} \div \frac{13.8}{200} = 0.136$$

Templin et al. (1996b); 4-day study in rats (nasal lesions and bone growth, periosteal proliferation):

$$NOAEL_{ADj} = NOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 2\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{4\ days}{7\ days} = 0.3\ ppm$$

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR = 0.3\ ppm \times 0.132 = 0.04\ ppm$$

Larson et al. (1996); 4-day study in mice (nasal lesions, periosteal proliferation):

$$NOAEL_{ADj} = NOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 2\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{4\ days}{7\ days} = 0.3\ ppm$$

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR = 0.3\ ppm \times 0.136 = 0.04\ ppm$$

Larson et al. (1994c) and Mery et al. (1994); 7-day study in rats (nasal lesions, bone growth, periosteal proliferation; olfactory neuron loss):

$$NOAEL_{ADJ} = NOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 3.1\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{7\ days}{7\ days} = 0.78\ ppm$$

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR = 0.78\ ppm \times 0.132 = 0.10\ ppm$$

Mery et al. (1994); 7-day study in mice (nasal periosteal proliferation):

$$NOAEL_{ADJ} = NOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 3\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{7\ days}{7\ days} = 0.8\ ppm$$

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR = 0.8\ ppm \times 0.136 = 0.1\ ppm$$

The LOAEL value for pulmonary effects in mice reported by de Oliveira et al. (2015) was adjusted to continuous exposure because it is unknown if the periodicity reported by Corley et al. (1990) for systemic effects is applicable to pulmonary effects. The $LOAEL_{ADJ}$ was then converted to a $LOAEL_{HEC}$ using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the RGDR for pulmonary effects ($RGDR_{PU}$). The $RGDR_{PU}$ is calculated using the following equation as defined by EPA (1994):

$$RGDR_{PU} = \frac{Q_{alv_a}}{SA_a} \div \frac{Q_{alv_h}}{SA_h} = \frac{0.028}{0.05} \div \frac{13.8}{54} = 2.19$$

where:
Q_{alv_a} = alveolar ventilation rate for B6C3F1 mice = 0.028 L/minute
SA_a = surface area of the pulmonary region in mice = 0.05 m^2
Q_{alv_h} = alveolar ventilation rate for humans = 13.8 L/minute
SA_h = surface area of the pulmonary region in humans = 54 m^2

Applying this equation results in an RGDR of 2.191304 for pulmonary effects in mice, and the HEC is calculated as shown below.

de Oliveira et al. (2015); 5-day study in mice (pulmonary effects):

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 7\ ppm \times \frac{1\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 0.2\ ppm$$

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 0.2\ ppm \times 2.19 = 0.4\ ppm$$

All candidate POD_{HEC} values are summarized in Table A-9. Based on POD_{HEC} values, the lowest POD identified was for nasal effects in rats and mice, with a $NOAEL_{HEC}$ value of 0.04 ppm. Therefore, nasal effects were selected as the critical effect.

Table A-9. Summary of Candidate Effects and POD Values Considered for Derivation of an Acute-Duration Inhalation MRL for Chloroform

Species	Duration	Effect	Candidate POD (ppm)	POD type	Reference
Hepatic effects					
B6C3F1 mouse	7 days 6 hours/day	Increased relative liver weight	9.9	$BMCL_{HEC}$	Larson et al. 1994c
B6C3F1 mouse	4 days 6 hours/day	Increased severity of hepatic lesions	2	$NOAEL_{HEC}$	Larson et al. 1996

Table A-9. Summary of Candidate Effects and POD Values Considered for Derivation of an Acute-Duration Inhalation MRL for Chloroform

Species	Duration	Effect	Candidate POD (ppm)	POD type	Reference
Respiratory effects					
C57BL/6 mouse	5 days 1 hour/day (20 minutes 3 times/day)	Increased white blood cells in BALF, increased alveolar area, and decreased density of alveolar septa; decreased relative lung weight in females	0.4	$LOAEL_{HEC}$	de Oliveira et al. 2015
Fischer 344 rat	4 days 6 hours/day	Nasal epithelial lesions, periosteal proliferation, new bone growth	0.04	$NOAEL_{HEC}$	Templin et al. 1996b
B6C3F1 mouse	4 days 6 hours/day	Nasal epithelial lesions, periosteal proliferation	0.04	$NOAEL_{HEC}$	Larson et al. 1996
B6C3F1 mouse	7 days 6 hours/day	Nasal periosteal proliferation	0.1	$NOAEL_{HEC}$	Mery et al. 1994
Fischer 344 rat	7 days 6 hours/day	Nasal epithelial lesions, periosteal proliferation, new bone growth	0.10	$NOAEL_{HEC}$	Larson et al. 1994c; Mery et al. 1994
Nervous system effects					
Fischer 344 rat	7 days 6 hours/day	Olfactory neuron loss	0.10	$NOAEL_{HEC}$	Larson et al. 1994c; Mery et al. 1994

BALF = bronchoalveolar lavage fluid; BMC = benchmark concentration (maximum likelihood estimate of the concentration associated with the selected benchmark response); BMCL = 95% lower confidence limit on the BMC; HEC = human equivalent concentration; LOAEL = lowest observed adverse effect level; MRL = Minimal Risk Level; NOAEL = no-observed-adverse-effect level; POD = point of departure

Selection of the Principal Study: Templin et al. (1996b) and Larson et al. (1996) were selected as co-principal studies because they provided the lowest candidate POD (0.04 ppm) for the critical effect (nasal lesions).

Summary of the Principal Study:

Templin MV, Larson JL, Butterworth BE, et al. 1996b. A 90-day chloroform inhalation study in F-344 rats: profile of toxicity and relevance to cancer studies. Fundam Appl Toxicol 32(1):109-125.

Larson JL, Templin MV, Wolf DC, et al. 1996. A 90-day chloroform inhalation study in female and male B6C3F1 mice: implications for cancer risk assessment. Fundam Appl Toxicol 30(1):118-137. https://doi.org/10.1006/faat.1996.0049.

Templin et al. (1996b) exposed 9-week-old male F344 rats (5/sex/group) to target concentrations of 0, 2, 10, 30, 90, or 300 ppm chloroform for 4 days (6 hours/day). In all animals, bromodeoxyuridine (BrdU) was administered 3.5 days prior to sacrifice. Endpoints evaluated included clinical signs, body weight, gross necropsy, histopathology (liver, kidney, nasal cavity, non-nasal bones (sternum, rib, vertebrae, tibia, femur), and cellular proliferation (BrdU labeling index) in liver, kidney, and bone.

Average analytical exposure concentrations were always within 4.5% of the target (quantitative values not reported). No deaths were reported. Body weight gains were significantly decreased compared to control in all exposure groups. Controls gained approximately 3% during the exposure period, while rats exposed to 2, 10, 30, 90, or 300 ppm lost approximately 2, 3, 3, 5, and 14% of their initial body weight (estimated based on graphically presented data). No histopathological lesions were observed in the liver, but the BrdU labelling index showed significantly increased hepatocellular proliferation at 300 ppm. Minimal vacuolation of proximal convoluted tubules was observed in 5/5 mice at 300 ppm; no renal cell proliferation was noted. In the nasal cavity, lesions were noted in at ≥10 ppm. The lesions were primarily observed in the ethmoid portion lined with olfactory epithelium (ventral and lateral regions of turbinates), including lesions within the lamina propria characterized by edema, loss of deep Bowman's glands, periosteal hypercellularity, and new bone growth in the proximal portions of the ethmoturbinates. The severity and relative distribution of the lesions were concentration-dependent, ranging from minimal involvement in rats exposed to 10 ppm to moderate to severe effects in rats exposed to 300 ppm. Focal atrophy of the olfactory epithelium was noted in rats exposed to 90 or 300 ppm.

Larson et al. (1996) investigated the ability of chloroform vapors to produce toxicity and regenerative cell proliferation in the liver, kidneys, and nasal passages of female B6C3F1 mice. Groups of five animals were exposed to target concentrations of 0, 0.3, 2, 10, 30, or 90 ppm chloroform (via inhalation for 6 hours/day for 4 consecutive days). At necropsy, livers and kidneys were removed, weighed, examined macroscopically, and prepared for microscopic evaluation. The nasal cavities and non-nasal bones (sternum with rib, vertebrae, tibia, femur) were also removed and prepared for microscopic evaluation. Animals were administered BrdU via an implanted osmotic pump for the last 3.5 days. Cell proliferation was quantitated as the percentage of cells in S-phase (labeling index [LI]) measured by immuno-histochemical detection of BrdU-labeled nuclei.

Analytical concentrations were 0, 0.3, 1.99, 10.0, 29.6, and 88 ppm. No clinical signs of toxicity were noted in females exposed to chloroform for 4 days. Relative kidney weights were similar to controls at all chloroform exposure levels; however, exposure to 90 ppm chloroform resulted in increased relative liver weights. Female mice exposed to chloroform for 4 days experienced a dose-dependent mild response of uniform hepatocyte lipid vacuolization. Scattered hepatocyte necrosis also occurred in a dose-dependent manner. Hepatic LI was significantly elevated in female mice in the 90-ppm dose group after 4 days exposure (9-fold; $p<0.05$). Kidneys of female mice exposed to chloroform were not different from those of controls at any dose. Exposure to chloroform did not significantly affect the kidney cortex LI in females at any dose. Mild, transient changes occurred in the posterior ventral areas of nasal tissue in female mice exposed to 10, 30, and 90 ppm chloroform. The lesions were characterized by mild proliferative responses in the periosteum consisting of a thickening of this bone. The adjacent lamina also exhibited loss of acini of Bowman's glands and vascular congestion. No microscopic changes were noted in non-nasal bones, nor were non-nasal bone LIs different from those of controls.

Selection of the Point of Departure for the MRL: The NOAEL of 2 ppm based on nasal lesions in rats and mice was selected as the POD as it provides the lowest POD for the critical effect. As mentioned above, data were either unavailable for BMD modeling (Templin et al. 1996b) or failed to produce any model fits (Larson et al. 1996).

Adjustment for Intermittent Exposure and Human Equivalent Concentration: As shown above in equations after Table A-8, the NOAEL of 2 ppm was adjusted for continuous exposure and converted into a $NOAEL_{HEC}$ of 0.04 ppm. The associated LOAEL of 10 ppm was adjusted for continuous exposure and converted into a $LOAEL_{HEC}$ of 0.19 ppm as shown below.

Templin et al. (1996b); rats:

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 10\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{4\ days}{7\ days} = 1.4\ ppm$$

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 1.4\ ppm \times 0.132 = 0.19\ ppm$$

Larson et al. (1996); mice:

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 10\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{4\ days}{7\ days} = 1.4\ ppm$$

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 1.4\ ppm \times 0.136 = 0.19\ ppm$$

Uncertainty Factors: The following uncertainty factors were applied to the $NOAEL_{HEC}$ to derive the MRL:

- Uncertainty factor of 3 for extrapolation from animals to humans with dosimetric adjustments
- Uncertainty factor of 10 for human variability

Subsequently, the MRL for acute-duration exposure to chloroform via inhalation is:

$$MRL = \frac{NOAEL_{HEC}}{(UF)} = \frac{0.04\ ppm}{30} = 0.001\ ppm$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that respiratory effects are a presumed health effect following exposure to chloroform based on inadequate evidence from human epidemiology studies and a high level of evidence from animal studies (Appendix C).

Lung damage has been reported in several fatal cases of inhalation exposure (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965). Case reports have also documented changes in respiratory rate and/or respiratory arrest at high exposure levels (Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965), but these effects are likely secondary to CNS depression. No data pertaining to potential nasal effects in humans following exposure to chloroform were identified. In animals, the nasal epithelium is a consistent target of toxicity in rodents following acute-, intermediate-, and chronic-duration inhalation exposure (Constan et al. 1999; Kasai et al. 2002; Larson et al. 1994c, 1996; Mery et al. 1994; Templin et al. 1996b; Yamamoto et al. 2002) and acute- and intermediate-duration gavage exposure (Dorman et al. 1997; Larson et al. 1995b; Templin et al. 1996a). Findings show a dose- and duration-dependent increase in the incidence and severity of lesions. The MRL based on nasal lesions is expected to be protective of lower respiratory effects, as damage to the lower respiratory tract in animals was generally only observed at lethal exposure levels (Bowman et al. 1978; Kasai et al. 2002; NCI 1976). Only minimal evidence of inflammatory responses has been reported in the lung at low inhalation exposure levels in mice (de Oliveira et al. 2015).

Agency Contacts (Chemical Managers): Rae T. Benedict, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Inhalation
Duration: Intermediate
MRL: 0.0008 ppm (0.004 mg/m^3)
Critical Effect: Nasal lesions
Reference: Templin et al. 1996b
Point of Departure: LOAEL of 2 ppm ($LOAEL_{HEC}$ of 0.07 ppm)
Uncertainty Factor: 90
LSE Graph Key: 37
Species: Rat

MRL Summary: An intermediate-duration inhalation MRL of 0.0008 ppm was derived for chloroform based on nasal lesions in rats exposed to concentrations ≥2 ppm for 13 weeks (7 days/week; 6 hours/day); a NOAEL was not identified (Templin et al. 1996b). The MRL is based on the LOAEL of 2 ppm, which was adjusted to continuous duration exposure and converted to a $LOAEL_{HEC}$ of 0.07 ppm and divided by a total uncertainty factor of 90 (3 for use of a minimal LOAEL, 3 for extrapolation from animals to humans after dosimetric adjustment, and 10 for human variability).

Selection of the Critical Effect: No intermediate-duration human studies with reliable exposure estimates were identified. The most sensitive effects following intermediate-duration inhalation exposure were respiratory effects, specifically damage to the nasal turbinates and overlying epithelial tissues (Table A-10).

Table A-10. Selected NOAEL and LOAEL Values in Animals Following Intermediate-Duration Inhalation Exposure to Chloroform

		Effect level (ppm)			
Species	Duration	NOAEL	LOAEL	Effect	Reference
Respiratory					
Fischer 344 rats	6 or 13 weeks 7 days/week 6 hours/day	ND	2	Loss of olfactory glands and edema in the nasal lamina propria; atrophy of ethmoid turbinate	Templin et al. 1996b
Fischer 344 rats	3 weeks 7 days/week 6 hours/day	2	10	Loss of olfactory glands; edema, and cellular proliferation in the nasal lamina propria	Templin et al. 1996b
BDF1 mouse	13 weeks 5 days/week 6 hours/day	ND	12	Eosinophilic change of olfactory and respiratory epithelia in females; thickening of nasal bones in both sexes	Kasai et al. 2002
Body weight					
BDF1 mouse	13 weeks 5 days/week 6 hours/day	ND	5	17% decrease in percent body weight gain	Templin et al. 1998

Table A-10. Selected NOAEL and LOAEL Values in Animals Following Intermediate-Duration Inhalation Exposure to Chloroform

Species	Duration	Effect level (ppm) NOAEL	LOAEL	Effect	Reference
Renal					
BDF1 mouse	13 weeks 5 days/week 6 hours/day	ND	12	Necrosis and cytoplasmic basophilia in the proximal tubules and proteinuria in males	Kasai et al. 2002

LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

Selection of the Principal Study: The 13-week study in rats by Templin et al. (1996b) was selected as the principal study because it provided the lowest candidate POD for the critical effect (nasal lesions).

Summary of the Principal Study:

Templin MV, Larson JL, Butterworth BE, et al. 1996b. A 90-day chloroform inhalation study in F-344 rats: profile of toxicity and relevance to cancer studies. Fundam Appl Toxicol 32(1):109-125.

Templin et al. (1996b) exposed nine-week-old male and female F344 rats (10/sex/group) to target concentrations of 0, 2, 10, 30, 90, or 300 ppm chloroform for 13 weeks via whole-body inhalation (7 days/week; 6 hours/day). BrdU was administered 3.5 days prior to sacrifice in 8/group in control and 30-, 90-, and 300-ppm groups. Endpoints evaluated included clinical signs, body weight, organ weights (liver and kidney), gross necropsy, histopathology on a complete set of tissues, and cell proliferation in the liver, kidney, and nasal tissues.

Average analytical exposure concentrations were always within 4.5% of the target (quantitative data not reported). No deaths were reported. Rats receiving the higher concentrations of chloroform exhibited signs of mild dehydration in the second week and, at the later time points, slight hair loss, discharge from the eyes and anogenital staining (data not shown). Body weight gains were significantly decreased in males at 90 ppm (40%) and 300 ppm (9%), compared to control (54%); estimated based on graphically presented data. In females, body weight gain was significantly decreased at 10 ppm (29%), 30 ppm (30%), 90 ppm (20%), and 300 ppm (5%), compared to control (36%); estimated based on graphically presented data. Relative liver weights were increased in males at 300 ppm (~30%) and in females increased at 90 ppm (~10%) and 300 ppm (~50%). Relative kidney weights were increased at 90 ppm in males (~10%) and females (~25%) and at 300 ppm in males (~30%) and females (~50%). Organ weight findings may be secondary to body weight effects (absolute organ weights were not reported); however, the study authors noted that increased female liver weight was associated with periductal fibrosis. Hepatocellular vacuolation and hepatocyte degeneration and/or necrosis was observed in both sexes at ≥90 ppm. Foci of adenofibrosis (intestinal-crypt-like ducts with periductular fibrosis) were observed in both sexes at 300 ppm (more severe in females). Hepatocellular proliferation was observed in both sexes at 300 ppm. In the kidney, vacuolation in the proximal convoluted tubule and scattered focal necrosis were observed in males at ≥90 ppm. Females showed scattered regenerating proximal convoluted tubules with anisokaryosis and megalokaryosis. Renal cell proliferation was observed in both sexes at ≥30 ppm. Nasal lesions were observed in 100% of exposed male rats; no nasal lesions were observed in control males. The most prevalent effects were atrophy of the ethmoid turbinates, loss of Bowman's glands, and mild-to-moderate edema in the lamina propria. Mineralization of the basal lamina was observed at 300 ppm and the olfactory epithelium showed focal edema and conversion to respiratory epithelium. Lesions were minimal at 2 ppm, mild at 10 and 30 ppm, mild-to-moderate at 90 ppm, and moderate-to-severe at

300 ppm. The study authors noted that nasal lesions in female rats were "similar to those found in the male;" however, quantitative data were not provided. Nasal cellular proliferation was noted at ≥10 ppm. No other tissue was affected by chloroform exposure.

Selection of the Point of Departure for the MRL: The LOAEL of 2 ppm based on nasal lesions in male rats was selected as the POD as it provides the lowest POD for the critical effect. The data are not amenable to BMD modeling because the response in male rats goes from 0% incidence in the control group to 100% incidence in all exposure groups.

Adjustment for Intermittent Exposure: The LOAEL of 2 ppm was adjusted for continuous exposure.

$$LOAEL_{ADJ} = LOAEL \times \frac{hours/day}{24\ hours} \times \frac{days/week}{7\ days} = 2\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{7\ days}{7\ days} = 0.5\ ppm$$

Human Equivalent Concentration: Sarangapani et al. (2002) is the only published chloroform model that simulates doses to the nasal cavity tissues. The model has been validated against observations made in rats, but not other laboratory animal models or humans. ATSDR typically requires that models used for dosimetry extrapolation in derivation of MRLs be validated in the species to which they are applied. As the principal study uses mice, the Sarangapani et al. (2002) model was not used for dosimetry extrapolation in deriving the intermediate-duration inhalation MRL. Instead, the $LOAEL_{ADJ}$ was converted to a $LOAEL_{HEC}$ using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the $RGDR_{ET}$. This $RGDR_{ET}$ is calculated using the following equation as defined by EPA (1994):

$$RGDR_{ET} = \frac{V_{E_a}}{SA_a} \div \frac{V_{E_h}}{SA_h} = \frac{0.137}{15} \div \frac{13.8}{200} = 0.132$$

where:
V_{E_a} = ventilation rate for male F344 rats = 0.137 L/minute
SA_a = surface area of the extrathoracic region in rats = 15 cm^2
V_{E_h} = ventilation rate for humans = 13.8 L/minute
SA_h = surface area of the extrathoracic region in humans = 200 cm^2

Applying this equation results in an RGDR of 0.13 for extrathoracic effects in F344 rats, and the HEC is calculated as:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 0.5\ ppm \times 0.132 = 0.07\ ppm$$

Uncertainty Factors: The following uncertainty factors were applied to the $LOAEL_{HEC}$ to derive the MRL:

- Uncertainty factor of 3 for use of a minimal LOAEL (nasal lesions of minimal severity)
- Uncertainty factor of 3 for animal to human extrapolation with applying dosimetric adjustment
- Uncertainty factor of 10 for human variability

Subsequently, the inhalation MRL for intermediate-duration exposure to chloroform is:

$$MRL = \frac{LOAEL_{HEC}}{(UF)} = \frac{0.07\ ppm}{90} = 0.0008\ ppm$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that respiratory effects are a presumed health effect following exposure to chloroform based on inadequate evidence from human epidemiology studies and a high level of evidence from animal studies (Appendix C).

Lung damage has been reported in several fatal cases of inhalation exposure (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965). Case reports have also documented changes in respiratory rate and/or respiratory arrest at high exposure levels (Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965), but these effects are likely secondary to CNS depression. No data pertaining to potential nasal effects in humans following exposure to chloroform were identified. In animals, the nasal epithelium is a consistent target of toxicity in rodents following acute-, intermediate-, and chronic-duration inhalation exposure (Constan et al. 1999; Kasai et al. 2002; Larson et al. 1994c, 1996; Mery et al. 1994; Templin et al. 1996b; Yamamoto et al. 2002) and acute- and intermediate-duration gavage exposure (Dorman et al. 1997; Larson et al. 1995b; Templin et al. 1996a). Findings show a dose- and duration-dependent increase in the incidence and severity of lesions.

Agency Contact (Chemical Manager): Rae T. Benedict, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Inhalation
Duration: Chronic
MRL: 0.0004 ppm (0.002 mg/m^3)
Critical Effect: Nasal lesions
Reference: Yamamoto et al. 2002
Point of Departure: LOAEL of 5.0 ppm ($LOAEL_{HEC}$ of 0.11 ppm)
Uncertainty Factor: 300
LSE Graph Key: 52
Species: Mouse

MRL Summary: A chronic-duration inhalation MRL of 0.0004 ppm was derived for chloroform based on nasal lesions in female mice exposed to concentrations ≥5 ppm for 104 weeks (5 days/week; 6 hours/day); a NOAEL was not identified (Yamamoto et al. 2002). The MRL is based on the LOAEL of 5.0 ppm, which was adjusted to continuous duration exposure and converted to a $LOAEL_{HEC}$ of 0.11 ppm and divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans after dosimetric adjustment, and 10 for human variability).

Selection of the Critical Effect: Both human and animal data were considered while determining the critical effects (Table A-11). The only chronic-duration human study with dose-response data is an occupational study by Li et al. (1993). A LOAEL of 2.76 ppm was identified for workers occupationally exposed to chloroform for 1–15 years based on impaired performance on the pursuit aiming task, indicating impaired hand-eye coordination. In animal studies, the most sensitive target of toxicity was nasal effects in mice at ≥5 ppm.

Table A-11. Selected NOAEL and LOAEL Values in Humans and Animals Following Chronic-Duration Inhalation Exposure to Chloroform

Species	Duration	Effect level (ppm) NOAEL	LOAEL	Effect	Reference
Neurological					
Human	1–15 years 5 days/week[a] 8 hours/day[a]	ND	2.76	Impaired hand-eye coordination	Li et al. 1993
Respiratory					
BDF1 mouse	104 weeks 5 days/week 6 hours/day	ND	5.0	Atrophy and respiratory metaplasia of the olfactory epithelium; thickening of nasal bones	Yamamoto et al. 2002
Fischer 344 rat	104 weeks 5 days/week 6 hours/day	ND	10.1	Atrophy and respiratory metaplasia of the olfactory epithelium; thickening of nasal bones	Yamamoto et al. 2002

Table A-11. Selected NOAEL and LOAEL Values in Humans and Animals Following Chronic-Duration Inhalation Exposure to Chloroform

Species	Duration	Effect level (ppm) NOAEL	LOAEL	Effect	Reference
Renal					
Fischer 344 rat	104 weeks 5 days/week 6 hours/day	10.1	30.0	Nuclear enlargement of the proximal tubules and dilation of the tubular lumen	Yamamoto et al. 2002

[a]Assuming a 40-day work week.

LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

In order to accurately compare PODs across study designs, species, and target tissues, candidate PODs were adjusted for continuous exposure in both studies, and a HEC value was calculated for the nasal effects in mice.

Li et al. (1993); human (neurological effects):

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 2.76\ ppm \times \frac{8\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 0.657\ ppm$$

Yamamoto et al. (2002); mouse (nasal effects):

The nasal LOAEL was adjusted for continuous exposure.

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 5.0\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 0.89\ ppm$$

The $LOAEL_{ADJ}$ was converted to a $LOAEL_{HEC}$ using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the $RGDR_{ET}$. This $RGDR_{ET}$ is calculated using the following equation as defined by EPA (1994):

$$RGDR_{ET} = \frac{V_{Ea}}{SA_a} \div \frac{V_{Eh}}{SA_h} = \frac{0.0245}{3} \div \frac{13.8}{200} = 0.118$$

where:
V_{E_a} = ventilation rate for female BDF1 mice = 0.0245 L/minute (Yamamoto et al. 2002)
SA_a = surface area of the extrathoracic region in mice = 3 cm^2 (EPA 1994)
V_{E_h} = ventilation rate for humans = 13.8 L/minute (EPA 1994)
SA_h = surface area of the extrathoracic region in humans = 200 cm^2 (EPA 1994)

Applying this equation results in an RGDR of 0.118 for extrathoracic effects in female BDF1 mice, and the HEC is calculated as shown below.

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 0.89\ ppm \times 0.118 = 0.11\ ppm$$

The candidate human and animal chronic-duration inhalation PODs are summarized in Table A-12.

Table A-12. Summary of Candidate Effects and POD Values Considered for Derivation of a Chronic-Duration Inhalation MRL for Chloroform

Species	Duration	Effect	Candidate POD (ppm)	POD type	Reference
Neurological effects					
Human	1–15 years 5 days/week[a] 8 hours/day[a]	Impaired hand-eye coordination	0.657	$LOAEL_{ADJ}$	Li et al. 1993
Respiratory effects					
BDF1 mouse	104 weeks 5 days/week 6 hours/day	Atrophy and respiratory metaplasia of the olfactory epithelium; thickening of nasal bones	0.11	$LOAEL_{HEC}$	Yamamoto et al. 2002

[a]Assuming a 40-hour work week.

ADJ = adjusted; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; MRL = Minimal Risk Level; POD = point of departure

Based on values in Table A-12, nasal lesions were selected as the critical effect because they provide the lowest candidate POD. Additionally, there is clear evidence of concentration- and duration-dependent increases in incidence and/or severity of nasal lesions in acute-, intermediate-, and chronic-duration animal studies. While selecting neurological effects from the human study would decrease uncertainty with regard to animal to human extrapolation, there are considerable uncertainties associated with the study by Li et al. (1993), including: 1) limited information regarding methods and timing of exposure assessment; 2) limited information regarding controls (identified only as individuals "without obvious exposure to occupational hazards"; 3) no information on potential concurrent exposures to other solvents or potentially neurotoxic compounds; and 4) relatively small group numbers, especially at the LOAEL (60 control, 14 low-exposure [2.76 ppm], 46 high-exposure [6.04 ppm]). Based on these limitations, systematic review determined that the Li et al. (1993) is a second-tier risk of bias study of low confidence (Appendix C).

Selection of the Principal Study: The 104-week study in mice by Yamamoto et al. (2002) was selected as the principal study because it provided the lowest candidate POD (0.11 ppm) for the critical effect (nasal lesions).

Summary of the Principal Study:

Yamamoto S, Kasai T, Matsumoto, et al. 2002. Carcinogenicity and chronic toxicity in rats and mice exposed to chloroform by inhalation. J Occup Health 44(5):283-293. https://doi.org/10.1539/joh.44.283.

Six-week-old male and female Crj:BDF1 mice (50/sex/group) were exposed to 0, 5, 30, or 90 ppm chloroform via whole-body inhalation for 6 hours/day, 5 days/week, for 104 weeks. Analytical concentrations were reported as 5.0, 10.1, 30.0, and 90.1 ppm. To avoid lethality, the 30- and 90-ppm exposure groups underwent stepwise exposure paradigms over the first 4–6 weeks. Time weighted averages (TWAs) were calculated from the analytical concentrations and exposure duration (2 weeks at 5.0 ppm, 2 weeks at 10.1 ppm, and 100 weeks at 30.0 ppm for the 30-ppm group; 2 weeks at 5.0 ppm,

2 weeks at 10.1 ppm, 2 weeks at 30.0 ppm, and 98 weeks at 90.1 ppm for the 90-ppm group), resulting in final TWA exposure concentrations of 0, 5.0, 29.1, and 85.8 ppm. Endpoints evaluated included lethality, clinical signs, body weight, food and water intake, hematology, blood chemistry, urinalysis, organ weights, gross necropsy, and histopathology. A complete set of tissues were examined.

Chloroform exposure did not affect survival rate (50–76%) or lead to any overt clinical signs of toxicity compared to control (using the stepwise protocol). Body weight was significantly decreased in males and females at all doses throughout the first year of the study, but subsequently recovered to control levels in the two lower dose female groups. The magnitude of decrease is unknown (data not reported). Food consumption was similar between exposed and control mice. No significant changes in hematological parameters were observed (data not shown). Serum chemistry changes included significant increases in serum AST, ALT, and BUN in males and females at 85.8 ppm. Serum ALP was also increased in males. No difference was seen in the urinalysis. Absolute, but not relative, kidney weight was significantly increased in males at 85.8 ppm (data not shown; attributed to tumors). No other organ weight data were reported. Gross examination showed increased incidences of renal nodules in males at 29.1 and 85.8 ppm, but not in the females (data not shown). Microscopic changes included significant increases in fatty change in the liver of males and females at 85.8 ppm and lesions in the renal proximal tubule (nuclear enlargement, cytoplasmic basophilia, hyperplasia) in males at ≥29.1 ppm. Kidney damage in females was markedly lower than in males, with the only change being a slight significant increase in cytoplasmic basophilia at 85.8 ppm. In the nasal cavity, thickening of bone was noted in both sexes at ≥5.0 ppm exposure with atrophy and respiratory metaplasia of the olfactory epithelium occurring in males at 85.8 ppm and in females at ≥5.0 ppm. In males, significant increases were seen in the incidence of renal adenoma or carcinoma (combined) at 29.1 ppm (7/50) and 85.8 ppm (12/48) and renal carcinoma at 85.8 ppm (11/48) compared to control (a significant positive trend for these tumors was noted). No renal tumors occurred in control males or female mice of any group. Incidence of liver tumors was not increased in any exposure group, although significant positive trends were found for hepatocellular adenoma or carcinoma (combined) and carcinoma in both males and females. No nonneoplastic or neoplastic lesions were increased in other organs.

Selection of the Point of Departure for the MRL: The LOAEL of 5.0 ppm for nasal lesions in mice was selected as it provided the lowest POD for the critical effect. The principal study only provided incidence data for neoplastic lesions; however, incidence data were available in unpublished Japanese-language reports with English tables (MHLW 1994b). Data for nasal lesions could not be BMD modeled because incidence went from 0% in controls to 100% at the lowest concentration.

Adjustment for Intermittent Exposure and Human Equivalent Concentration: Sarangapani et al. (2002) is the only published chloroform model that simulates doses to the nasal cavity tissues. The model has been validated against observations made in rats, but not other laboratory animal models or humans. ATSDR typically requires that models used for dosimetry extrapolation in derivation of MRLs be validated in the species to which they are applied. As the principal study uses mice, the Sarangapani et al. (2002) model was not used for dosimetry extrapolation in deriving the chronic-duration inhalation MRL. Therefore, the LOAEL was converted to a $LOAEL_{HEC}$ using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the $RGDR_{ET}$, as shown in the equations after Table A-11. The LOAEL of 5.0 ppm was adjusted for continuous exposure and converted into a $LOAEL_{HEC}$ of 0.11 ppm.

Uncertainty Factors: The following uncertainty factors were then applied to the $LOAEL_{HEC}$ to derive the MRL.

- 10 for use of a LOAEL
- 3 for extrapolation from animals to humans with dosimetric adjustments

- 10 for human variability

Subsequently, the inhalation MRL for chronic-duration exposure to chloroform is:

$$MRL = \frac{LOAEL_{HEC}}{UFs} = \frac{0.11\ ppm}{300} = 0.00037\ ppm\ \approx 0.0004\ ppm$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that respiratory effects are a presumed health effect following exposure to chloroform based on inadequate evidence from human epidemiology studies and a high level of evidence from animal studies (Appendix C).

Lung damage has been reported in several fatal cases of inhalation exposure (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965). Case reports have also documented changes in respiratory rate and/or respiratory arrest at high exposure levels (Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965), but these effects are likely secondary to CNS depression. In animals, the nasal epithelium is a consistent target of toxicity in rodents following acute-, intermediate-, and chronic-duration inhalation exposure (Constan et al. 1999; Kasai et al. 2002; Larson et al. 1994c, 1996; Mery et al. 1994; Templin et al. 1996b; Yamamoto et al. 2002) and acute- and intermediate-duration gavage exposure (Dorman et al. 1997; Larson et al. 1995b; Templin et al. 1996a). Findings show a dose- and duration-dependent increase in the incidence and severity of lesions.

Agency Contact (Chemical Manager): Rae T. Benedict, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Oral
Duration: Acute
MRL: 0.3 mg/kg/day
Critical Effect: Hepatotoxicity (hepatic lesions)
Reference: Larson et al. 1994b
Point of Departure: NOAEL of 26 mg/kg/day
Uncertainty Factor: 100
LSE Graph Key: 33
Species: Mouse

MRL Summary: An acute-duration oral MRL of 0.3 mg/kg/day was derived for chloroform based on hepatic effects (centrilobular hepatocyte eosinophilic cytoplasm) in B6C3F1 mice following exposure to chloroform in drinking water for 4 days (Larson et al. 1994b). The MRL is based on a NOAEL of 26 mg/kg/day, which was divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Selection of the Critical Effect: No adequate acute-duration human data were available. Experimental acute-duration oral data in animals clearly show that rodents are more susceptible to chloroform toxicity via gavage exposure than drinking water exposure. The lowest acute-duration LOAELs identified in rats and mice via gavage exposure range from 10 to 34 mg/kg/day for respiratory, hepatic, renal, neurological, and developmental effects (Table A-13). In contrast, the lowest acute-duration LOAELs identified in rats and mice exposed via drinking water range from 53 to 81 mg/kg/day for hepatic effects and decreased body weights (Table A-14).

Table A-13. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Gavage Exposure to Chloroform

Species	Duration	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Renal					
Osborne-Mendel rat	Once	ND	10	Regenerative cell proliferation in the epithelial cells of the proximal tubules of the renal cortex	Templin et al. 1996a
Fischer 344 rat	4 days	10	34	Mild-to-moderate degeneration of renal proximal tubules and tubule epithelial cell proliferation	Larson et al. 1993
Fischer 344 rat	Once	ND	34	Scattered necrosis of the renal proximal tubule	Larson et al. 1993
B6C3F1 mouse	4 days	ND	34	Extensive acute necrosis of the proximal convoluted tubule, regenerative cell proliferation in the renal cortex and medulla	Larson et al. 1994d

Table A-13. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Gavage Exposure to Chloroform

Species	Duration	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Developmental					
Dutch belted rabbit	13 days GDs 6–18	ND	20	8% decrease in fetal body weight, delayed ossification	Thompson et al. 1974
Neurological					
CD-1 mouse	10 days	10	30	Conditioned taste aversion to saccharin	Landauer et al. 1982
Hepatic					
Fischer 344 rat	4 days	10	34	Increased relative liver weight	Larson et al. 1993
Respiratory					
Fischer 344 rat	4 days	ND	34	Degeneration of the olfactory epithelium and superficial Bowman's glands; periosteal hypercellularity	Larson et al. 1995b

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

Table A-14. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Drinking Water Exposure to Chloroform

Species	Duration	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Renal					
Fischer 344 rat	Drinking water	68.1	ND		Larson et al. 1995a
B6C3F1 mouse	Drinking water	105	ND		Larson et al. 1994b
Hepatic					
B6C3F1 mouse	Drinking water	26	53	Centrilobular hepatocyte eosinophilic cytoplasm	Larson et al. 1994b
Fischer 344 rat	Drinking water	68.1	ND		Larson et al. 1995a
Body weight					
Fischer 344 rat	Drinking water	33.2	57.5	17% decrease in body weight gain	Larson et al. 1995a
B6C3F1 mouse	Drinking water	53	81	20% body weight loss	Larson et al. 1994b

LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

Increased toxicity in rodents following acute-duration gavage exposure, compared to drinking water, is likely due to saturation of detoxification pathways following bolus gavage exposure, which exacerbates toxicity due to accumulation of toxic metabolites in hepatic and renal tissues. Specifically, it is proposed that the reaction of chloroform metabolites with GSH acts as a detoxifying mechanism. This is supported by observations that chloroform doses that caused liver GSH depletion produced liver necrosis (Docks and Krishna 1976). Additionally, exposure to chloroform via drinking water over the course of the day, rather than in a single bolus dose, may result in adaptive mechanisms. In support, hepatotoxicity in female mice associated with a 3-day gavage exposure to 263 mg/kg/day was attenuated if mice were exposed to chloroform at doses up to 520 mg/kg/day in drinking water for 3 weeks prior to gavage exposure (Pereira and Grothaus 1997). No literature was identified indicating similar adaptive changes regarding detoxification capacity following gavage exposure. Considering these chloroform-specific data regarding differential toxicity and toxicokinetics via gavage versus drinking water exposure, basing an oral MRL on the most sensitive endpoint following gavage exposure (renal toxicity) may be overly conservative and not applicable to lower, environmentally relevant exposure levels. Based on this rationale, findings from drinking water studies are considered more relevant to environmental exposure levels and scenarios. The most sensitive effects following drinking water exposure are hepatic effects in mice at 53 mg/kg/day (Table A-14). Therefore, hepatic effects are selected as the critical effect for derivation of the acute-duration oral MRL.

Selection of the Principal Study: The 4-day study in mice by Larson et al. (1994b) is selected as the principal study because it provides the highest NOAEL below the lowest LOAEL for the critical effect (hepatotoxicity).

Summary of the Principal Study:

Larson JL, Wolf DC, Butterworth BE. 1994b. Induced cytotoxicity and cell proliferation in the hepatocarcinogenicity of chloroform in female B6C3F1 mice: comparison of administration by gavage in corn oil vs ad libitum in drinking water. Fund Appl Toxicol 22:90-102.

Groups of female B6C3F1 mice (14/group) were exposed to chloroform at drinking water concentrations of 0, 60, 200, 400, 900, or 1,800 ppm for 4 days. Mice were housed individually so accurate dose calculations could be made based on individual water consumption data. Body weights were recorded. After the 4-day exposure period, mice were sacrificed, and all animals were examined for macroscopic changes in the liver and kidney prior to being divided into three groups for analysis. Group 1 (five animals per group) was evaluated for serum clinical chemistry (ALT, SDH), liver and kidney weight, and liver and kidney histology. Group 2 (four animals per group) was evaluated for kidney histology. Group 3 (five animals per group) was evaluated for cellular proliferation (via BrdU labelling) in the liver and kidney.

Based on measured water intake and body weights, the study authors calculated average chloroform intakes of 0, 16.0, 26.4, 53.5, 80.9, and 105 mg/kg/day at 0, 60, 200, 400, 900, and 1,800 ppm, respectively. Dose-related decreases in body weights and water intake were observed, with an approximate 20% body weight loss during the exposure period at ≥900 ppm (data presented graphically). At necropsy, no exposure-related changes in serum ALT or SDH, gross pathology, or liver or kidney weights were observed. The study authors reported tinctorial changes, characterized by pale cytoplasmic eosinophilic staining of centrilobular hepatocytes, in 2/5, 8/10, and 4/5 mice, respectively; it is noted that the methods section indicates that only five per group were evaluated for liver histology. Liver histology at ≤200 ppm was reportedly not different from control (incidence data not reported). No exposure-related histopathological changes were noted in the kidney. Chloroform exposure via drinking water did not induce cell proliferation in either the liver or kidney.

Selection of the Point of Departure for the MRL: The NOAEL of 26 mg/kg/day for hepatic effects in the study by Larson et al. (1994b) was selected as the POD. While the study authors reported incidence data at ≥400 ppm (53.5 mg/kg/day), incidence data for ≤200 ppm (≤26 mg/kg/day) were not provided; therefore, BMD modeling was not used to derive this MRL.

Calculations: None. Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al. (1990) and Reitz et al. (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Both studies relied on data from studies of a single gavage dose (or in the case of humans, gelatin capsule dosing) of chloroform in oil-based vehicles. The models have not been validated for simulating dosimetry of repeated continuous exposures, such as daily ingestion of chloroform in drinking water. Application of either model to dosimetry extrapolation in the derivation of the acute MRL would be highly uncertain. The major uncertainty would be in extrapolating the internal doses from delivery of a large bolus dose to the liver from an oil gavage dose to the internal dose expected for repeated ingestion of chloroform in water. This extrapolation has not been validated. Therefore, the models were not used for dosimetry extrapolation in deriving the acute-duration MRL.

Uncertainty Factors: The following uncertainty factors were applied to the NOAEL to derive the MRL:

- Uncertainty factor of 10 for extrapolation from animals to humans
- Uncertainty factor of 10 for human variability

Subsequently, the oral MRL for acute-duration exposure to chloroform is:

$$Provisional\ MRL = \frac{NOAEL}{(UF)} = \frac{26\ mg/kg/day}{100} = 0.26\ mg/kg/day \approx 0.3\ mg/kg/day$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that hepatic effects are a known health effect following exposure to chloroform based on a low level of evidence from human epidemiology studies, high level of evidence from animal studies, and other relevant data consisting of the extensive database of case reports and case series documenting hepatic effects of chloroform in exposed humans (Appendix C).

Numerous case series and case reports indicate that the liver is a clear target of toxicity in humans following oral and inhalation exposure to high levels of chloroform (Section 2.9). In fatal ingestion cases, acute liver failure and/or severe liver damage have been found at autopsy (Dettling et al. 2016; Piersol et al. 1933). In nonfatal cases of oral ingestion, clinical signs of hepatotoxicity manifested within 1–7 days of exposure (Choi et al. 2006; Dell'Aglio et al. 2010; Hakim et al. 1992; Jayaweera et al. 2017; Kim 2008; Rao et al. 1993; Schroeder 1965; Sridhar et al. 2011; Storms 1973). Impaired liver function was observed in a man who ingested 21 mg/kg/day chloroform in a cough medicine for 10 years (Wallace 1950).

In animal studies, the liver is also a clear target of toxicity following acute-, intermediate-, and chronic-duration inhalation and oral studies in rodents; intermediate- and chronic-duration oral studies in dogs; and an acute-duration oral study in rabbits (Section 2.9). Findings in rodents following gavage exposure range from changes in clinical chemistry and mild histopathological damage after lower, shorter exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation), with widespread and severe necrosis and degeneration with higher and/or longer durations. As discussed above, the rodent liver is less susceptible to toxicity following drinking water exposure, presumably due to saturation of metabolic detoxification pathways with bolus gavage exposure.

Agency Contact (Chemical Manager): Rae T. Benedict, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL: 0.1 mg/kg/day
Critical Effect: Hepatotoxicity (increased serum ALT)
Reference: Heywood et al. 1979
Point of Departure: NOAEL of 15 mg/kg/day ($NOAEL_{ADJ}$ of 13 mg/kg/day)
Uncertainty Factor: 100
LSE Graph Key: 74
Species: Dog

MRL Summary: An intermediate-duration oral MRL of 0.1 mg/kg/day was derived for chloroform based on hepatic effects (~2-fold increase in serum ALT) in Beagle dogs following exposure to chloroform for 26–52 weeks (6 days/week) via toothpaste capsule (Heywood et al. 1979). The MRL is based on a NOAEL of 15 mg/kg/day, which was adjusted to a continuous duration dose ($NOAEL_{ADJ}$) of 13 mg/kg/day and divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Selection of the Critical Effect: No adequate intermediate-duration oral studies in humans were identified. As discussed in the acute-duration oral MRL worksheet above, rodents are more susceptible to chloroform toxicity via gavage exposure than drinking water exposure following intermediate-duration oral exposure. This pattern is clearly shown in a series of 21-day studies in rats and mice by Larson et al. (1994b, 1995a; Table A-15).

Table A-15. Comparison of Toxicity in Rodents Following a 21-Day Exposure to Chloroform via Gavage versus Drinking Water Exposure

Species	Route	Effect level (mg/kg/day) NOAEL	Effect level (mg/kg/day) LOAEL	Effect	Reference
Hepatic					
Fischer 344 rat	Gavage in oil	34	100	Increased hepatocellular proliferation	Larson et al. 1995a
Fischer 344 rat	Drinking water	106	ND		
B6C3F1 mouse	Gavage in oil	10	34	Mild vacuolation of hepatocytes, increased serum ALT and SDH	Larson et al. 1994b
B6C3F1 mouse	Drinking water	43	82	Increased relative liver weight	

Table A-15. Comparison of Toxicity in Rodents Following a 21-Day Exposure to Chloroform via Gavage versus Drinking Water Exposure

Species	Route	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Renal					
Fischer 344 rat	Gavage in oil	34	100	Increased proliferation of proximal tubule epithelial cells in renal cortex	Larson et al. 1995a
Fischer 344 rat	Drinking water	106	ND		

ALT = alanine aminotransferase; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level; SDH = sorbitol dehydrogenase

Based on the rationale discussed in the acute-duration oral MRL worksheet above, gavage studies in rodents were not considered for intermediate-duration oral MRL derivation. The most sensitive effects in drinking water studies in rodents and oral exposure studies in other species are hepatic effects in dogs at ≥30 mg/kg/day and renal and gastrointestinal effects in Eker rats at ≥27 mg/kg/day (Table A-16). Findings in Eker rats is not considered an appropriate basis for the MRL since it is an animal model of hereditary renal cancer (McDorman et al. 2003a). Additionally, no additional drinking water studies in rats or mice report adverse renal or gastrointestinal effects (Table A-16). Therefore, hepatic effects are selected as the critical effect for derivation of the intermediate-duration oral MRL.

Table A-16. Selected NOAEL and LOAEL Values in Animals Following Intermediate-Duration Oral Exposure to Chloroform

Species	Duration (route)	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Hepatic effects					
Beagle dog	26–52 weeks (C) 6 days/week	15	30	~2-fold increase in serum ALT	Heywood et al. 1979
B6C3F1 mouse	3 weeks (W)	43	82	Increased relative liver weight	Larson et al. 1994b
Fischer 344 rat	3 weeks (W)	106	ND		Larson et al. 1995a
B6C3F1 mouse	90 days (W)	145	290	Increased fat content of the liver; centrilobular fatty changes	EPA 1980
Osborne-Mendel rat	90 days (W)	160	ND		EPA 1980
Fischer 344 rat	28 or 90 days (W)	200	ND		Chu et al. 1982a, 1982b

Table A-16. Selected NOAEL and LOAEL Values in Animals Following Intermediate-Duration Oral Exposure to Chloroform

Species	Duration (route)	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Renal effects					
Eker[a] rat	10 months (W)	ND	27	Increased incidence of atypical tubules and hyperplasia	McDorman et al. 2003a
Fischer 344 rat	3 weeks (W)	106	ND		Larson et al. 1995a
Osborne-Mendel rat	90 days (W)	160	ND		EPA 1980
Fischer 344 rat	28 or 90 days (W)	200	ND		Chu et al. 1982a, 1982b
B6C3F1 mouse	3 weeks (W)	329	ND		Larson et al. 1994b
B6C3F1 mouse	90 days (W)	435	ND		EPA 1980
Gastrointestinal effects					
Eker[a] rat	10 months (W)	ND	27	Increased incidence of aberrant crypt foci in the colon	McDorman et al. 2003a
Fischer 344 rat	13 weeks (W)	34	ND		DeAngelo et al. 2002
Fischer 344 rat	26 weeks (W)	35	ND		Geter et al. 2004b

[a]Animal model of hereditary renal cancer.

ALT = alanine aminotransaminase; (C) = capsule; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level; (W) = drinking water

Selection of the Principal Study: The 26–52-week study in dogs by Heywood et al. (1979) is selected as the principal study because it provides the highest NOAEL below the lowest LOAEL for the critical effect (hepatotoxicity). In the study by Heywood et al. (1979), dose delivery was via toothpaste-containing gelatin capsule. This route of exposure is not expected to mimic the bolus dose conditions of gavage administration. The capsule will disintegrate over time, resulting in a slower release of contents compared to bolus administration; thus, this mode of administration was considered to be relevant to human exposure conditions.

Summary of the Principal Study:

Heywood R, Sortwell RJ, Noel PRB, et al. 1979. Safety evaluation of toothpaste containing chloroform. III. Long-term study in beagle dogs. J Environ Pathol Toxicol 2:835-851.

In order to assess safety of toothpaste containing chloroform, groups of male and female Beagle dogs (8/sex/group) were exposed to chloroform in toothpaste-containing capsules at doses of 15 or 30 mg/kg/day for 6 days/week for up to 7.5 years. Control groups included untreated controls (8/sex),

vehicle (capsule) controls (16/sex), and an alternative non-chloroform toothpaste control (8/sex). During the intermediate-phase of the study (<1 year), blood was collected to measure hematology and clinical chemistry parameters at 6 and 13 weeks of exposure and at intervals of 8–32 weeks thereafter. Body weight, food intake, water intake, and clinical signs were monitored throughout the exposure period.

No dogs died during the first year of the study. No clinical signs of toxicity or body weight effects were observed. Serum ALT was significantly increased in males and females exposed to 30 mg/kg/day beginning at 6 weeks and at every interval thereafter. The observed increase was approximately 2-fold starting on week 26. ALT activity was not increased in dogs exposed to 15 mg/kg/day group until week 130. Therefore, 15 mg/kg/day is considered a NOAEL for intermediate-duration exposure. No additional changes in serum clinical chemistry or hematology were noted.

Selection of the Point of Departure for the MRL: The NOAEL of 15 mg/kg/day for hepatic effects in the study by Heywood et al. (1979) was selected as the POD. While study authors reported mean ALT activity values and results of the statistical analysis, a measure of variance was not provided; therefore, BMD modeling could not be used to derive this MRL.

Calculations: The NOAEL of 15 mg/kg/day was adjusted for a daily exposure scenario:

$$NOAEL_{ADJ} = NOAEL \times \frac{days\ exposed}{7\ days} = 15\ mg/kg/day \times \frac{6\ days}{7\ days} = 13\ mg/kg/day$$

Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al. (1990) and Reitz et al. (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Neither of these studies evaluated dogs and are therefore not suitable for dose extrapolation.

Uncertainty Factors: The following uncertainty factors were applied to the $NOAEL_{ADJ}$ to derive the MRL:

- Uncertainty factor of 10 for extrapolation from animals to humans
- Uncertainty factor of 10 for human variability

Subsequently, the oral MRL for intermediate-duration exposure to chloroform is:

$$MRL = \frac{NOAEL_{ADJ}}{(UF)} = \frac{13\ mg/kg/day}{100} = 0.13\ mg/kg/day \approx 0.1\ mg/kg/day$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that hepatic effects are a known health effect following exposure to chloroform based on a low level of evidence from human epidemiology studies, high level of evidence from animal studies, and other relevant data consisting of the extensive database of case reports and case series documenting hepatic effects of chloroform in exposed humans (Appendix C).

Numerous case series and case reports indicate that the liver is a clear target of toxicity in humans following oral and inhalation exposure to high levels of chloroform (Section 2.9). In fatal ingestion cases, acute liver failure and/or severe liver damage have been found at autopsy (Piersol et al. 1933; Dettling et al. 2016). In nonfatal cases of oral ingestion, clinical signs of hepatotoxicity manifest within 1–7 days of exposure (Choi et al. 2006; Dell'Aglio et al. 2010; Hakim et al. 1992; Jayaweera et al. 2017; Kim 2008; Rao et al. 1993; Schroeder 1965; Sridhar et al. 2011; Storms 1973). Impaired liver function was observed in a man who ingested 21 mg/kg/day chloroform in a cough medicine for 10 years (Wallace 1950).

In animal studies, the liver is also a clear target of toxicity following acute-, intermediate-, and chronic-duration inhalation and oral studies in rodents; intermediate- and chronic-duration oral studies in dogs; and an acute-duration oral study in rabbits (Section 2.9). Findings in rodents following oral gavage exposure range from changes in clinical chemistry and mild histopathological damage after lower, shorter exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation), with widespread and severe necrosis and degeneration with higher and/or longer exposure durations. As discussed above, the rodent liver is less susceptible to toxicity following drinking water exposure, presumably due to saturation of metabolic detoxification pathways with bolus gavage exposure.

Agency Contact (Chemical Manager): Rae T. Benedict, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Oral
Duration: Chronic
MRL: 0.02 mg/kg/day
Critical Effect: Hepatotoxicity (moderate-to-marked fatty cysts)
Reference: Heywood et al. 1979
Point of Departure: $BMDL_{10}$ of 2.15 mg/kg/day ($BMDL_{ADJ}$ of 1.84 mg/kg/day)
Uncertainty Factor: 100
LSE Graph Key: 84
Species: Dog

MRL Summary: A chronic-duration oral MRL of 0.02 mg/kg/day was derived for chloroform based on hepatic effects (moderate-to-marked fatty cysts) in Beagle dogs following exposure to chloroform for up to 7.5 years (6 days/week) via toothpaste capsule (Heywood et al. 1979). The MRL is based on a $BMDL_{10}$ of 2.15 mg/kg/day in male dogs, which was adjusted to a continuous duration dose ($BMDL_{ADJ}$) of 1.84 mg/kg/day and divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Selection of the Critical Effect: No adequate chronic-duration oral studies in humans were identified. The most sensitive chronic-duration oral LOAELs are shown in Table A-17. In contrast to findings in acute- and intermediate-duration studies, a clear increase in susceptibility was not observed in rodents exposed via gavage, compared to those exposed via drinking water (i.e., comparable lowest LOAEL values following chronic exposure). Several factors may contribute to this finding, including: (1) adaptive metabolic changes with chronic-duration exposure leading to blunting or attenuation of bolus effects; (2) lack of evaluation at low gavage doses in some studies (which may have potentially identified lower LOAELs); and/or (3) evaluation of different strains in chronic versus shorter-duration studies that may have differential susceptibility. However, dogs are more sensitive than rodents, regardless of oral exposure methodology. Therefore, the most sensitive endpoint in dogs (hepatotoxicity) is selected as the critical effect for derivation of the intermediate-duration oral MRL.

Table A-17. Selected NOAEL and LOAEL Values in Animals Following Chronic-Duration Oral Exposure to Chloroform

Species	Duration (route)	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Hepatic					
Beagle dog	7.5 years (C)	ND	15	Moderate-to-marked fatty cysts; ~2-fold increase in serum ALT	Heywood et al. 1979
ICI mouse	80 weeks (GO)	60	ND		Roe et al. 1979
Osborne-Mendel rat	78 weeks (GO)	100	200	Necrosis of hepatic parenchyma	NCI 1976

Table A-17. Selected NOAEL and LOAEL Values in Animals Following Chronic-Duration Oral Exposure to Chloroform

Species	Duration (route)	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
B6C3F1 mouse	78 weeks (GO)	ND	M: 138 F: 238	Nodular hyperplasia	NCI 1976
Renal					
Beagle dog	7.5 years (C)	15	30	Fat deposition in glomeruli	Heywood et al. 1979
Fischer 344 rat	104 weeks (W)	ND	45	Increased incidences of cytoplasmic basophilia and tubular lumen dilation in the proximal tubule	Nagano et al. 2006
ICI mouse	80 weeks (GO)	F: 60	M: 60	Moderate-to-severe kidney disease	Roe et al. 1979
Osborne-Mendel rat	104 weeks (W)	38	81	Renal tubule cell alterations	Hard et al. 2000; Jorgenson et al. 1985
Osborne-Mendel rat	78 weeks (GO)	200	ND		NCI 1976
B6C3F1 mouse	78 weeks (GO)	M: 277 F: 477	ND		NCI 1976

ALT = alanine aminotransaminase; (C) = capsule; F = females; (GO) = gavage in oil; LOAEL = lowest-observed-adverse-effect level; M = males; ND = not determined; NOAEL = no-observed-adverse-effect level; (W) = drinking water

Selection of the Principal Study: The 7.5-year study in dogs by Heywood et al. (1979) is selected as the principal study because it provides the highest NOAEL below the lowest LOAEL for the critical effect (hepatotoxicity). In the study by Heywood et al. (1979), dose delivery was via toothpaste-containing gelatin capsule. This route of exposure is not expected to mimic the bolus dose conditions of gavage administration. The capsule will disintegrate over time, resulting in a slower release of contents compared to bolus administration; thus, this mode of administration was considered to be relevant to human exposure conditions.

Summary of the Principal Study:

Heywood R, Sortwell RJ, Noel PRB, et al. 1979. Safety evaluation of toothpaste containing chloroform. III. Long-term study in beagle dogs. J Environ Pathol Toxicol 2:835-851.

In order to assess safety of toothpaste containing chloroform, groups of male and female Beagle dogs (8/sex/group) were exposed to chloroform in toothpaste orally via gelatin capsules at doses of 15 or 30 mg/kg/day for 6 days/week for up to 7.5 years followed by a 20–24-week observation period. Control groups included untreated controls (8/sex), vehicle (capsule) controls (16/sex), and an alternative non-chloroform toothpaste control (8/sex). Survival, clinical signs, food intake, and water intake were monitored throughout the exposure period. Blood was collected to measure hematology and clinical chemistry parameters at 6 and 13 weeks of exposure and at intervals of 8–32 weeks thereafter.

Ophthalmoscopy was performed prior to exposure and at 3-month intervals thereafter. During the 6th year of the study, bromosulfalein retention tests were conducted to assess liver function. At natural death or scheduled sacrifice, main organs (brain, pituitary, spinal cord, heart, lungs, liver, spleen, pancreas, thymus, prostate, uterus, kidneys, thyroids, adrenals, testes, ovaries) were removed and weighed, and a full microscopic examination was conducted on these tissues and all abnormalities. Electron microscopy was performed on liver and kidney sections from two untreated controls and three high-dose dogs (sex unspecified).

Several dogs died prior to scheduled sacrifice between week 87 and 328; however, mortalities were not exposure related. In male dogs, observed deaths included one from each of the following groups: untreated control, vehicle control, low-exposure, and high-exposure groups. In female dogs, three untreated controls and four vehicle controls died; all exposed animals survived until scheduled sacrifice. The study authors noted that about 20% of the dogs were hyperexcitable, mainly during the first 2–3 years. Some had convulsions, and 10 of the 11 reported fatalities occurred after such an attack. While study authors did not indicate which animal groups showed excitability, based on a lack of dose-related mortality it is assumed that neurological signs were not dose-related. No exposure-related changes were observed for body weight, food intake, or water intake. No exposure-related ophthalmological or hematological changes were noted. Serum ALT levels were significantly increased at 15 and 30 mg/kg/day starting on week 130 and 6, respectively. Elevations of ~2-fold were observed at week 260 and 26, respectively. Approximate 2-fold elevations in serum AST and serum ALP were also observed at the end of the exposure period (no statistical analysis provided). Serum enzyme levels recovered somewhat during the recovery period. The bromosulfalein retention test during the 6th year did not reveal any liver impairment. No organ weight changes were found in the exposed groups. Exposure-related nonneoplastic histopathological changes were observed in the liver and kidney. Fatty cysts were observed in the liver in all groups; however, incidence and severity increased in a dose-related manner, with moderate-to-marked fatty cysts significantly elevated in treated groups, compared to control. In males, moderate-to-marked fatty cysts were observed in 1/15, 6/7, and 6/7 dogs at 0 (vehicle control), 15, and 30 mg/kg/day, respectively. In females, moderate-to-marked fatty cysts were observed in 0/12, 3/8, and 7/8 dogs at 0 (vehicle control), 15, and 30 mg/kg/day, respectively. No moderate-to-marked fatty cysts were observed in untreated or non-chloroform toothpaste controls. Fat deposition in renal glomeruli was reportedly higher in the 30 mg/kg/day chloroform group (incidence data were not provided). No remarkable nonneoplastic histopathological differences were observed in other evaluated tissues. No exposure-related tumors were observed.

Selection of the Point of Departure for the MRL: In order to identify the most sensitive POD, BMD modeling was attempted for the incidence data for fatty cysts in male and female dogs (Heywood et al. 1979). BMD modeling was not conducted for serum ALT data because the study authors did not report a measure of variance for the means. The incidence data were fit to all available dichotomous models in EPA's BMDS (version 3.3) using a BMR of 10% extra risk. Adequate model fit was judged by four criteria: goodness-of-fit statistics (p-value >0.1), visual inspection of the dose-response curve, a 95% lower confidence limit on the BMD (BMDL) that is not 10 times lower than the lowest non-zero dose, and a scaled residual within ±2 units at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest BMDL was selected as the POD when the difference between the BMDLs estimated from these models was >3-fold; otherwise, the BMDL from the model with the lowest AIC was chosen.

The datasets used for BMD modeling are presented in Table A-18. Details of the modeling results for the model predictions for hepatic lesions in male and female dogs are in Tables A-19 and A-20, respectively. In accordance with the selection criteria mentioned above, the Logistic model, a frequentist, unrestricted model, was selected for males and the Probit model, a frequentist, unrestricted model, was selected for females.

Table A-18. Moderate-to-Marked Hepatic Fatty Cysts in Dogs Following Oral Exposure to Chloroform for up to 7.5 years

	Dose (mg/kg/day)		
	0 (vehicle)	15	30
Males Incidence (percent incidence)	1/15 (7%)	6/7[a] (86%)	6/7[a] (86%)
Females Incidence (percent incidence)	0/12 (0%)	3/8[a] (38%)	7/8[a] (88%)

[a]p<0.05 (2-tailed Fisher's Exact Probability Test, conducted for this review).

Source: Heywood et al. 1979

Table A-19. Model Predictions for Increased Incidence of Moderate-to-Marked Hepatic Fatty Cysts in Male Dogs Following Oral Exposure to Chloroform for up to 7.5 Years (Heywood et al. 1979)

					Scaled residuals[c]	
Model	BMD_{10}[a] (mg/kg/day)	$BMDL_{10}$[a] (mg/kg/day)	p-Value[b]	AIC	Dose below BMD	Dose above BMD
Dichotomous Hill			NA	26.83	-5.25×10^{-9}	1.25×10^{-8}
Gamma[d]			0.69	23.81	-0.04	0.35
Log-Logistic[e]			0.87	23.03	-0.002	0.12
Multistage Degree 2[f]			0.69	23.81	-0.04	0.35
Multistage Degree 1[f]			0.69	23.81	-0.04	0.35
Weibull[d]			0.69	23.81	-0.04	0.35
Logistic[g]	**3.83**	**2.15**	**0.35**	**26.09**	**-0.56**	**0.69**
Log-Probit			NA	NA	NA	NA
Probit	3.83	2.36	0.27	26.50	-0.58	0.89
Quantal Linear			0.69	23.81	-0.04	0.35

[a]BMD and BMDLs values for models that do not provide adequate fit are not included in this table.
[b]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD.
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. Only Logistic and Probit modes provided an adequate fit to the data. BMDLs were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected (Logistic).

AIC = Akaike Information Criterion; BMD = benchmark dose (maximum likelihood estimate of the dose associated with the selected benchmark response); $BMDL_{10}$ = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., 10 = dose associated with 10% extra risk; NA = computation failed

Table A-20. Model Predictions for Increased Incidence of Moderate-to-Marked Hepatic Fatty Cysts in Female Dogs Following Oral Exposure to Chloroform for up to 7.5 Years (Heywood et al. 1979)

Model	BMD_{10}[a] (mg/kg/day)	$BMDL_{10}$[a] (mg/kg/day)	p-Value[b]	AIC	Scaled residuals[c]	
					Dose below BMD	Dose above BMD
Dichotomous Hill			NA	22.61	-4.28×10^{-4}	3.05×10^{-9}
Gamma[d]			1.00	20.61	-4.28×10^{-4}	5.54×10^{-10}
Log-Logistic[e]			1.00	20.61	-4.28×10^{-4}	3.49×10^{-9}
Multistage Degree 2[f]			1.00	18.63	-4.28×10^{-4}	-0.08
Multistage Degree 1[f]			0.80	19.87	-4.28×10^{-4}	-0.56
Weibull[d]			1.00	20.61	-4.28×10^{-4}	-4.68×10^{-9}
Logistic	9.04	4.86	0.55	21.35	-0.50	0.32
Log-Probit			1.00	20.61	-4.28×10^{-4}	3.20×10^{-10}
Probit[g]	**8.70**	**4.63**	**0.62**	**21.09**	**-0.39**	**0.30**
Quantal Linear			0.80	19.87	-4.28×10^{-4}	-0.56

[a]BMD and BMDLs values for models that do not provide adequate fit are not included in this table.
[b]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD.
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. Only Logistic and Probit modes provided an adequate fit to the data. BMDLs were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected (Probit).

AIC = Akaike Information Criterion; BMD = benchmark dose (maximum likelihood estimate of the dose associated with the selected benchmark response); $BMDL_{10}$ = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., 10 = dose associated with 10% extra risk; NA = computation failed

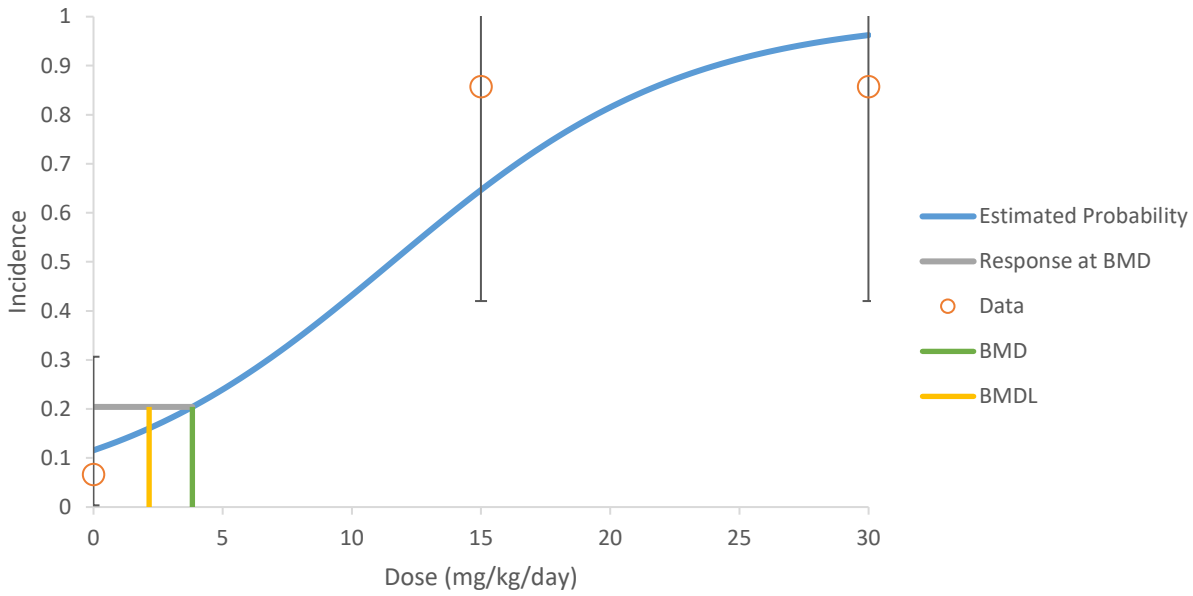
The candidate PODs for hepatic effects in dogs are summarized in Table A-21. Confidence is higher in the PODs based on BMD modeling; from these, the lowest POD identified was 2.15 mg/kg/day. Therefore, the $BMDL_{10}$ of 2.15 mg/kg/day for increased incidence of moderate-to-marked fatty cysts in the liver of male dogs was selected as the POD for the chronic-duration oral MRL. Model fit for the hepatic lesions in male dogs is shown in Figure A-1 (Logistic model).

Table A-21. Summary of Candidate POD Values Considered for Derivation of a Chronic-Duration Oral MRL for Chloroform

Species (sex)	Duration	Effect	Candidate POD (mg/kg/day)	POD type	Reference
Dog (male and female)	7.5 years (6 days/week)	>2-fold increase in serum ALT	15	LOAEL	Heywood et al. 1979
Dog (male)	7.5 years (6 days/week)	Increased incidence of moderate-to-marked hepatic fatty cysts	2.15	BMDL	Heywood et al. 1979
Dog (female)	7.5 years (6 days/week)	Increased incidence of moderate-to-marked hepatic fatty cysts	4.63	BMDL	Heywood et al. 1979

ALT = alanine aminotransferase; BMD = maximum likelihood estimate of the exposure dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD; LOAEL = lowest observed adverse effect level; MRL = Minimal Risk Level; POD = point of departure

Figure A-1. Fit of Logistic Model to Incidence Data for Moderate-to-Marked Hepatic Fatty Cysts in Male Dogs Following Oral Exposure to Chloroform for up to 7.5 Years (Heywood et al. 1979)



Calculations: The $BMDL_{10}$ of 2.15 mg/kg/day was adjusted for a daily exposure scenario:

$$BMDL_{ADJ} = BMDL_{10} \times \frac{days\ exposed}{7\ days} = 2.15\ mg/kg/day \times \frac{6\ days}{7\ days} = 1.84\ mg/kg/day$$

Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al. (1990) and Reitz et al. (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Neither of these studies evaluated dogs and are therefore not suitable for dose extrapolation.

Uncertainty Factors: The following uncertainty factors were applied to the $BMDL_{ADJ}$ to derive the MRL:

- Uncertainty factor of 10 for extrapolation from animals to humans
- Uncertainty factor of 10 for human variability

Subsequently, the oral MRL for chronic-duration exposure to chloroform is:

$$MRL = \frac{BMDL_{ADJ}}{(UF)} = \frac{1.84\ mg/kg/day}{100} = 0.0184\ mg/kg/day \approx 0.02\ mg/kg/day$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that hepatic effects are a known health effect following exposure to chloroform based on a low level of evidence from human epidemiology studies, high level of evidence from animal studies, and other relevant data consisting of the extensive database of case reports and case series documenting hepatic effects of chloroform in exposed humans (Appendix C).

Numerous case series and case reports indicate that the liver is a clear target of toxicity in humans following oral and inhalation exposure to high levels of chloroform (Section 2.9). In fatal ingestions cases, acute liver failure and/or severe liver damage have been found at autopsy (Dettling et al. 2016; Piersol et al. 1933). In nonfatal cases of oral ingestion, clinical signs of hepatotoxicity manifest within 1–7 days of exposure (Choi et al. 2006; Dell'Aglio et al. 2010; Hakim et al. 1992; Jayaweera et al. 2017; Kim 2008; Rao et al. 1993; Schroeder 1965; Sridhar et al. 2011; Storms 1973). Impaired liver function was observed in a man who ingested 21 mg/kg/day chloroform in a cough medicine for 10 years (Wallace 1950).

In animal studies, the liver is also a clear target of toxicity following acute-, intermediate-, and chronic-duration inhalation and oral studies in rodents; intermediate- and chronic-duration oral studies in dogs; and an acute-duration oral study in rabbits (Section 2.9). Findings in rodents following gavage exposure range from changes in clinical chemistry and mild histopathological damage after lower, shorter exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation), with widespread and severe necrosis and degeneration with higher and/or longer durations. As discussed above, the rodent liver is less susceptible to toxicity following drinking water exposure, presumably due to saturation of metabolic detoxification pathways with bolus gavage exposure.

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APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR CHLOROFORM

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to chloroform.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen were conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for chloroform. ATSDR primarily focused on peer-reviewed articles without language restrictions. Foreign language studies are reviewed based on available English-language abstracts and/or tables (or summaries in regulatory assessments, such as International Agency for Research on Cancer [IARC] documents). If the study appears critical for hazard identification or MRL derivation, translation into English is requested. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of chloroform have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of chloroform are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen[a]

Health Effects
Species
Human
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
In vitro (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects
Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects
Renal effects
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects

Table B-1. Inclusion Criteria for the Literature Search and Screen[a]

Reproductive effects
Developmental effects
Other noncancer effects
Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

[a]Physical-chemical properties are not generally obtained from literature searches, but rather from curated governmental databases such as PubChem.

Prioritization of Human Data. Numerous epidemiological studies evaluate potential associations between exposure to chlorinated drinking water and adverse health outcomes, particularly developmental endpoints and cancer. Epidemiological studies evaluating associations with consumption of chlorinated water or total trihalomethane exposure only were not included in the profile due to availability of studies with chloroform-specific exposure estimates and analyses. Additionally, human epidemiological studies without monitoring data, such as ecological studies based on proximity to emission sources or cohort studies with only self-reported ever/never exposed classifications, were not included in the profile. These studies have limited usefulness due to high risk of exposure misclassification and no information on intensity of potential exposure.

Prioritization of Animal Data. The acute- and intermediate-duration databases for hepatic and renal endpoints in animals following inhalation or oral exposure are extensive. Therefore, animal studies evaluating hepatic and renal endpoints were prioritized for efficient review. Inclusion of hepatic and renal animal studies in Chapter 2 (and the systematic review) was based on the following criteria:

- Acute- and intermediate-duration, single-dose studies that focused only on hepatic and renal endpoints were excluded. All chronic-duration studies and studies evaluating multiple systems were retained regardless of number of dose groups. Lethality data were retained from all studies.
- Only acute- and intermediate-duration studies that evaluated at least one dose within the same order of magnitude (e.g., 0–9, 10–99, 100–999, etc.) of the lowest identified LOAEL for hepatic or renal effects in the 1997 toxicological profile were included. Route- and duration-specific lowest LOAELs are shown in Table B-2. Based on these LOAEL values, only acute-duration inhalation studies evaluating at least one concentration <10 or <100 ppm were included for hepatic and renal endpoints, respectively. For intermediate-duration inhalation studies, only studies evaluating at least one concentration <100 ppm were included for hepatic and renal endpoints. For acute- and intermediate-duration oral studies, only studies evaluating at least one concentration <100 mg/kg/day were included for hepatic and renal endpoints. All chronic-duration studies and studies that evaluated multiple systems were retained regardless of the lowest dose level. Lethality data were retained from all studies.

Table B-2. Lowest LOAELs for Hepatic and Renal Endpoints Reported in 1997 Toxicological Profile

System	Inhalation (ppm)	Oral (mg/kg/day)
Hepatic		
Acute	3	34
Intermediate	25	30
Renal		
Acute	29	34
Intermediate	10	17.4

B.1.1 Literature Search

The literature search was conducted to update the Toxicological Profile for Chloroform released in 1997. All literature cited in the previous (1997) toxicological profile were considered for inclusion in the updated profile. The initial literature search, which was performed in September 2020, was restricted to studies added to databases since January 1995. An updated literature search was performed after the Toxicological Profile for Chloroform Draft for Public Comment was released in January 2024 to identify any additional studies added to databases between September 2020 and February 2024. The following main databases were searched in September 2020 and February 2024:

- PubMed
- National Technical Reports Library (NTRL)
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for chloroform. The query strings used for the literature search are presented in Table B-3.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures

and Results (NIH RePORTER) databases using the queries presented in Table B-4. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to chloroform were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-3. Database Query Strings

Database search date	Query string
PubMed	
02/2024	(("Chloroform"[mh] OR 67-66-3[rn]) AND (2020/09/01:3000[mhda])) OR ((("1,1,1-Trichloromethane"[tw] OR "chloroform"[tw] OR "methane trichloro"[tw] OR "Trichloromethane"[tw] OR "Formyl trichloride"[tw] OR "carbon trichloride"[tw] OR "Freon 20"[tw] OR "HCC 20"[tw] OR "Methane trichloride"[tw] OR "Methenyl chloride"[tw] OR "Methenyl trichloride"[tw] OR "Methyl trichloride"[tw] OR "Methylidyne trichloride"[tw] OR "Trichloroform"[tw] OR (("F 20"[tw] OR "F20"[tw]) AND "freon*") OR (("R 20"[tw] OR "R20"[tw]) AND "refrigerant*")) NOT medline[sb]) AND (2020/09/01:3000[dp] OR 2020/09/01:3000[crdt] OR 2020/09/01:3000[edat]))
09/2020	((((("Chloroform/toxicity"[mh] OR "Chloroform/adverse effects"[mh] OR "Chloroform/poisoning"[mh] OR "Chloroform/pharmacokinetics"[mh]) OR ("Chloroform"[mh] AND ("environmental exposure"[mh] OR ci[sh])) OR ("Chloroform"[mh] AND toxicokinetics[mh:noexp]) OR ("Chloroform/blood"[mh] OR "Chloroform/cerebrospinal fluid"[mh] OR "Chloroform/urine"[mh]) OR ("Chloroform"[mh] AND ("endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh])) OR ("Chloroform"[mh] AND ("computational biology"[mh] OR "medical informatics"[mh] OR genomics[mh] OR genome[mh] OR proteomics[mh] OR proteome[mh] OR metabolomics[mh] OR metabolome[mh] OR genes[mh] OR "gene expression"[mh] OR phenotype[mh] OR genetics[mh] OR genotype[mh] OR transcriptome[mh] OR ("systems biology"[mh] AND ("environmental exposure"[mh] OR "epidemiological monitoring"[mh] OR analysis[sh])) OR "transcription, genetic "[mh] OR "reverse transcription"[mh] OR "transcriptional activation"[mh] OR "transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, messenger"[mh] OR "RNA, transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "reverse transcriptase polymerase chain reaction"[mh] OR "base sequence"[mh] OR "trans-activators"[mh] OR "gene expression profiling"[mh])) OR ("Chloroform/antagonists and inhibitors"[mh]) OR ("Chloroform/metabolism"[mh] AND ("humans"[mh] OR "animals"[mh])) OR ("Chloroform"[mh] AND cancer[sb]) OR ("Chloroform/pharmacology"[majr])) OR (("1,1,1-Trichloromethane"[tw] OR "CARBON TRICHLORIDE"[tw] OR "chloroform"[tw] OR "Formyl trichloride"[tw] OR "Freon 20"[tw] OR "HCC 20"[tw] OR "Methane trichloride"[tw] OR "Methane, trichloro-"[tw] OR "Methenyl chloride"[tw] OR "Methenyl trichloride"[tw] OR "Methyl trichloride"[tw] OR "Methylidyne trichloride"[tw] OR "Trichloroform"[tw] OR "Trichloromethane"[tw] OR (("F 20"[tw] OR "F20"[tw]) AND freon*[tw]) OR (("R 20"[tw] OR "R20"[tw]) AND refrigerant*[tw])) NOT medline[sb]))) AND (1995:3000[dp] OR 1995:3000[mhda] OR 1995:3000[crdt] OR 1995:3000[edat])

Table B-3. Database Query Strings

Database search date	Query string
NTRL	
02/2024	Terms in Title or Keyword; limited to 2020-present "1,1,1-Trichloromethane" OR "chloroform" OR "methane trichloro" OR "Trichloromethane" OR "Formyl trichloride" OR "carbon trichloride" OR "Freon 20" OR "HCC 20" OR "Methane trichloride" OR "Methenyl chloride" OR "Methenyl trichloride" OR "Methyl trichloride" OR "Methylidyne trichloride" OR "Trichloroform"
09/2020	Date Published 1995 to 2020 Fields: Title or Keyword "1,1,1-Trichloromethane" OR "CARBON TRICHLORIDE" OR "chloroform" OR "Formyl trichloride" OR "Freon 20" OR "HCC 20" OR "Methane trichloride" OR "Methane, trichloro-" OR "Methenyl chloride" OR "Methenyl trichloride" OR "Methyl trichloride" OR "Methylidyne trichloride" OR "Trichloroform" OR "Trichloromethane"
Toxcenter	
02/2024	FILE 'TOXCENTER' ENTERED AT 13:04:32 ON 14 FEB 2024 L1 38527 SEA FILE=TOXCENTER 67-66-3 L2 27919 SEA FILE=TOXCENTER L1 NOT PATENT/DT L3 4855 SEA FILE=TOXCENTER L2 AND ED>=20200901 ACT TOXQUERY/Q --------- L4 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L5 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L6 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L7 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L8 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L9 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L10 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L11 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L12 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L13 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L14 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L15 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L16 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L17 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)

Table B-3. Database Query Strings

Database search date	Query string
	L18 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?) L19 QUE (ENDOCRIN? AND DISRUPT?) L20 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L21 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) L22 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L23 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?) L24 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?) L25 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?) L26 QUE (NEPHROTOX? OR HEPATOTOX?) L27 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?) L28 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?) L29 QUE L4 OR L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 L30 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?) L31 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE) L32 QUE L29 OR L30 OR L31 L33 QUE (NONHUMAN MAMMALS)/ORGN L34 QUE L32 OR L33 L35 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?) L36 QUE L34 OR L35 --------- L37 2780 SEA FILE=TOXCENTER L3 AND L36 L38 104 SEA FILE=TOXCENTER L37 AND MEDLINE/FS L39 2676 SEA FILE=TOXCENTER L37 NOT MEDLINE/FS L40 2687 DUP REM L38 L39 (93 DUPLICATES REMOVED) L*** DEL 104 S L37 AND MEDLINE/FS L*** DEL 104 S L37 AND MEDLINE/FS L41 104 SEA FILE=TOXCENTER L40 L*** DEL 2676 S L37 NOT MEDLINE/FS L*** DEL 2676 S L37 NOT MEDLINE/FS L42 2583 SEA FILE=TOXCENTER L40 L43 2583 SEA FILE=TOXCENTER (L41 OR L42) NOT MEDLINE/FS D SET D SEL D CLUSTER

Table B-3. Database Query Strings

Database search date	Query string
	D SCAN L43
09/2020	FILE 'TOXCENTER' ENTERED AT 12:58:25 ON 25 SEP 2020 CHARGED TO COST=EH038.05.01.LB.03 L1 31178 SEA FILE=TOXCENTER 67-66-3 L2 30902 SEA FILE=TOXCENTER L1 NOT TSCATS/FS L3 21961 SEA FILE=TOXCENTER L2 NOT PATENT/DT L4 13834 SEA FILE=TOXCENTER L3 AND PY>1994 ACT TOXQUERY/Q --------- L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L7 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L10 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L11 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L12 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L13 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L14 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L15 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L16 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L17 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L18 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?) L19 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?) L20 QUE (ENDOCRIN? AND DISRUPT?) L21 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L22 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) L23 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L24 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?) L25 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)

Table B-3. Database Query Strings

Database search date	Query string
	L26 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L27 QUE (NEPHROTOX? OR HEPATOTOX?)
	L28 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L29 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L30 QUE L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29
	L31 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L32 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L33 QUE L30 OR L31 OR L32
	L34 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L35 QUE L33 OR L34

	L36 6707 SEA FILE=TOXCENTER L4 AND L35
	L37 732 SEA FILE=TOXCENTER L36 AND MEDLINE/FS
	L40 1002 SEA FILE=TOXCENTER L36 AND BIOSIS/FS
	L41 4927 SEA FILE=TOXCENTER L36 AND CAPLUS/FS
	L42 46 SEA FILE=TOXCENTER L36 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L43 5991 DUP REM L37 L40 L42 L41 (716 DUPLICATES REMOVED) ANSWERS '1-5991' FROM FILE TOXCENTER
	L*** DEL 732 S L36 AND MEDLINE/FS
	L*** DEL 732 S L36 AND MEDLINE/FS
	L44 732 SEA FILE=TOXCENTER L43
	L*** DEL 1002 S L36 AND BIOSIS/FS
	L*** DEL 1002 S L36 AND BIOSIS/FS
	L45 794 SEA FILE=TOXCENTER L43
	L*** DEL 4927 S L36 AND CAPLUS/FS
	L*** DEL 4927 S L36 AND CAPLUS/FS
	L46 4428 SEA FILE=TOXCENTER L43
	L*** DEL 46 S L36 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L*** DEL 46 S L36 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L47 37 SEA FILE=TOXCENTER L43
	L48 5259 SEA FILE=TOXCENTER (L44 OR L45 OR L46 OR L47) NOT MEDLINE/FS
	L53 732 SEA FILE=TOXCENTER (L49 OR L50 OR L51 OR L52) AND MEDLINE/FS
	L54 769 SEA FILE=TOXCENTER L48 AND PY<=2000
	L56 740 SEA FILE=TOXCENTER L48 AND PY>2000 AND PY<=2005
	L58 1008 SEA FILE=TOXCENTER L48 AND PY>2005 AND PY<=2010
	L60 1449 SEA FILE=TOXCENTER L48 AND PY>2010 AND PY<=2015
	L61 1293 SEA FILE=TOXCENTER L48 AND PY>2015
	L62 5259 SEA FILE=TOXCENTER L54 OR L56 OR L58 OR L60 OR L61

Table B-3. Database Query Strings

Database search date	Query string
	D SCAN L54 D SCAN L56 D SCAN L58 D SCAN L60 D SCAN L61

Table B-4. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS via ChemView	
02/2024; 09/2020	67-66-3
NTP	
02/2024	Date limited: 2020-2024; not dated 67-66-3 "chloroform" "Trichloromethane" "1,1,1-Trichloromethane" "methane trichloro" "Formyl trichloride" "carbon trichloride" "Freon 20" "HCC 20" "Methane trichloride" "Methenyl chloride" "Methenyl trichloride" "Methyl trichloride" "Methylidyne trichloride" "Trichloroform"
09/2020	Limited to content types: Reports & Publications; Systematic Reviews; ROC Profiles, Reviews, or Candidates; and Testing Status 67-66-3
NPIRS	
02/2024	EPA Registration #: 020701
Regulations.gov	
02/2024	Dockets and Documents (limited to 01/01/2020-02/12/2024 and Notices) 67-66-3 "chloroform" "Trichloromethane" "1,1,1-Trichloromethane" "methane trichloro" "Formyl trichloride" "carbon trichloride" "Freon 20" "HCC 20" "Methane trichloride" "Methenyl chloride" "Methenyl trichloride" "Methyl trichloride" "Methylidyne trichloride" "Trichloroform"

Table B-4. Strategies to Augment the Literature Search

Source	Query and number screened when available
09/2020	Limited to rules, proposed rules, notices, other 67-66-3
NIH RePORTER	
05/2024	"1,1,1-Trichloromethane" OR "chloroform" OR "methane trichloro" OR "Trichloromethane" OR "Formyl trichloride" OR "carbon trichloride" OR "Freon 20" OR "HCC 20" OR "Methane trichloride" OR "Methenyl chloride" OR "Methenyl trichloride" OR "Methyl trichloride" OR "Methylidyne trichloride" OR "Trichloroform"
02/2023	Text Search: "1,1,1-Trichloromethane" OR "CARBON TRICHLORIDE" OR "chloroform" OR "Formyl trichloride" OR "Freon 20" OR "HCC 20" OR "Methane trichloride" OR "Methane, trichloro-" OR "Methenyl chloride" OR "Methenyl trichloride" OR "Methyl trichloride" OR "Methylidyne trichloride" OR "Trichloroform" OR "Trichloromethane" (advanced) Limit to: Project Title, Project Terms, Project Abstracts
Other	Identified throughout the assessment process[a]

[a]References identified throughout the assessment process may include studies found by tree searching; recommended by intraagency, interagency, peer, or public reviewers; or published more recently than the date of the literature search (February 2024). Additional references include those for specific regulations or guidelines and publications found by targeted searches for specific information (e.g., searches for reviews of general [not chemical-specific] mechanisms of toxicity).

The 2020 pre-public comment search results were:

- Number of records identified from PubMed, NTRL, and TOXCENTER (after duplicate removal): 10,710
- Number of records identified from other strategies: 133
- Total number of records to undergo literature screening: 10,843

The 2024 post-public comment search results were:

- Number of records identified from PubMed, NTRL, and TOXCENTER (after duplicate removal): 4,578
- Number of records identified from other strategies: 166
- Total number of records to undergo literature screening: 4,744

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on chloroform during the pre- and post-public comment drafts:

- Title and abstract screen
- Full text screen

Pre-Public Comment Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

- Number of titles and abstracts screened: 10,843

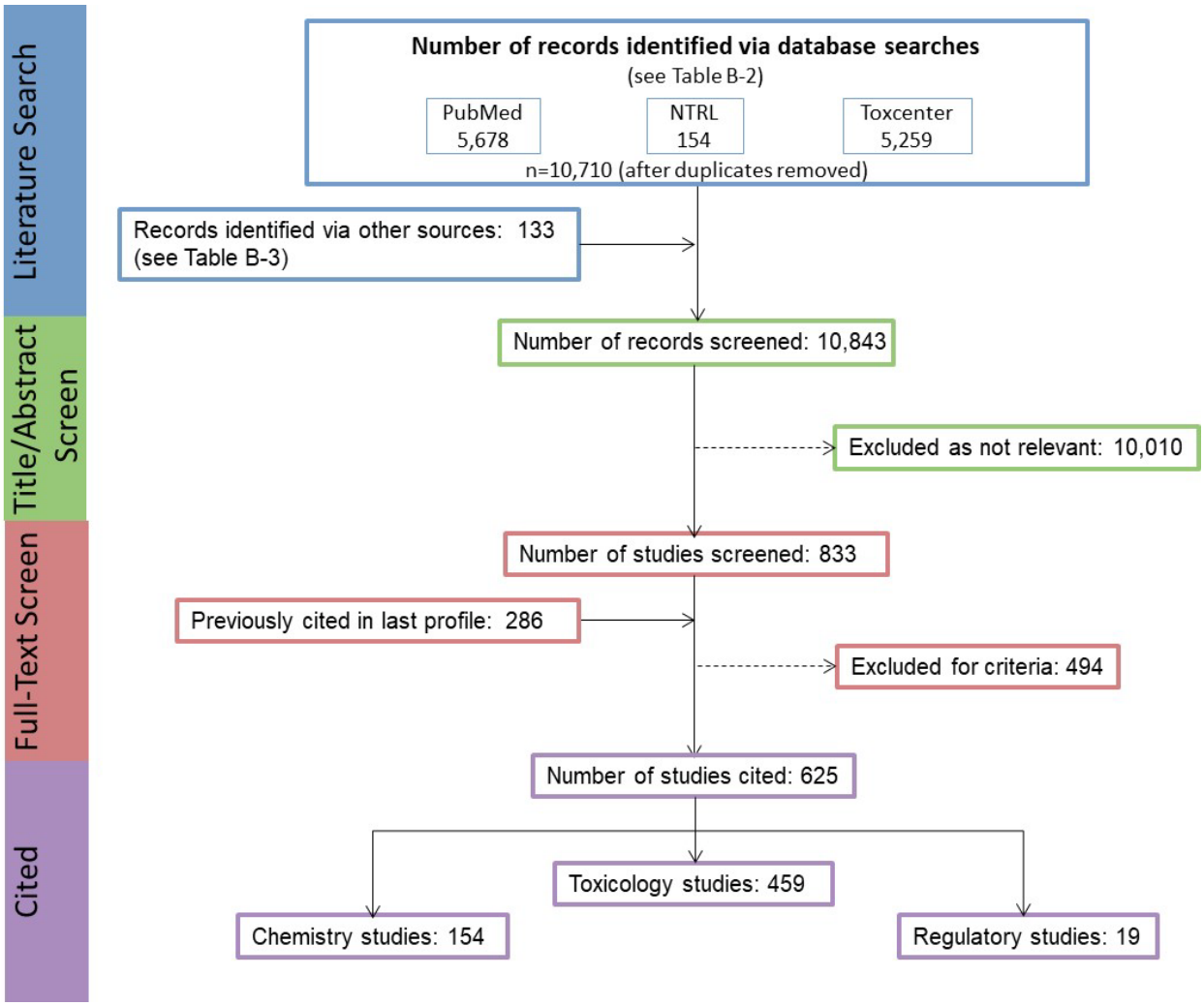
- Number of studies considered relevant and moved to the next step: 833

Pre-Public Comment Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 833
- Number of studies cited in the previous toxicological profile: 286
- Total number of studies cited in the profile: 625

A summary of the results of the pre-public literature search and screening is presented in Figure B-1.

Figure B-1. September 2020 Pre-Public Comment Literature Search Results and Screen for Chloroform



*The chemistry studies category includes studies pertaining to the potential for human exposure (Table B-1). The toxicology studies category includes human and animal studies of health effects as well as studies of toxicokinetics, biomarkers, and interactions with other chemicals (Table B-1). The regulatory studies category includes those studies cited in Chapter 7.

Post-Public Comment Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

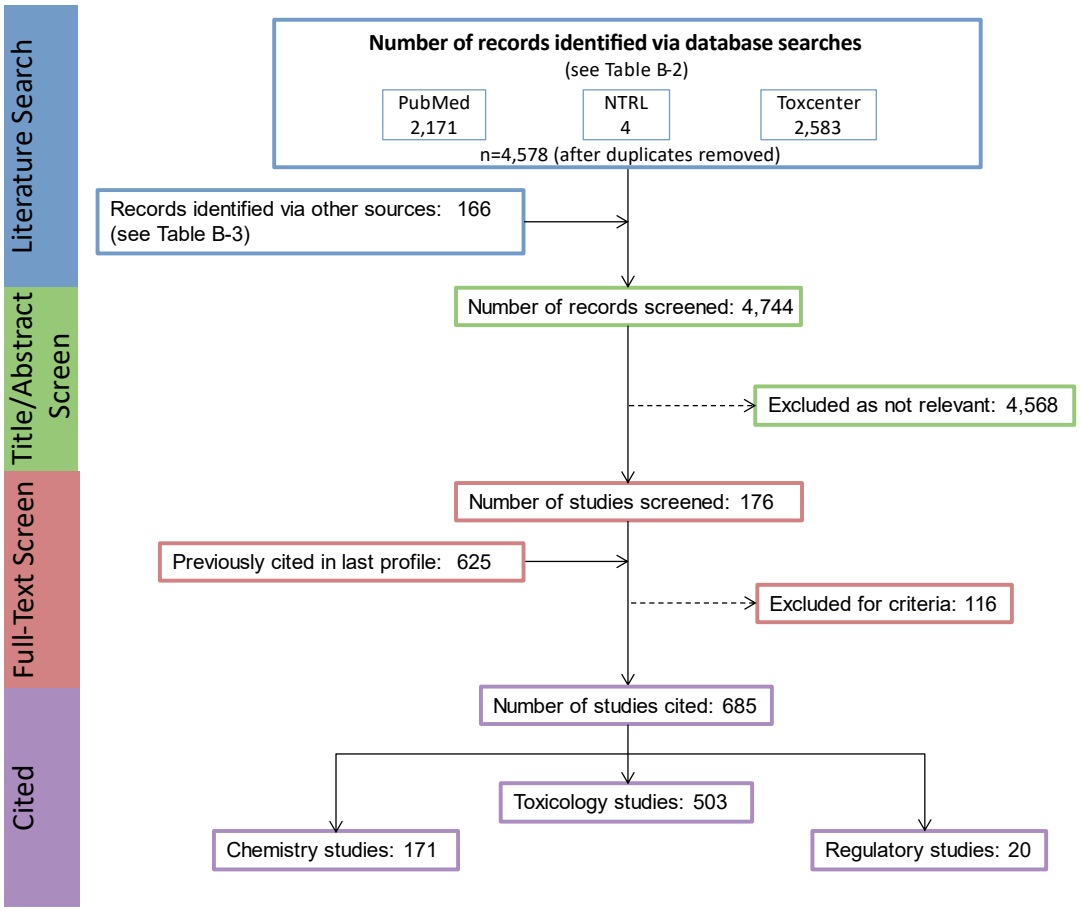
- Number of titles and abstracts screened: 4,744
- Number of studies considered relevant and moved to the next step: 176

Post-Public Comment Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 176
- Number of studies cited in the pre-public draft of the toxicological profile: 625
- Total number of studies cited in the profile: 685

A summary of the results of the post-public comment literature search and screening is presented in Figure B-2

Figure B-2. May 2024 Post-Public Comment Literature Search Results and Screen for Chloroform*



*The chemistry studies category includes studies pertaining to the potential for human exposure (Table B-1). The toxicology studies category includes human and animal studies of health effects as well as studies of toxicokinetics, biomarkers, and interactions with other chemicals (Table B-1). The regulatory studies category includes those studies cited in Chapter 7.

APPENDIX C. FRAMEWORK FOR ATSDR'S SYSTEMATIC REVIEW OF HEALTH EFFECTS DATA FOR CHLOROFORM

To increase the transparency of ATSDR's process of identifying, evaluating, synthesizing, and interpreting the scientific evidence on the health effects associated with exposure to chloroform, ATSDR utilized a slight modification of NTP's Office of Health Assessment and Translation (OHAT) systematic review methodology (NTP 2013, 2015; Rooney et al. 2014). ATSDR's framework is an eight-step process for systematic review with the goal of identifying the potential health hazards of exposure to chloroform:

- Step 1. Problem Formulation
- Step 2. Literature Search and Screen for Health Effects Studies
- Step 3. Extract Data from Health Effects Studies
- Step 4. Identify Potential Health Effect Outcomes of Concern
- Step 5. Assess the Risk of Bias for Individual Studies
- Step 6. Rate the Confidence in the Body of Evidence for Each Relevant Outcome
- Step 7. Translate Confidence Rating into Level of Evidence of Health Effects
- Step 8. Integrate Evidence to Develop Hazard Identification Conclusions

C.1 PROBLEM FORMULATION

The objective of the toxicological profile and this systematic review was to identify the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to chloroform. The inclusion criteria used to identify relevant studies examining the health effects of chloroform are presented in Table C-1.

Data from human and laboratory animal studies were considered relevant for addressing this objective. Human studies were divided into two broad categories: observational epidemiology studies and controlled exposure studies. The observational epidemiology studies were further divided: cohort studies (retrospective and prospective studies), population studies (with individual data or aggregate data), and case-control studies.

Table C-1. Inclusion Criteria for Identifying Health Effects Studies

Species
Human[a]
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects

Table C-1. Inclusion Criteria for Identifying Health Effects Studies

Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects[b]
Renal effects[b]
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects
Reproductive effects
Developmental effects
Other noncancer effects
Cancer

[a]Inclusion criteria were refined for human studies as described in Section B.1.1, *Prioritization of Human Data*.
[b]Inclusion criteria were refined for animal studies evaluating hepatic and renal effects as described in Section B.1.1, *Prioritization of Animal Data*.

C.2 LITERATURE SEARCH AND SCREEN FOR HEALTH EFFECTS STUDIES

A literature search and screen were conducted to identify studies examining the health effects of chloroform. The literature search framework for the toxicological profile is discussed in detail in Appendix B.

C.2.1 Literature Search

As noted in Appendix B, the current literature search was intended to update the Draft Toxicological Profile for Chloroform released for public comment in 2024; thus, the literature search was restricted to studies published between September 2020 and February 2024. See Appendix B for the databases searched and the search strategy.

A total of 10,843 and 4,744 records relevant to all sections of the toxicological profile were identified in the initial and update literature search, respectively (after duplicate removal).

C.2.2 Literature Screening

As described in Appendix B, a two-step process was used to screen the literature search to identify relevant studies examining the health effects of chloroform.

Title and Abstract Screen. In the Title and Abstract Screen step, 107 documents (inclusive of both literature searches) were considered to meet the health effects inclusion criteria in Table C-1 and were moved to the next step in the process.

Full Text Screen. In the second step in the literature screening process for the systematic review, a full text review of 191 health effect documents (documents identified in the update literature search and

documents cited in older versions of the profile) was performed. From those 191 documents (258 studies), 88 documents (137 studies) were included in the qualitative review.

C.3 EXTRACT DATA FROM HEALTH EFFECTS STUDIES

Relevant data extracted from the individual studies selected for inclusion in the systematic review were collected in customized data forms. A summary of the type of data extracted from each study is presented in Table C-2. For references that included more than one experiment or species, data extraction records were created for each experiment or species.

Table C-2. Data Extracted from Individual Studies

Citation
Chemical form
Route of exposure (e.g., inhalation, oral, dermal)
Specific route (e.g., gavage in oil, drinking water)
Species
Strain
Exposure duration category (e.g., acute, intermediate, chronic)
Exposure duration
Frequency of exposure (e.g., 6 hours/day, 5 days/week)
Exposure length
Number of animals or subjects per sex per group
Dose/exposure levels
Parameters monitored
Description of the study design and method
Summary of calculations used to estimate doses (if applicable)
Summary of the study results
Reviewer's comments on the study
Outcome summary (one entry for each examined outcome)
No-observed-adverse-effect level (NOAEL) value
Lowest-observed-adverse-effect level (LOAEL) value
Effect observed at the LOAEL value

A summary of the extracted data for each study is presented in the Supplemental Document for Chloroform and overviews of the results of the inhalation, oral, and dermal exposure studies are presented in Sections 2.2–2.19 of the profile and in the Levels Significant Exposures tables in Section 2.1 of the profile (Tables 2-1, 2-2, and 2-3, respectively).

C.4 IDENTIFY POTENTIAL HEALTH EFFECT OUTCOMES OF CONCERN

Overviews of the potential health effect outcomes for chloroform identified in human and animal studies are presented in Tables C-4 and C-5, respectively. Available human studies evaluating noncancer effects include numerous case studies and case-series reports, a limited number of occupational exposure studies, and general population exposure studies (primarily focusing on exposure to chloroform as a disinfection byproduct in residential water supplies). When evaluated together, these studies suggest that the respiratory, hepatic, renal, and neurological systems and the developing fetus may be susceptible to

chloroform toxicity. Animal studies evaluated a comprehensive set of endpoints following inhalation and oral exposure; dermal studies were limited to two acute-duration studies evaluating a limited number of endpoints. Respiratory and hepatic effects were considered sensitive outcomes following inhalation exposure in animals, and hepatic, renal, and developmental effects were considered sensitive outcomes following oral exposure in animals (i.e., effects were observed at low concentrations or doses; see Tables 2-1 and 2-2 and Figures 1-3, 1-4, 2-1, and 2-2 for further detail). Based on effects noted in human and animal studies, epidemiological and experimental studies examining these respiratory, hepatic, renal, neurological, and developmental outcomes were carried through to Steps 4–8 of the systematic review. Case studies and case-series reports were not included in the formal systematic review due to inherent high risk of bias and low confidence based on study design. However, consistent findings from numerous case studies were considered during the adjustment of the confidence rating (with regards to consistency and/or severity of observed effects). There were 136 studies (published in 88 documents) examining these potential outcomes carried through to Steps 4–8 of the systematic review.

APPENDIX C

Table C-3. Overview of the Health Outcomes for Chloroform Evaluated in Human Studies

	Body weight	Respiratory	Cardiovascular	Gastrointestinal	Hematological	Musculoskeletal	Hepatic	Renal	Dermal	Ocular	Endocrine	Immunological	Neurological	Reproductive	Developmental	Other Noncancer	Caner
Inhalation studies																	
Cohort			1	2			3	1				1	2			1	
			1	2			1	0				1	2			1	
Case-control		6	4	4	1	3	13	3				1	3		1		10
		6	4	4	1	3	13	3				1	3		0		2
Population		1					1	1				1					
		0					0	0				0					
Case series			2	3	1		4						2				
			2	3	1		3						2				
Oral studies																	
Cohort														6	15		3
														2	3		3
Case-control		5	4	4	2	1	12	4				1	9	3	6	1	8
		5	4	4	2	1	12	4				1	9	0	3	1	3
Population					1		1	1						2	2	1	2
					1		1	1						0	1	1	0
Case series																	
Dermal studies																	
Cohort																	
Case-control				1			1		6				1				
				1			1		6				1				
Population																	
Case series																	

Number of studies examining endpoint	0	1	2	3	4	5–9	≥10
Number of studies reporting outcome	0	1	2	3	4	5–9	≥10

APPENDIX C

Table C-4. Overview of the Health Outcomes for Chloroform Evaluated in Experimental Animal Studies

	Body weight	Respiratory	Cardiovascular	Gastrointestinal	Hematological	Musculoskeletal	Hepatic	Renal	Dermal	Ocular	Endocrine	Immunological[a]	Neurological[a]	Reproductive[a]	Developmental	Other Noncancer	Caner
Inhalation studies																	
Acute-duration	14	9					11	11				6	8	7	6		
	9	8					10	9				3	8	5	6		
Intermediate-duration	15	10	4	4	2	8	15	15	2	4	4	6	4	6			
	7	6	0	0	0	0	14	12	0	0	0	0	0	0			
Chronic-duration	3	2	2	2	2	2	2	3	2	2	2	2	2	2			3
	2	2	0	0	0	0	2	3	0	0	0	0	0	0			1
Oral studies																	
Acute-duration	21	3	1	3	3		22	18	2	1		1	7	3	5		1
	12	3	1	1	2		19	14	2	1		1	7	3	4		
Intermediate-duration	22	8	4	8	6	2	19	16		2	5	6	7	5	4		7
	8	3	1	1	1	0	15	6		0	1	1	1	1	2		3
Chronic-duration	10	3	3	2	4	2	4	7	2	1	2	2	4	3			9
	4	1	1	0	0	0	3	3	0	0	0	0	0	0			7
Dermal studies																	
Acute-duration	1						1	1	2								
	1						0	1	2								
Intermediate-duration																	
Chronic-duration																	

Number of studies examining endpoint	0	1	2	3	4	5–9	≥10
Number of studies reporting outcome	0	1	2	3	4	5–9	≥10

[a]Number of studies examining endpoint includes study evaluating histopathology, but not evaluating function.

C.5 ASSESS THE RISK OF BIAS FOR INDIVIDUAL STUDIES

C.5.1 Risk of Bias Assessment

The risk of bias of individual studies was assessed by reviewers using the guidelines provided in OHAT's Risk of Bias Tool (NTP 2015). The risk of bias questions for observational epidemiology studies, human-controlled exposure studies, and animal experimental studies are presented in Tables C-6, C-7, and C-8, respectively. Each risk of bias question was answered on a four-point scale:

- **Definitely low risk of bias (++)**
- **Probably low risk of bias (+)**
- **Probably high risk of bias (-)**
- **Definitely high risk of bias (– –)**

In general, "definitely low risk of bias" or "definitely high risk of bias" were used if the question could be answered with information explicitly stated in the study report. If the response to the question could be inferred, then "probably low risk of bias" or "probably high risk of bias" responses were typically used.

Table C-5. Risk of Bias Questionnaire for Observational Epidemiology Studies

Selection bias
Were the comparison groups appropriate?
Confounding bias
Did the study design or analysis account for important confounding and modifying variables?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

Table C-6. Risk of Bias Questionnaire for Human-Controlled Exposure Studies

Selection bias
Was administered dose or exposure level adequately randomized?
Was the allocation to study groups adequately concealed?
Performance bias
Were the research personnel and human subjects blinded to the study group during the study?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

Table C-7. Risk of Bias Questionnaire for Experimental Animal Studies

Selection bias
Was administered dose or exposure level adequately randomized?
Was the allocation to study groups adequately concealed?
Performance bias
Were experimental conditions identical across study groups?
Were the research personnel blinded to the study group during the study?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

After the risk of bias questionnaires were completed for the health effects studies, the studies were assigned to one of three risk of bias tiers based on the responses to the key questions listed below and the responses to the remaining questions.

- Is there confidence in the exposure characterization? (only relevant for observational studies)
- Is there confidence in the outcome assessment?
- Does the study design or analysis account for important confounding and modifying variables? (only relevant for observational studies)

First Tier. Studies placed in the first tier received ratings of "definitely low" or "probably low" risk of bias on the key questions **AND** received a rating of "definitely low" or "probably low" risk of bias on the responses to at least 50% of the other applicable questions.

Second Tier. A study was placed in the second tier if it did not meet the criteria for the first or third tiers.

Third Tier. Studies placed in the third tier received ratings of "definitely high" or "probably high" risk of bias for the key questions **AND** received a rating of "definitely high" or "probably high" risk of bias on the response to at least 50% of the other applicable questions.

The results of the risk of bias assessment for the different types of chloroform health effects studies (observational epidemiology and animal experimental studies) are presented in Tables C-9 and C-10, respectively.

Table C-8. Summary of Risk of Bias Assessment for Chloroform—Observational Epidemiology Studies

Reference	Risk of bias criteria and ratings						Risk of bias tier
	Selection bias	Confounding bias	Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Were the comparison groups appropriate?	**Did the study design or analysis account for important confounding and modifying variables?***	Were outcome data complete without attrition or exclusion from analysis?	**Is there confidence in the exposure characterization?***	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Outcome: Respiratory effects							
Cross-sectional studies							
Font-Ribera et al. 2010	+	–	+	+	++	++	Second
Outcome: Hepatic effects							
Cohort studies							
Aiking et al. 1994	+	–	+	+	–	++	Second
Bomski et al. 1967	+	–	–	–	–	–	Third
Challen et al. 1958	+	+	+	+	+	+	First
Li et al. 1993	+	–	–	+	–	–	Second
Outcome: Renal effects							
Cohort studies							
Aiking et al. 1994	+	–	+	+	–	++	Second
Li et al. 1993	+	–	–	+	–	–	Second
Outcome: Neurological effects							
Cohort studies							
Challen et al. 1958	+	+	+	+	–	+	Second
Li et al. 1993	+	–	–	+	+	–	Second

Table C-8. Summary of Risk of Bias Assessment for Chloroform—Observational Epidemiology Studies

Reference	Risk of bias criteria and ratings						Risk of bias tier
	Selection bias	Confounding bias	Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Were the comparison groups appropriate?	**Did the study design or analysis account for important confounding and modifying variables?***	Were outcome data complete without attrition or exclusion from analysis?	**Is there confidence in the exposure characterization?***	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Outcome: Developmental effects							
Cohort studies							
Botton et al. 2015	+	–	++	+	+	++	Second
Cao et al. 2016	+	–	++	++	+	++	Second
Costet et al. 2011	+	–	++	+	+	++	Second
Dodds and King 2001	+	–	–	–	+	++	Second
Grazuleviciene et al. 2011	+	–	++	++	+	++	Second
Grazuleviciene et al. 2013	+	–	++	++	+	++	Second
Hinckley et al. 2005	+	–	++	–	+	++	Second
Hoffman et al. 2008	+	–	++	–	+	++	Second
Liu et al. 2021	+	–	++	+	+	+	Second
Rivera-Núñez and Wright 2013	+	+	+	–	+	++	Second
Sun et al. 2020	+	–	++	+	+	++	Second
Villanueva et al. 2018	+	–	++	++	++	++	Second
Villanueva et al. 2011	–	–	++	–	+	++	Second
Zhu et al. 2022	+	–	++	–	+	++	Second

Table C-8. Summary of Risk of Bias Assessment for Chloroform—Observational Epidemiology Studies

Reference	Risk of bias criteria and ratings						Risk of bias tier
	Selection bias	Confounding bias	Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Were the comparison groups appropriate?	**Did the study design or analysis account for important confounding and modifying variables?***	Were outcome data complete without attrition or exclusion from analysis?	**Is there confidence in the exposure characterization?***	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Population studies							
Porter et al. 2005	+	–	++	–	+	–	Second
Wright et al. 2004	+	–	++	–	+	++	Second
Case-control studies							
Bonou et al. 2017	++	–	++	+	+	++	Second
Kaufman et al. 2018	++	–	++	–	++	++	Second
Kaufman et al. 2020	++	–	++	–	++	++	Second
Kramer et al. 1992	–	–	++	–	–	–	Third
Levallois et al. 2012	+	–	++	+	+	++	Second
Summerhayes et al. 2012	+	–	++	–	+	++	Second
Swartz et al. 2015a, 2015b	+	++	++	–	+	++	Second
Zaganjor et al. 2020	+	–	++	+	++	++	Second

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias

*Key question

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Outcome: Respiratory effects									
Inhalation acute-duration exposure									
Constan et al. 1999 (Sv/129 mice)	+	+	++	++	++	++	++	++	First
Constan et al. 1999 (B6C3F1 mice)	+	+	++	++	++	++	++	++	First
de Oliveira et al. 2015	–	+	++	+	++	+	+	++	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	–	First
Larson et al. 1994c; Mery et al. 1994 (mouse)	–	+	++	+	++	+	+	++	First
Larson et al. 1994c; Mery et al. 1994 (rat)	–	+	++	+	++	+	+	++	First
Larson et al. 1996	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (4 days)	–	+	++	+	++	+	+	++	First
Inhalation intermediate-duration exposure									
Kasai et al. 2002 (mouse)	–	+	++	+	++	++	+	–	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	–	First
Larson et al. 1996 (13 weeks; 5 days/week)	–	+	++	+	++	–	+	++	First
Larson et al. 1996 (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (3 weeks)	–	+	++	+	++	+	+	++	First

APPENDIX C

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias: Was administered dose or exposure level adequately randomized?	Selection bias: Was the allocation to study groups adequately concealed?	Performance bias: Were the experimental conditions identical across study groups?	Performance bias: Were the research personnel blinded to the study group during the study?	Attrition / exclusion bias: Were outcome data complete without attrition or exclusion from analysis?	Detection bias: Is there confidence in the exposure characterization?	Detection bias: **Is there confidence in the outcome assessment?***	Selective reporting bias: Were all measured outcomes reported?	Risk of bias tier
Larson et al. 1996 (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 5 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (3 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (6 weeks)	–	+	++	+	++	+	+	++	First
Inhalation chronic-duration exposure									
Yamamoto et al. 2002 (mouse)	–	+	++	+	++	++	+	+	First
Yamamoto et al. 2002 (rat)	–	+	++	+	++	++	+	+	First
Oral acute-duration exposure									
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Templin et al. 1996a (Fischer 344)	+	+	++	+	++	+	+	++	First
Templin et al. 1996a (Osborne-Mendel)	+	+	++	+	++	+	+	++	First
Oral intermediate-duration exposure									
Chu et al. 1982a	–	+	+	+	–	+	+	–	First
Chu et al. 1982b	–	+	+	+	+	+	+	+	First
Dorman et al. 1997	– –	+	–	+	–	+	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
NTP 1988a	++	+	++	+	++	+	+	++	First
EPA 1980 (mouse)	++	+	–	+	+	+	+	++	First
EPA 1980 (rat)	++	+	–	+	+	+	+	++	First
Larson et al. 1995b	+	+	++	+	++	+	++	+	First
Sehata et al. 2002 (CB6F1)	–	+	++	+	+	+	++	++	First
Oral chronic-duration exposure									
Dunnick and Melnick 1993; NCI 1976 (mouse)	+	+	++	+	++	++	+	++	First
Dunnick and Melnick 1993; NCI 1976 (rat)	+	+	++	+	++	++	+	++	First
Roe et al. 1979 (Experiment 1)	+	+	–	+	–	++	–	+	Second
Outcome: Liver effects									
Inhalation acute-duration exposure									
Baeder and Hofmann 1988	–	+	++	+	++	+	–	++	Second
Constan et al. 1999 (Sv/129 mice)	+	+	++	+	++	+	+	++	First
Constan et al. 1999 (B6C3F1 mice)	+	+	++	+	++	+	+	++	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	++	First
Larson et al. 1994c; Mery et al. 1994 (mouse)	–	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Larson et al. 1994c; Mery et al. 1994 (rat)	–	+	++	+	++	+	+	++	First
Larson et al. 1996	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (4 days)	–	+	++	+	++	+	+	++	First
Templin et al. 1996c (2 weeks)	+	+	++	+	+	+	+	++	First
Templin et al. 1996c (4 days)	+	+	++	+	+	+	+	++	First
Inhalation intermediate-duration exposure									
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	++	First
Larson et al. 1996 (13 weeks; 5 days/week)	–	+	++	+	++	–	+	++	First
Larson et al. 1996 (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (3 weeks)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 5 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (3 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1998 (13 weeks)	+	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Templin et al. 1998 (3 weeks)	+	+	++	+	++	+	+	++	First
Templin et al. 1998 (7 weeks)	+	+	++	+	++	+	+	++	First
Torkelson et al. 1976 (rat 1–4 hours/day)	–	+	++	+	–	++	+	++	First
Torkelson et al. 1976 (rat 7 hours/day)	–	+	++	+	–	++	+	++	First
Inhalation chronic-duration exposure									
Yamamoto et al. 2002 (mouse)	–	+	++	+	++	++	++	+	First
Yamamoto et al. 2002 (rat)	–	+	++	+	++	++	++	+	First
Oral acute-duration exposure									
Chu et al. 1982b	–	+	++	+	–	+	+	+	First
Ewaid et al. 2020	+	+	–	+	–	+	+	+	First
Jones et al. 1958	– –	+	–	+	–	–	+	+	Second
Keegan et al. 1998	–	+	++	+	++	+	++	++	First
Larson et al. 1993 (mouse)	+	+	++	+	++	+	+	+	First
Larson et al. 1993 (rat)	+	+	++	+	++	+	+	+	First
Larson et al. 1994b (GO)	+	+	+	+	+	+	+	+	First
Larson et al. 1994b (W)	+	+	+	+	+	+	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Larson et al. 1994d	+	+	++	+	+	+	+	+	First
Larson et al. 1995a (DW)	+	+	++	+	+	+	+	+	First
Larson et al. 1995a (G)	+	+	++	+	+	+	+	+	First
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Lilly et al. 1997	–	+	++	+	++	+	++	+	First
Miyagawa et al. 1998	–	+	+	+	+	–	+	+	First
Moore et al. 1982 (G)	+	+	+	+	+	–	+	++	First
Moore et al. 1982 (GO)	+	+	+	+	+	–	+	++	First
Munson et al. 1982	–	+	+	+	–	–	++	+	First
Templin et al. 1996a (Fischer 344)	+	+	++	+	++	+	+	++	First
Templin et al. 1996a (Osborne-Mendel)	+	+	++	+	++	+	+	++	First
Thompson et al. 1974 (Experiment 2, 6 F)	–	+	+	+	++	–	+	+	First
Wada et al. 2015	+	+	+	+	++	+	+	++	First
Wang et al. 1997	–	+	++	+	++	–	++	++	First
Oral intermediate-duration exposure									
Bull et al. 1986 (GO)	+	+	++	+	+	–	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Bull et al. 1986 (GW)	+	+	++	+	+	–	+	+	First
Chu et al. 1982a	–	+	+	+	–	–	+	++	First
Chu et al. 1982b	–	+	+	+	+	–	+	+	First
Eschenbrenner and Miller 1945	–	+	+	+	+	–	–	+	Second
NTP 1988a	++	+	++	+	++	+	+	++	First
Heywood et al. 1979	–	+	+	+	+	–	+	–	First
EPA 1980 (mouse)	++	+	–	+	+	+	+	++	First
EPA 1980 (rat)	++	+	–	+	+	+	+	++	First
Larson et al. 1994b (GO)	+	+	+	+	+	+	+	+	First
Larson et al. 1994b (W)	+	+	+	+	+	+	+	+	First
Larson et al. 1994d	+	+	++	+	+	+	+	++	First
Larson et al. 1995a (GO)	+	+	++	+	+	+	+	+	First
Larson et al. 1995a (W)	+	+	++	+	+	+	+	+	First
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Melnick et al. 1998	–	+	++	+	+	+	+	++	First
Mostafa et al. 2009	–	+	– –	+	++	–	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias — Was administered dose or exposure level adequately randomized?	Selection bias — Was the allocation to study groups adequately concealed?	Performance bias — Were the experimental conditions identical across study groups?	Performance bias — Were the research personnel blinded to the study group during the study?	Attrition / exclusion bias — Were outcome data complete without attrition or exclusion from analysis?	Detection bias — Is there confidence in the exposure characterization?	Detection bias — **Is there confidence in the outcome assessment?***	Selective reporting bias — Were all measured outcomes reported?	Risk of bias tier
Munson et al. 1982	–	+	+	+	–	–	+	+	First
Sehata et al. 2002 (CB6F1)	–	+	++	+	+	+	++	++	First
Oral chronic-duration exposure									
Heywood et al. 1979	–	+	+	+	–	++	–	++	Second
Dunnick and Melnick 1993; NCI 1976 (mouse)	+	+	++	+	++	+	+	++	First
Dunnick and Melnick 1993; NCI 1976 (rat)	+	+	++	+	++	+	+	++	First
Roe et al. 1979 (Experiment 1)	+	+	–	+	–	++	+	+	First
Outcome: Kidney effects									
Inhalation acute-duration exposure									
Baeder and Hofmann 1988	–	+	++	+	++	+	–	++	Second
Constan et al. 1999 (Sv/129 mice)	+	+	++	+	++	+	+	++	First
Constan et al. 1999 (B6C3F1 mice)	+	+	++	+	++	+	+	++	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	++	First
Larson et al. 1994c; Mery et al. 1994 (mouse)	–	+	++	+	++	+	+	++	First
Larson et al. 1994c; Mery et al. 1994 (rat)	–	+	++	+	++	+	+	++	First
Larson et al. 1996	–	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Templin et al. 1996b (4 days)	–	+	++	+	++	+	+	++	First
Templin et al. 1996c (2 weeks)	+	+	++	+	+	+	+	++	First
Templin et al. 1996c (4days)	+	+	++	+	+	+	+	++	First
Inhalation intermediate-duration exposure									
Kasai et al. 2002 (mouse)	–	+	++	+	++	++	+	++	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	++	First
Larson et al. 1996 (13 weeks; 5 days/week)	–	+	++	+	++	–	+	++	First
Larson et al. 1996 (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (3 weeks)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 5 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (3 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1998 (13 weeks)	+	+	++	+	++	+	+	++	First
Templin et al. 1998 (3 weeks)	+	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Templin et al. 1998 (7 weeks)	+	+	++	+	++	+	+	++	First
Torkelson et al. 1976 (rat 1–4 hours/day)	–	+	++	+	–	++	+	++	First
Torkelson et al. 1976 (rat 7 hours/day)	–	+	++	+	–	++	+	++	First
Inhalation chronic-duration exposure									
Yamamoto et al. 2002 (mouse)	–	+	++	+	++	++	++	+	First
Yamamoto et al. 2002 (rat)	–	+	++	+	++	++	++	+	First
Oral acute-duration exposure									
Chu et al. 1982b	–	+	++	+	–	+	+	+	First
Ewaid et al. 2020	+	+	–	+	–	+	+	+	First
Keegan et al. 1998	–	+	++	+	++	+	+	++	First
Larson et al. 1993 (rat)	+	+	++	+	++	+	+	+	First
Larson et al. 1993 (mouse)	+	+	++	+	++	+	+	+	First
Larson et al. 1994b (GO)	+	+	+	+	+	+	++	+	First
Larson et al. 1994b (W)	+	+	+	+	+	+	++	+	First
Larson et al. 1994d	+	+	++	+	+	+	+	++	First
Larson et al. 1995a (DW)	+	+	++	+	+	+	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Larson et al. 1995a (G)	+	+	++	+	+	+	+	+	First
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Lilly et al. 1997	–	+	++	+	++	+	++	+	First
Liu et al. 2013	–	+	+	+	+	–	++	++	First
Miyagawa et al. 1998	–	+	+	+	+	–	+	+	First
Moore et al. 1982 (G)	+	+	+	+	+	–	+	++	First
Moore et al. 1982 (GO)	+	+	+	+	+	–	+	++	First
Potter et al. 1996	+	+	++	+	+	–	+	++	First
Templin et al. 1996a (Fischer 344)	+	+	++	+	++	+	+	++	First
Templin et al. 1996a (Osborne-Mendel)	+	+	++	+	++	+	+	++	First
Thompson et al. 1974 (Experiment 2, 6 F)	–	+	+	+	++	–	+	+	First
Oral intermediate-duration exposure									
Chu et al. 1982a	–	+	+	+	–	–	+	–	Second
Chu et al. 1982b	–	+	+	+	+	–	+	+	First
NTP 1988a	++	+	++	+	++	+	+	++	First
Heywood et al. 1979	–	+	+	+	+	–	–	–	Second

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Hooth et al. 2002; McDorman et al. 2003a, 2003b	+	+	++	+	++	+	+	++	First
EPA 1980 (mouse)	++	+	–	+	+	+	+	++	First
EPA 1980 (rat)	++	+	–	+	+	+	+	++	First
Larson et al. 1994b (GO)	+	+	+	+	+	+	++	+	First
Larson et al. 1994b (W)	+	+	+	+	+	+	++	+	First
Larson et al. 1994d	+	+	++	+	+	+	+	++	First
Larson et al. 1995a (GO)	+	+	++	+	+	+	+	+	First
Larson et al. 1995a (W)	+	+	++	+	+	+	+	+	First
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Lipsky et al. 1993 (GO)	–	+	++	+	+	–	+	+	First
Lipsky et al. 1993 (GW)	–	+	++	+	+	–	+	+	First
Sehata et al. 2002 (CB6F1)	–	+	++	+	+	+	++	+	First
Oral chronic-duration exposure									
Heywood et al. 1979	–	+	+	+	–	++	–	++	Second
Hard et al. 2000; Jorgenson et al. 1985 (rat)	+	+	++	+	–	++	++	++	First
Nagano et al. 2006	+	+	++	+	+	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Dunnick and Melnick 1993; NCI 1976 (rat)	+	+	++	+	++	+	+	++	First
Dunnick and Melnick 1993; NCI 1976 (mouse)	+	+	++	+	++	+	+	++	First
Roe et al. 1979 (Experiment 1)	+	+	–	+	–	++	+	++	First
Roe et al. 1979 (Experiment 3)	+	+	–	+	–	++	+	+	First
Outcome: Neurological effects									
Inhalation acute-duration exposure									
Constan et al. 1999 (Sv/129 mice)	+	–	++	–	++	+	–	++	Second
Constan et al. 1999 (B6C3F1 mice)	+	–	++	–	++	+	–	++	Second
DHA 2022	–	+	++	+	++	++	+	++	First
EPA 1978	–	+	++	–	++	–	–	++	Second
Gehring 1968	–	+	++	–	++	–	–	++	Second
Larson et al. 1994c; Mery et al. 1994 (rat)	–	+	++	+	++	+	+	++	First
Lehmann and Flury 1943 (cat)	–	–	–	–	–	–	–	–	Third
Lehmann and Flury 1943 (mouse)	–	–	–	–	–	–	–	–	Third
Inhalation intermediate-duration exposure									
Larson et al. 1996 (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Larson et al. 1996 (3 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (3 weeks)	–	+	++	+	++	+	+	++	First
Inhalation chronic-duration exposure									
Yamamoto et al. 2002 (mouse)	–	+	++	–	++	++	+	+	First
Yamamoto et al. 2002 (rat)	–	+	++	–	++	++	+	+	First
Oral acute-duration exposure									
Balster and Borzelleca 1982 (14 days)	+	+	+	+	++	–	++	++	First
Balster and Borzelleca 1982 (once)	+	+	+	+	+	–	++	+	First
Bowman et al. 1978	–	+	+	+	–	–	–	–	Third
NTP 1988a	++	+	++	+	+	+	+	–	First
Jones et al. 1958	– –	+	–	+	–	–	–	+	Third
Landauer et al. 1982	+	+	++	+	+	–	+	+	First
Oral intermediate-duration exposure									
Balster and Borzelleca 1982 (30 days)	+	+	+	+	–	–	++	+	First
Balster and Borzelleca 1982 (60 days)	+	+	+	+	–	–	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Balster and Borzelleca 1982 (90 days)	+	+	+	+	–	–	++	+	First
Chu et al. 1982a	–	+	–	+	–	–	+	–	Second
Chu et al. 1982b	–	+	–	+	+	–	+	+	First
Dorman et al. 1997	– –	+	++	+	+	+	+	++	First
Sehata et al. 2002 (CB6F1)	–	+	++	+	+	+	+	+	First
Wada et al. 2015	+	++	+	++	++	+	++	+	First
Oral chronic-duration exposure									
Heywood et al. 1979	–	–	+	–	–	++	–	+	Third
Dunnick and Melnick 1993; NCI 1976 (rat)	+	+	++	–	++	++	+	++	First
Dunnick and Melnick 1993; NCI 1976 (mouse)	+	+	++	–	++	++	+	++	First
Roe et al. 1979 (Experiment 1)	+	–	–	–	–	++	+	+	Second
Outcome: Developmental Effects									
Inhalation acute-duration exposure									
Baeder and Hofmann 1988	–	+	++	+	++	+	+	++	First
EPA 1978	–	+	++	+	++	–	+	–	Second
Murray et al. 1979 (GDs 1–7)	–	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Murray et al. 1979 (GDs 6–15)	–	+	++	+	++	+	+	++	First
Murray et al. 1979 (GDs 8–15)	–	+	++	+	++	+	+	++	First
Schwetz et al. 1974	–	+	++	+	–	+	+	++	First
Oral acute-duration exposure									
Ruddick et al. 1983	+	+	++	+	++	+	++	–	First
Thompson et al. 1974 (Experiment 1, 25 F)	–	+	++	+	++	–	+	++	First
Thompson et al. 1974 (Experiment 2, 6 F)	–	+	++	+	++	–	+	++	First
Thompson et al. 1974 (rabbit, 1 time/day)	–	+	++	+	++	–	+	++	First
Thompson et al. 1974 (rabbit, 2 times/day)	–	+	++	+	++	–	+	++	First
Oral intermediate-duration exposure									
Burkhalter and Balster 1979	+	+	++	++	–	+	++	++	First
NTP 1988a	+	+	++	+	+	++	++	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Lim et al. 2004 (5 weeks)	+	+	++	+	–	– –	++	++	First
Lim et al. 2004 (8 weeks)	+	+	++	+	–	– –	++	++	First

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias; (DW) = drinking water; F = females; (G) = gavage; GD = gestation day; (GO) = gavage in oil; (GW) = gavage in water; (W) = water

*Key question

C.6 RATE THE CONFIDENCE IN THE BODY OF EVIDENCE FOR EACH RELEVANT OUTCOME

Confidences in the bodies of human and animal evidence were evaluated independently for each potential outcome. ATSDR did not evaluate the confidence in the body of evidence for carcinogenicity; rather, the Agency defaulted to the cancer weight-of-evidence assessment of other agencies including HHS, EPA, and IARC. The confidence in the body of evidence for an association or no association between exposure to chloroform and a particular outcome was based on the strengths and weaknesses of individual studies. Four descriptors were used to describe the confidence in the body of evidence for effects or when no effect was found:

- **High confidence:** the true effect is highly likely to be reflected in the apparent relationship
- **Moderate confidence:** the true effect may be reflected in the apparent relationship
- **Low confidence:** the true effect may be different from the apparent relationship
- **Very low confidence:** the true effect is highly likely to be different from the apparent relationship

Confidence in the body of evidence for a particular outcome was rated for each type of study: case-control, case series, cohort, population, human-controlled exposure, and experimental animal. In the absence of data to the contrary, data for a particular outcome were collapsed across animal species, routes of exposure, and exposure durations. If species (or strain), route, or exposure duration differences were noted, then the data were treated as separate outcomes.

C.6.1 Initial Confidence Rating

In ATSDR's modification to the OHAT approach, the body of evidence for an association (or no association) between exposure to chloroform and a particular outcome was given an initial confidence rating based on the key features of the individual studies examining that outcome. The presence of these key features of study design was determined for individual studies using four "yes or no" questions, which were customized for epidemiology, human controlled exposure, or experimental animal study designs. Separate questionnaires were completed for each outcome assessed in a study. The key features for observational epidemiology (cohort, population, and case-control) studies, human controlled exposure, and experimental animal studies are presented in Tables C-11, C-12, C-13, respectively. The initial confidence in the study was determined based on the number of key features present in the study design:

- **High Initial Confidence:** Studies in which the responses to the four questions were "yes".

- **Moderate Initial Confidence:** Studies in which the responses to only three of the questions were "yes".

- **Low Initial Confidence:** Studies in which the responses to only two of the questions were "yes".

- **Very Low Initial Confidence:** Studies in which the response to one or none of the questions was "yes".

Table C-10. Key Features of Study Design for Observational Epidemiology Studies

Exposure was experimentally controlled
Exposure occurred prior to the outcome
Outcome was assessed on individual level rather than at the population level
A comparison group was used

Table C-11. Key Features of Study Design for Human-Controlled Exposure Studies

A comparison group was used or the subjects served as their own control
A sufficient number of subjects were tested
Appropriate methods were used to measure outcomes (i.e., clinically-confirmed outcome versus self-reported)
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis

Table C-12. Key Features of Study Design for Experimental Animal Studies

A concurrent control group was used
A sufficient number of animals per group were tested
Appropriate parameters were used to assess a potential adverse effect
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis

The presence or absence of the key features and the initial confidence levels for studies examining respiratory, hepatic, renal, neurological, and developmental endpoints observed in the observational epidemiology and animal experimental studies are presented in Tables C-14 and C-15, respectively.

Table C-13. Presence of Key Features of Study Design for Chloroform—Observational Epidemiology Studies

	Key features				
Reference	Controlled exposure	Exposure prior to outcome	Outcomes assessed on an individual level	Comparison group	Initial study confidence
Outcome: Respiratory effects					
Cross-sectional studies					
Font-Ribera et al. 2010	No	Yes	Yes	Yes	Moderate

Table C-13. Presence of Key Features of Study Design for Chloroform—Observational Epidemiology Studies

Reference	Key features				Initial study confidence
	Controlled exposure	Exposure prior to outcome	Outcomes assessed on an individual level	Comparison group	
Outcome: Hepatic effects					
Cohort studies					
Aiking et al. 1994	No	Yes	Yes	Yes	Moderate
Bomski et al. 1967	No	Yes	Yes	Yes	Moderate
Challen et al. 1958	No	Yes	Yes	Yes	Moderate
Li et al. 1993	No	Yes	Yes	Yes	Moderate
Outcome: Renal effects					
Cohort studies					
Aiking et al. 1994	No	Yes	Yes	Yes	Moderate
Li et al. 1993	No	Yes	Yes	Yes	Moderate
Outcome: Neurological effects					
Cohort studies					
Challen et al. 1958	No	Yes	Yes	Yes	Moderate
Li et al. 1993	No	Yes	Yes	Yes	Moderate
Outcome: Developmental effects					
Cohort studies					
Botton et al. 2015	No	No	Yes	Yes	Low
Cao et al. 2016	No	No	Yes	Yes	Low
Costet et al. 2011	No	Yes	Yes	Yes	Moderate
Dodds and King 2001	No	Yes	Yes	Yes	Moderate
Grazuleviciene et al. 2011	No	Yes	Yes	Yes	Moderate
Grazuleviciene et al. 2013	No	Yes	Yes	Yes	Moderate
Hinckley et al. 2005	No	No	Yes	Yes	Low
Hoffman et al. 2008	No	Yes	Yes	Yes	Moderate
Liu et al. 2021	No	Yes	Yes	Yes	Moderate
Rivera-Núñez and Wright 2013	No	No	Yes	Yes	Low
Sun et al. 2020	No	Yes	Yes	Yes	Moderate
Villanueva et al. 2018	No	Yes	Yes	Yes	Moderate
Villanueva et al. 2011	No	No	Yes	Yes	Low
Zhu et al. 2022	No	Yes	Yes	Yes	Moderate
Population studies					
Porter et al. 2005	No	Yes	Yes	Yes	Moderate
Wright et al. 2004	No	Yes	Yes	Yes	Moderate

Table C-13. Presence of Key Features of Study Design for Chloroform—Observational Epidemiology Studies

Reference	Key features				Initial study confidence
	Controlled exposure	Exposure prior to outcome	Outcomes assessed on an individual level	Comparison group	
Case-control studies					
Bonou et al. 2017	No	Yes	Yes	Yes	Moderate
Kaufman et al. 2018	No	Yes	Yes	Yes	Moderate
Kaufman et al. 2020	No	Yes	Yes	Yes	Moderate
Kramer et al. 1992	No	Yes	Yes	Yes	Moderate
Levallois et al. 2012	No	Yes	Yes	Yes	Moderate
Summerhayes et al. 2012	No	Yes	Yes	Yes	Moderate
Swartz et al. 2015a, 2015b	No	No	Yes	Yes	Low
Zaganjor et al. 2020	No	Yes	Yes	Yes	Moderate

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				Initial study confidence
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	
Outcome: Respiratory effects					
Inhalation acute-duration exposure					
Constan et al. 1999 (Sv/129 mice)	Yes	Yes	Yes	Yes	High
Constan et al. 1999 (B6C3F1 mice)	Yes	Yes	Yes	Yes	High
de Oliveira et al. 2015	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (mouse)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (4 days)	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Inhalation intermediate-duration exposure					
Kasai et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 5 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 7 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (3 weeks)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (6 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (3 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (6 weeks)	Yes	Yes	Yes	Yes	High
Inhalation chronic-duration exposure					
Yamamoto et al. 2002 (mouse); additional information from unpublished study (MHLW 1994a, 1994b)	Yes	Yes	Yes	Yes	High
Yamamoto et al. 2002 (rat); additional information from unpublished study (MHLW 1994a, 1994b)	Yes	Yes	Yes	Yes	High
Oral acute-duration exposure					
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Templin et al. 1996a (Fischer 344)	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Osborne-Mendel)	Yes	Yes	Yes	Yes	High
Oral intermediate-duration exposure					
Chu et al. 1982a	Yes	Yes	Yes	No	Moderate
Chu et al. 1982b	Yes	Yes	Yes	No	Moderate
Dorman et al. 1997	Yes	Yes	Yes	Yes	High
NTP 1988a	Yes	Yes	Yes	Yes	High
EPA 1980 (mouse)	Yes	Yes	Yes	Yes	High
EPA 1980 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Sehata et al. 2002 (CB6F1)	Yes	Yes	Yes	Yes	High
Oral chronic-duration exposure					
Dunnick and Melnick 1993; NCI 1976 (mouse)	Yes	Yes	Yes	No	Moderate

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features: Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Dunnick and Melnick 1993; NCI 1976 (rat)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 1)	Yes	Yes	No	No	Low
Outcome: Liver effects					
Inhalation acute-duration exposure					
Baeder and Hofmann 1988	Yes	Yes	No	Yes	Moderate
Constan et al. 1999 (Sv/129 mice)	Yes	Yes	Yes	Yes	High
Constan et al. 1999 (B6C3F1 mice)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (mouse)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (4 days)	Yes	Yes	Yes	Yes	High
Templin et al. 1996c (2 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1996c (4 days)	Yes	No	Yes	Yes	Moderate
Inhalation intermediate-duration exposure					
Kasai et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 5 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 7 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (3 weeks)	Yes	No	Yes	Yes	Moderate
Larson et al. 1996 (6 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (3 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1996b (6 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1998 (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1998 (3 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1998 (7 weeks)	Yes	No	Yes	Yes	Moderate
Torkelson et al. 1976 (rat 1–4 hours/day)	Yes	Yes	Yes	Yes	High
Torkelson et al. 1976 (rat 7 hours/day)	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Inhalation chronic-duration exposure					
Yamamoto et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Yamamoto et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Oral acute-duration exposure					
Chu et al. 1982b	Yes	Yes	Yes	No	Moderate
Ewaid et al. 2020	Yes	No	Yes	No	Low
Jones et al. 1958	No	Yes	Yes	No	Low
Keegan et al. 1998	Yes	Yes	Yes	Yes	High
Larson et al. 1993 (mouse)	Yes	Yes	Yes	No	Moderate
Larson et al. 1993 (rat)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994d	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (DW)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (G)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Lilly et al. 1997	Yes	Yes	Yes	Yes	High
Miyagawa et al. 1998	Yes	Yes	Yes	No	Moderate
Moore et al. 1982 (G)	Yes	Yes	Yes	Yes	High
Moore et al. 1982 (GO)	Yes	Yes	Yes	Yes	High
Munson et al. 1982	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Fischer 344)	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Osborne-Mendel)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (Experiment 2, 6 F)	Yes	Yes	Yes	No	Moderate
Wada et al. 2015	Yes	Yes	Yes	Yes	High
Wang et al. 1997	Yes	Yes	Yes	Yes	High
Oral intermediate-duration exposure					
Bull et al. 1986 (GO)	Yes	Yes	Yes	No	Moderate
Bull et al. 1986 (GW)	Yes	Yes	Yes	No	Moderate
Chu et al. 1982a	Yes	Yes	Yes	Yes	High
Chu et al. 1982b	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Eschenbrenner and Miller 1945	Yes	Yes	No	Yes	Moderate
NTP 1988a	Yes	Yes	Yes	Yes	High
Heywood et al. 1979	Yes	Yes	Yes	No	Moderate
EPA 1980 (mouse)	Yes	Yes	Yes	Yes	High
EPA 1980 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994b (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994d	Yes	Yes	Yes	Yes	High
Larson et al. 1995a (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Melnick et al. 1998	Yes	Yes	Yes	Yes	High
Mostafa et al. 2009	No	Yes	Yes	No	Low
Munson et al. 1982	Yes	Yes	Yes	No	Moderate
Sehata et al. 2002 (CB6F1)	Yes	Yes	Yes	Yes	High
Oral chronic-duration exposure					
Heywood et al. 1979	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (mouse)	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (rat)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 1)	Yes	Yes	Yes	No	Moderate
Outcome: Kidney effects					
Inhalation acute-duration exposure					
Baeder and Hofmann 1988	Yes	Yes	No	Yes	Moderate
Constan et al. 1999 (Sv/129 mice)	Yes	Yes	Yes	Yes	High
Constan et al. 1999 (B6C3F1 mice)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (mouse)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (4 days)	Yes	Yes	Yes	Yes	High
Templin et al. 1996c (2 weeks)	Yes	No	Yes	Yes	Moderate

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features: Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Templin et al. 1996c (4 days)	Yes	No	Yes	Yes	Moderate
Inhalation intermediate-duration exposure					
Kasai et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 5 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 7 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (3 weeks)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (6 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (3 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (6 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1998 (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1998 (3 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1998 (7 weeks)	Yes	Yes	Yes	Yes	High
Torkelson et al. 1976 (rat 1–4 hours/day)	Yes	Yes	Yes	Yes	High
Torkelson et al. 1976 (rat 7 hours/day)	Yes	Yes	Yes	Yes	High
Inhalation chronic-duration exposure					
Yamamoto et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Yamamoto et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Oral acute-duration exposure					
Chu et al. 1982b	Yes	Yes	Yes	No	Moderate
Ewaid et al. 2020	Yes	No	Yes	No	Low
Keegan et al. 1998	Yes	Yes	No	Yes	Moderate
Larson et al. 1993 (rat)	Yes	Yes	Yes	No	Moderate
Larson et al. 1993 (mouse)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994d	Yes	Yes	Yes	Yes	High
Larson et al. 1995a (DW)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (G)	Yes	Yes	Yes	No	Moderate

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Lilly et al. 1997	Yes	Yes	Yes	Yes	High
Liu et al. 2013	Yes	Yes	Yes	Yes	High
Miyagawa et al. 1998	Yes	Yes	Yes	No	Moderate
Moore et al. 1982 (G)	Yes	Yes	Yes	Yes	High
Moore et al. 1982 (GO)	Yes	Yes	Yes	Yes	High
Potter et al. 1996	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Fischer 344)	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Osborne-Mendel)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (Experiment 2, 6 F)	Yes	Yes	Yes	No	Moderate
Oral intermediate-duration exposure					
Chu et al. 1982a	Yes	Yes	Yes	Yes	High
Chu et al. 1982b	Yes	Yes	Yes	Yes	High
NTP 1988a	Yes	Yes	Yes	Yes	High
Heywood et al. 1979	Yes	Yes	No	No	Low
Hooth et al. 2002; McDorman et al. 2003a, 2003b	Yes	Yes	Yes	Yes	High
EPA 1980 (mouse)	Yes	Yes	Yes	Yes	High
EPA 1980 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994b (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994d	Yes	Yes	Yes	Yes	High
Larson et al. 1995a (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Lipsky et al. 1993 (GO)	Yes	Yes	Yes	No	Moderate
Lipsky et al. 1993 (GW)	Yes	Yes	Yes	No	Moderate
Sehata et al. 2002 (CB6F1)	Yes	Yes	Yes	Yes	High
Oral chronic-duration exposure					
Heywood et al. 1979	Yes	Yes	Yes	No	Moderate
Hard et al. 2000; Jorgenson et al. 1985 (rat)	Yes	Yes	Yes	Yes	High
Nagano et al. 2006	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features: Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Dunnick and Melnick 1993; NCI 1976 (rat)	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (mouse)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 1)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 3)	Yes	Yes	Yes	Yes	High
Outcome: Neurological effects					
Inhalation acute-duration exposure					
Constan et al. 1999 (Sv/129 mice)	Yes	Yes	No	Yes	Moderate
Constan et al. 1999 (B6C3F1 mice)	Yes	Yes	No	Yes	Moderate
DHA 2022	Yes	Yes	Yes	Yes	High
EPA 1978	Yes	Yes	No	Yes	Moderate
Gehring 1968	Yes	Yes	No	Yes	Moderate
Larson et al. 1994c; Mery et al. 1994 (rat)	Yes	Yes	Yes	Yes	High
Lehmann and Flury 1943 (cat)	No	No	No	No	Very low
Lehmann and Flury 1943 (mouse)	No	No	No	No	Very low
Inhalation intermediate-duration exposure					
Larson et al. 1996 (13 weeks; 7 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (3 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (3 weeks)	Yes	No	Yes	Yes	Moderate
Inhalation chronic-duration exposure					
Yamamoto et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Yamamoto et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Oral acute-duration exposure					
Balster and Borzelleca 1982 (14 days)	Yes	Yes	Yes	Yes	High
Balster and Borzelleca 1982 (once)	No	Yes	Yes	Yes	Moderate
Bowman et al. 1978	No	Yes	No	No	Low
NTP 1988a	Yes	Yes	Yes	No	Moderate
Jones et al. 1958	No	Yes	Yes	No	Low
Landauer et al. 1982	Yes	Yes	Yes	Yes	High
Oral intermediate-duration exposure					
Balster and Borzelleca 1982 (30 days)	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Balster and Borzelleca 1982 (60 days)	Yes	Yes	Yes	Yes	High
Balster and Borzelleca 1982 (90 days)	Yes	Yes	Yes	Yes	High
Chu et al. 1982a	Yes	Yes	Yes	Yes	High
Chu et al. 1982b	Yes	Yes	Yes	Yes	High
Dorman et al. 1997	Yes	Yes	Yes	Yes	High
Sehata et al. 2002 (CB6F1)	Yes	Yes	Yes	Yes	High
Wada et al. 2015	Yes	Yes	Yes	Yes	High
Oral chronic-duration exposure					
Heywood et al. 1979	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (rat)	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (mouse)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 1)	Yes	Yes	Yes	No	Moderate
Outcome: Developmental Effects					
Inhalation acute-duration exposure					
Baeder and Hofmann 1988	Yes	Yes	Yes	Yes	High
EPA 1978	Yes	Yes	Yes	No	Moderate
Murray et al. 1979 (GDs 1–7)	Yes	Yes	Yes	Yes	High
Murray et al. 1979 (GDs 6–15)	Yes	Yes	Yes	Yes	High
Murray et al. 1979 (GDs 8–15)	Yes	Yes	Yes	Yes	High
Schwetz et al. 1974	Yes	No	Yes	Yes	Moderate
Oral acute-duration exposure					
Ruddick et al. 1983	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (Experiment 1, 25 F)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (Experiment 2, 6 F)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (rabbit, 1 time/day)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (rabbit, 2 times/day)	Yes	Yes	Yes	Yes	High
Oral intermediate-duration exposure					
Burkhalter and Balster 1979	Yes	No	Yes	Yes	Moderate
NTP 1988a	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features: Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Lim et al. 2004 (5 weeks)	Yes	Yes	Yes	Yes	High
Lim et al. 2004 (8 weeks)	Yes	Yes	Yes	Yes	High

(DW) = drinking water; F = females; (G) = gavage; GD = gestation day; (G) = gavage in water; (GW) = gavage in water; (W) = water

A summary of the initial confidence ratings for each outcome is presented in Table C-15. If individual studies for a particular outcome and study type had different study quality ratings, then the highest confidence rating for the group of studies was used to determine the initial confidence rating for the body of evidence; any exceptions were noted in Table C-16.

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Outcome: Respiratory effects		
Inhalation acute-duration exposure		
Human studies		
Font-Ribera et al. 2010	Moderate	Moderate
Animal studies		
Constan et al. 1999 (Sv/129 mice)	High	High
Constan et al. 1999 (B6C3F1 mice)	High	
de Oliveira et al. 2015	High	
Kasai et al. 2002 (rat)	High	
Larson et al. 1994c; Mery et al. 1994 (mouse)	High	
Larson et al. 1994c; Mery et al. 1994 (rat)	High	
Larson et al. 1996	High	
Templin et al. 1996b (4 days)	High	
Inhalation intermediate-duration exposure		
Animal studies		
Kasai et al. 2002 (mouse)	High	High
Kasai et al. 2002 (rat)	High	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Larson et al. 1996 (13 weeks; 5 days/week)	High	
Larson et al. 1996 (13 weeks; 7 days/week)	High	
Larson et al. 1996 (3 weeks)	High	
Larson et al. 1996 (6 weeks)	High	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (3 weeks)	High	
Templin et al. 1996b (6 weeks)	High	
Inhalation chronic-duration exposure		
Animal studies		
Yamamoto et al. 2002 (mouse)	Moderate	Moderate
Yamamoto et al. 2002 (rat)	Moderate	
Oral acute-duration exposure		
Animal studies		
Larson et al. 1995b	Moderate	
Templin et al. 1996a (Fischer 344)	High	High
Templin et al. 1996a (Osborne-Mendel)	High	
Oral intermediate-duration exposure		
Animal studies		
Chu et al. 1982a	Moderate	
Chu et al. 1982b	Moderate	
Dorman et al. 1997	High	
NTP 1988a	High	
EPA 1980 (mouse)	High	High
EPA 1980 (rat)	High	
Larson et al. 1995b	Moderate	
Sehata et al. 2002 (CB6F1)	High	
Oral chronic-duration exposure		
Animal studies		
Dunnick and Melnick 1993; NCI 1976 (mouse)	Moderate	
Dunnick and Melnick 1993; NCI 1976 (rat)	Moderate	Moderate
Roe et al. 1979 (Experiment 1)	Low	
Outcome: Hepatic effects		
Inhalation acute-duration exposure		
Human studies		
Aiking et al. 1994	Moderate	Moderate
Animal studies		
Baeder and Hofmann 1988	Moderate	
Constan et al. 1999 (Sv/129 mice)	High	
Constan et al. 1999 (B6C3F1 mice)	High	High
Kasai et al. 2002 (rat)	High	
Larson et al. 1994c; Mery et al. 1994 (mouse)	High	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Larson et al. 1994c; Mery et al. 1994 (rat)	High	
Larson et al. 1996	High	
Templin et al. 1996b (4 days)	High	
Templin et al. 1996c (2 weeks)	Moderate	
Templin et al. 1996c (4 days)	Moderate	
Inhalation intermediate-duration exposure		
Animal studies		
Kasai et al. 2002 (mouse)	High	
Kasai et al. 2002 (rat)	High	
Larson et al. 1996 (13 weeks; 5 days/week)	High	
Larson et al. 1996 (13 weeks; 7 days/week)	High	
Larson et al. 1996 (3 weeks)	Moderate	
Larson et al. 1996 (6 weeks)	Moderate	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (13 weeks)	High	High
Templin et al. 1996b (3 weeks)	Moderate	
Templin et al. 1996b (6 weeks)	Moderate	
Templin et al. 1998 (13 weeks)	High	
Templin et al. 1998 (3 weeks)	Moderate	
Templin et al. 1998 (7 weeks)	Moderate	
Torkelson et al. 1976 (rat 1–4 hours/day)	High	
Torkelson et al. 1976 (rat 7 hours/day)	High	
Inhalation chronic-duration exposure		
Human studies		
Bomski et al. 1967	Moderate	
Challen et al. 1958	Moderate	Moderate
Li et al. 1993	Moderate	
Animal studies		
Yamamoto et al. 2002 (mouse)	High	High
Yamamoto et al. 2002 (rat)	High	
Oral acute-duration exposure		
Animal studies		
Chu et al. 1982b	Moderate	
Ewaid et al. 2020	Low	
Jones et al. 1958	Low	
Keegan et al. 1998	High	
Larson et al. 1993 (mouse)	Moderate	High
Larson et al. 1993 (rat)	Moderate	
Larson et al. 1994b (GO)	Moderate	
Larson et al. 1994b (W)	Moderate	
Larson et al. 1994d	Moderate	
Larson et al. 1995a (DW)	Moderate	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Larson et al. 1995a (G)	Moderate	
Larson et al. 1995b	Moderate	
Lilly et al. 1997	High	
Miyagawa et al. 1998	Moderate	
Moore et al. 1982 (G)	High	
Moore et al. 1982 (GO)	High	
Munson et al. 1982	High	
Templin et al. 1996a (Fischer 344)	High	
Templin et al. 1996a (Osborne-Mendel)	High	
Thompson et al. 1974 (Experiment 2, 6 F)	Moderate	
Wada et al. 2015	High	
Wang et al. 1997	High	
Oral intermediate-duration exposure		
Animal studies		
Bull et al. 1986 (GO)	Moderate	
Bull et al. 1986 (GW)	Moderate	
Chu et al. 1982a	High	
Chu et al. 1982b	High	
Eschenbrenner and Miller 1945	Moderate	
NTP 1988a	High	
Heywood et al. 1979	Moderate	
EPA 1980 (mouse)	High	
EPA 1980 (rat)	High	
Larson et al. 1994b (GO)	Moderate	High
Larson et al. 1994b (W)	Moderate	
Larson et al. 1994d	High	
Larson et al. 1995a (GO)	Moderate	
Larson et al. 1995a (W)	Moderate	
Larson et al. 1995b	Moderate	
Melnick et al. 1998	High	
Mostafa et al. 2009	Low	
Munson et al. 1982	Moderate	
Sehata et al. 2002 (CB6F1)	High	
Oral chronic-duration exposure		
Animal studies		
Heywood et al. 1979	Moderate	
Dunnick and Melnick 1993; NCI 1976 (mouse)	Moderate	Moderate
Dunnick and Melnick 1993; NCI 1976 (rat)	Moderate	
Roe et al. 1979 (Experiment 1)	Moderate	
Outcome: Renal effects		
Inhalation acute-duration exposure		
Animal studies		

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Baeder and Hofmann 1988	Moderate	High
Constan et al. 1999 (Sv/129 mice)	High	
Constan et al. 1999 (B6C3F1 mice)	High	
Kasai et al. 2002 (rat)	High	
Larson et al. 1994c; Mery et al. 1994 (mouse)	High	
Larson et al. 1994c; Mery et al. 1994 (rat)	High	
Larson et al. 1996	High	
Templin et al. 1996b (4 days)	High	
Templin et al. 1996c (2 weeks)	Moderate	
Templin et al. 1996c (4 days)	Moderate	
Inhalation intermediate-duration exposure		
Animal studies		
Kasai et al. 2002 (mouse)	High	High
Kasai et al. 2002 (rat)	High	
Larson et al. 1996 (13 weeks; 5 days/week)	High	
Larson et al. 1996 (13 weeks; 7 days/week)	High	
Larson et al. 1996 (3 weeks)	High	
Larson et al. 1996 (6 weeks)	High	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (3 weeks)	High	
Templin et al. 1996b (6 weeks)	High	
Templin et al. 1998 (13 weeks)	High	
Templin et al. 1998 (3 weeks)	High	
Templin et al. 1998 (7 weeks)	High	
Torkelson et al. 1976 (rat 1–4 hours/day)	High	
Torkelson et al. 1976 (rat 7 hours/day)	High	
Inhalation chronic-duration exposure		
Human studies		
Aiking et al. 1994	Moderate	Moderate
Li et al. 1993	Moderate	
Animal studies		
Yamamoto et al. 2002 (mouse)	High	High
Yamamoto et al. 2002 (rat)	High	
Oral acute-duration exposure		
Animal studies		
Chu et al. 1982b	Moderate	High
Ewaid et al. 2020	Low	
Keegan et al. 1998	Moderate	
Larson et al. 1993 (rat)	Moderate	
Larson et al. 1993 (mouse)	Moderate	
Larson et al. 1994b (GO)	Moderate	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Larson et al. 1994b (W)	Moderate	
Larson et al. 1994d	High	
Larson et al. 1995a (DW)	Moderate	
Larson et al. 1995a (G)	Moderate	
Larson et al. 1995b	Moderate	
Lilly et al. 1997	High	
Liu et al. 2013	High	
Miyagawa et al. 1998	Moderate	
Moore et al. 1982 (G)	High	
Moore et al. 1982 (GO)	High	
Potter et al. 1996	High	
Templin et al. 1996a (Fischer 344)	High	
Templin et al. 1996a (Osborne-Mendel)	High	
Thompson et al. 1974 (Experiment 2, 6 F)	Moderate	
Oral intermediate-duration exposure		
Animal studies		
Chu et al. 1982a	High	
Chu et al. 1982b	High	
NTP 1988a	High	
Heywood et al. 1979	Low	
Hooth et al. 2002; McDorman et al. 2003a, 2003b	High	
EPA 1980 (mouse)	High	
EPA 1980 (rat)	High	
Larson et al. 1994b (GO)	Moderate	High
Larson et al. 1994b (W)	Moderate	
Larson et al. 1994d	High	
Larson et al. 1995a (GO)	Moderate	
Larson et al. 1995a (W)	Moderate	
Larson et al. 1995b	Moderate	
Lipsky et al. 1993 (GO)	Moderate	
Lipsky et al. 1993 (GW)	Moderate	
Sehata et al. 2002 (CB6F1)	High	
Oral chronic-duration exposure		
Animal studies		
Heywood et al. 1979	Moderate	
Hard et al. 2000; Jorgenson et al. 1985 (rat)	High	
Nagano et al. 2006	High	
Dunnick and Melnick 1993; NCI 1976 (rat)	Moderate	Moderate
Dunnick and Melnick 1993; NCI 1976 (mouse)	Moderate	
Roe et al. 1979 (Experiment 1)	Moderate	
Roe et al. 1979 (Experiment 3)	High	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Outcome: Neurological effects		
Inhalation acute-duration exposure		
Animal studies		
Constan et al. 1999 (Sv/129 mice)	Moderate	High
Constan et al. 1999 (B6C3F1 mice)	Moderate	
DHA 2022	High	
EPA 1978	Moderate	
Gehring 1968	Moderate	
Larson et al. 1994c; Mery et al. 1994 (rat)	High	
Lehmann and Flury 1943 (cat)	Very low	
Lehmann and Flury 1943 (mouse)	Very low	
Inhalation intermediate-duration exposure		
Animal studies		
Larson et al. 1996 (13 weeks; 7 days/week)	High	High
Larson et al. 1996 (3 weeks)	Moderate	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (3 weeks)	Moderate	
Inhalation chronic-duration exposure		
Human studies		
Challen et al. 1958	Moderate	Moderate
Li et al. 1993	Moderate	
Animal studies		
Yamamoto et al. 2002 (mouse)	High	High
Yamamoto et al. 2002 (rat)	High	
Oral acute-duration exposure		
Animal studies		
Balster and Borzelleca 1982 (14 days)	High	High
Balster and Borzelleca 1982 (once)	Moderate	
Bowman et al. 1978	Low	
NTP 1988a	Moderate	
Jones et al. 1958	Low	
Landauer et al. 1982	High	
Oral intermediate-duration exposure		
Animal studies		
Balster and Borzelleca 1982 (30 days)	High	High
Balster and Borzelleca 1982 (60 days)	High	
Balster and Borzelleca 1982 (90 days)	High	
Chu et al. 1982a	High	
Chu et al. 1982b	High	
Dorman et al. 1997	High	
Sehata et al. 2002 (CB6F1)	High	
Wada et al. 2015	High	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Oral chronic-duration exposure		
Animal studies		
Heywood et al. 1979	Moderate	Moderate
Dunnick and Melnick 1993; NCI 1976 (rat)	Moderate	
Dunnick and Melnick 1993; NCI 1976 (mouse)	Moderate	
Roe et al. 1979 (Experiment 1)	Moderate	
Outcome: Developmental effects		
Inhalation acute-duration exposure		
Animal studies		
Baeder and Hofmann 1988	High	High
EPA 1978	Moderate	
Murray et al. 1979 (GDs 1–7)	High	
Murray et al. 1979 (GDs 6–15)	High	
Murray et al. 1979 (GDs 8–15)	High	
Schwetz et al. 1974	Moderate	
Inhalation chronic-duration exposure		
Human studies		
Swartz et al. 2015a, 2015b	Low	Low
Oral acute-duration exposure		
Animal studies		
Ruddick et al. 1983	High	High
Thompson et al. 1974 (Experiment 1, 25 F)	High	
Thompson et al. 1974 (Experiment 2, 6 F)	High	
Thompson et al. 1974 (rabbit, 1 time/day)	High	
Thompson et al. 1974 (rabbit, 2 times/day)	High	
Oral chronic-duration exposure		
Human studies		
Bonou et al. 2017	Moderate	Moderate
Botton et al. 2015	Low	
Cao et al. 2016	Low	
Costet et al. 2011	Moderate	
Dodds and King 2001	Moderate	
Grazuleviciene et al. 2011	Moderate	
Grazuleviciene et al. 2013	Moderate	
Hinckley et al. 2005	Low	
Hoffman et al. 2008	Moderate	
Kaufman et al. 2018	Moderate	
Kaufman et al. 2020	Moderate	
Kramer et al. 1992	Moderate	
Levallois et al. 2012	Moderate	
Liu et al. 2021	Moderate	
Porter et al. 2005	Moderate	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Rivera-Núñez and Wright 2013	Low	
Summerhayes et al. 2012	Moderate	
Sun et al. 2020	Moderate	
Villanueva et al. 2011	Moderate	
Villanueva et al. 2018	Low	
Wright et al. 2004	Moderate	
Zaganjor et al. 2020	Moderate	
Zhu et al. 2022	Moderate	
Animal studies		
Burkhalter and Balster 1979	Moderate	High
NTP 1988a	High	
Lim et al. 2004 (5 weeks)	High	
Lim et al. 2004 (8 weeks)	High	

(DW) = drinking water; F = females; (G) = gavage; GD = gestation day; (G) = gavage in water; (GW) = gavage in water; (W) = water

Table C-16. Adjustments to the Initial Confidence in the Body of Evidence

	Initial confidence	Adjustments to the initial confidence rating	Final confidence
Outcome: Respiratory effects			
Human studies	Moderate	-1 Risk of bias	Low
Animal studies	High		High
Outcome: Hepatic effects			
Human studies	Moderate	-1 Risk of bias	Low
Animal studies	High	+1 Consistency in the body of evidence	High
Outcome: Renal effects			
Human studies	Moderate	-1 Risk of bias	Low
Animal studies	High	+1 Consistency in the body of evidence	High
Outcome: Neurological effects			
Human studies	Moderate	-1 Risk of bias	Low
Animal studies	High	-1 Risk of bias +1 Large magnitude of effect	High
Outcome: Developmental effects			
Human studies	Moderate	-1 Risk of bias -1 Unexplained inconsistencies	Very low
Animal studies	High	-1 Unexplained inconsistencies	Moderate

C.6.2 Adjustment of the Confidence Rating

The initial confidence rating was then downgraded or upgraded depending on whether there were substantial issues that would decrease or increase confidence in the body of evidence. The nine properties of the body of evidence that were considered are listed below. The summaries of the assessment of the confidence in the body of evidence for respiratory, hepatic, renal, neurological, and developmental effects are presented in Table C-17. For epidemiological data, if the confidence ratings for a particular outcome were based on more than one type of human study, then the highest confidence rating was used for subsequent analyses. The initial confidence reflects the body of evidence for the health outcome across all exposure routes and durations. Adjustments to the initial confidence are based on the properties discussed below and shown in Table C-16. If a property is not shown in Table C-16, ATSDR concluded that the property neither increases nor decreases confidence in the corresponding health outcome.

Table C-17. Confidence in the Body of Evidence for Chloroform

	Confidence in body of evidence	
Outcome	Human studies	Animal studies
Respiratory effects	Low	High
Hepatic effects	Low	High
Renal effects	Low	High
Neurological effects	Low	High
Developmental effects	Very low	Moderate

Five properties of the body of evidence were considered to determine whether the confidence rating should be downgraded:

- **Risk of bias.** Evaluation of whether there is substantial risk of bias across most of the studies examining the outcome. This evaluation used the risk of bias tier groupings for individual studies examining a particular outcome (Tables C-9 and C-10). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for risk of bias:
    - No downgrade if most studies are in the risk of bias first tier
    - Downgrade one confidence level if most studies are in the risk of bias second tier
    - Downgrade two confidence levels if most studies are in the risk of bias third tier

- **Unexplained inconsistency.** Evaluation of whether there is inconsistency or large variability in the magnitude or direction of estimates of effect across studies that cannot be explained. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for unexplained inconsistency:
    - No downgrade if there is little inconsistency across studies or if only one study evaluated the outcome
    - Downgrade one confidence level if there is variability across studies in the magnitude or direction of the effect
    - Downgrade two confidence levels if there is substantial variability across studies in the magnitude or direct of the effect

- **Indirectness.** Evaluation of four factors that can affect the applicability, generalizability, and relevance of the studies:
    - Relevance of the animal model to human health—unless otherwise indicated, studies in rats, mice, and other mammalian species are considered relevant to humans
    - Directness of the endpoints to the primary health outcome—examples of secondary outcomes or nonspecific outcomes include organ weight in the absence of histopathology or clinical chemistry findings in the absence of target tissue effects
    - Nature of the exposure in human studies and route of administration in animal studies—inhalation, oral, and dermal exposure routes are considered relevant unless there are compelling data to the contrary
    - Duration of treatment in animal studies and length of time between exposure and outcome assessment in animal and prospective human studies—this should be considered on an outcome-specific basis

  Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for indirectness:
    - No downgrade if none of the factors are considered indirect
    - Downgrade one confidence level if one of the factors is considered indirect
    - Downgrade two confidence levels if two or more of the factors are considered indirect

- **Imprecision.** Evaluation of the narrowness of the effect size estimates and whether the studies have adequate statistical power. Data are considered imprecise when the ratio of the upper to lower 95% CIs for most studies is ≥10 for tests of ratio measures (e.g., odds ratios) and ≥100 for absolute measures (e.g., percent control response). Adequate statistical power is determined if the study can detect a potentially biologically meaningful difference between groups (20% change from control response for categorical data or risk ratio of 1.5 for continuous data). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for imprecision:
    - No downgrade if there are no serious imprecisions
    - Downgrade one confidence level for serious imprecisions
    - Downgrade two confidence levels for very serious imprecisions

- **Publication bias.** Evaluation of the concern that studies with statistically significant results are more likely to be published than studies without statistically significant results.
    - Downgrade one level of confidence for cases where there is serious concern with publication bias

Four properties of the body of evidence were considered to determine whether the confidence rating should be upgraded:

- **Large magnitude of effect.** Evaluation of whether the magnitude of effect is sufficiently large so that it is unlikely to have occurred as a result of bias from potential confounding factors.
    - Upgrade one confidence level if there is evidence of a large magnitude of effect in a few studies, provided that the studies have an overall low risk of bias and there is no serious unexplained inconsistency among the studies of similar dose or exposure levels; confidence can also be upgraded if there is one study examining the outcome, provided that the study has an overall low risk of bias

- **Dose response.** Evaluation of the dose-response relationships measured within a study and across studies. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
    - Upgrade one confidence level for evidence of a monotonic dose-response gradient
    - Upgrade one confidence level for evidence of a non-monotonic dose-response gradient where there is prior knowledge that supports a non-monotonic dose-response and a non-monotonic dose-response gradient is observed across studies

- **Plausible confounding or other residual biases.** This factor primarily applies to human studies and is an evaluation of unmeasured determinants of an outcome such as residual bias towards the null (e.g., "healthy worker" effect) or residual bias suggesting a spurious effect (e.g., recall bias). Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
    - Upgrade one confidence level for evidence that residual confounding or bias would underestimate an apparent association or treatment effect (i.e., bias toward the null) or suggest a spurious effect when results suggest no effect

- **Consistency in the body of evidence.** Evaluation of consistency across animal models and species, consistency across independent studies of different human populations and exposure scenarios, and consistency across human study types. Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
    - Upgrade one confidence level if there is a high degree of consistency in the database

C.7 TRANSLATE CONFIDENCE RATING INTO LEVEL OF EVIDENCE OF HEALTH EFFECTS

In the seventh step of the systematic review of the health effects data for chloroform, the confidence in the body of evidence for specific outcomes was translated to a level of evidence rating. The level of evidence rating reflected the confidence in the body of evidence and the direction of the effect (i.e., toxicity or no toxicity); route-specific differences were noted. The level of evidence for health effects was rated on a five-point scale:

- **High level of evidence:** High confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Moderate level of evidence:** Moderate confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Low level of evidence:** Low confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Evidence of no health effect:** High confidence in the body of evidence that exposure to the substance is not associated with the health outcome
- **Inadequate evidence:** Low or moderate confidence in the body of evidence that exposure to the substance is not associated with the health outcome OR very low confidence in the body of evidence for an association between exposure to the substance and the health outcome

A summary of the level of evidence of health effects for chloroform is presented in Table C-18.

Table C-18. Level of Evidence of Health Effects for Chloroform

Outcome	Confidence in body of evidence	Direction of health effect	Level of evidence for health effect
Human studies			
Respiratory effects	Low	No Effect	Inadequate evidence
Hepatic effects	Low	Effect	Low
Renal effects	Low	Effect	Low
Neurological effects	Low	Effect	Low
Developmental effects	Very low	Effect	Inadequate evidence
Animal studies			
Respiratory effects	High	Effect	High
Hepatic effects	High	Effect	High
Renal effects	High	Effect	High
Neurological effects	High	Effect	High
Developmental effects	Moderate	Effect	Moderate

C.8 INTEGRATE EVIDENCE TO DEVELOP HAZARD IDENTIFICATION CONCLUSIONS

The final step involved the integration of the evidence streams for the human studies and animal studies to allow for a determination of hazard identification conclusions. For health effects, there were four hazard identification conclusion categories:

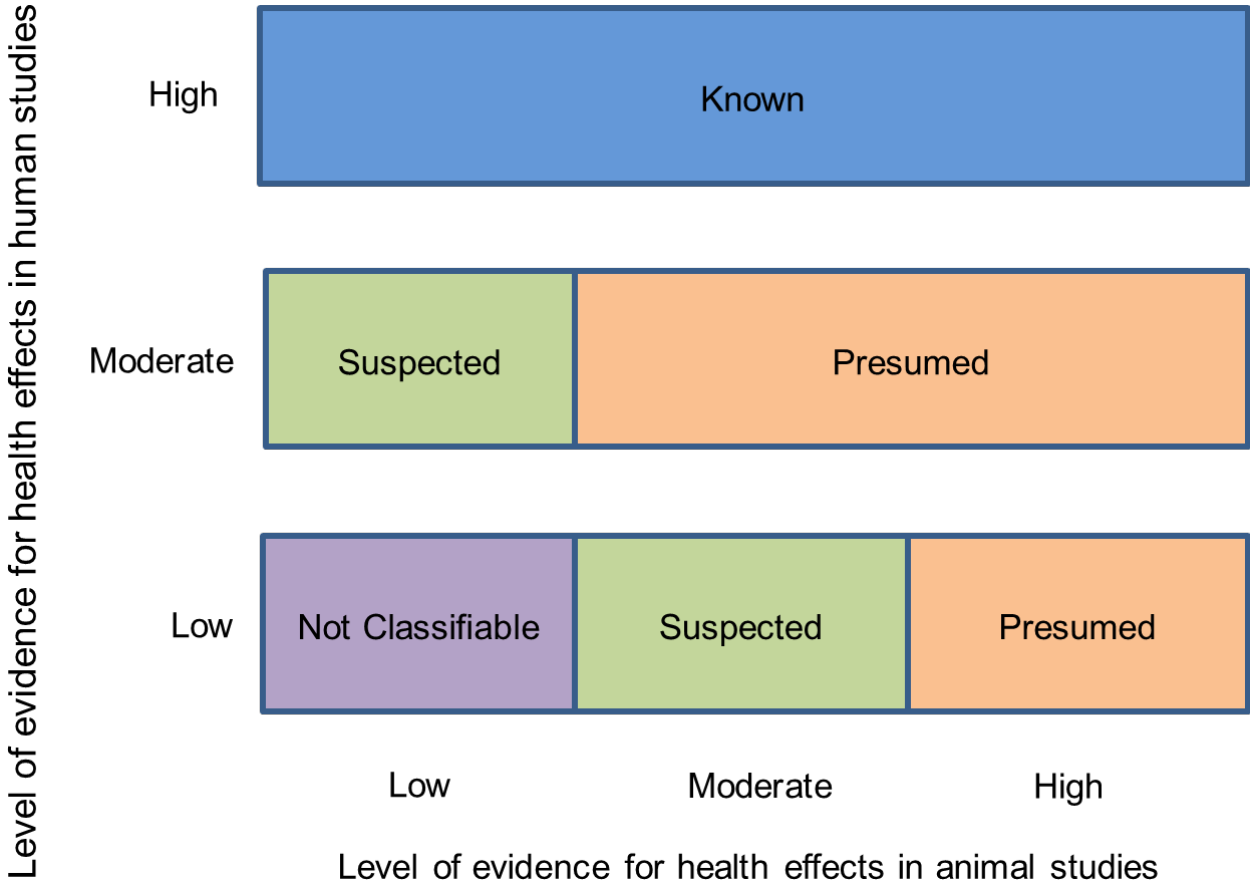
- **Known** to be a hazard to humans
- **Presumed** to be a hazard to humans
- **Suspected** to be a hazard to humans
- **Not classifiable** as to the hazard to humans

The initial hazard identification was based on the highest level of evidence in the human studies and the level of evidence in the animal studies; if there were no data for one evidence stream (human or animal), then the hazard identification was based on the one data stream (equivalent to treating the missing evidence stream as having low level of evidence). The hazard identification scheme is presented in Figure C-1 and described below:

- **Known:** A health effect in this category would have:
  - High level of evidence for health effects in human studies **AND** a high, moderate, or low level of evidence in animal studies.
- **Presumed:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** high or moderate level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** high level of evidence in animal studies
- **Suspected:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** low level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** moderate level of evidence in animal studies

- **Not classifiable:** A health effect in this category would have:
  - Low level of evidence in human studies **AND** low level of evidence in animal studies

Figure C-1. Hazard Identification Scheme



Other relevant data such as mechanistic or mode-of-action data were considered to raise or lower the level of the hazard identification conclusion by providing information that supported or opposed biological plausibility.

Two hazard identification conclusion categories were used when the data indicated that there may be no health effect in humans:

- **Not identified** to be a hazard in humans
- **Inadequate** to determine hazard to humans

If the human level of evidence conclusion of no health effect was supported by the animal evidence of no health effect, then the hazard identification conclusion category of "not identified" was used. If the human or animal level of evidence was considered inadequate, then a hazard identification conclusion category of "inadequate" was used. As with the hazard identification for health effects, the impact of other relevant data was also considered for no health effect data.

The hazard identification conclusions for chloroform are listed below and summarized in Table C-19.

Known Health Effects

- Hepatic effects: There is a low level of evidence for hepatic effects from limited epidemiological data and a high level of evidence from a large number of animal studies with consistent findings. The Hazard Identification conclusion for hepatic effects was increased from "Presumed" to "Known" based on other relevant data consisting of the extensive database of case reports and case series documenting hepatic effects of chloroform in exposed humans.
  - Evidence from epidemiological studies: There is some evidence of adverse hepatic effects in humans with occupational exposure to chloroform (Bomski et al. 1967), while other studies of occupational exposure did not find any hepatic effects (Challen et al. 1958; Li et al. 1993).
  - Evidence from animal studies: Hepatic lesions have been observed in numerous animal studies, including acute-, intermediate-, and chronic-duration inhalation and oral studies in rodents; intermediate- and chronic-duration oral studies in dogs; and an acute-duration oral study in rabbits (Section 2.9). Typical lesion progression begins with mild histopathological damage after low and/or brief exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation) and progresses to widespread and severe necrosis and degeneration with high and/or long-term exposure.
  - Other relevant data: The hepatic findings are strengthened by a large number of case reports and case-series reports indicating that the liver is a primary target following high-level chloroform exposure. Acute liver failure and/or severe liver damage are common findings in fatal exposures via inhalation (Giusti and Chiarotti 1981; Lionte 2010; Royston 1924; Townsend 1939) or oral (Dettling et al. 2016; Piersol et al. 1933) exposure. Reversible clinical signs of hepatotoxicity are commonly observed in nonfatal case studies of chloroform toxicity following inhalation exposure (Dettling et al. 2016; Gosselink et al. 2012; Hutchens and Kung 1985; Kang et al. 2014; Lin et al. 2005; Lunt 1953; Minor et al. 2018; Phoon et al. 1983; Smith et al. 1973). Similarly, reversible hepatotoxicity is a common finding in nonfatal cases of attempted suicide via chloroform ingestion (Choi et al. 2006; Dell'Aglio et al. 2010; Jayaweera et al. 2017; Kim 2008; Rao et al. 1993; Schroeder 1965;) and other cases of accidental or unspecified oral poisoning (Hakim et al. 1992; Sridhar et al. 2011; Storms 1973). One nonfatal dermal case also reported reversible hepatotoxicity (Vlad et al. 2014). Experimental studies demonstrate that hepatic effects are attributable to reactive intermediates produced during metabolism of chloroform (Brown et al. 1974a; Constan et al. 1999; Fang et al. 2008; Gopinath and Ford 1975).
- Neurological effects: There is a low level of evidence for neurological effects from limited epidemiological data and a high level of evidence from a large number of animal studies with consistent findings. The Hazard Identification conclusion for neurological effects was increased from "Presumed" to "Known" based on: (a) the historical use of chloroform as a general anaesthetic; (b) case reports and case series documenting marked neurological effects of chloroform in exposed humans; and (c) a plausible mechanism of action.
  - Evidence from epidemiological studies: There is limited evidence of neurological impairments (e.g., impaired hand-eye coordination, slowed reaction time) and subjective neurological complaints (e.g., dizziness, fatigue, depression) following occupational exposure to chloroform (Challen et al. 1958; Li et al. 1993).
  - Evidence from animal studies Chloroform is a CNS depressant in animals exposed via inhalation (Constan et al. 1999; EPA 1978; Gehring 1968; Lehmann and Flury 1943) or oral routes (Bowman et al. 1978; NTP 1988a; Jones et al. 1958). At exposure levels below those associated with CNS depression, there is limited evidence for altered neurobehavior following oral exposure in animals, including altered motor activity, impaired coordination, and altered operant learning (Balster and Borzelleca 1982; DHA 2022; Landauer et al. 1982; Wada et al. 2015). The only histopathological change reported in the neurological system is olfactory nerve loss in rats following acute-duration inhalation exposure (Larson et al. 1994c;

Mery et al. 1994); this finding is likely in response to degeneration of the nasal olfactory epithelial tissue observed at the same exposure levels.

- Other relevant data: Chloroform was used as a general anesthetic beginning in the late 1800s and was widely used for more than 100 years (Davison 1959), providing clear evidence for its neurological effects after inhalation exposure. Case reports also show that chloroform induces CNS depression at high inhalation exposure levels in humans (Featherstone 1947; Smith et al. 1973; Whitaker and Jones 1965). CNS depression has also been reported in individuals who intentionally or accidentally ingested the chemical (Piersol et al. 1933; Schroeder 1965; Storms 1973). Chloroform may cause CNS depression via perturbation of the lipophilic cell membrane, which results in alterations in proteins that function as ion channels and/or neurotransmitter receptors (Harris and Groh 1985; Jenkins et al. 2001; Nakagawa et al. 2000).

Presumed Health Effects

- Respiratory effects: There is inadequate evidence for respiratory effects from a single epidemiological study and a high level of evidence from several animal studies with consistent findings. Other relevant data (case reports) were not sufficient to merit an increase in the hazard identification conclusion.
  - Evidence from epidemiological studies: A single epidemiological study reported no change in respiratory function in adults after a 40-minute swim in a chlorinated pool (Font-Ribera et al. 2010); no other epidemiology studies of this endpoint were located, and no studies evaluating nasal effects in humans following exposure to chloroform were identified.
  - Evidence from animal studies: In animals, the nasal epithelium is a sensitive target of toxicity following inhalation and oral exposure (Section 2.4). Damage to the lower respiratory tract in animals was generally only observed at lethal exposure levels (Bowman et al. 1978; Kasai et al. 2002; NCI 1976). There is limited evidence of inflammatory responses in the lung at low inhalation exposure levels in mice (de Oliveira et al. 2015).
  - Other relevant data: Lung damage has been reported in several fatal cases of inhalation or oral exposure (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965). Changes in respiratory rate and/or respiratory arrest have been reported in human case reports of high exposure (Cui et al. 2022; Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965) but these effects are likely secondary to CNS depression.
- Renal effects: There is inadequate evidence for renal effects from limited epidemiological data and a high level of evidence from a large number of animal studies with consistent findings. Other relevant data (case reports) were not sufficient to merit an increase in the hazard identification conclusion.
  - Evidence from epidemiological studies: No changes in renal clinical chemistry values were observed in one occupational cohort (Li et al. 1993) or in a group of competitive swimmers exposed to chlorinated water during training (Aiking et al. 1994).
  - Evidence from animal studies: The kidney is a clear target of toxicity in animals. Renal lesions have been observed in numerous studies following acute-, intermediate-, and chronic-duration inhalation and oral studies in rodents; intermediate- and chronic-duration oral studies in dogs; and acute-duration oral and dermal studies in rabbits (Section 2.10). Typical lesion progression begins with mild histopathological damage after low and/or brief exposures (e.g., tubular dilation, single-cell necrosis, renal cell proliferation) and progresses to severe nephropathy characterized by widespread necrosis and degeneration with higher and/or long-term exposure.
  - Other relevant data: Case reports of fatal chloroform exposures have reported renal damage (Piersol et al. 1933; Royston 1924). Additionally, reversible changes in renal clinical chemistry and urinalysis have been reported in nonfatal cases (Dettling et al. 2016; Gosselink

et al. 2012; Piersol et al. 1933; Schroeder 1965; Sridhar et al. 2011; Wallace 1950). Experimental studies demonstrate that renal effects are attributable to reactive intermediates produced during metabolism of chloroform (Constan et al. 1999; Culliford and Hewitt 1957; Liu et al. 2013; Weir et al. 2005).

Suspected Health Effects

- Developmental effects: There is inadequate evidence for developmental effects from epidemiological data and a moderate level of evidence from animal studies with some inconsistent findings. Other relevant data were limited and did influence the hazard conclusion.
  - Evidence from epidemiological studies: Impaired growth (e.g., low birth weight, small for gestational age, decreased postnatal weight gain) has been associated with chloroform exposure from tap water in some epidemiological studies (Botton et al. 2015; Grazuleviciene et al. 2011; Kramer et al. 1992; Summerhayes et al. 2012; Sun et al. 2020; Wright et al. 2004; Zaganjor et al. 2020). However, these findings were not observed in other studies (Bonou et al. 2017; Cao et al. 2016; Hinckley et al. 2005; Liu et al. 2021; Porter et al. 2005; Villanueva et al. 2011). No clear associations were observed between chloroform exposure and birth defects (Dodds and King 2001; Grazuleviciene et al. 2013; Hoffman et al. 2008; Kaufman et al. 2018, 2020; Levallois et al. 2012; Rivera-Núñez and Wright 2013) or neurodevelopmental outcomes (Villanueva et al. 2018). A meta-analysis identified a slight (5%) increase in risk of small for gestational age associated with increased chloroform levels in maternal drinking water; however, this increase in risk paralleled the increase (7%) observed for total trihalomethanes in drinking water (Summerhayes et al. 2021).
  - Evidence from animal studies: In animals, maternal inhalation during gestation was associated with birth defects in rats, such as missing ribs and acaudate fetuses with imperforate anus, and cleft palate in mice (Murray et al. 1979; Schwetz et al. 1974). These defects were not observed in additional developmental studies in rats exposed via inhalation (Baeder and Hofmann 1988; EPA 1978) or rats or rabbits exposed orally (Ruddick et al. 1983; Thompson et al. 1974). However, delayed ossification and decreased fetal growth were reported in many developmental studies after inhalation or oral exposure, generally at maternally toxic exposure levels (Baeder and Hofmann 1988; Murray et al. 1979; Ruddick et al. 1983; Schwetz et al. 1974; Thompson et al. 1974).
  - Other relevant data: Chloroform is known to cross the placenta (Danielsson et al. 1986).

Table C-19. Hazard Identification Conclusions for Chloroform

Outcome	Hazard identification
Respiratory effects	Presumed
Hepatic effects	Known
Renal effects	Presumed
Neurological effects	Known
Developmental effects	Suspected

APPENDIX D. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page D-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), food intake (FI), gross necropsy (GN), hematology (HE), histopathology (HP), immune function (IX), lethality (LE), neurological function (NX), organ function (OF), ophthalmology (OP), organ weight (OW), reproductive function (RX), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page D-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(14) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(15) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(17) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

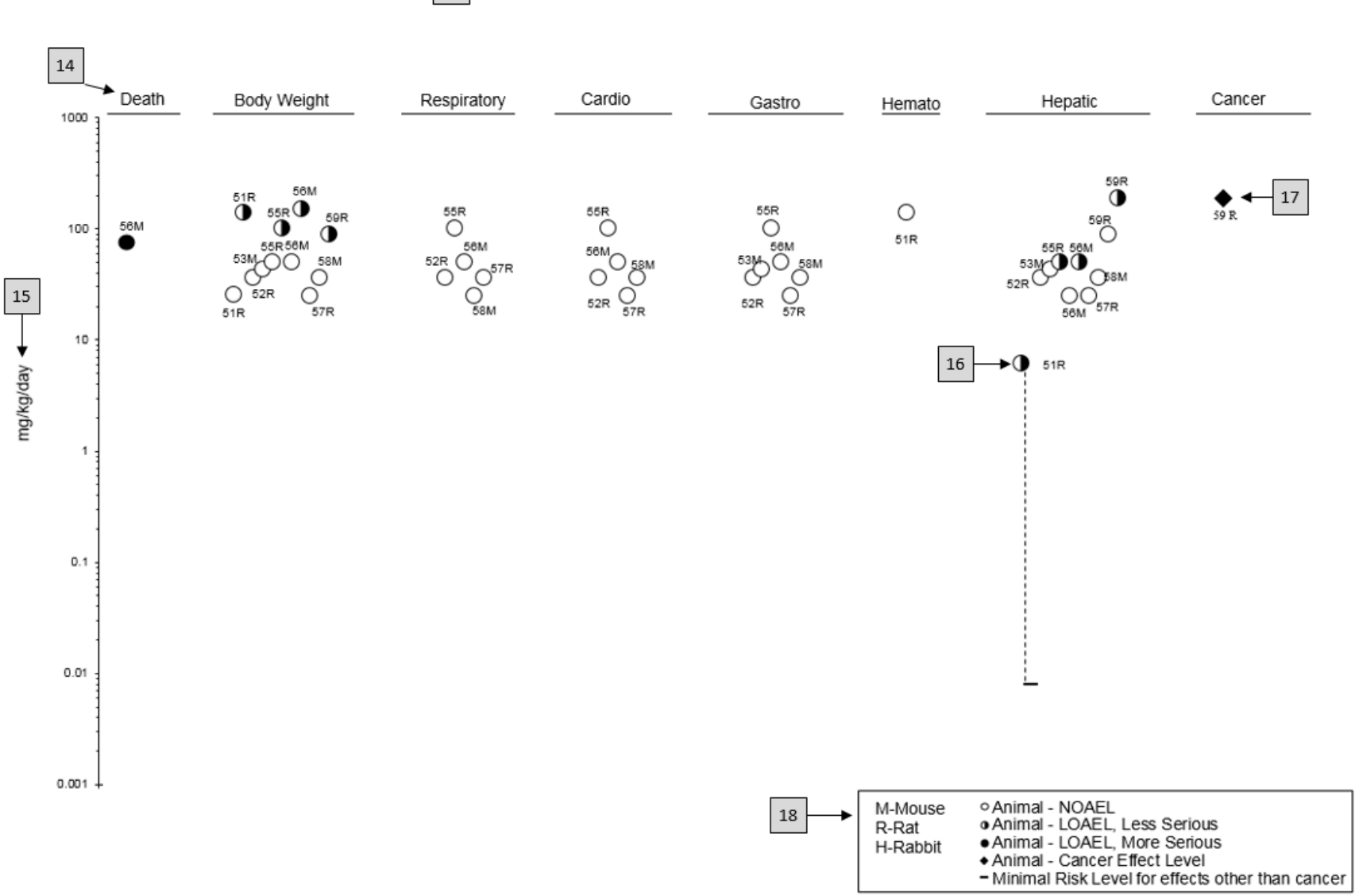
(18) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral (1)

Figure key[a] (4)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
(2) **CHRONIC EXPOSURE**									
51 (3)	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
(10) **Aida et al. 1992**									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

(11) [a]The number corresponds to entries in Figure 2-x.
[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).
[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral



APPENDIX E. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:
Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

ATSDR develops educational and informational materials for health care providers categorized by hazardous substance, clinical condition, and/or by susceptible population. The following additional materials are available online:

Clinician Briefs and Overviews discuss health effects and approaches to patient management in a brief/factsheet style. They are narrated PowerPoint presentations with Continuing Education credit available (see https://www.atsdr.cdc.gov/emes/health_professionals/clinician-briefs-overviews.html).

Managing Hazardous Materials Incidents is a set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp).

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 400 7th Street, S.W., Suite 5W, Constitution Center, Washington, DC 20024 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX F. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or malignant tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal LOAEL—Indicates a minimal adverse effect or a reduced capacity of an organ or system to absorb additional toxic stress that does not necessarily lead to the inability of the organ or system to function normally.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The exposure level of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this exposure level, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Serious LOAEL—A dose that evokes failure in a biological system and can lead to morbidity or mortality.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX G. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register

FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences

NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
USNRC	U.S. Nuclear Regulatory Commission

VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result