# Carbon Tetrachloride

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Carbon Tetrachloride

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# TABLE OF CONTENTS

**DSD HISTORY** ..... I

**TABLE OF CONTENTS** ..... II

**LIST OF TABLES** ..... IV

**LIST OF FIGURES** ..... V

**ACRONYMS AND ABBREVIATIONS** ..... VII

**CHAPTER 1 SUMMARY TABLES** ..... 1

**CHAPTER 2 BACKGROUND INFORMATION** ..... 5

2.1 PHYSICAL/CHEMICAL PROPERTIES ..... 5
2.2 SOURCES AND USES ..... 5

**CHAPTER 3 ACUTE EVALUATION** ..... 6

3.1 HEALTH-BASED ACUTE REV AND $^{ACUTE}$ESL ..... 6
*3.1.1 Key and Supporting Studies* ..... *6*
3.1.1.1 Human Studies ..... 6
3.1.1.1.1 Davis (1934) Study ..... 7
3.1.1.1.2 Lehmann and Schmidt-Kehl (1936) Study ..... 9
3.1.1.1.3 Kazantzis and Bomford (1960) Study ..... 9
3.1.1.2 Animal Studies ..... 10
3.1.1.2.1 Adams et al (1952) Study ..... 10
3.1.1.2.2 David et al. (1981) Study ..... 10
3.1.1.2.3 Paustenbach et al. (1986) Studies ..... 11
3.1.1.2.4 Wang et al. (1997) Study ..... 11
3.1.1.3 Reproductive and Developmental Studies ..... 11
3.1.1.3.1 Human Studies ..... 11
3.1.1.3.2 Animal Studies ..... 11
*3.1.2 Health-Based Acute 1-h ReV and ESL* ..... *12*
3.1.2.1 Selection of the Key Study, Point of Departure (POD), and Critical Effect ..... 12
3.1.2.2 MOA and Dose Metric for Critical Effect ..... 13
3.1.2.3 Adjustments to the POD ..... 13
3.1.2.3.1 Default Exposure Duration Adjustments ..... 13
3.1.2.3.1.1 For the 4-h NOAEL of 76 ppm ..... 13
3.1.2.3.1.2 For the 30-min LOAEL of 158 ppm ..... 14
3.1.2.3.2 Default Dosimetry Adjustments from Animal-to-Human Exposure ..... 14
3.1.2.4 Adjustments to the $POD_{HEC}$ ..... 14
3.1.2.5 Health-Based 1-h Acute ReV and $^{acute}$ESL ..... 15
3.1.2.6 Acute Inhalation Observed Adverse Effect Level (IOAEL) ..... 16
*3.1.3 Health-Based Acute 24-h ReV* ..... *16*
3.1.3.1 Selection of the Key Study, POD, and Critical Effect ..... 16
3.1.3.2 MOA Analysis and Dose Metric for the Critical Effect ..... 17
3.1.3.3 Adjustments to the POD ..... 17
3.1.3.3.1 Default Exposure Duration Adjustments ..... 17
3.1.3.3.1.1 For the 8-h LOAEL of 45 ppm ..... 17
3.1.3.3.1.2 For the 4-h NOAEL of 76 ppm ..... 17
3.1.3.3.2 Default Dosimetry Adjustments from Animal-to-Human Exposure ..... 17

3.1.3.4 Adjustments to the $POD_{HEC}$ ..... 18
3.1.3.5 Health-Based Acute 24-h ReV ..... 18
3.1.3.6 Subacute IOAEL ..... 19
3.2 WELFARE-BASED ACUTE EVALUATION ..... 20
*3.2.1 Odor Perception* ..... *20*
*3.2.2 Vegetation Effects* ..... *20*
3.3 SUMMARY OF THE ACUTE VALUES ..... 20

**CHAPTER 4 CHRONIC EVALUATION ..... 21**

4.1 NONCARCINOGENIC POTENTIAL ..... 21
*4.1.1 Key and Supporting Studies* ..... *21*
4.1.1.1 Human Study, Tomenson et al. (1995) ..... 21
4.1.1.2 Animal Studies ..... 22
4.1.1.2.1 Nagano et al. (2007a) ..... 22
4.1.1.2.2 Adams et al. (1952) ..... 24
4.1.1.2.3 Prendergast et al. (1967) ..... 24
4.1.1.2.4 Nagano et al. (2007b) ..... 24
4.1.1.3 Reproductive and Developmental Studies ..... 25
*4.1.2 Selection of the Key Study and Critical Effect* ..... *26*
*4.1.3 MOA Analysis and Dose Metric* ..... *27*
*4.1.4 Derivation of the POD* ..... *28*
4.1.4.1 PBPK Modeling for Internal Dose Metrics for the Key Study (Nagano et al. 2007a) ..... 28
4.1.4.2 Benchmark Dose Modeling ..... 29
4.1.4.3 PBPK Modeling to derive Human Equivalent Concentrations ..... 30
*4.1.5 Default Exposure Duration Adjustments* ..... *32*
*4.1.6 Default Dosimetry Adjustments from Animal-to-Human Exposure* ..... *32*
*4.1.7 Adjustments to the $POD_{HEC}$* ..... *32*
*4.1.6 Health-Based Chronic ReV and $^{chronic}ESL_{nonlinear(nc)}$* ..... *33*
*4.1.7 Chronic Noncarcinogenic IOAEL* ..... *34*
*4.1.8 Comparison of Results* ..... *34*
4.2 CARCINOGENIC POTENTIAL ..... 36
*4.2.1 Carcinogenic Weight of Evidence* ..... *36*
*4.2.2 Key and Supporting Studies* ..... *37*
4.2.2.1 Human/Epidemiological Studies ..... 37
4.2.2.2 Animal Studies – Nagano et al. 2007a, JBRC 1998 ..... 38
*4.2.3 Carcinogenic MOA and Dose Metric* ..... *40*
*4.2.4 Selection of the Key Study and Critical Effects* ..... *41*
*4.2.5 Derivation of the POD* ..... *42*
4.2.5.1 PBPK Modeling for Internal Dose Metrics for the Key Study ..... 42
4.2.5.2 BMD Modeling ..... 43
4.2.5.3 PBPK Modeling of HECs ..... 46
4.2.5.3.1 Default Exposure Duration Adjustments ..... 49
*4.2.6 Calculation of a Unit Risk Factor* ..... *49*
*4.2.7 Calculation of an Air Concentration at $1x10^{-5}$ Excess Cancer Risk* ..... *50*
*4.2.8 Comparison of Cancer Potency Factors* ..... *50*
*4.2.9 Evaluating Susceptibility from Early-Life Exposures* ..... *51*
*4.2.10 Chronic Carcinogenic IOAEL* ..... *51*
4.3 WELFARE-BASED CHRONIC EVALUATION ..... 52

4.4 SUMMARY OF THE CHRONIC VALUES ..... 52

**CHAPTER 5 REFERENCES ..... 53**

**APPENDIX A BENCHMARK DOSE MODELING FOR DERIVING THE CHRONIC REFERENCE VALUE ..... 56**

**APPENDIX B BENCHMARK DOSE MODELING OUTPUTS FOR LOW-DOSE LINEAR EXTRAPOLATION APPROACH ..... 65**

# LIST OF TABLES

Table 1. Acute health and welfare-based screening values for carbon tetrachloride ..... 2
Table 2. Chronic health and welfare-based screening values for carbon tetrachloride ..... 3
Table 3. Chemical and physical data ..... 4
Table 4: Summary of experiments 1 through 8: time, dose, and degree of central nervous system effects [a] from $CCl_4$ exposure [b] ..... 9
Table 5. Derivation of the 1-h acute ReV and $^{acute}$ESL ..... 15
Table 6. Derivation of the 24-h acute ReV ..... 19
Table 7. Incidences of selected histopathological liver lesions in rats exposed to $CCl_4$ vapor by inhalation for 2 yr ..... 23
Table 8. Incidences of selected histopathological liver lesions in mice exposed to $CCl_4$ vapor by inhalation for 2 yr ..... 23
Table 9. Summary of subchronic and chronic inhalation toxicity studies for $CCl_4$ ..... 27
Table 10. Comparisons of internal dose metrics predicted from PBPK rat models ..... 29
Table 11. Summary of $BMD_{10}$ and $BMDL_{10}$ values for male and female rats using MCA or MRAMKL dose metrics ..... 30
Table 12. $BMDL_{10}$, and corresponding HEC values for fatty changes of liver in male rats exposed to $CCl_4$ 31
Table 13. Derivation of the chronic ReV and $^{chronic}ESL_{threshold(nc)}$ ..... 33
Table 14. $BMD_{10}$ and corresponding $HEC_{BMD10}$ values for fatty changes of liver in male rats exposed to $CCl_4$ ..... 34
Table 15. Comparison of the chronic toxicity factors for $CCl_4$ among agencies ..... 34
Table 16. Incidences of liver tumors in rats exposed to $CCl_4$ vapor ..... 39
Table 17. Incidences of liver tumors in mice exposed to $CCl_4$ vapor ..... 40
Table 18. Comparison of internal dose metrics predicted from Fisher et al. (2004) and Thrall et al. (2000) PBPK mouse models[a] ..... 43
Table 19. Summary of $BMD_{10}$ and $BMDL_{10}$ values for incidence data using MRAMKL as the dose metric for combined liver tumors in female rats ..... 44
Table 20. Summary of $BMD_{10}$ and $BMDL_{10}$ values for combined liver tumors in mice using the MRAMKL dose metric ..... 45
Table 21. Summary of $BMD_{10}$ and $BMDL_{10}$ values for pheochromocytomas in mice using the MCA dose metric ..... 46
Table 22. $BMDL_{10}$ values and corresponding HEC values for combined liver tumors in female rats ..... 47
Table 23. $BMDL_{10}$ values and corresponding HEC values for combined liver tumors in male and female mice ..... 48

Table 24. $BMDL_{10}$ values and corresponding HEC values for pheochromocytomas in male and female mice ..... 48
Table 25. Summary of URF estimates and calculated air concentration corresponding to 1 in 100,000 excess cancer risk ..... 49
Table 26. Summary of $BMD_{10}$ values and corresponding $HEC_{BMD10}$ values for adrenal pheochromocytomas in male and female mice ..... 51
Table 27. Male F344 rat using MCA dose metric ..... 56
Table 28. Male F344 rat using MRAMKL dose metric ..... 57
Table 29. Female F344 rat using MCA dose metric ..... 60
Table 30. Female F344 rat using MRAMKL dose metric ..... 61
Table 31. Female F344 rat using MCA dose metric (highest dose dropped) ..... 62
Table 32. Female F344 rat using MRAMKL dose metric (highest dose dropped) ..... 63
Table 33. Female rat combined liver tumors using MRAMKL dose metric ..... 65
Table 34. Male mice combined liver tumors using MRAMKL dose metric ..... 67
Table 35. Male mice combined liver tumors using MRAMKL dose metric, highest dose dropped ..... 68
Table 36. Female mice combined liver tumors frequentist multistage model using MRAMKL dose metric ..... 71
Table 37. Female mice combined liver tumors frequentist multistage model using MRAMKL dose metric (highest dose dropped) ..... 71
Table 38. Male and female mice adrenal pheochromocytomas frequentist multistage model using MCA dose metric ..... 74
Table 39. Male mice adrenal pheochromocytomas using MCA dose metric and VmaxC by Thrall et al. 2000 ..... 75

# LIST OF FIGURES

Figure 1. Male rat using MCA dose metric: Vmax = 0.4 mg/hour/kg $BW^{0.07}$, log-logistic model ..... 58
Figure 2. Male rat using MCA dose metric: Vmax = 0.65 mg/hour/kg $BW^{0.07}$, log-logistic model ..... 58
Figure 3. Male rat using MRAMKL dose metric: Vmax = 0.4 mg/hour/kg $BW^{0.07}$, logistic model ..... 59
Figure 4. Male rat using MRAMKL dose metric: Vmax = 0.65 mg/hour/kg $BW^{0.07}$, logistic model ..... 59
Figure 5. Female rat using MCA dose metric: Vmax = 0.4 mg/hour/kg $BW^{0.07}$ (highest dose dropped), multistage degree 2 model ..... 64
Figure 6. Female rat using MCA dose metric: Vmax = 0.65 mg/hour/kg $BW^{0.07}$ (highest dose dropped), multistage degree 2 model ..... 64
Figure 7. Female rat combined liver tumors using MRAMKL dose metric: Vmax = 0.4 mg/hour/kg $BW^{0.07}$, Weibull model ..... 66
Figure 8. Female rat combined liver tumors using MRAMKL dose metric: Vmax = 0.65 mg/hour/kg $BW^{0.07}$, Weibull model ..... 66
Figure 9. Male mice combined liver tumors using MRAMKL dose metric: VmaxC by Fisher et al., dichotomous Hill model ..... 69
Figure 10. Male mice combined liver tumors using MRAMKL dose metric: VmaxC by Thrall et al., dichotomous Hill model ..... 69
Figure 11. Male mice combined liver tumors using MRAMKL dose metric: VmaxC by Fisher et al. (highest dose dropped), log-logistic model ..... 70

Carbon Tetrachloride

Figure 12. Male mice combined liver tumors using MRAMKL dose metric: VmaxC by Thrall et al. (highest dose dropped), Weibull model ..... 70
Figure 13. Female mice combined liver tumors using MRAMKL dose metric: VmaxC by Fisher et al., multistage degree 2 model ..... 71
Figure 14. Female mice combined liver tumors using MRAMKL dose metric: VmaxC by Thrall et. al., multistage degree 2 model ..... 72
Figure 15. Female mice combined liver tumors using MRAMKL dose metric: VmaxC by Fisher et al. (highest dose dropped), multistage degree 2 model ..... 72
Figure 16. Female mice combined liver tumors using MRAMKL dose metric: VmaxC by Thrall et al. (highest dose dropped), multistage degree 2 model ..... 73
Figure 17. Male mice adrenal pheochromocytomas using MCA dose metric: VmaxC by Fisher et al., multistage degree 1 model ..... 76
Figure 18. Male mice adrenal pheochromocytomas using MCA dose metric: VmaxC by Thrall et al., dichotomous Hill model ..... 76
Figure 19. Female mice adrenal pheochromocytomas using MCA dose metric: VmaxC by Fisher et al., multistage degree 2 model ..... 77
Figure 20. Female mice adrenal pheochromocytomas using MCA dose metric: VmaxC by Thrall et al., multistage degree 2 model ..... 77

# Acronyms and Abbreviations

| Acronyms and Abbreviations | Definition |
|---|---|
| ACGIH | American Conference of Governmental Industrial Hygienists |
| AEGL | Acute Exposure Guideline Levels |
| AIC | Akaike information criterion |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferase, also referred to as serum glutamic-pyruvic transaminase (SGPT) |
| AST | aspartate aminotransferase, also referred to as serum glutamic-oxaloacetic transaminase (SGOT) |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| $^{\circ}$C | degrees Celsius |
| BMR | benchmark response |
| BMD | Benchmark dose |
| BMDL | Benchmark dose lower confidence limit |
| BUN | blood urea nitrogen |
| bw | body weight |
| $CCl_4$ | carbon tetrachloride |
| CNS | central nervous system |
| d | day |
| DSD | development support document |
| ESL | Effects Screening Level |
| $^{acute}ESL$ | acute health-based Effects Screening Level for chemicals meeting minimum database requirements |
| $^{acute}ESL_{generic}$ | acute health-based Effects Screening Level for chemicals not meeting minimum database requirements |
| $^{acute}ESL_{odor}$ | acute odor-based Effects Screening Level |
| $^{acute}ESL_{veg}$ | acute vegetation-based Effects Screening Level |
| $^{chronic}ESL_{threshold(c)}$ | chronic health-based Effects Screening Level for threshold dose response cancer effect |

| Acronyms and Abbreviations | Definition |
|---|---|
| $^{chronic}ESL_{threshold(nc)}$ | chronic health-based Effects Screening Level for threshold dose response noncancer effects |
| $^{chronic}ESL_{nonthreshold(c)}$ | chronic health-based Effects Screening Level for nonthreshold dose response cancer effects |
| $^{chronic}ESL_{nonthreshold(nc)}$ | chronic health-based Effects Screening Level for nonthreshold dose response noncancer effects |
| $^{chronic}ESL_{veg}$ | chronic vegetation-based Effects Screening Level |
| GI | gastrointestinal |
| h | hour |
| $H_{b/g}$ | blood:gas partition coefficient |
| $(H_{b/g})_A$ | blood:gas partition coefficient, animal |
| $(H_{b/g})_H$ | blood:gas partition coefficient, human |
| HEC | human equivalent concentration |
| HQ | hazard quotient |
| HSDB | Hazardous Substance Data Base |
| IARC | International Agency for Research on Cancer |
| IOAEL | inhalation observed adverse effect level |
| $^{acute}IOAEL$ | acute inhalation observed adverse effect level |
| $^{subacute}IOAEL$ | subacute inhalation observed adverse effect level |
| $^{chronic}IOAEL_{(nc)}$ | chronic inhalation observed adverse effect level (noncancer effects) |
| $^{chronic}IOAEL_{(c)}$ | chronic inhalation observed adverse effect level (cancer effects) |
| IRIS | USEPA Integrated Risk Information System |
| kg | kilogram |
| $K_{ow}$ | n-octanol-water partition coefficient |
| $LC_{50}$ | concentration causing lethality in 50% of test animals |
| $LD_{50}$ | dose causing lethality in 50% of test animals |
| LOAEL | lowest-observed-adverse-effect-level |
| LTD | limited toxicity data |
| MCA | time-averaged arterial blood concentration of carbon tetrachloride (µmol/L blood) |

| Acronyms and Abbreviations | Definition |
|---|---|
| mmHg | A millimeter of mercury; approximately 1 torr, or 1/760 of standard atmospheric pressure |
| MRAMKL | time-averaged rate of metabolism of carbon tetrachloride (µmol/h/kg liver weight) |
| MRL | minimal risk level |
| MW | molecular weight |
| µg | microgram |
| µg/$m^3$ | micrograms per cubic meter of air |
| mg | milligrams |
| mg/$m^3$ | milligrams per cubic meter of air |
| min | minute |
| MOA | mode of action |
| n | number |
| NOAEL | no-observed-adverse-effect-level |
| NOEL | no-observed-effect-level |
| NRC | National Research Council |
| OSHA | Occupational Safety and Health Administration |
| PBPK | physiologically based pharmacokinetic |
| POD | point of departure |
| $POD_{ADJ}$ | point of departure adjusted for exposure duration |
| $POD_{HEC}$ | point of departure adjusted for human equivalent concentration |
| ppb | parts per billion |
| ppm | parts per million |
| REL | reference exposure level |
| ReV | reference value |
| Acute ReV | acute (e.g., 1-hour) health-based reference value for chemicals meeting minimum database requirements |
| Acute ReV-24hr | acute 24-hour health-based reference value for chemicals meeting minimum database requirements |

| Acronyms and Abbreviations | Definition |
|---|---|
| Chronic $ReV_{threshold(nc)}$ | chronic health-based reference value for threshold dose response noncancer effects |
| RGDR | Regional Gas Dose Ratio |
| SD | Sprague-Dawley |
| SDH | sorbitol dehydrogenase |
| SGOT | serum glutamic-oxaloacetic transaminase, also referred to aspartate aminotransferase (AST) |
| SGPT | serum glutamic-pyruvic transaminase, also referred to as alanine aminotransferase (ALT) |
| TCEQ | Texas Commission on Environmental Quality |
| TD | Toxicology, Risk Assessment, and Research Division |
| UF | uncertainty factor |
| $UF_H$ | interindividual or intraspecies human uncertainty factor |
| $UF_A$ | animal to human uncertainty factor |
| $UF_{Sub}$ | subchronic to chronic exposure uncertainty factor |
| $UF_L$ | LOAEL to NOAEL uncertainty factor |
| $UF_D$ | incomplete database uncertainty factor |
| USEPA | United States Environmental Protection Agency |
| wk | week |
| yr | year |

# Chapter 1 Summary Tables

Table 1 and Table 2 provide a summary of health- and welfare-based values from an acute and chronic evaluation of carbon tetrachloride ($CCl_4$), respectively, for use in air permitting and air monitoring. Please refer to Section 1.6.2 of the *TCEQ Guidelines to Develop Toxicity Factors* (TCEQ 2015a) for an explanation of air monitoring comparison values (AMCVs), reference values (ReVs) and effects screening levels (ESLs) used for review of ambient air monitoring data and air permitting. Table 3 provides summary information and the physical/chemical data of $CCl_4$.

**Table 1. Acute health and welfare-based screening values for carbon tetrachloride**

| Screening Level Type | Duration | Value 1 (µg/m³) | Value 2 (ppb) | Usage | Flags | Surrogated/ RPF | Critical Effect(s) | Notes |
|---|---|---|---|---|---|---|---|---|
| Acute ReV | 1 h | 8,200 | 1,300 | M | A | --- | Central nervous system (CNS) depression (headache, dizziness, and sleepiness) and nausea and vomiting in humans | --- |
| Acute ReV-24hr | 24 h | 520 | 83 | M | A | --- | Nausea, anorexia, vomiting, gastrointestinal (GI) distress, and CNS effects in humans | --- |
| **$^{acute}$ESL** | **1 h** | **2,400** | **390** | **P** | **S,D** | **---** | **CNS depression (headache, dizziness, and sleepiness) and nausea and vomiting in humans** | **---** |
| $^{acute}$IOAEL | 30 min | 990,000 | 158,000 | N | none | --- | CNS depression (headache, dizziness, and sleepiness) and nausea and vomiting in humans | --- |
| $^{subacute}$IOAEL | 8 h | 280,000 | 45,000 | N | none | --- | Nausea, anorexia, vomiting, GI distress, and CNS effects in humans | --- |
| $^{acute}ESL_{odor}$ | --- | --- | --- | --- | --- | --- | -- | --- |
| $^{acute}ESL_{veg}$ | --- | --- | --- | --- | --- | --- | -- | --- |

Bold values used for air permit reviews

[a] Based on the acute ReV multiplied by 0.3 (i.e., HQ = 0.3) to account for cumulative and aggregate risk during the air permit review.

Usage:
P = Used in Air Permitting
M = Used to Evaluate Air Monitoring Data
R = Used to Calculate Remediation Cleanup Levels
N = Usage Not Defined

Flags:
A = AMCV report
S = ESL Summary Report
D = ESL Detail Report

**Table 2. Chronic health and welfare-based screening values for carbon tetrachloride**

| Screening Level Type | Duration | Value 1 (µg/m³) | Value 2 (ppb) | Usage | Flags | Surrogated/ RPF | Critical Effect(s) | Notes |
|---|---|---|---|---|---|---|---|---|
| Chronic $ReV_{threshold(nc)}$ | Annual | 160 | 25 | M | A | --- | Fatty changes in the liver of rats | --- |
| $^{chronic}ESL_{threshold(nc)}$ [a] | Annual | 48 | 7.5 | --- | S,D | --- | Same as above | --- |
| $^{chronic}IOAEL_{(nc)}$ | Annual | 18,000 | 2,900 | N | none | --- | Same as above | --- |
| $^{chronic}ESL_{threshold(c)}$ | --- | --- | --- | --- | --- | --- | --- | --- |
| **$^{chronic}ESL_{nonthreshold(c)}$ [b]** | **Annual** | **2.8** | **0.44** | **P** | **S,D** | **---** | **Hepatocellular tumors in rats and mice; adrenal gland pheochromocytomas in mice** | **---** |
| $^{chronic}IOAEL_{(c)}$ | Annual | 52,000 | 8,300 | N | none | --- | Hepatocellular tumors in rats and mice; adrenal gland pheochromocytomas in mice | --- |
| $^{chronic}ESL_{veg}$ | --- | --- | --- | --- | --- | --- | --- | --- |
| $^{chronic}ESL_{animal}$ | --- | --- | --- | --- | --- | --- | --- | --- |

Bold values used for air permit reviews

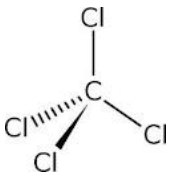
[a] Based on the chronic ReV multiplied by 0.3 (i.e., HQ = 0.3) to account for cumulative and aggregate risk during the air permit review.

[b] Based on the URF of $3.6 \times 10^{-6}$ $(\mu g/m^3)^{-1}$ or $2.3 \times 10^{-5}$ $(ppb)^{-1}$ and a no significant risk level of 1 in 100,000 excess cancer risk.

Usage:
P = Used in Air Permitting
M = Used to Evaluate Air Monitoring Data
R = Used to Calculate Remediation Cleanup Levels
N = Usage Not Defined

Flags:
A = AMCV report
S = ESL Summary Report
D = ESL Detail Report

**Table 3. Chemical and physical data**

| Parameter | Value | Reference |
|---|---|---|
| Chemical Structure |  | ATSDR (2005) |
| Molecular Formula | $CCl_4$ | ATSDR (2005) |
| Molecular Weight | 153.82 g/mole | ATSDR (2005) |
| Physical State at 25°C | liquid | ATSDR (2005) |
| Color | colorless | ATSDR (2005) |
| Odor | aromatic, sweet | ATSDR (2005) |
| Odor threshold | 4.6 ppm | Nagata (2003) |
| CAS Registry Number | 56-23-5 | ATSDR (2005) |
| Common Synonym(s) | carbon chloride, tetrachloromethane, carbon tet, methane tetrachloride, perchloromethane, tetrachlorocarbon | ATSDR (2005) |
| Tradenames | Benzinoform, Fasciolin, Freon 10, Halon 104, Tetraform, Tetrafinol, Carbona | ATSDR (2005) |
| Solubility in water | 800 mg/L | ATSDR (2005) |
| Log $K_{ow}$ | 2.64 | ATSDR (2005) |
| Vapor Pressure | 90 mmHg at 20°C | ATSDR (2005) |
| Vapor Density (air = 1) | 5.32 to 5.41 g/L | USEPA (2010) |
| Density (water = 1) | 1.594 g/ml at 20°C | USEPA (2010) |
| Melting Point | -23°C | ATSDR (2005) |
| Boiling Point | 76.5°C | ATSDR (2005) |
| Conversion Factors | 1 µg/m$^3$ = 0.16 ppb<br>1 ppb = 6.29 µg/m$^3$ | USEPA (2010) |

# Chapter 2 Background Information

## *2.1 Physical/Chemical Properties*

The main chemical and physical properties of $CCl_4$ are summarized in Table 3. $CCl_4$ is a halogenated hydrocarbon with an aromatic sweet odor (ATSDR 2005). $CCl_4$ is a colorless and volatile liquid at room temperature, and evaporates quickly due to its high vapor pressure, thus allowing for inhalation. $CCl_4$ has an octanol-water partition coefficient (Log $K_{ow}$) of 2.64, meaning $CCl_4$ has an affinity for fat (lipid-containing) tissue and molecules.

## *2.2 Sources and Uses*

$CCl_4$ does not occur naturally. Most atmospheric $CCl_4$ is a result of direct industrial releases to the atmosphere. $CCl_4$ is produced by exhaustive chlorination of a variety of low molecular weight hydrocarbons such as carbon disulfide, methane, ethane, propane, and ethylene dichloride. $CCl_4$ is also produced by thermal chlorination of methyl chloride (OEHHA 2000, USEPA 2017).

$CCl_4$ has been produced in large quantities to make refrigeration fluid and propellants for aerosol cans. It was also used in fire extinguishers and as a fumigant to kill insects in grain. Most of these uses were discontinued in the mid-1960s. Information on $CCl_4$ historical uses, taken from ATSDR (2005), is given below. See ATSDR (2005) for the cited references and additional information.

Prior to institution of the Montreal Protocol, which banned the manufacture and use of chlorofluorocarbons in several countries including the United States, $CCl_4$ was used as a chemical intermediate and as a feedstock in the production of chlorofluorocarbons, such as the Freons dichlorodifluoromethane (F-12) and trichlorofluoromethane (F-11), which were used primarily as refrigerants. $CCl_4$ is also used in petroleum refining, in pharmaceutical manufacturing, as an industrial solvent, in the processing of fats, oils, and rubber, and in laboratory applications (IARC 1999, ATSDR 2005, Health Canada 2010). $CCl_4$ currently is not permitted in products intended for home use (NTP 2011). There are a number of designated essential uses for $CCl_4$ in pharmaceutical and agrochemical applications together with a large list of process agent (solvent) uses for $CCl_4$.

$CCl_4$ may be present in trace amounts in adhesive remover, paint, coatings, adhesives, and brake cleaners and is used as a feedstock in the production of perchloroethylene, hydrochlorofluorocarbons, hydrofluorocarbons, and hydrofluoroolefins (USEPA 2017, USEPA 2020). Because the production of $CCl_4$ for most uses has been phased out due to the Montreal Protocol and the Federal Clean Air Act, $CCl_4$ is only available for those uses for which no effective substitute has been found, such as chemical feedstock use, use as a processing agent, and laboratory or analytical use (USEPA 2017, USEPA 2020). $CCl_4$ evaporates easily and releases

to the environment during its production and use. Shah and Heyerdahl (1988, as cited in ATSDR 2005) reported an average concentration of $CCl_4$ of 0.168 ppb (1.1 µg/$m^3$) in ambient air in the United States based on 4,913 ambient air samples taken at various sites, and these levels have since been decreasing (Health Canada 2009). Ambient air data collected by the TCEQ from 2007-2011 indicate that annual average air concentrations of $CCl_4$ at monitoring sites around Texas range from 0.07 to 0.19 ppb with a statewide mean annual average of 0.10 ppb. From 2012 to 2016, $CCl_4$ annual averages ranged from 0.07 to 0.15 ppb. From 2012 through June 2017, the 24-hour (h) concentrations ranged from 0.051 to 0.153 ppb.

# Chapter 3 Acute Evaluation

## *3.1 Health-Based Acute ReV and $^{acute}$ESL*

Few studies of the effects of $CCl_4$ have been performed on humans. Occupational reports indicate nausea, dyspepsia, central nervous system (CNS) depression, narcosis, headache, weakness, lethargy, nausea, and vomiting via inhalation. However, these studies were not well conducted with controlled exposures. In one study, Davis (1934) used human subjects, controlled exposure dose and duration, and performed eight experiments that developed both no-observed-adverse-effect-level (NOAEL) and lowest-observed-adverse-effect-level (LOAEL) values. Therefore, this study was used as the key study for development of an acute reference value (ReV) and effects screening level (ESL). Acute studies in animals at higher concentrations showed hepatic effects from inhalation exposure. See USEPA (2010), ASTDR (2005), and Health Canada (2011) for additional studies and details on acute inhalation exposure to $CCl_4$ in humans and animals.

### 3.1.1 Key and Supporting Studies

#### *3.1.1.1 Human Studies*

Acute inhalation exposure in humans to approximately ≥ 150 ppm caused depression of the CNS and gastrointestinal (GI) effects (ATSDR 2005, USEPA 2010). Acute exposure to $CCl_4$ (1,191 ppm for 15 minutes [min] or 6,400 ppm for 3 min) has been shown to cause short-term CNS effects (drowsiness, headache, dizziness, and weakness) and GI effects (nausea, vomiting, diarrhea, and abdominal pain). These effects dissipate after cessation of exposure. Single exposures of 200 ppm for ≤ 3 h resulted in hepatic effects (increased serum bilirubin), renal effects (proteinuria), and GI effects (nausea) (ATSDR 2005, USEPA 2010). At lower doses, mucosal irritation and CNS effects have been reported following $CCl_4$ exposure. For example, in one of the first controlled human studies on the effects of $CCl_4$, Davis (1934) observed headaches, nausea, and vomiting in subjects exposed to 317 ppm (1,994 mg/$m^3$) for 30 min.

### 3.1.1.1.1 Davis (1934) Study

Davis (1934) reported the results of several experiments in which human subjects were exposed to $CCl_4$. The $CCl_4$ concentrations were determined based on the room volume and the amount of $CCl_4$ necessary to achieve the desired concentration (i.e., nominal concentration). There was no mention of air flow rate or ventilation in the test room. Both CNS and GI effects were recorded; other parameters evaluated included: blood cell count, hemoglobin, blood pressure, urinalysis, respiration rate, and pulse rate.

In experiment one, 4 individuals (ages 20, 28, 28, and 30 yrs; gender not specified) were exposed to 158 ppm $CCl_4$ for 30 min. One subject experienced nervousness and slight nausea but the remaining three were asymptomatic. There were no physiologically significant alterations in blood pressure, heart rate, respiratory rate, blood cell counts, or hemoglobin concentration. Urinalyses at 24 h post-exposure revealed no signs of toxicity.

In experiment two, 4 different subjects (ages 22, 30, 35, and 48; gender not specified) were exposed to a $CCl_4$ concentration of 76 ppm for 2.5 h. There were no symptoms or signs of toxicity in any of the subjects (respiration, blood pressure, blood count, and hemoglobin).

In experiment three, the same subjects used in the previous experiment were exposed 24 h later to 76 ppm $CCl_4$ for 4 h without signs or symptoms. Urinalyses at 72 h post-exposure were normal. These results along with those from experiment two suggest a NOAEL for neurological effects of 76 ppm for 2.5- to 4-h exposures.

In experiment four, three additional subjects (ages 20, 36 and 45, gender not specified) were exposed to 317 ppm $CCl_4$ for 30 min. Although clinical tests (blood pressure, hemoglobin, blood cell counts, pulse, and urinalysis at 48 h post-exposure) were normal, one subject experienced nausea, another nausea and vomiting, and the third complained of headache. These results along with those from experiment one suggests a LOAEL for neurological effects in the range of 158 and 317 ppm for a 30-min exposure.

In experiment five, four subjects (ages 19, 21, 28, and 40; gender not specified) were exposed for 15 min to 1,191 ppm $CCl_4$. Two of the subjects experienced headache, nausea and vomiting and could only tolerate a 9- or 10-minute exposure each; another experienced nausea and vomiting and could only tolerate a 12-min exposure; and another experienced nausea and headache. Blood pressure was similar to those exposed at lower concentrations. The pulse rate appeared somewhat elevated, although no baseline data were provided for comparison. Urinalyses at 48 h post-exposure were negative (details not given) except for slightly increased acidity and phosphates.

In experiment six, three subjects (ages, 40, 26, and 19, gender not specified) were exposed to 12,800 ppm $CCl_4$ for 3, 5, and 7 min, respectively. The first subject became dizzy, nauseated,

sleepy, and experienced a throbbing headache. The second subject became nervous, nauseated, and listless, and the third subject experienced nausea, vomiting, dizziness, and became sleepy. Blood pressure was similar to those exposed at lower concentrations. The pulse rate appeared somewhat elevated, although no baseline data were provided for comparison. Urinalyses at 48 h post-exposure were negative (details not given) except for slightly increased acidity and phosphates. Clinical examination 2 weeks (wk) post-exposure revealed no adverse effects.

In experiment seven, Davis (1934) measured the $CCl_4$ concentration near the faces of three men (age not specified) asked to use pure $CCl_4$ by painting it on fabric in an enclosed room which had exhaust ventilation about 6 feet away from the table upon which the fabric was placed. Using an alcohol potassium hydroxide and combustion method, the $CCl_4$ concentration was found to be 0.23% (2,300 ppm). None of the three subjects could work for more than 10 min without becoming nauseated and sleepy. One of the three experienced vomiting, dizziness, and a throbbing headache.

In experiment eight, the air within a working area was analyzed (as above) and found to contain 0.02% (200 ppm) of $CCl_4$. Several workers (number, age and gender not given) had complained of nausea and vomiting. Several had indicated they felt tired and sleepy after their shift. One man who had been working in the cement house was found to have albumin in his urine. He was removed to a work area without $CCl_4$ and his urine was examined weekly; at the end of 2 months the albumin had disappeared. The phenolsulphonphthalein (Phenol Red) kidney function test showed him to have 90% function. He was not returned to a work-area containing $CCl_4$. His urinary albumin levels were normal and there was no microscopic evidence of any pathologic condition in his urine.

A summary of all eight experiments from Davis (1934) is presented in Table 4.

**Table 4: Summary of experiments 1 through 8: time, dose, and degree of central nervous system effects [a] from $CCl_4$ exposure [b]**

| Duration of Exposure (Minutes) | 76 ppm [c] | 158 ppm | 200 ppm | 317 ppm | 1,191 ppm | 2,300 ppm | 12,800 ppm |
|---|---|---|---|---|---|---|---|
| **3 - 7** | | | | | | | 3 |
| **10** | | | | | | 3 | |
| **9 - 15** | | | | | 3 | | |
| **30** | | 2 | | 3 | | | |
| **150** | 1 | | | | | | |
| **240** | 1 | | | | | | |
| **480** | | | 3 | | | | |

[a] Degree of effect: 1 = no effects noted; 2 = mild effects such as dizziness and headache; 3 = moderate effects (level 2) plus nausea, vomiting, and sleepiness
[b] Blank cells indicate no tests were performed
[c] Concentration of $CCl_4$

Adverse neurological effects such as nausea and headache occurred at the exposure duration of 30 min and exposure concentrations of 158 and 317 ppm, which can be considered the LOAEL range for these effects. The NOAEL for these effects was 76 ppm with exposure durations of 150 and 240 min (2.5 and 4 h, respectively).

In summary, similar effects occur at higher concentrations and shorter durations and at lower concentrations and longer durations, as shown in Table 4.

### 3.1.1.1.2 Lehmann and Schmidt-Kehl (1936) Study

Lehmann and Schmidt-Kehl (1936, as cited in USEPA 2010) described the neurological symptoms in humans exposed briefly to $CCl_4$ vapor. No effects were observed following exposure at 3,200 ppm for 5 min. Exposure to 4,800 ppm for 2.5 min resulted in slight drowsiness after 5 min. Exposure to 6,400 ppm for 3 min resulted in tremor and drowsiness, followed by staggering. The highest tested exposure, 14,100 ppm for 0.8 min, resulted in loss of consciousness. Due to the brief exposure durations, these results were not considered to be relevant for derivation of a 1-h ReV.

### 3.1.1.1.3 Kazantzis and Bomford (1960) Study

Kazantzis and Bomford (1960) examined workers exposed to $CCl_4$ vapors while cleaning quartz crystals used in electronic components. Fourteen men and four women, 16-54 yrs of age, were exposed to $CCl_4$ for about 8 h/day (d) to concentrations of approximately 45-97 ppm. The

atmospheric samples were taken, after running the equipment for 3 h, in two impingers in series with a hand pump and analyzed. Sampling was repeated after control measures were implemented to determine if a reduction in $CCl_4$ was achieved.

Fifteen workers complained of neurological and GI symptoms (nausea, anorexia, vomiting, depression, headache, and GI discomfort). Complaints were made of irritability and unusual shortness of temper. Generally, symptoms could not be attributed to a single 8-h exposure as symptoms typically developed in the middle of the workweek and increased in severity as the week progressed, and then disappeared over the weekend. One person when examined in the hospital had elevated serum glutamic-oxaloacetic transaminase (SGOT), but this test was normal five days later. After lowering $CCl_4$ workplace levels all workers were symptom-free within one wk and no recurrences occurred up to six months later. The levels of 45-97 ppm can be considered a LOAEL for neurological and GI symptoms for repeated 8-h exposure over a few consecutive days.

### *3.1.1.2 Animal Studies*

Inhalation exposure to 10-100 ppm $CCl_4$, 6–7 h/d in rats for up to 2 wks generally resulted in mild to moderate signs of liver injury (fatty degeneration) (Adams et al. 1952, David et al. 1981, Paustenbach et al. 1986, Wang et al. 1997).

#### 3.1.1.2.1 Adams et al (1952) Study

Adams et al. (1952) exposed groups of three or four male albino rats to $CCl_4$ for up to 7 h to determine the range of acute toxic effects (non-toxic to toxic, but non-lethal). Rats exposed to 50 ppm showed no adverse effects, whereas rats exposed to 100 ppm showed adverse effects (increase of weight and total lipid in liver, fatty degeneration).

#### 3.1.1.2.2 David et al. (1981) Study

Wistar male rats (7 months old, 12/group) exposed to 50 ppm $CCl_4$ for 6 h/d for 4 d [concentration x time (C x T) = 300 ppm-hours/d] had a maximum 1.7-fold increase in serum glutamic-pyruvic transaminase (SGPT) (David et al. 1981). A concentration of 1,615 mg/m$^3$ (250 ppm) $CCl_4$ for 72 min/d for 4 d (C x T = 300 ppm-hours) resulted in a maximum 2-fold increase in SGPT activity. A concentration of 6,500 mg/m$^3$ (1,000 ppm) for 3 min six times at 1-h intervals (total of intermittent exposure is 300 ppm-hours) had no effect on SGPT activity, while exposure to 6,500 mg/m$^3$ (1,000 ppm) for 18 min for 4 d (300 ppm-hours) resulted in a maximum 4-fold effect in SGPT activity. The authors also described mild liver effects (altered glycogen distribution, hepatocytic steatosis, hydropic degeneration, and/or necrosis) in rats exposed to $CCl_4$. These results indicate that the severity of liver lesions is more influenced by the concentration of $CCl_4$ in the inhaled air than by the total inhaled over time.

#### 3.1.1.2.3 Paustenbach et al. (1986) Studies

Sixteen groups of adult male Sprague-Dawley rats (4 rats/group) were exposed to 100 ± 12 ppm of $CCl_4$ for 8 or 11.5 h/d, 5 d/wk for 1 to 10 d (Paustenbach et al. 1986). Serum sorbitol dehydrogenase (SDH) analysis and histopathology were performed on the liver and kidney. The SDH activities for nearly all groups exposed to the 11.5-hr/day dosage regimen were significantly higher than the comparable group exposed to the 8-hr/day schedule. When compared to the control values stated by the authors (mean of 8.5 international units [IU]/mL), rats exposed to 100 ppm for 8 h/d had a 2.5-fold increase in SDH after 3 days of exposure and a 4.6-fold increase in SDH after 10 exposure days. When compared to the control values, rats exposed to 100 ppm for 11.5 h/d had 1.7-, 3.4-, 8.0-, and 13-fold increases in SDH after 1, 3, 4, and 8 days of exposure, respectively. Additionally, exposures of rats to 100 ppm for 8 or 11.5 hours/day for 5 or more days resulted in fatty changes in the liver and nephrosis.

#### 3.1.1.2.4 Wang et al. (1997) Study

Wang et al. (1997) exposed groups of male Wistar rats (8 weeks old, 5 rats/group) to 0, 50, or 500 ppm $CCl_4$ for 6 h. Liver enzymes (serum SGOT and SGPT) were measured. Rats exposed to 50 ppm did not exhibit any sign of liver injury, as there were no statistically significant differences in SGOT and SGPT versus controls. Statistically significant increases in SGOT (1.4- to 2.0-fold) and SGPT activities (1.9-fold) compared to the control group were observed in rats exposed to 500 ppm $CCl_4$.

### *3.1.1.3 Reproductive and Developmental Studies*

#### 3.1.1.3.1 Human Studies

No studies were available regarding reproductive effects in humans after inhalation or oral exposure to $CCl_4$ (ASTDR 2005). No studies were available on developmental effects in humans after known inhalation exposure to $CCl_4$. A questionnaire-based study of 3,418 pregnant women in West Germany found no association between probable occupational exposure to $CCl_4$ (as estimated from a job exposure matrix) and the birth of infants who were small for their gestational age (ASTDR 2005; Seidler et al. 1999, as cited in USEPA 2010).This human study, however, is not adequate for consideration as a possible basis for the calculation of the acute ReV (e.g., lack of a causation or a demonstrated dose-response for $CCl_4$ specifically) and is not useful in providing perspective as to the potential for such effects (lower birth weight) due to inhalation exposure.

#### 3.1.1.3.2 Animal Studies

A 2010 toxicological review of $CCl_4$ by USEPA (2010) does not provide more current information than ATSDR (2005) on acute and developmental/reproductive studies in laboratory animals, as no additional studies have been performed since 2005. ATSDR (2005) cites no LOAELs for

reproductive effects in animals and provides the following information on developmental effects in animals following short- and long-term exposure "*Ultimately, ATSDR concluded that data suggest developmental toxicity due to $CCl_4$ exposure is not a major area of concern."* See ATSDR (2005) for the cited references.

Groups of 22–23 pregnant female Sprague-Dawley rats were exposed by inhalation to $CCl_4$ vapor at concentrations of 0, 334, or 1,004 ppm (0, 2,101, or 6,316 mg/$m^3$) for 7 h/d on gestational days (GD) 6–15. Exposures to the two different exposure levels of $CCl_4$ were not performed concurrently, so two separate control groups were used. Additionally, nonpregnant rats also were exposed for evaluation of potential for hepatotoxicity (serum ALT and gross examination of livers). Exposure to both concentrations of $CCl_4$ resulted in reduced feed intake by dams and maternal weight loss, and hepatotoxicity in nonpregnant rats (4-fold increase in serum ALT relative to control, and increased relative liver weight [26% and 44% in rats exposed to 334 and 1,004 ppm, respectively]) (Schwetz et al. 1974, as cited in USEPA 2010). In the 334- and 1,004-ppm groups, significant reductions in fetal body weight (7 and 14%, respectively) and crown-rump length (3.5 and 4.5%, respectively), but no gross anomalies, were observed. There were no reproductive effects on conception, number of implants, or number of resorptions. The incidence of delayed ossification of sternebrae, a skeletal variation associated with developmental delay, was significantly increased at 1,004 ppm (13%) compared with the concurrent control (2%). However, as maternal toxicity (e.g., weight loss, reduced food intake) occurred at the same exposure levels as fetotoxicity (e.g., weight loss, reduced growth), effect levels from this study are not appropriate developmental toxicity LOAELs and do not demonstrate developmental concerns as the observed effects are commonly secondary to maternal toxicity. Again, ATSDR (2005) concluded that data suggest developmental toxicity due to $CCl_4$ exposure is not a major area of concern.

No adequate reproductive toxicity studies have been conducted in animals exposed by the oral route (USEPA 2010). Total litter loss has been described at maternally toxic doses that are higher than those associated with liver and kidney toxicity (Narotsky and Kavlock, 1995).

Please refer to ATSDR (2005) for additional discussion of short-term animal studies.

## 3.1.2 Health-Based Acute 1-h ReV and ESL

### *3.1.2.1 Selection of the Key Study, Point of Departure (POD), and Critical Effect*

The Davis (1934) study used human subjects, controlled exposure dose and duration; and performed eight experiments and developed both a 150-240 min NOAEL (76 ppm) and a 30-min minimal LOAEL (158 ppm) for central nervous system (CNS) effects. Acute inhalation studies in rats indicate that CNS effects were observed at exposure levels (≥ 4,600 ppm) much higher than those observed in human studies (Adams et al. 1952, as cited in USEPA 2010) and were

associated with death. Thus, the Davis (1934) human study was used as the key study for development of an acute ReV and ESL. The longer exposure period of a 4-h NOAEL (76 ppm) for CNS effects identified in the Davis (1934) study was considered as a candidate point-of-departure (POD) to derive the acute (1-h) ReV and $^{acute}$ESL. In addition, since the exposure duration for the identified LOAEL (30 min) is shorter than that of interest (1 h) for calculating the acute ReV, the 30-min LOAEL of 158 ppm will also be selected as a candidate POD to derive the 1-h toxicity factors. Additionally, the observed 2.5-h and 4-h NOAEL of 76 ppm without exposure adjustment also will be selected as a candidate POD to derive the 1-h toxicity factors.

CNS effects are considered the most sensitive endpoint for acute human inhalation exposure to $CCl_4$. Because of the CNS depressant properties of $CCl_4$, common signs include headache, giddiness, weakness, lethargy, nervousness, nausea, and vomiting. Several of these CNS effects (e.g., nausea, headache) noted in the key study (Davis 1934) serve as the critical effects for derivation of the acute ReV.

### *3.1.2.2 MOA and Dose Metric for Critical Effect*

While metabolism of $CCl_4$ is required for chronic hepatotoxic effects, acute effects are produced directly and without metabolic activation. The majority of research on the mode of action (MOA) for $CCl_4$ has focused on hepatotoxic processes. Consequently, although the CNS effects of $CCl_4$ are well documented, the precise MOA is unknown (NRC 2010, as cited in USEPA 2010). However, consistent with available dose-response data, these noncarcinogenic acute effects would be expected to exhibit a threshold (TCEQ 2015a). In the key study (Davis 1934), data on $CCl_4$ air concentrations are available. Exposure concentration of the parent chemical will be used as the default dose metric for the key study.

### *3.1.2.3 Adjustments to the POD*

#### 3.1.2.3.1 Default Exposure Duration Adjustments

##### *3.1.2.3.1.1 For the 4-h NOAEL of 76 ppm*

Time-series data from Davis (1934) indicate that $CCl_4$-induced CNS effects are both concentration- and time-dependent. The NOAEL (76 ppm) from the Davis (1934) key study is for a longer exposure period (150-240 min) than that of interest (60 min) for calculating the acute ReV. The longer exposure period of 240 min (4 h) NOAEL (POD) will be used for exposure duration adjustment to 1 h ($POD_{ADJ}$). The duration adjustment will use Haber's rule as modified by ten Berge (1986) with an "n" of 3, consistent with Section 4.2.2 of TCEQ (2015).

$POD_{ADJ} = C_2 = [(C_1)^3 \times (T_1 / T_2)]^{1/3} = [(76\ ppm)^3 \times (4\ h/1\ h)]^{1/3} = 120.642\ ppm$

#### *3.1.2.3.1.2 For the 30-min LOAEL of 158 ppm*

The 30-min LOAEL (POD) of 158 ppm was adjusted to 1 h ($POD_{ADJ}$). The duration adjustment will use Haber's rule as modified by ten Berge (1986) with an "n" of 1.

$POD_{ADJ} = C_2 = [(C_1) \times (T_1 / T_2)] = [158 \text{ ppm} \times (30 \text{ min}/60 \text{ min})] = 79 \text{ ppm}$

### 3.1.2.3.2 Default Dosimetry Adjustments from Animal-to-Human Exposure

No dosimetry adjustment from animal-to-human exposure is needed as the $POD_{ADJ}$ was identified from human study. Thus,

$POD_{ADJ}$ from the 4-h NOAEL = $POD_{HEC}$ = 120.642 ppm,

$POD_{ADJ}$ from the 30-min LOAEL = $POD_{HEC}$ = 79 ppm, and

POD from the 2.5-h and 4-h NOAEL without duration adjustment = $POD_{HEC}$ = 76 ppm

The $POD_{HEC}$ of 79 ppm from the 30-min LOAEL is lower than that from the 4-h NOAEL even before adjusting to a NOAEL through use of a $UF_L$, and obviously had an associated 4-h LOAEL been identified (for a direct LOAEL-based $POD_{HEC}$ comparison) it would have been even higher. However, the $POD_{HEC}$ of 76 ppm from the observed 2.5-h and 4-h NOAEL is lower than the $POD_{HEC}$ of 79 ppm from the 30-min LOAEL, and is lower than the $POD_{HEC}$ adjusted from the 4-h NOAEL. Thus, the 2.5-h and 4-h NOAEL of 76 ppm was selected to derive the acute ReV.

### *3.1.2.4 Adjustments to the $POD_{HEC}$*

The MOA by which $CCl_4$ may produce CNS effects is discussed in Section 3.1.2.2. CNS depression by $CCl_4$ is a noncarcinogenic effect which exhibits a threshold MOA. Thus, the lowest $POD_{HEC}$ of 76 ppm is selected and appropriate uncertainty factors (UFs) are applied to derive a ReV (TCEQ 2015a).

The following UFs were applied to the $POD_{HEC}$ of 76 ppm:

- A $UF_H$ of 10 was used for intrahuman variability because the study population comprised healthy adults and it was not known if potentially sensitive subpopulations were included (i.e., individuals with pre-exposure to alcohol or pre-existing liver disease).
- A $UF_D$ of 6 was used because the acute toxicological database for $CCl_4$ indicates a potentially steep dose-response curve in human and animal studies (AEGL 2008, ATSDR 2005), the MOA for $CCl_4$ causing CNS effects is unknown, and the quality of the available studies is low to medium.

A total UF of 60 was applied to the $POD_{HEC}$ of 76 ppm to derive the acute ReV of 1.3 ppm (rounded to two significant figures).

acute ReV = $POD_{HEC}$ / ($UF_H$ × $UF_D$)
= 76 ppm / (10 × 6) = 76 ppm / 60
= 1.267 ppm = 1.3 ppm or 1,300 ppb (rounded to two significant figures)

### *3.1.2.5 Health-Based 1-h Acute ReV and $^{acute}$ESL*

The resulting 1-h acute ReV was rounded to two significant figures at the end of all calculations. The rounded acute ReV was then used to calculate the $^{acute}$ESL at the target hazard quotient (HQ) of 0.3 (Table 5).

**Table 5. Derivation of the 1-h acute ReV and $^{acute}$ESL**

| Parameter | Summary |
|---|---|
| Study | Davis (1934) |
| Study Population | At least 21 healthy adult volunteers in eight experiments |
| Study Quality | Medium |
| Exposure Method | Inhalation exposure chamber study with testing at various exposure levels and time intervals |
| Exposure Duration | 3 – 480 min |
| Critical Effects | CNS depression as indicated by nausea, vomiting, headache, dizziness, and sleepiness |
| NOAEL | 76 ppm (2.5 h) (POD) |
| LOAEL | 158 ppm (30 min) |
| $POD_{ADJ}$ | 76 ppm (from 2.5-h NOAEL) |
| $POD_{HEC}$ | 76 ppm (from 2.5-h NOAEL) |
| Total uncertainty factors (UFs) | 60 |
| *Interspecies UF* | N/A |
| *Intraspecies UF* | 10 |
| *LOAEL-to-NOAEL UF* | N/A |
| *Incomplete Database UF*<br>*Database Quality* | 6<br>Low to medium |
| **Acute ReV [1 h] (HQ = 1)** | **8,200 µg/m$^3$ (1,300 ppb)** |
| **$^{acute}$ESL [1 h] (HQ = 0.3)** | **2,400 µg/m$^3$ (390 ppb)** |

#### *3.1.2.6 Acute Inhalation Observed Adverse Effect Level (IOAEL)*

Risk assessors and the general public often ask to have information on the levels in air where health effects would be expected to occur. So, when possible, the TCEQ provides chemical-specific observed adverse effects levels in DSDs (TCEQ 2015a). As the basis for development of IOAELs is limited to available data, future studies could possibly identify a lower POD for this purpose. The acute IOAEL is provided for informational purposes only (TCEQ 2015a). The 30-min LOAEL of 158 ppm based on an acute inhalation human study (Davis 1934) was used as the POD to develop the acute inhalation observed adverse effect level($^{acute}$IOAEL). The human LOAEL of 158 ppm was not adjusted for exposure duration. The 30-min LOAEL of 158 ppm is then used as the $^{acute}$IOAEL.

The margin of exposure between the estimated acute IOAEL (158,000 ppb or 990,000 µg/m$^3$) and the acute ReV (1,300 ppb or 8,200 µg/m$^3$) for $CCl_4$ is a factor of approximately 120.

### 3.1.3 Health-Based Acute 24-h ReV

The TCEQ collects 24-h canister volatile organic compound (VOC) data based on USEPA's every sixth-day schedule. These data are used to calculate annual averages for comparison to chronic, health-protective ReVs (typically based on noncarcinogenic effects) and ESLs (typically based on carcinogenic effects) as well as welfare-based ESLs (vegetation). In regard to acute exposure durations, however, the TCEQ typically has only 1-h health-based ReVs and welfare-based ESLs (odor, vegetation) for the evaluation of ambient air data, which, while conservative, are not designed to evaluate 24-h sample results. Thus, the development of 24-h, health-protective ReVs would allow the TCEQ to more fully utilize 24-h VOC ambient air data for the evaluation of potential public health concerns. This section documents the derivation of a 24-h AMCV for $CCl_4$, although in a briefer format than the previous section.

#### *3.1.3.1 Selection of the Key Study, POD, and Critical Effect*

Kazantzis and Bomford (1960) reported on a group of factory workers exposed to lower levels of $CCl_4$, approximately 45-97 ppm for 8 h/d, 5 d/wk (see Section 3.1.1.1.3). This study showed that workers developed nausea, anorexia, vomiting, GI distress, and CNS effects including headache. Symptoms typically developed in the latter half of the workweek and cleared over the weekend. The lowest exposure level (45 ppm) can be considered a free-standing LOAEL. Despite possible confounding factors (e.g., co-existed pollutants, smoking status), the study will be used as a key candidate study for calculating a 24-h ReV. The POD is the LOAEL of 45 ppm.

From available controlled human studies, the 240 min (4 h) NOAEL (76 ppm) for CNS effects identified from the key candidate Davis (1934) study will also be used as a candidate POD.

### *3.1.3.2 MOA Analysis and Dose Metric for the Critical Effect*

Similar to the acute (1-h) ReV, CNS effects (e.g., nausea, headache) were noted in the candidate key studies for the 24-h ReV (Kazantzis and Bomford 1960, Davis 1934) and serve as the critical effects for the derivation of 24-ReV. The MOA by which $CCl_4$ may produce such effects is discussed in Section 3.1.2.2. These $CCl_4$-induced noncarcinogenic effects exhibit a threshold MOA.

### *3.1.3.3 Adjustments to the POD*

#### 3.1.3.3.1 Default Exposure Duration Adjustments

##### *3.1.3.3.1.1 For the 8-h LOAEL of 45 ppm*

Exposure in Kazantzis and Bomford (1960) was not continuous and occurred over several days. Therefore, based on toxicokinetic considerations (e.g., some clearance between each 8-h exposure day), a duration adjustment that adjusts the POD is justified. The duration adjustment will use Haber's rule as modified by ten Berge (1986) with an "n" of 1, consistent with Section 4.2.1 of TCEQ (2015a). The POD of 45 ppm was conservatively duration-adjusted (Haber's rule with n = 1) from 8 h to 24 h to calculate the 24-h $POD_{ADJ}$ of 15 ppm. Thus,

$$\text{24-h } POD_{ADJ} = [(45 \text{ ppm}) \times (8 \text{ h}/24 \text{ h})] = 15 \text{ ppm}$$

##### *3.1.3.3.1.2 For the 4-h NOAEL of 76 ppm*

For the Davis (1934) study, the 240-min (4-h) NOAEL (POD) will be used for exposure duration adjustment to 24 h ($POD_{ADJ}$). The POD of 76 ppm was adjusted (Haber's rule with n = 1) from 4 h to 24 h to calculate the 24-h $POD_{ADJ}$ of 12.667 ppm.

$$\text{24-h } POD_{ADJ} = C_2 = [(C_1) \times (T_1 / T_2)] = [(76 \text{ ppm}) \times (4 \text{ h}/24 \text{ h})] = 12.667 \text{ ppm}$$

As this NOAEL-based $POD_{ADJ}$ is just below the LOAEL-based $POD_{ADJ}$ value above, it can be said that a $POD_{ADJ}$ value based on an associated 4-h LOAEL (had one been identified) would be higher than that cited above (15 ppm) and would not identify the critical effect(s)/study based on comparison of LOAEL-based values.

#### 3.1.3.3.2 Default Dosimetry Adjustments from Animal-to-Human Exposure

No dosimetry adjustment from animal-to-human exposure is needed as the $POD_{ADJ}$ was identified from human study. Thus,

24-h $POD_{ADJ}$ from the 8-h LOAEL = 24-h $POD_{HEC}$ = 15 ppm, and

24-h $POD_{ADJ}$ from the 4-h NOAEL = 24-h $POD_{HEC}$ = 12.667 ppm

The $POD_{HEC}$ of 12.667 ppm based on the Davis (1934) 4-h NOAEL is lower than that from the 8-h LOAEL, but critical effects generally are not selected based on comparisons of NOAELs to LOAELs (TCEQ 2015a). Additionally, as mentioned above, the NOAEL-based $POD_{ADJ}$ is just below the LOAEL-based $POD_{ADJ}$ value above, and it can be said that a $POD_{ADJ}$ value based on an associated 4-h LOAEL (had one been identified) would have been higher and would not have identified the critical effect(s)/study. Thus, the $POD_{HEC}$ of 15 ppm from the 8-h LOAEL from Kazantzis and Bomford (1960) was selected to derive the 24-h ReV. This selection not only considers the process for identifying critical effects under TCEQ (2015a), but is also the more conservative selection (i.e., results in a lower 24-h ReV).

### *3.1.3.4 Adjustments to the $POD_{HEC}$*

CNS depression by $CCl_4$ is a noncarcinogenic effect that exhibits a threshold, nonlinear MOA. Thus, the 24-h $POD_{HEC}$ of 15 ppm is selected and appropriate UFs are applied to derive a ReV (TCEQ 2015a).

The following uncertainty factors (UFs) were applied to the $POD_{HEC}$ of 15 ppm:

- A $UF_H$ of 10 was used for intrahuman variability because the study population comprised healthy adults and it was not known if potentially sensitive subpopulations were included (i.e., individuals with pre-exposure to alcohol or pre-existing liver disease).
- A $UF_L$ of 3, to account for uncertainty in extrapolating from a LOAEL to a NOAEL, was used as the effects observed were relatively mild and the duration-adjusted LOAEL (24-h $POD_{ADJ}$ of 15 ppm) is close to the duration-adjusted NOAEL (24-h $POD_{ADJ}$ of 12.7 ppm) from the supporting study (Davis 1934).
- A $UF_D$ of 6 was used for database uncertainty because fewer 24-h studies were available than 1-h studies, the MOA for $CCl_4$ causing CNS effects is unknown, and the quality of the available studies is low to medium.

$$\text{acute ReV} = POD_{HEC} / (UF_H \times UF_L \times UF_D)$$

$$= 15 \text{ ppm} / (10 \times 3 \times 6)$$
$$= 15 \text{ ppm} / 180$$
$$= 0.083 \text{ ppm}$$
$$= 0.083 \text{ ppm or } 83 \text{ ppb (rounded to two significant figures)}$$

### *3.1.3.5 Health-Based Acute 24-h ReV*

The resulting 24-h acute ReV was rounded to two significant figures at the end of all calculations (Table 6).

**Table 6. Derivation of the 24-h acute ReV**

| Parameter | Summary |
|---|---|
| Study | Kazantzis and Bomford (1960) |
| Study Population | 18 workers (14 men and 4 women, 16-54 yrs old) |
| Study Quality | Medium |
| Exposure Method | 8 h/d for 5 consecutive days during the workweek |
| Exposure Duration | 8 h |
| Critical Effects | Nausea, anorexia, vomiting, GI distress, and CNS effects including headache |
| NOAEL | 76 ppm (4 h) |
| LOAEL | 45 ppm (POD) |
| $POD_{ADJ}$ | 15 ppm (from 8-h LOAEL) |
| $POD_{HEC}$ | 15 ppm (from 8-h LOAEL) |
| Total uncertainty factors (UFs) | 180 |
| *Interspecies UF* | 1 |
| *Intraspecies UF* | 10 |
| *LOAEL-to-NOAEL UF* | 3 |
| *Incomplete Database UF*<br>*Database Quality* | 6<br>Low to medium |
| **Acute ReV [24 h] (HQ = 1)** | **520 µg/m$^3$ (83 ppb)** |

### *3.1.3.6 Subacute IOAEL*

Risk assessors and the general public often ask to have information on the levels in air where health effects would be expected to occur. So, when possible, the TCEQ provides chemical-specific observed adverse effects levels in DSDs (TCEQ 2015a). As the basis for development of IOAELs is limited to available data, future studies could possibly identify a lower POD for this purpose. The IOAELs are provided for informational purposes only (TCEQ 2015a). The 8-h LOAEL of 45 ppm for minimal CNS effects (e.g., nausea, headache) identified from the Kazantzis and Bomford (1960) occupational study will be used as a POD to derive a subacute IOAEL. The human LOAEL of 45 ppm was not adjusted from an exposure duration. The $LOAEL_{HEC}$ of 45 ppm is then used as the $^{subacute}$IOAEL.

The margin of exposure between the observed 8-h IOAEL (45,000 ppb or 280,000 µg/m$^3$) and the 24-h ReV (83 ppb or 520 µg/m$^3$) for $CCl_4$ is a factor of approximately 540.

## *3.2 Welfare-Based Acute Evaluation*

### 3.2.1 Odor Perception

$CCl_4$ has a sweet, aromatic odor. However, according to TCEQ Approaches to Derive Odor-Based Values Guidelines, the TCEQ develops odor-based values only for chemicals that are malodorous. (i.e., that smell very unpleasant, obnoxious, disagreeable, or pungent) (TCEQ 2015b). $CCl_4$ is not malodorous and thus no $^{acute}ESL_{odor}$ was derived for $CCl_4$.

### 3.2.2 Vegetation Effects

No significant data were found concerning $CCl_4$ in air and effects on plants. Vegetation may take up and store small amounts of $CCl_4$ when they grow in contaminated soil with no adverse effects. Moreover, $CCl_4$ does not cause damage to plants even when used as a fumigant (Health Canada 2010). No direct effects of airborne $CCl_4$ (atmospheric, spray, etc.) on plants have been reported. $CCl_4$ has been used in many countries as a fumigant either alone, or, more usually, mixed with other chemicals with no reported adverse effects. Since $CCl_4$ does not interact with vegetation, it has been used as a carrier for chemicals with herbicidal activity. When $CCl_4$ was tested for effects on seed germination, results indicate $CCl_4$ is safe (seeds germinated) at dose levels up to 135 ppm (Field 2002). Because $CCl_4$ has not been shown to produce adverse effects on plants or grain, no acute vegetation-based ESL ($^{acute}ESL_{veg}$) was derived.

## *3.3 Summary of the Acute Values*

The acute evaluation resulted in the derivation of the following values:

- Acute 1-h ReV = 1,300 ppb (8,200 µg/m$^3$)
- $^{acute}$ESL [1 h] = 390 ppb (2,400 µg/m$^3$)
- Acute 24-h ReV = 83 ppb (520 µg/m$^3$)

For the evaluation of ambient air monitoring data, the acute 1-h ReV will be used to evaluate 1-h monitoring data. The health-based $^{acute}$ESL will be used as the 1-h ESL for air permitting. The 24-h ReV of 83 ppb (520 µg/m$^3$) will be available for comparison to 24-h canister data.

The $^{acute}$ESL (HQ = 0.3) is not used to evaluate ambient air monitoring data and will be used in air permitting applications.

# Chapter 4 Chronic Evaluation

## *4.1 Noncarcinogenic Potential*

Available data indicate that the liver is the primary target of chronic inhalation exposure to $CCl_4$ (USEPA 2010, USEPA 2020, ATSDR 2005). While human data are preferred for derivation of a chronic noncarcinogenic ReV and ESL ($^{chronic}ESL_{threshold(nc)}$), information on the long-term toxicity of inhaled $CCl_4$ in humans that can be used for a dose-response assessment is limited. Thus, an animal study serves as the key study for derivation of the chronic ReV and $^{chronic}ESL_{threshold(nc)}$.

### 4.1.1 Key and Supporting Studies

$CCl_4$ has been known for many years to be an adverse hepatotoxic agent in humans and animals. The principal clinical signs of liver injury in humans who inhale sufficiently high levels of $CCl_4$ for an adequate duration are jaundice, increased serum enzyme levels (see Tomenson et al. 1995 below), and, in fatal cases, necrosis of the liver. In cases of lethal acute or repeated exposures, autopsy generally reveals marked liver necrosis with pronounced steatosis, and repeated or chronic exposure leads in some cases to fibrosis and/or cirrhosis. In animals, the liver and kidney are the most prominent target organs of toxicity in subchronic and chronic inhalation studies of $CCl_4$. The hepatic effects seen with inhalation exposure to relatively high $CCl_4$ levels in laboratory animals are much the same as in humans: elevated serum enzyme levels, steatosis, and centrilobular necrosis progressing to fibrosis. Nephrotoxic effects were reported less frequently in these animal studies and generally at higher concentrations than those causing hepatotoxic effects (USEPA 2010, USEPA 2020, ATSDR 2005).

#### *4.1.1.1 Human Study, Tomenson et al. (1995)*

A cross sectional study of hepatic function was conducted on 135 workers occupationally exposed to $CCl_4$ and 276 nonexposed controls who were employed in three plants in northern England. Workers were categorized according to their duration of employment (< 1 yr, 1–5 yrs, and > 5 yrs), but the serum enzyme results were not presented by estimated duration of exposure because statistical analysis showed that duration of exposure had no significant effect. Exposures were estimated from historical personal monitoring data for each job category, and exposure groups were categorized as low (≤ 1 ppm), medium (2.5 ppm; with a range of 1.1–3.9 ppm), or high (8 ppm; with a range of 4.0–11.9 ppm). Alcohol consumption was considered equivalent across groups. Blood samples were taken from all participants and analyzed for a variety of biochemical and hematological variables.

Multivariate analysis of four liver function variables, ALT, aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT), showed a significant difference ($p < 0.05$) between exposed and non-exposed workers. However, there were no significant differences between different exposure groups. The univariate analysis of variance did not

show significant differences between the exposed and non-exposed workers in these four variables, but there was a suggestion of a dose-response for ALP and GGT. Pairwise comparisons showed a significant increase ($p < 0.05$) in ALP and GGT in the medium exposure group (+9% and +23%, respectively) and a similar, but non-significant increase in the high exposure group. There were no similar patterns in AST and ALT. These changes were not considered appropriate endpoints for toxicity factor derivation as they did not show a significant dose-response relationship. Moreover, none of the exposed subjects had related (e.g., hepatic) disease that could be attributed to exposure to $CCl_4$. In summary, the data collected did not clearly show that exposure to $CCl_4$ was the cause for these changes. Furthermore, the enzyme differences were not associated with clinical disease.

This study does not present conclusive evidence of a NOAEL or LOAEL for potential toxic effects in humans based on serum enzyme levels or related disease.

### *4.1.1.2 Animal Studies*

#### 4.1.1.2.1 Nagano et al. (2007a)

The objective of the Nagano et al. (2007) study was to investigate the toxicity of inhaled $CCl_4$ on F344 rats and BDF1 mice (50/sex/species) at 0, 5, 25, or 125 ppm (nominal concentrations) for 6 h/d, 5 d/wk, for 104 wk. Chamber concentrations of $CCl_4$ were monitored by gas chromatography every 15 min, and were maintained at 5.0 ± 0.1 (mean ± standard deviation), 25.1 ± 0.4, and 125.1 ± 1.1 ppm for the exposure of rats and at 5.0 ± 0.1, 25.1 ± 0.4, and 125.2 ± 1.2 ppm for the exposure of mice throughout the 2-yr exposure period.

All organs (thymus, adrenal gland, ovary, testis, heart, lung, kidney, spleen, liver, and brain) were examined for macroscopic lesions and histopathologic changes. Concentration-related hepatic effects were noted in rats (both sexes) at 25 and 125 ppm consisting of chronic hepatotoxicity characterized by cirrhosis, fibrosis, and fatty change. In mice, ceroid deposition, bile-duct proliferation, and hydropic changes were observed in both sexes at 25 and 125 ppm. Relative liver, kidney, and lung weights were increased (> 10%) in rats (both sexes) at 25 and/or 125 ppm. In mice, mean body weights of 25 and 125 ppm-exposed males and females were significantly decreased (22–39% lower than controls at termination) during the course of the 2-yr exposure period. Decreased body weights (≥ 10% lower than controls at termination) also were observed in male and female rats.

Alterations in some serum biomarkers of hepatotoxicity were not statistically significant in rats exposed at 5 ppm. Statistically significant increases in liver weight and serum parameters (ALT, AST, lactate dehydrogenase [LDH], and/or GGT) were observed at ≥ 25 ppm in rats and mice (increases over control ranged from 1.6- to 13-fold and 1.5- to 18-fold in rats and mice, respectively). Hepatic lesions at ≥ 25 ppm included basophilic, eosinophilic, clear, and mixed cell

foci, deposition of ceroid, fibrosis, and cirrhosis, and increased severity of fatty change and granulation. Incidences of fatty change in the liver were significantly increased in both male and female rats exposed to 25 and 125 ppm. The incidence of liver cirrhosis was significantly increased in the 125 ppm-exposed male and female rats, while the incidence of liver fibrosis was significantly increased in the 25 ppm-exposed male and female rats.

In mice, statistically significant decreases in some serum parameters at 5 ppm were not considered to be biologically significant because the control values for serum chemistry parameters in males were higher than historical control values. Incidences of deposition of ceroid, bile-duct proliferation, and hydropic change in centrilobular hepatocytes were significantly increased in both male and female mice exposed to 25 and 125 ppm.

Table 7 and Table 8 list the incidences of selected histopathological liver lesions in rats and mice, respectively.

**Table 7. Incidences of selected histopathological liver lesions in rats exposed to $CCl_4$ vapor by inhalation for 2 yr**

| Group | Male Control | Male 5 ppm | Male 25 ppm | Male 125 ppm | Female Control | Female 5 ppm | Female 25 ppm | Female 125 ppm |
|---|---|---|---|---|---|---|---|---|
| **No. of animals examined** | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Fatty change | 4 | 7 | 39** | 49** | 6 | 7 | 49** | 46** |
| Fibrosis | 0 | 0 | 43** | 2 | 0 | 0 | 45** | 0 |
| Cirrhosis | 0 | 0 | 1 | 40** | 0 | 0 | 2 | 50** |

*p ≤ 0.05, **p ≤ 0.01

**Table 8. Incidences of selected histopathological liver lesions in mice exposed to $CCl_4$ vapor by inhalation for 2 yr**

| Group | Male Control | Male 5 ppm | Male 25 ppm | Male 125 ppm | Female Control | Female 5 ppm | Female 25 ppm | Female 125 ppm |
|---|---|---|---|---|---|---|---|---|
| **No. of animals examined** | 50 | 50 | 50 | 50 | 50 | 49 | 50 | 49 |
| Deposition of ceroid | 2 | 1 | 36** | 36** | 0 | 0 | 28** | 35** |
| Proliferation: bile duct | 0 | 0 | 19** | 22** | 0 | 0 | 5* | 9** |
| Hydropic change: centrilobular | 1 | 0 | 8* | 9** | 1 | 0 | 13** | 12** |

*p ≤ 0.05, **p ≤ 0.01

The incidences of non-neoplastic liver lesions in rats and mice show a dose-response effect. Therefore, it was determined that 25 ppm is the LOAEL based on adverse hepatic effects (liver histopathology and liver weight) and 5 ppm is the NOAEL in rats and mice.

### 4.1.1.2.2 Adams et al. (1952)

Adams et al. (1952) exposed Wistar rats (15-25/sex/group), guinea pigs (5-9/sex/group), rabbits (1-2/sex/group), and rhesus monkeys (1-2 of either sex/group) to $CCl_4$ at 5, 10, 25, 50, 100, and/or 400 ppm for 7 h/d, 5 d/wk for approximately 6 to 9 months. Each group was observed for general appearance and behavior, and hematology parameters, body and organ weights, and histopathology of various organs were evaluated for evidence of acute toxicity. The primary target of $CCl_4$ in all species was the liver.

The authors identified NOAEL and LOAEL values of approximately 5 and 10 ppm, respectively, for potential hepatic effects in rats and guinea pigs, 10 and 25 ppm in rabbits, and 50 and 100 ppm in monkeys, respectively. The effects at the LOAELs included: (1) rats: increased liver weight; slight to moderate fatty degeneration (no cirrhosis); and increased total lipid, neutral fat, and serum esterified cholesterol values in liver; (2) guinea pigs: increased liver weight, slight to moderate fatty degeneration (no cirrhosis), and increased total lipid, neutral fat, and serum esterified cholesterol values in liver; (3) rabbits: increased liver weight, slight to moderate fatty degeneration and slight cirrhosis; (4) monkeys: slight depression in growth, slight cloudy swelling in a few central areas of the liver and slight fatty degeneration throughout the liver with increased total lipid content of the liver.

### 4.1.1.2.3 Prendergast et al. (1967)

Prendergast et al. (1967, as cited in USEPA 2010, 2020) exposed groups of 15 Sprague-Dawley or Long-Evans rats, 15 Hartley guinea pigs, 3 New Zealand rabbits, 2 beagle dogs, and 3 squirrel monkeys to 10 ppm n-octane (as vehicle control group); or 1 or 10 ppm $CCl_4$ vapor (diluted in 10 ppm of n-octane) for 24 h/d for 90 d. Serum chemistry and liver lipid analyses were performed on some animals. No statistical tests were conducted. Exposure to 10 ppm $CCl_4$ resulted in the deaths of 3/15 guinea pigs. Body weight gain was depressed in all species relative to the controls. Fatty changes in the liver were observed most prominently in rats and guinea pigs but were present in the other species as well. Exposure to 1 ppm $CCl_4$ produced no mortality or clinical signs of toxicity. No effects were noted in the n-octane vehicle control group. The results of this study suggest a NOAEL of 1 ppm and a LOAEL of 10 ppm for hepatotoxicity identified in rats, guinea pigs, and other tested species.

### 4.1.1.2.4 Nagano et al. (2007b)

Nagano et al. (2007b) also conducted a 13-wk inhalation toxicity study of $CCl_4$ in rats and mice. Groups of F344/DuCrj rats and Crj:BDF1 mice (10/sex/species/group) were exposed (whole body) to 0, 10, 30, 90, 270, or 810 ppm of $CCl_4$ (99.8% pure) vapor for 6 h/d, 5 d/wk for 13 wks.

Hematology and serum chemistry analyses were performed at the end of the 13-wk exposure period. All organs (thymus, adrenal gland, ovary, testis, heart, lung, kidney, spleen, liver, and brain) were examined for macroscopic lesions and histopathologic findings.

Significant and dose-related increases ($p < 0.01$) in relative liver weights were observed in male rats exposed to ≥ 10 ppm and in female rats and mice exposed to ≥ 30 ppm. Statistically significant exposure-related decreases in hemoglobin and hematocrit were observed at ≥ 90 ppm in male and female rats. Exposure-related increases in the incidence and severity of histopathological lesions of the liver were observed at ≥ 10 ppm in both male and female rats. Statistically significant and exposure-related increases in AST and ALT were observed in female rats at ≥ 30 ppm, respectively, by 1.5- to 14-fold and 2- to 17-fold; and increases in ALT and ALP were observed in male rats at ≥ 90 ppm, respectively, by 2.7- to 19-fold and 1.1- to 4.1-fold. In mice, serum chemistry changes of note included significant increases in ALT in males and females at ≥ 90 ppm by 2.5- to 5.1-fold; and increases in ALP in males at ≥ 30 ppm by 1.3- to 1.6-fold. Exposure-related increases in the incidence and severity of histopathological lesions of the liver were observed at ≥ 10 ppm in rats and mice in both sexes. At the 10 ppm concentration, chemical-related liver lesions included slight fatty change, cytological alteration, and granulation.

The results of this study showed that the most sensitive endpoint of $CCl_4$-induced toxicity was fatty change with large droplets in rats of both sexes and in male mice, and cytoplasmic globules in male mice, as well as increased relative liver weight in male rats. The lowest exposure level of 10 ppm is a minimal LOAEL for hepatic effects (increased liver weight and histopathology) in rats and slight cytological alterations in male mice. No NOAEL was identified from this study.

### *4.1.1.3 Reproductive and Developmental Studies*

For the acute evaluation (Chapter 3), consideration of potential developmental and/or reproductive effects did not result in significant concern for these effects as potential critical effects for derivation of the ReV. More specifically, consistent with ATSDR (2005), available data suggest developmental toxicity due to $CCl_4$ exposure is not a major area of concern, and no reproductive effects (e.g., number of implants or resorptions, conception) occurred in rats exposed up to 1,004 ppm for 7 h/d on GD 6–15 (Schwetz et al. 1974, as cited in USEPA 2010, 2020). Dams exhibited reduced food consumption, decreased bodyweight, and nonpregnant rats showed signs of hepatotoxicity (increased SGPT activity, pale and mottled livers, and increased relative liver weight).

There are also data specifically relevant to the chronic evaluation. For example, in rats that inhaled $CCl_4$ vapors for three generations, there was a decrease in fertility in animals exposed to concentrations of 200 ppm or higher for 8 h/d, 5 d/wk, for 10.5 months (Smyth et al. 1936,

as cited in ATSDR 2005). Moderate to marked degeneration of testicular germinal epithelium was observed in rats exposed repeatedly (7 h/d, 5 d/wk) to 200 ppm for 192 d (Adams et al. 1952). Additionally, deposition of ceroid was observed in the ovaries of mice that were exposed to 125 ppm of $CCl_4$ vapor, 6 h/d, 5 d/wk for 2 yrs (Japan Bioassay Research Center [JBRC] 1998, as cited in ATSDR 2005). However, these chronic PODs (e.g., LOAELs ≥ 125 ppm) are higher than the rodent PODs (NOAEL = 5 ppm and LOAEL = 25 ppm) for hepatic effects (Section 4.1.1.2). Consequently, similar to the acute evaluation, developmental/reproductive toxicity due to $CCl_4$ exposure is not a major area of concern for the chronic evaluation because setting a toxicity factor that protects against hepatotoxicity will also protect against the reproductive/developmental effects that occur at substantially higher exposure concentrations.

### 4.1.2 Selection of the Key Study and Critical Effect

Table 9 is a summary of the subchronic and chronic inhalation toxicity studies for $CCl_4$ exposure. The table shows that the most robust study on the long-term toxicity of $CCl_4$ is a 2-yr inhalation toxicity and oncogenicity rat and mouse study (Nagano et al. 2007a). The Nagano et al. (2007a) study identified a NOAEL and LOAEL of 5 and 25 ppm, respectively, for hepatic effects (liver histopathology and liver weight). Adams et al (1952) also identified a NOAEL of 5 ppm for potential hepatic effects (serum enzyme levels) in rats and guinea pigs. A NOAEL and LOAEL of 1 and 10 ppm for hepatotoxicity (fatty changes in the liver), respectively, were identified from the Prendergast et al. (1967) 13-wk study. However, the exposure durations utilized in both the Adams et al. and Prendergast et al. studies are less than one year. In a 13-wk study, Nagano et al. (2007b) identified a minimal LOAEL of 10 ppm for similar histopathological effects to those identified in the Nagano et al. (2007a) 2-yr study. The hepatic effects observed in the Nagano et al. (2007a) chronic inhalation bioassay were considered the most appropriate basis for derivation of the chronic ReV, and while there are lower LOAELs from other studies (representing a departure from TCEQ 2015a) likely due to dose selection, the chronic NOAEL from this study is lower than the LOAELs from the other studies and is therefore expected to result in a sufficiently conservative and health-protective value. Fatty change in the liver of rats was selected as the specific endpoint for exposure-response analysis. Thus, the identified NOAEL of 5 ppm for liver lesions (fatty changes) in rats and mice will be used as a POD for the derivation of a chronic noncarcinogenic ReV and $^{chronic}ESL_{threshold(nc)}$. This study was also used by ATSDR (2005) for derivation of the chronic inhalation minimal risk level (MRL) and by USEPA (2010, 2020) for derivation of their reference concentration (RfC). The hepatic effects (fatty changes) were selected as critical effects because this histopathologic lesion is indicative of cellular damage and appears to be a more sensitive endpoint than other changes (e.g., fibrosis and cirrhosis).

**Table 9. Summary of subchronic and chronic inhalation toxicity studies for $CCl_4$**

| Species | Duration and Concentration | NOAEL (ppm) | LOAEL (ppm) | Effects at the LOAEL | Reference |
|---|---|---|---|---|---|
| Rat (15-25/sex/ group) | 7 h/d, 5 d/wk for 6 months: 0, 5, 10, 25, 50, 100, 200, or 400 ppm | 5 | 10 | Increased liver weight; fatty degeneration in liver | Adams et al. 1952 |
| Guinea pig (5–9/sex/ group) | | 5 | 10 | Increased liver weight; fatty degeneration in liver | |
| Rabbit (1-2/sex/ group) | 7 h/d, 5 d/wk for 6 months: 0, 5, 10, 25, 50, 100, or 200 ppm | 10 | 25 | Increased liver weight; fatty degeneration and slight cirrhosis in liver | |
| Monkey (1-2/group) | | 50 | 100 | Slight fatty degeneration and increased lipid content in liver | |
| Rat or Guinea pig (15/group); rabbit or (3/group); dog (2/group) | 24 h/d, 7 d/wk for 13 wks: 10 ppm n-octane (control group), 1 or 10 ppm $CCl_4$ (diluted in 10 ppm n-octane) | 1 | 10 | Reduced body weight gain; enlarged liver with fatty change | Prendergast et al. 1967 |
| Rat (10/ sex/group) | 6 h/d, 5 d/wk for 13 wks: 0, 10, 30, 90, 270, or 810 ppm | --- | 10 | Increased liver weight; fatty change in liver | Nagano et al. 2007b |
| Mouse (10/ sex/group) | | --- | 10 | Slight cytological alterations in the liver | |
| Rat (50/sex/ group) | 6 h/d, 5 d/wk for 104 wks: 0, 5, 25, or 125 ppm | 5 | 25 | Concentration-related hepatic effects: increased serum enzyme activities; lesions in the liver (fatty changes, fibrosis, cirrhosis) | Nagano et al. 2007a |
| Mouse (50/sex/ group | | 5 | 25 | Increased serum enzyme activities; lesions in the liver (degeneration) | |

## 4.1.3 MOA Analysis and Dose Metric

A MOA is generally defined as a sequence of key events and processes (starting with interaction of an agent with a cell and proceeding through operational and anatomical changes) resulting in toxicity. While the primary acute effect of lower levels of exposure to $CCl_4$ are CNS-related, which does not require metabolic activation, additional adverse effects (e.g., hepatotoxicity) and long-term effects (e.g., cancer) do require that $CCl_4$ be metabolized (USEPA 2010, 2020). $CCl_4$ metabolism occurs primarily in the liver. The metabolism of $CCl_4$ has been extensively

studied in in vivo and in vitro mammalian systems (USEPA 2010). Metabolism of $CCl_4$ via cytochrome P450 (primarily by CYP2E1 to generate highly reactive free radical metabolites [trichloromethyl radical ($CCl_3$·) and trichloromethylperoxy radical ($CCl_3OO$·)] plays a role in its hepatotoxicity MOA. The primary metabolites, $CCl_3$· and $CCl_3OO$·, are capable of covalently binding and causing damage to cellular macromolecules (Weber et al. 2003). The longer-term hepatotoxicity of $CCl_4$ is secondary to its metabolism and thus, the liver is expected to be an important target organ on the basis of its high CYP2E1 content. The adverse hepatic effects resulting from the MOA in operation are expected to exhibit a threshold (e.g., such as a NOAEL). For such threshold effects, a POD is determined and divided by UFs to derive the chronic ReV (TCEQ 2015a).

## 4.1.4 Derivation of the POD

The USEPA toxicological assessment of $CCl_4$ (USEPA 2010, 2020) utilized physiologically based pharmacokinetic (PBPK) modeling estimates of the internal liver dose of metabolites. Exposure levels studied in the 2-yr rat bioassay (Nagano et al. 2007a) were converted to estimates of internal doses by application of a PBPK model. The rat PBPK model was used to simulate internal dose metrics corresponding to intermittent exposure (6 h/d, 5 d/wk) to concentrations of 5, 25, and 125 ppm, as studied in the 2-yr bioassay. Liver metabolism rate was selected as the primary dose metric for liver effects, based on evidence that metabolism of $CCl_4$ via CYP2E1 to highly reactive free radical metabolites plays a crucial role in its MOA in producing liver toxicity. Then, benchmark dose (BMD) modeling was used to analyze the relationship between the estimated internal doses and response (i.e., fatty change of the liver). Lastly, the resulting benchmark dose lower confidence limit (BMDL) values were converted to estimates of human equivalent exposure concentrations (HECs) by applying a human PBPK model.

### *4.1.4.1 PBPK Modeling for Internal Dose Metrics for the Key Study (Nagano et al. 2007a)*

Metabolism of $CCl_4$ via CYP2E1 to highly reactive free radical metabolites ($CCl_3$· and $CCl_3OO$·) plays a role in its MOA in producing liver toxicity. The rate of hepatic metabolism of $CCl_4$ was considered a reasonable internal dose surrogate for these radical species in liver (USEPA 2010).

Internal dose metrics corresponding to the exposure concentrations studied in the Nagano et al. (2007a) 2-yr rat inhalation bioassay were predicted by the rat PBPK model (Thrall et al. 2000; Benson and Springer 1999; Paustenbach et al. 1988, as cited in USEPA 2010, 2020). The two dose metrics, time-averaged arterial blood concentration of $CCl_4$ (µmol/L blood) (MCA) and time-averaged rate of metabolism of $CCl_4$ (µmol/h/kg liver weight) (MRAMKL), were simulated in the rat PBPK model as time-averaged values, with the averaging time being the chronic exposure period (e.g., 2 yrs). Internal dose metrics corresponding to the exposure concentrations studied in the 2-yr rat inhalation bioassay (Nagano et al. 2007a) are presented in Table 10. Two values for $V_{maxC}$ (maximum rate of hepatic metabolism of $CCl_4$) have been

reported for the rat; Gargas et al. (1986, as cited in USEPA 2010) derived a value for $V_{maxC}$ of 0.4 mg/h/kg $BW^{0.70}$, based on the results of gas uptake studies in rats. Paustenbach et al. (1988) derived a value of 0.65 mg/h/kg $BW^{0.70}$, based on a reanalysis of data for a subset of the rats used in the Gargas et al. (1986) study. Increasing $V_{maxC}$ from 0.4 to 0.65 mg/h/kg $BW^{0.70}$ resulted in lower values for the mean MCA and higher values for the mean MRAMKL in the liver dose metric. The effect of varying $V_{maxC}$ on MRAMKL becomes more pronounced as exposure concentration increases.

**Table 10. Comparisons of internal dose metrics predicted from PBPK rat models**

| | **MCA (µmol/L blood)** | **MCA (µmol/L blood)** | **MRAMKL (µmol/h/kg liver)** | **MRAMKL (µmol/h/kg liver)** |
|---|---|---|---|---|
| Exposure concentration (ppm) | $V_{maxC}$ = 0.40 | $V_{maxC}$ = 0.65 | $V_{maxC}$ = 0.40 | $V_{maxC}$ = 0.65 |
| 5 | 0.128 | 0.116 | 3.813 | 4.991 |
| 25 | 0.708 | 0.653 | 12.092 | 17.626 |
| 125 | 3.892 | 3.775 | 24.320 | 36.266 |

Source: Thrall et al. (2000); Paustenbach et al. (1988); Gargas et al. (1986), as cited in USEPA 2010 (Table 5-5) and USEPA 2020.

### *4.1.4.2 Benchmark Dose Modeling*

The TCEQ performed benchmark dose modeling using USEPA (2023) BMD software (desktop version 3.3.2 in Excel) to analyze data on estimated internal doses (i.e., MCA and MRAMKL) and incidence data (i.e., fatty changes of the rat liver) from the 2-yr rat bioassay (Nagano et al. 2007a) (Table 7 and Table 10). Data were used to predict 95% lower confidence limits on the BMDs using dichotomous models. A default benchmark response (BMR) of 10% was selected as the critical effect size ($BMD_{10}$ and $BMDL_{10}$). All the available dichotomous models were run (Appendix A), and the best fit models based on $p > 0.1$, absolute value of scaled residual values < 2, BMDL values within 3-fold of each other for viable models, and lowest AIC value are listed in Table 11.

In the male rat, the best fit of the data was provided by the log-logistic model using MCA as the dose metric and the logistic model using MRAMKL as the dose metric. For female rats, no models fit the data when all dose groups were included and using MCA or MRAMKL as the dose metric. After dropping the highest dose, the best fit of the data was provided by the multistage degree 2 model using MCA as the dose metric. With the highest dose dropped for female rats, the models using MRAMKL as the dose metric either had p values < 0.1 and or had p values that could not be calculated; therefore there were no models with adequate fit. Summaries of the resulting $BMD_{10}$ and $BMDL_{10}$ values for male and female rats are shown in Table 11.

**Table 11. Summary of $BMD_{10}$ and $BMDL_{10}$ values for male and female rats using MCA or MRAMKL dose metrics**

| | | $V_{maxC}$ = 0.40[a] | | $V_{maxC}$ = 0.65[b] | |
|---|---|---|---|---|---|
| **Dose Metric (Sex)** | **Best Fit Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **$BMD_{10}$** | **$BMDL_{10}$** |
| MCA (Male) | Log-Logistic | 0.137 | 0.079 | 0.123 | 0.071 |
| MCA (Female) | No model fit (all p values < 0.1) | --- | --- | --- | --- |
| MCA[c] (Female) | Multistage Degree 2 | 0.124 | 0.085 | 0.114 | 0.078 |
| MRAMKL (Male) | Logistic | 3.257 | 2.586 | 4.601 | 3.653 |
| MRAMKL (Female) | No model fit (all p values < 0.1) | --- | --- | --- | --- |
| MRAMKL[c] (Female) | No model fit (all p values < 0.1) | --- | --- | --- | --- |

MCA: time-averaged arterial blood concentration of $CCl_4$ (µmol/L blood)
MRAMKL: time-averaged rate of metabolism of $CCl_4$ (µmol/h/kg liver weight)
[a] Exposure internal dose for MCA: 0, 0.128, 0.708, 3.892 µmol/L blood; for MRAMKL: 0, 3.813, 12.092, 24.32 µmol/h/kg liver (exposure concentrations of 5, 25 and 125 ppm, respectively).
[b] Exposure internal dose for MCA: 0, 0.116, 0.653, 3.775 µmol/L blood; for MRAMKL: 0, 4.991, 17.626 36.266 µmol/h/kg liver (exposure concentrations of 5, 25 and 125 ppm, respectively).
[c] Benchmark dose modeling after highest dose dropped.

### *4.1.4.3 PBPK Modeling to derive Human Equivalent Concentrations*

Interspecies extrapolation (i.e., rat-to-human) of $CCl_4$ inhalation dosimetry was accomplished using a human PBPK model described in Thrall et al. (2000), Benson and Springer (1999), and Paustenbach et al. (1988), as cited in USEPA (2010, 2020). The human PBPK model was used to estimate the continuous exposure HECs (in $mg/m^3$) shown in Table 12, which would result in values for the internal dose metrics (MCA or MRAMKL) equal to the $BMDL_{10}$ values for fatty changes of the liver in rats. For complete details see Section C.3. of Appendix C of USEPA (2010).

Estimates of the dose metrics, MCA and MRAMKL, were sensitive to the value assigned to the $V_{maxC}$ parameter. Several values for $V_{maxC}$ in animals and humans have been reported: a value of 1.49 mg/h/kg $BW^{0.70}$ for humans derived by Thrall et al. (2000) and Benson and Springer (1999); a value of 1.7 mg/h/kg $BW^{0.70}$ for hamsters derived by Thrall et al. (2000); and the two values estimated for the rat (0.4, 0.65 mg/h/kg $BW^{0.70}$ by Paustenbach et al. (1988) and Gargas et al. (1986) were used by the USEPA in the estimation of HECs [all aforementioned references are cited by USEPA 2010]). A human $V_{maxC}$ estimated from *in vitro* human data ($V_{maxC(H)}$) can reasonably be presumed to be more relevant than a human $V_{maxC}$ based entirely on rodent

data. In addition, because the MOA for $CCl_4$-induced hepatotoxicity involves metabolism to reactive metabolites in the liver, HECs based on the MRAMKL dose metric is the most proximate to the critical effect (USEPA 2010). Therefore, $V_{maxC(H)}$ of 1.49 mg/h/kg $BW^{0.70}$ and the dose metric MRAMKL are considered to yield the best estimates of the HEC. Based on this $V_{maxC(H)}$ and the MRAMKL ($BMDL_{10}$) dose metric, the HEC values were calculated based on a trend equation developed by USEPA (see the third figure in Figure C-12 of Appendix C, USEPA 2010):

HEC (ppm) = 0.49547 × MRAMKL + ([15.078 × MRAMKL] / [67.700 – MRAMKL])

Table 12 provides estimated HEC values, based on a $V_{maxC(H)}$ = 1.49 mg/h/kg $BW^{0.70}$, corresponding to $BMDL_{10}$ values for fatty changes in the liver as reported in the 2-yr rat inhalation bioassay (Nagano et al. 2007a). The range of $BMDL_{10\text{-}HEC}$ values based on the most appropriate $V_{maxC(H)}$ and dose metric is 11.83-16.79 mg/$m^3$.

USEPA (2010, 2020) indicated that no information is available to establish a rat $V_{maxC}$ of 0.4 or 0.65 mg/h/kg $BW^{0.70}$ as the more scientifically defensible value for this parameter. Therefore, HECs derived using these two rat $V_{maxC}$ values were averaged as the POD for the USEPA $CCl_4$ RfC (USEPA 2010, 2020). Accordingly, the average POD based on male rat data was 14.31 mg/$m^3$. The POD based on data for the male rat (14.31 mg/$m^3$) was selected by the TCEQ as the $POD_{HEC}$ for the health-based chronic ReV derivation.

**Table 12. $BMDL_{10}$, and corresponding HEC values for fatty changes of liver in male rats exposed to $CCl_4$**

| | **$BMD_{10}$[a] $V_{MAXc(R)}$ = 0.4** | **$BMD_{10}$[a] $V_{MAXc(R)}$ = 0.65** | **$BMDL_{10}$[a] $V_{MAXc(R)}$ = 0.4** | **$BMDL_{10}$[a] $V_{MAXc(R)}$ = 0.65** | **HEC (ppm)[b] $V_{MAX}$ = 0.4** | **HEC (ppm)[b] $V_{MAX}$ = 0.65** | **HEC (ppm)[d] Average** |
|---|---|---|---|---|---|---|---|
| MRAMKL | 3.257 | 4.601 | 2.586 | 3.653 | 1.88 ppm (11.83 mg/$m^3$)[c] | 2.67 ppm (16.79 mg/$m^3$)[c] | 2.28 ppm (14.31 mg/$m^3$)[c] |

MRAMKL: time-averaged rate of metabolism of $CCl_4$ (µmol/h/kg liver weight)
[a] Rats were exposed to $CCl_4$ vapor for 104 wks (6 h/d, 5 d/wk). Doses modeled correspond to exposure concentrations of 0, 5, 25, and 125 ppm.
[b] HEC (ppm) = 0.49547 x MRAMKL + ([15.078 x MRAMKL] / [67.700 – MRAMKL]); based on a $V_{maxC(H)}$ of 1.49 mg/h/kg $BW^{0.70}$
[c] HEC (mg/$m^3$) = 6.29 x HEC (ppm)
[d] The average of these values is the basis for the chronic ReV POD.

### 4.1.5 Default Exposure Duration Adjustments

Because the human PBPK model was used to estimate continuous human equivalent exposures, no further duration adjustments are necessary. Dose-response analysis of the data from the Nagano et al. (2007a) study, which included BMD and PBPK modeling, yielded a POD of 14.31 mg/m$^3$. Thus, POD = $POD_{ADJ}$ = 14.31 mg/m$^3$ (2.28 ppm).

### 4.1.6 Default Dosimetry Adjustments from Animal-to-Human Exposure

Because the human PBPK model was used to estimate continuous HECs from internal dose metrics in rats, no further animal-to-human adjustments are necessary. Thus,

$POD_{ADJ}$ = continuous exposure modeled $POD_{HEC}$ = 14.31 mg/m$^3$

### 4.1.7 Adjustments to the $POD_{HEC}$

Determining a POD and applying appropriate UFs (i.e., assume a threshold MOA) are the default procedures for noncarcinogenic effects (TCEQ 2015a). UFs account for inherent uncertainties in dose-response assessments. Therefore, UFs were applied to the $POD_{HEC}$ value from the key study in deriving the chronic noncarcinogenic ReV.

The following uncertainty factors (UFs) were applied to the $POD_{HEC}$ of 14.31 mg/m$^3$:

- A $UF_H$ of 10 was used to account for potential intrahuman variability (i.e., potentially sensitive subpopulations such as children, those with pre-existing health conditions, etc.) and for known genetic polymorphisms of CYP2E1 in the human population which may affect the ability to metabolize $CCl_4$ and, therefore, may confer increased susceptibility to the hepatotoxic effects.
- A $UF_A$ of 3 was used for potential toxicodynamic differences between rats and humans since toxicokinetic (dosimetric) adjustments from rats to humans have already been made. Although the MOA for $CCl_4$-related hepatotoxicity is believed to be the same in rats and humans, the $UF_A$ of 3 for potential interspecies toxicodynamics is considered appropriate because of uncertainty in the interspecies differences in cellular protective mechanisms (USEPA, 2010).
- A $UF_D$ of 3 was used because the inhalation database for $CCl_4$ relevant for a chronic evaluation is well documented and includes subchronic and chronic exposure in animals, 2-yr chronic inhalation bioassays in rats and mice, and one human epidemiological study. Although there are limited inhalation reproductive/developmental studies in rats that did not use an exposure concentration low enough to identify a NOAEL for either maternal or fetal toxicity, developmental/reproductive toxicity due to $CCl_4$ exposure is not a major area of concern (ATSDR 2005, USEPA 2010). The database lacks an adequate multigeneration study of reproductive function by any route of exposure; therefore, a $UF_D$ of 3 was applied.

$$\text{chronic ReV} = POD_{HEC} / (UF_H \times UF_A \times UF_D)$$

$$= 14.31 \text{ mg/m}^3 / (10 \times 3 \times 3)$$
$$= 14.31 \text{ mg/m}^3 \ / \ 90$$
$$= 0.159 \text{ mg/m}^3$$
$$= 0.16 \text{ mg/m}^3 \text{ or } 160 \text{ µg/m}^3 \text{ (rounded to two significant figures)}$$

### 4.1.6 Health-Based Chronic ReV and $^{chronic}ESL_{nonlinear(nc)}$

In deriving the chronic ReV, no numbers were rounded between equations until the ReV was calculated. The chronic ReV was rounded to two significant figures, and then used to calculate the $^{chronic}ESL_{threshold(nc)}$ using a target hazard quotient of 0.3 (Table 13).

**Table 13. Derivation of the chronic ReV and $^{chronic}ESL_{threshold(nc)}$**

| Parameter | Summary |
|---|---|
| Study | Nagano et al. (2007a) |
| Study Population | 50 F344 rats/sex/exposure group |
| Study Quality | High |
| Exposure Method | 0, 5, 25, or 125 ppm of $CCl_4$ |
| Critical Effects | Significant fatty changes in the liver of male rats |
| Exposure Duration | 104 wks |
| $BMDL_{10\text{-}HEC}$ (internal PBPK modeled $POD_{HEC}$) | 14.31 mg/m$^3$ |
| $POD_{ADJ}$ | 14.31 mg/m$^3$ (no adjustment because a continuous exposure human PBPK model already adjusted for discontinuous animal exposure regimen) |
| $POD_{HEC}$ | 14.31 mg/m$^3$ |
| Total UFs | 90 |
| *Interspecies UF* | 3 |
| *Intraspecies UF* | 10 |
| *LOAEL UF* | N/A |
| *Subchronic to chronic UF* | N/A |
| *Incomplete Database UF*<br>*Database Quality* | 3<br>Medium to High |
| **Chronic ReV (HQ = 1)** | **160 µg/m$^3$ (25 ppb)** |

| Parameter | Summary |
|---|---|
| **$^{chronic}ESL_{threshold(nc)}$ (HQ = 0.3)** | **48 µg/m³ (7.5 ppb)** |

## 4.1.7 Chronic Noncarcinogenic IOAEL

Estimated HEC values, based on a $V_{maxC(H)}$ = 1.49 mg/h/kg $BW^{0.70}$, corresponding to $BMDL_{10}$ values (Table 12) for fatty changes in the liver as reported in the 2-yr rat inhalation bioassay (Nagano et al. 2007a) are selected as a POD ($HEC_{BMD10}$) for derivation of a chronic IOAEL. Based on the equation HEC (ppm) = 0.49547 x MRAMKL + ([15.078 x MRAMKL] / [67.700 – MRAMKL]), the corresponding $HEC_{BMD10}$ values for fatty changes in liver using a $V_{max\ C(R)}$ of 0.4 and 0.65 were calculated (Table 14).

**Table 14. $BMD_{10}$ and corresponding $HEC_{BMD10}$ values for fatty changes of liver in male rats exposed to $CCl_4$**

| | **$BMD_{10}$ $V_{MAXc(R)}$ = 0.4** | **$BMD_{10}$ $V_{MAXc(R)}$ = 0.65** | **$HEC_{BMD10}$ (mg/m³) $V_{MAX}$ = 0.4** | **$HEC_{BMD10}$ (mg/m³) $V_{MAX}$ = 0.65** | **$HEC_{BMD10}$ (mg/m³) Average** |
|---|---|---|---|---|---|
| MRAMKL | 3.257 | 4.601 | 14.95 | 21.26 | 18.11 (2.88 ppm) |

MRAMKL: time-averaged rate of metabolism of $CCl_4$ (µmol/h/kg liver weight)

The average $HEC_{BMD10}$ based on male rat data (18.11 mg/m³) was selected as a POD to derive the chronic IOAEL. The POD($HEC_{BMD10}$) of 2.9 ppm (18 mg/m³) (rounded to 2 significant figures) is then used as the $^{chronic}$IOAEL. The chronic IOAEL is provided for informational purposes only (TCEQ 2015a). The margin of exposure between the estimated chronic IOAEL (2,900 ppb or 18,000 µg/m³) and the chronic ReV (25 ppb or 160 µg/m³) for $CCl_4$ is a factor of approximately 110.

## 4.1.8 Comparison of Results

Table 15 is a summary of the chronic toxicity factors for $CCl_4$ derived by the TCEQ and other agencies.

**Table 15. Comparison of the chronic toxicity factors for $CCl_4$ among agencies**

| | **TCEQ** | **ATSDR (2005)** | **USEPA (2010)** | **CalEPA (2000)** |
|---|---|---|---|---|
| **Toxicity Factor** | 160 µg/m³ (25 ppb) | 190 µg/m³ (30 ppb) | 100 µg/m³ (16 ppb) | 40 µg/m³ (6 ppb) |
| **Study** | Nagano et al. (2007a) | Nagano et al. (2007a) | Nagano et al. (2007a) | Adams et al. (1952) |

| | TCEQ | ATSDR (2005) | USEPA (2010) | CalEPA (2000) |
|---|---|---|---|---|
| **Critical Effects** | Significant fatty changes in the liver of rats | Significant fatty changes in the liver of rats | Significant fatty changes in the liver of rats | Increased liver weight and hepatic fatty infiltration in female guinea pigs |
| **POD** | 5 ppm (NOAEL) | 5 ppm (NOAEL) | 5 ppm (NOAEL) | 5 ppm (LOAEL) |
| **$POD_{ADJ}$** | --- | 0.9 ppm | --- | 1 ppm |
| **RGDR** | --- | 1.7 (default of 1) | --- | 1.7 |
| **$POD_{HEC}$ ($POD_{ADJ}$ x RGDR)** | --- | 0.9 ppm (0.9 ppm x 1) | --- | 1.7 ppm (1 ppm x 1.7) |
| **$BMDL_{10\text{-}HEC}$ (internal PBPK modeled $POD_{HEC}$ dose)** | 14.31 $mg/m^3$ | --- | 14.3 $mg/m^3$ | --- |
| **Total UFs** | 90 | 30 | 100 | 300 |
| **Interspecies UF** | 3 | 3 | 3 | 3 |
| **Intraspecies UF** | 10 | 10 | 10 | 10 |
| **LOAEL to NOAEL UF** | --- | | --- | 3 |
| **Subchronic to chronic UF** | | | | 3 |
| **Incomplete Database UF** | 3 | --- | 3 | |

The TCEQ-derived chronic ReV is similar to those derived by ATSDR (2005) and USEPA (2010). ATSDR employed the same $CCl_4$ 2-yr study (Nagano et al. 2007a) in developing a chronic inhalation minimal risk level (MRL) value of 0.03 ppm (30 ppb). The methodology used by ATSDR to derive their MRL used the NOAEL of 5 ppm as a POD and adjusted that value to continuous exposure ($POD_{ADJ}$ = 0.9 ppm). A $POD_{HEC}$ of 0.9 ppm was calculated by multiplying a default value of regional gas dose ratio (RGDR) of 1. The chronic minimal risk level (MRL) was derived by applying a total UF of 30. Despite these differences, the chronic inhalation MRL of 30 ppb is similar to the TCEQ chronic noncarcinogenic ReV of 25 ppb. The USEPA calculated a reference concentration (RfC) of 100 $\mu g/m^3$ (16 ppb) also based on the Nagano et al. (2007a) study and applied PBPK and benchmark dose modeling to estimate a $BMDL_{10\text{-}HEC}$ (internal PBPK modeled $POD_{HEC}$) of 14.3 $mg/m^3$. A total of UF of 100 was applied to $POD_{HEC}$ to derive the RfC.

The CalEPA (2000) chronic reference exposure level (REL) of 40 μg/$m^3$ (6 ppb) is based on a POD (LOAEL) of 5 ppm for increases in liver weight and liver lipid content in female guinea pigs from the Adams et al. (1952) study. The level of 5 ppm, however, was considered a NOAEL by the ATSDR and USEPA. The exposure duration-adjusted POD ($POD_{ADJ}$ = 1 ppm) was then adjusted using a RGDR of 1.7 resulting in a $POD_{HEC}$ of 1.7 ppm. A total UF of 300 was applied to $POD_{HEC}$ to derive the chronic REL.

## *4.2 Carcinogenic Potential*

Epidemiological reports have noted the occurrence of liver cancer in workers exposed to $CCl_4$ by inhalation exposure; however, the data are not sufficient to establish a cause-and-effect relationship in humans. In laboratory animals, however, oral and inhalation studies of $CCl_4$ administered for 2 yrs caused liver tumors in rats and mice. Mice that inhaled $CCl_4$ also developed tumors of the adrenal gland. The lowest cancer effect levels were observed for mice at an exposure concentration of 25 ppm by inhalation and at a dose of 20 mg/kg-d administered orally (ATSDR 2005).

### 4.2.1 Carcinogenic Weight of Evidence

IARC (1999) has classified $CCl_4$ in Group 2B (possibly carcinogenic to humans) based on inadequate evidence of carcinogenicity in humans and sufficient evidence in experimental animals. IARC states:

"The risk of cancer from $CCl_4$ has been examined in five occupational populations. In three of four studies that collected information on non-Hodgkin lymphoma (two cohort investigations and one independent nested case-control study), associations with exposure to $CCl_4$ were suggested. However, not all of these studies distinguished exposure to $CCl_4$ specifically, and the associations were not strong statistically. In the fourth study (another cohort investigation), few men were exposed to $CCl_4$ and the risk of non-Hodgkin lymphoma was not reported.

A nested case-control study of lung cancer in a cohort of chemical workers showed no association with exposure to $CCl_4$. Four population-based case-control studies have examined associations of $CCl_4$ with chronic lymphocytic leukemia, brain cancer, female breast cancer, and intraocular melanoma. Findings were generally unremarkable. In a fifth case-control study, which examined several cancers, no association was found with non-Hodgkin lymphoma, although the power to detect an increased risk was low.

$CCl_4$ was tested for carcinogenicity by various routes of administration. $CCl_4$ produced liver neoplasms in mice and rats and mammary neoplasms in rats following subcutaneous injection. In one study in mice by inhalation, an increased incidence of pheochromocytomas was reported. In experiments involving administration of $CCl_4$ after known carcinogens, the

occurrence of tumors and/or pre-neoplastic lesions of the liver in mice, rats, and hamsters were enhanced."

The Department of Health and Human Services (NTP 2011, as cited in ATSDR 2005) determined that $CCl_4$ may reasonably be anticipated to be a human carcinogen. $CCl_4$ caused tumors in several species of experimental animals, at two different tissue sites, and by several different routes of exposure.

The National Institute of Occupational Safety and Health (NIOSH 2007) indicates that $CCl_4$ is a potential occupational carcinogen based on positive carcinogenic findings in chronic bioassays in rodents.

The American Conference of Governmental Industrial Hygienists (ACGIH 2011) classifies $CCl_4$ as a suspected human carcinogen.

Under the Guidelines for Carcinogen Risk Assessment (USEPA 2005), $CCl_4$ is "likely to be carcinogenic to humans" based on: (1) inadequate evidence of carcinogenicity in humans and (2) sufficient evidence in animals by oral and inhalation exposure, i.e., hepatic tumors in multiple species (rat, mouse, and hamster) and pheochromocytomas (adrenal gland tumors) in mice."

TCEQ also considers the weight of evidence (WOE) for $CCl_4$ as consistent with "likely to be carcinogenic to humans". TCEQ performs carcinogenic dose-response assessments for chemicals considered "likely to be carcinogenic to humans" based on the WOE if there are adequate data (TCEQ 2015a).

## 4.2.2 Key and Supporting Studies

### *4.2.2.1 Human/Epidemiological Studies*

Studies in humans are inadequate to show a good correlation between exposure to $CCl_4$ and carcinogenicity. There is some evidence for certain types of cancer in occupational populations thought to have had some exposure to $CCl_4$, including non-Hodgkin's lymphoma (Blair et al. 1998, Spirtas et al. 1991), lymphosarcoma and lymphatic leukemia (Checkoway et al. 1984, Wilcosky et al. 1984), esophageal and cervical cancer (Blair et al. 1990, 1979), breast cancer (Cantor et al. 1995), astrocytic brain cancer (Heineman et al. 1994, as cited in 2010), and rectal cancer (Dumas et al. 2000) [all aforementioned references are cited by USEPA 2010]. In these cases, exposure to $CCl_4$ was poorly characterized and confounded by simultaneous exposures to other chemicals. Additionally, these studies were designed to evaluate tetrachloroethylene and trichloroethylene and had only limited ability to examine other chemical exposures such as $CCl_4$. None of the human epidemiology studies reported associations with cancer of the liver or adrenal gland, which are the main sites of carcinogenicity in animal studies, but these negative

results may be because of a lack of power to detect human tumors and/or the exposures to $CCl_4$ may have been too low. Moreover, subjects in the studies were exposed to multiple chemicals and exposures were estimated qualitatively based on historical information.

No positive association was found between likely occupational exposure to $CCl_4$ and the risk of increased mortality from pancreatic cancer among residents of 24 states (Kernan et al. 1999, as cited by USEPA 2010). No significantly elevated risk of cancer was detected in various cohorts of Finnish workers who were exposed to chemicals including $CCl_4$, but follow-up time was too short (< 16 yrs) to detect cancers with longer latency periods (Kauppinen et al. 1995, 2003, as cited by USEPA 2010).

#### *4.2.2.2 Animal Studies – Nagano et al. 2007a, JBRC 1998*

In an inhalation study, groups of 50 BDF1 mice/sex/group and 50 F344 rats/sex/group were exposed to 0, 5, 25, or 125 ppm $CCl_4$ 6 h/d, 5 d/wk, for 104 wk. In rats, the incidence of hepatocellular adenomas and hepatocellular carcinomas was significantly increased at 125 ppm $CCl_4$ in both sexes of rats. The incidence of tumors in rats exposed to 5 or 25 ppm $CCl_4$ was the same as controls (1 animal per group). For the male rats, only the animals receiving 125 ppm $CCl_4$ showed a positive response (40/50). Due to the dose-response observed, no meaningful modeling could be performed (e.g., data appear inadequate to define the dose-response curve). The incidence of tumors was not increased in female rats exposed to 5 or 25 ppm by the same protocol, although the incidence of liver carcinomas (3/50) in 25-ppm-exposed females exceeded the range of historical control incidence from Japan Bioassay Research Centre (JBRC 1988) bioassays. Multiple occurrences of hepatocellular tumors were found in the 125-ppm-exposed rats of both sexes. Incidences of hepatocellular adenomas and carcinomas and the combined incidences of these liver tumors were increased in an exposure concentration-dependent manner, as evidenced by a significant positive trend by Peto's test (Table 16).

**Table 16. Incidences of liver tumors in rats exposed to $CCl_4$ vapor**

| Group | Male Control | Male 5 ppm | Male 25 ppm | Male 125 ppm | Female Control | Female 5 ppm | Female 25 ppm | Female 125 ppm |
|---|---|---|---|---|---|---|---|---|
| **No. of animals examined** | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Hepatocellular adenoma | 0^^ | 1 | 1 | 21** | 0^^ | 0 | 0 | 40** |
| Hepatocellular carcinoma | 1^^ | 0 | 0 | 32** | 0^^ | 0 | 3 | 15** |
| Hepatocellular adenoma and hepatocellular carcinoma, combined | 1^^ | 1 | 1 | 40** | 0^^ | 0 | 3 | 44** |

**Tumor incidence significantly elevated when compared to controls by Fisher's exact test ($p \leq 0.01$).
^^Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).

In mice, the incidence of hepatocellular carcinomas was significantly increased at 25 and 125 ppm in both males and females. The tumors often occupied almost all areas of the entire liver in the 125 ppm-exposed mice. The incidence of hepatocellular adenomas was significantly increased at 25 and 125 ppm in males and at 5 and 25 ppm in females. A statistically significant increase in the incidence of liver adenomas in female mice at 5 ppm was observed compared to the concurrent control. However, the incidence of hepatocellular carcinomas in the 5 ppm-exposed female mice was not significantly increased. The incidences of combined hepatocellular adenoma and carcinoma were significantly increased in the 25 and 125 ppm-exposed mice of both sexes by Fisher's exact test. Incidences of hepatocellular adenomas and carcinomas and their combined incidences were increased in an exposure concentration-dependent manner, as evidenced by a significant positive trend by Peto's test (Table 17).

Pheochromocytomas in the adrenal gland in mice occurred in an exposure concentration-dependent manner, as evidenced by a significant positive trend by Peto's test. Significant increases (Fisher's exact test) were also observed in the incidence of benign adrenal pheochromocytomas in male mice at 25 or 125 ppm and female mice at 125 ppm. Specifically, pheochromocytomas were identified in 32/50 male mice in the 125 ppm group, only one of which was classified as malignant (the remaining 31 pheochromocytomas were benign) (JBRC 1998). Benign pheochromocytomas were identified in 22/49 female mice in the 125 ppm group (Table 17). In addition to the potential cancer risk suggested by these tumors, benign pheochromocytomas may represent a noncancer health risk because of the excessive secretion of catecholamines, leading to sustained and unregulated sympathetic nervous system hyperactivity.

**Table 17. Incidences of liver tumors in mice exposed to $CCl_4$ vapor**

| Group | Male Control | Male 5 ppm | Male 25 ppm | Male 125 ppm | Female Control | Female 5 ppm | Female 25 ppm | Female 125 ppm |
|---|---|---|---|---|---|---|---|---|
| **No. of animals examined** | 50 | 50 | 50 | 50 | 50 | 49 | 50 | 49 |
| Hepatocellular adenoma | 9^^ | 10 | 27** | 16 | 2^^ | 8* | 17** | 5 |
| Hepatocellular carcinoma | 17^^ | 12 | 44** | 47** | 2^^ | 1 | 33** | 48** |
| Hepatocellular adenoma and carcinoma | 24^^ | 20 | 49** | 47** | 4^^ | 9 | 44** | 48** |
| Adrenal gland pheochromocytomas [a] | 0^^ | 0 | 16** | 32** | 0^^ | 0 | 0 | 22** |

[a] All pheochromocytomas were benign with the exception of one malignant pheochromocytoma in the 125 ppm male mouse group.
*Tumor incidence significantly elevated compared with controls by Fisher's exact test ($p \leq 0.05$).
**Tumor incidence significantly elevated compared with controls by Fisher's exact test ($p \leq 0.01$).
^^ Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).

## 4.2.3 Carcinogenic MOA and Dose Metric

A MOA is generally defined as a sequence of key events and processes (starting with interaction of an agent with a cell and proceeding through operational and anatomical changes) resulting in toxicity. The Nagano (2007a) study provided clear evidence of carcinogenicity for $CCl_4$ in rats and mice. Both a cytotoxic-proliferative MOA and a genotoxic MOA for $CCl_4$-induced hepatocarcinogenesis have been suggested. USEPA (2010, 2020) proposed a hypothetical MOA for $CCl_4$-induced liver tumors involving metabolism to reactive intermediates ($CCl_3$· and $CCl_3OO$·), radical-induced hepatocellular toxicity, and sustained regenerative and proliferative changes. It is important to note that in female mice exposed to 5 ppm $CCl_4$, non-neoplastic lesions and pre-neoplastic lesions were not observed in the liver, yet there was a statistically significant increase in the incidence of hepatocellular adenomas (Nagano 2007a).

Because humans exhibit the same signs of liver toxicity that have been observed in animal studies and the types of hepatocellular tumor expressed consistently in several animal species exposed to $CCl_4$ are also found in the human population, the hypothesized MOA and tumors are considered to be relevant to humans. However, the MOA for $CCl_4$-induced liver tumors at concentrations lower than those utilized in the carcinogenicity studies has not been sufficiently demonstrated and accepted by the scientific community, and the MOA for the

pheochromocytomas in mice exposed to $CCl_4$ is unknown (USEPA 2010). An area of uncertainty is mutagenicity at concentrations below those associated with observed tumors. Because (1) the MOA for liver tumors is unknown at concentrations below those associated with observed hepatocellular tumors in rodents, (2) the MOA for pheochromocytomas is unknown, and (3) a statistically significant increase in hepatocellular adenomas was observed in female mice at the lowest concentration of 5 ppm without the findings of non-neoplastic or pre-neoplastic liver lesions, a linear low-dose extrapolation was performed.

Consideration was given for a threshold for development of liver tumors. If the liver tumors form via a threshold MOA, then because the MOA for fatty liver changes and carcinogenesis both involve generation of reactive $CCl_4$ metabolites and covalent binding to macromolecules and that fatty liver precedes development of liver tumors, the $^{chronic}ReV_{threshold(nc)}$ of 160 µg/m$^3$ should also protect against liver cancer. If one applies a hazard quotient of 0.3 for air permitting purposes, the chronic $ESL_{threshold(c)}$ would be 48 µg/m$^3$ (7.5 ppb). This toxicity factor is higher than the ambient air concentration of 2.8 µg/m$^3$ associated with TCEQ's inhalation unit risk factor based on adrenal pheochromocytomas in male mice, with an excess cancer risk of 1 in 100,000. Because the MOA for $CCl_4$-induced tumors is not entirely clear and may include a genotoxic component (which is conservatively considered to have a linear dose-response), the $^{chronic}ESL_{nonthreshold(c)}$ of 2.8 µg/m$^3$ (0.44 ppb) will be used for evaluation of long-term ambient air data and for air permit reviews.

## 4.2.4 Selection of the Key Study and Critical Effects

As noted previously, epidemiological studies of populations exposed to $CCl_4$ provide only limited evidence for an association between $CCl_4$ exposure and human cancer and are not adequate for dose-response analysis. However, animal carcinogenicity data are available; a chronic bioassay of $CCl_4$ by the inhalation route is the 104-wk inhalation bioassay in rats and mice conducted by JBRC (Nagano et al. 2007a, JBRC 1998). This bioassay provides data adequate for dose-response modeling between hepatic cytotoxicity and tumor formation. The Nagano et al. (2007a) study showed that $CCl_4$ produced a statistically significant increase in hepatocellular adenomas and carcinomas in rats and mice of both sexes, and adrenal gland pheochromocytomas in mice of both sexes. Incidences of hepatocellular adenomas and carcinomas and their combined incidences were increased in an exposure concentration-dependent manner. The bioassay generally supports a prominent role for cytotoxicity, regeneration, and proliferation in the MOA for $CCl_4$-induced liver tumors at higher exposure levels. However, the MOA for liver tumors is unknown at lower exposure concentrations, the MOA is unknown for pheochromocytomas, and hepatocellular tumors were observed in mice at the lowest concentration without findings of non-neoplastic and pre-neoplastic liver lesions in female mice. Additionally, there is uncertainty regarding mutagenicity at concentrations of $CCl_4$ below those associated with observed tumors. Therefore, a nonthreshold linear model was used. The Nagano et al. (2007a) study was selected as key study for the calculation of an URF

and $^{chronic}ESL_{nonthreshold(c)}$. Increase in hepatocellular tumors in rats and mice as well as adrenal gland pheochromocytomas in mice in both sexes are the critical carcinogenic effects.

## 4.2.5 Derivation of the POD

As described in Section 4.1.4, the USEPA utilized PBPK modeling estimates of internal liver dose of metabolites. Internal dose metrics were selected as they were considered the most relevant to the toxicity endpoints of interest (i.e., liver tumors and pheochromocytomas). Exposure concentrations studied in the 2-yr rat bioassay (Nagano et al. 2007a) were converted to estimates of internal doses by application of a PBPK model. The rat and mouse PBPK models were used to simulate internal dose metrics corresponding to intermittent exposure (6 h/d, 5 d/wk) to concentrations of 5, 25, and 125 ppm, as studied in the 2-yr bioassay. Then, benchmark dose modeling was used to analyze the relationship between the estimated internal doses and response (i.e., pheochromocytomas and hepatocellular tumors). Lastly, the resulting BMDL values were converted to estimates of HECs by applying a human PBPK model.

### *4.2.5.1 PBPK Modeling for Internal Dose Metrics for the Key Study*

As described in Section 4.1.4.1, the two dose metrics are: (1) time-averaged arterial blood concentration of $CCl_4$ (MCA, µmol/L), and (2) time-averaged rate of metabolism of $CCl_4$ (MRAMKL, µmol/hour/kg liver). These dose metrics were simulated in the rat or mouse PBPK model as time-averaged values, with the averaging time being the chronic exposure period (e.g., 2 yr). MRAMKL was selected as the primary dose metric for liver tumors based on evidence that metabolism of $CCl_4$ via CYP2E1 to highly reactive free radical metabolites plays a crucial role in its MOA in producing liver tumors.

Data on incidence of adrenal gland pheochromocytomas in mice were also analyzed as the USEPA considers mouse pheochromocytomas to be relevant to humans. All pheochromocytomas in the mouse were benign with the exception of one malignant pheochromocytoma in the 125 ppm male mouse group (Table 17). USEPA (2010) states that the presence of one observed malignant tumor in the mouse study suggests the potential for these benign tumors to progress to malignancy. The USEPA considers MCA the appropriate dose metric to represent internal doses in modeling pheochromocytoma incidence in mice. The MRAMKL dose metric was excluded from consideration in the analysis of pheochromocytomas on the basis that reactive metabolites of $CCl_4$ formed in the liver are unlikely to be sufficiently stable to contribute to toxicity or transformations of cells in the adrenal gland. Therefore, the MCA dose metric was used to represent the internal dose in BMD modeling of pheochromocytoma incidence in mice.

For the rat, the internal dose metrics corresponding to the exposure concentrations studied in the 2-yr rat inhalation bioassay (Nagano et al. 2007a) for two values of $V_{maxC}$ (i.e., 0.40 and 0.65 mg/h/kg $BW^{0.70}$) were provided previously in Table 10 (Section 4.1.4.1).

For the mouse, the internal dose metrics corresponding to the exposure concentrations were derived from the Fisher et al. (2004, as cited in USEPA 2010, 2020) and Thrall et al. (2000). Mouse PBPK models are presented in Table 18. The USEPA states that because whether the Fisher et al. (2004) or Thrall et al. (2000) model provides the more accurate prediction of the internal dose for the mouse cannot be established, the cancer risk values derived using these two mouse models were averaged to derive the final cancer risk values for $CCl_4$ (details are presented in USEPA 2010, 2020).

**Table 18. Comparison of internal dose metrics predicted from Fisher et al. (2004) and Thrall et al. (2000) PBPK mouse models[a]**

| | MCA (µmol/L blood) | MCA (µmol/L blood) | MRAMKL (µmol/h/kg liver) | MRAMKL (µmol/h/kg liver) |
|---|---|---|---|---|
| **Exposure (ppm)** | **Fisher et al. 2004** | **Thrall et al. 2000** | **Fisher et al. 2004** | **Thrall et al. 2000** |
| 5 | 0.111 | 0.213 | 12.666 | 15.456 |
| 25 | 0.603 | 1.226 | 41.675 | 43.599 |
| 125 | 3.315 | 6.856 | 71.589 | 63.596 |

[a] Values are for a 0.036 kg mouse.
Source: USEPA 2010 (Table 5-10) and 2020

### *4.2.5.2 BMD Modeling*

The TCEQ performed BMD modeling using USEPA BMD software (desktop version 3.3.2 in Excel) to analyze data on estimated internal doses (i.e., MCA for pheochromocytomas and MRAMKL for liver tumors) and incidence data of adrenal gland and liver tumors. First, multistage modeling was used for the liver tumor and pheochromocytoma incidence data for rats and mice. When adequate fit could not be achieved with the multistage model, other models (i.e., regular dichotomous models) from the BMDS suite of models were used. A default BMR of 10%, as per TCEQ guidelines (TCEQ 2015a) was selected as the critical effect size ($BMD_{10}$ and $BMDL_{10}$).

Dose-response modeling was performed for tumor responses from the Nagano (2007a) inhalation bioassay: combined liver tumors (adenoma and carcinoma) in female rats (see Table 16); combined liver tumors in male and female mice (see Table 17); and combined pheochromocytomas in male and female mice (see Table 17). The tumor incidence reflects that of benign or malignant tumors combined (i.e., hepatocellular adenomas or carcinomas; benign or malignant pheochromocytomas). The male rat incidence data for combined liver tumors were not modeled because this data set lacked the resolution desired for dose-response modeling (see Table 16 and discussion in Section 4.2.2.2). The results of the BMD modeling are summarized below (Tables 19-22); detailed model outputs are provided in Appendix B.

Multistage modeling for combined liver tumors (adenoma and carcinoma) in female rats using MRAMKL as a dose metric did not provide an adequate fit; therefore, for this data set, other models for dichotomous data in BMDS were run. The Weibull model was the best fit model for female rat combined liver tumors at $V_{maxC}$ of 0.4 and 0.65 (Table 19).

**Table 19. Summary of $BMD_{10}$ and $BMDL_{10}$ values for incidence data using MRAMKL as the dose metric for combined liver tumors in female rats**

| Dose Metric | Best Fit Model | $BMD_{10}$ $V_{maxC}$ = 0.40 [a] | $BMDL_{10}$ $V_{maxC}$ = 0.40 [a] | $BMD_{10}$ $V_{maxC}$ = 0.65 [b] | $BMDL_{10}$ $V_{maxC}$ = 0.65 [b] |
|---|---|---|---|---|---|
| MRAMKL | Weibull | 13.45 | 11.28 | 19.70 | 16.39 |

[a] MRAMKL values modeled were 0, 3.813, 12.092, 24.32 µmol/h/kg liver for the 0, 5, 25 and 125 ppm exposures, respectively.
[b] MRAMKL values modeled were 0, 4.991, 17.626, 36.266 µmol/h/kg liver for the 0, 5, 25 and 125 ppm exposures, respectively.

For combined liver tumors in male mice, multistage modeling did not provide an adequate fit of the data with all doses included or with the highest dose dropped. For male mice, BMD dichotomous modeling using all doses showed the best fit of the combined liver tumor data with the Dichotomous Hill model for $V_{maxC}$ by Fisher at al. (2004) and for $V_{maxC}$ by Thrall et al. (2000). For combined liver tumors in female mice, multistage modeling did provide an adequate fit of the data with all doses included using MRAMKL as the dose metric (Table 20).

As shown in Table 17, the male mouse data provided poor resolution of the dose-response relationship for combined liver tumors. Combined liver tumor incidence in 5 ppm-exposed male mice (20/50) was below that of the control males (24/50), and the incidences in the 25 and 125 ppm groups were close to maximal response (49/50), without any intervening exposure concentrations having submaximal responses. For the female mouse, the bioassay data set contained two exposure concentrations (25 and 125 ppm) at which close to maximal responses (44/50 and 48/49, respectively) were seen. Therefore, multistage model fits were also conducted without use of the highest exposure group (125 ppm) data for both male and female mice combined liver tumors.

$BMD_{10}$ and $BMDL_{10}$ values for combined liver tumor incidences after dropping the highest dose in the mouse study are shown in Table 20. With the highest dose data dropped, multistage modeling MRAMKL as the dose metric provided an adequate fit in female mice, but not in male mice. For male mice, BMD dichotomous modeling with the highest dose dropped showed the best fit of the combined liver tumor data with the Log-Logistic model for $V_{maxC}$ by Fisher at al. (2004) and the Weibull model for $V_{maxC}$ by Thrall et al. (2000).

**Table 20. Summary of $BMD_{10}$ and $BMDL_{10}$ values for combined liver tumors in mice using the MRAMKL dose metric**

| Dose Metric (Sex) | Best Fit Model | $BMD_{10}$ $V_{maxC}$ by Fisher et al. 2004 [a] | $BMDL_{10}$ $V_{maxC}$ by Fisher et al. 2004 [a] | $BMD_{10}$ $V_{maxC}$ by Thrall et al. 2000 [b] | $BMDL_{10}$ $V_{maxC}$ by Thrall et al. 2000 [b] |
|---|---|---|---|---|---|
| MRAMKL (male) | Dichotomous Hill | 23.36 | 11.23 | 25.21 | 13.90 |
| MRAMKL (male) [c] | Log-Logistic | 30.71 | 10.95 | --- | --- |
| MRAMKL (male) [c] | Weibull | --- | --- | 35.99 | 12.67 |
| MRAMKL (female) | Multistage Degree 2 | 10.30 | 5.57 | 10.48 | 7.59 |
| MRAMKL (female) [c] | Multistage Degree 2 | 9.71 | 6.32 | 10.46 | 7.59 |

[a] MRAMKL values modeled were 0, 12.666, 41.675, 71.589 µmol/h/kg liver for the 0, 5, 25 and 125 ppm exposures, respectively.
[b] MRAMKL values modeled were 0, 15.456, 43.599, 63.596 µmol/h/kg liver for the 0, 5, 25 and 125 ppm exposures, respectively.
[c] Benchmark dose modeling after highest dose dropped.

For mouse pheochromocytoma, a multistage model was used to fit pheochromocytoma data using MCA as the dose metric (Table 21). The multistage model provided an adequate fit of male mouse date with $V_{maxC}$ by Fisher et al. 2004, and for female mouse data with $V_{maxC}$ by Fisher et al. 2004 and with $V_{maxC}$ by Thrall et al. 2000. For male mice, BMD dichotomous modeling showed the best fit of the combined pheochromocytoma data with the Dichotomous Hill model for $V_{maxC}$ by Thrall et al. (2000).

**Table 21. Summary of $BMD_{10}$ and $BMDL_{10}$ values for pheochromocytomas in mice using the MCA dose metric**

| Dose Metric (Sex) | Best Fit Model | $BMD_{10}$ $V_{maxC}$ by Fisher et al. 2004 [a] | $BMDL_{10}$ $V_{maxC}$ by Fisher et al. 2004 [a] | $BMD_{10}$ $V_{maxC}$ = by Thrall et al. 2000 [b] | $BMDL_{10}$ $V_{maxC}$ = by Thrall et al. 2000 [b] |
|---|---|---|---|---|---|
| MCA (male) | Multistage Degree 1 | 0.292 | 0.230 | --- | --- |
| MCA (male) | Dichotomous Hill | --- | --- | 1.04 | 0.470 |
| MCA (female) | Multistage Degree 2 | 1.43 | 1.14 | 2.95 | 2.34 |

[a] MCA values modeled were 0, 0.111, 0.603, 3.315 µmol/L blood for the 0, 5, 25 and 125 ppm exposures, respectively.
[b] MCA values modeled were 0, 0.213, 1.226, 6.856 µmol/L blood for the 0, 5, 25 and 125 ppm exposures, respectively.

### *4.2.5.3 PBPK Modeling of HECs*

PBPK models were used to estimate HECs (in ppm or $mg/m^3$) that would result in values for the internal dose metrics (MCA or MRAMKL) equal to the respective internal BMDL values based on liver tumor or pheochromocytoma incidence data. As discussed in Section 4.2.5.1, the dose metric MRAMKL was used to represent internal doses in modeling liver tumor incidence in rats and mice, and MCA was considered to be the appropriate dose metric to represent internal doses in modeling pheochromocytoma incidence in mice; these dose metrics were used as the basis for the cancer risk estimate. As in the derivation of the chronic ReV, the human $V_{maxC}$ ($V_{maxC(H)}$) estimated from *in vitro* human data (1.49 mg/hour/kg $BW^{0.70}$) was considered to yield the most appropriate estimate of the HEC and was used as the basis for cancer risk estimates. Based on this $V_{maxC(H)}$ (1.49 mg/h/kg $BW^{0.70}$) and the MRAMKL or MCA dose metrics (BMDLs), the HEC values were calculated using trend equations for any value of MRAMKL (Equation 1, see third figure in Figure C-12 of Appendix C , USEPA 2010)) and MCA (Equation 2 and 3) developed by USEPA (Figure C-6 of Appendix C, USEPA 2010):

$$HEC\ (ppm) = 0.49547 \times MRAMKL + ([15.078 \times MRAMKL] / [67.700 - MRAMKL]) \quad \text{[Equation 1]}$$

$$HEC\ (mg/m^3) = 78.6179 \times MCA^{0.9937} \text{ for MCA values (0.01-1)} \quad \text{[Equation 2]}$$

$$HEC\ (mg/m^3) = 78.4969 \times MCA^{0.9342} \text{ for MCA values (1-5)} \quad \text{[Equation 3]}$$

Estimated HEC values, based on a $V_{maxC(H)}$ = 1.49 mg/h/kg $BW^{0.70}$, corresponding to $BMDL_{10}$ values for the 2-yr rat and mouse inhalation bioassays (Nagano et al. 2007b, JBRC 1998) for different tumor types and alternative values of $V_{maxC}$ are presented in Tables 22 to 24.

For the rat model, no information is available to establish whether a rat $V_{maxC}$ of 0.4 or 0.65 mg/hour/kg $BW^{0.70}$ is the more scientifically defensible value for this parameter. Therefore, the cancer risk values derived using these two rat $V_{maxC}$ values were averaged to derive the final cancer risk values for $CCl_4$ (Table 22). Similarly, for the mouse, it cannot be established whether the Fisher et al. (2004) or Thrall et al. (2000) model provides the more accurate prediction of the internal dose for the mouse. Therefore, the cancer risk values derived using these two mouse models were averaged to derive the final cancer risk values for $CCl_4$. The calculated average HEC values from tumor response modeling runs (four for liver tumors and two for pheochromocytomas; Table 23 and Table 24) were used as PODs to estimate the respective inhalation unit risk factor (URF) and $10^{-5}$ risk air concentrations.

**Table 22. $BMDL_{10}$ values and corresponding HEC values for combined liver tumors in female rats**

| | **$BMDL_{10}$ $V_{MAX}$ = 0.4** | **$BMDL_{10}$ $V_{MAX}$ = 0.65** | **HEC (ppm) $V_{MAX}$ = 0.4** | **HEC (ppm) $V_{MAX}$ = 0.65** | **HEC (ppm) Average** |
|---|---|---|---|---|---|
| MRAMKL (dichotomous data) | 11.28 (Weibull model) | 16.39 (Weibull model) | 8.61 (54.15 mg/$m^3$) | 12.94 (81.40 mg/$m^3$) | 10.78 (67.78 mg/$m^3$) |

MRAMKL: time-averaged rate of metabolism of $CCl_4$ (µmol/h/kg liver weight)
HEC (ppm) = 0.49547 x MRAMKL + ([15.078 x MRAMKL] / [67.700 – MRAMKL]); based on a $V_{maxC(H)}$ of 1.49 mg/h/kg $BW^{0.70}$
HEC (mg/$m^3$) = 6.29 x HEC (ppm)

**Table 23. $BMDL_{10}$ values and corresponding HEC values for combined liver tumors in male and female mice**

| | **$BMDL_{10}$ Fisher et al. 2004** | **$BMDL_{10}$ Thrall et al. 2000** | **HEC (ppm) Fisher et al. 2004** | **HEC (ppm) Thrall et al. 2000** | **HEC (ppm) Average** |
|---|---|---|---|---|---|
| MRAMKL (male) | 11.23 (Dichotomous Hill) | 13.90 (Dichotomous Hill) | 8.56 (53.87 $mg/m^3$) | 10.79 (67.84 $mg/m^3$) | 9.67 (60.86 $mg/m^3$) |
| MRAMKL (male) [a] | 10.95 (Log-logistic) | 12.67 (Weibull) | 8.33 (52.42 $mg/m^3$) | 9.75 (61.33 $mg/m^3$) | 9.04 (56.88 $mg/m^3$) |
| MRAMKL (female) | 5.57 (multistage degree 2) | 7.59 (multistage degree 2) | 4.12 (25.89 $mg/m^3$) | 5.67 (35.66 $mg/m^3$) | 4.89 (30.78 $mg/m^3$) |
| MRAMKL (female) [a] | 6.32 (multistage degree 2) | 7.59 (multistage degree 2) | 4.68 (29.47 $mg/m^3$) | 5.67 (35.65 $mg/m^3$) | 5.18 (32.56 $mg/m^3$) |

[a] Benchmark dose modeling after highest dose dropped
MRAMKL: time-averaged rate of metabolism of $CCl_4$ (µmol/h/kg liver weight)
HEC (ppm) = 0.49547 x MRAMKL + ([15.078 x MRAMKL] / [67.700 – MRAMKL]); based on a $V_{maxC(H)}$ of 1.49 mg/h/kg $BW^{0.70}$
HEC ($mg/m^3$) = 6.29 x HEC (ppm)

**Table 24. $BMDL_{10}$ values and corresponding HEC values for pheochromocytomas in male and female mice**

| | **$BMDL_{10}$ Fisher et al. 2004** | **$BMDL_{10}$ Thrall et al. 2000** | **HEC (ppm) Fisher et al. 2004** | **HEC (ppm) Thrall et al. 2000** | **HEC (ppm) Average** |
|---|---|---|---|---|---|
| MCA (male) | 0.230 (multistage degree 1) | 0.470 (Dichotomous Hill) | 2.90 (18.26 $mg/m^3$) | 5.90 (37.13 $mg/m^3$) | 4.40 (27.69 $mg/m^3$) |
| MCA (female) | 1.14 (multistage degree 2) | 2.34 (multistage degree 2) | 14.07 (88.50 $mg/m^3$) | 27.62 (174 $mg/m^3$) | 20.85 (131 $mg/m^3$) |

MCA: time-averaged arterial blood concentration of $CCl_4$ (µmol/L blood)
HEC ($mg/m^3$) = 78.6179 x $MCA^{0.9937}$ for MCA values (0.01-1)
HEC ($mg/m^3$) = 78.4969 x $MCA^{0.9342}$ for MCA values (1-5)
HEC (ppm) = HEC ($mg/m^3$) / 6.29

#### 4.2.5.3.1 Default Exposure Duration Adjustments

Because the human PBPK model was used to estimate continuous human equivalent exposures from internal dose metrics in rats and mice, no further duration adjustments are necessary. Thus,

$POD_{HEC} = POD_{ADJ}$ ($HEC_{ADJ}$)

### 4.2.6 Calculation of a Unit Risk Factor

Inhalation URF estimates based on the seven tumor data sets analyzed in Section 4.2.5.3 were calculated as follows (Table 25):

URF = BMR / HEC = 0.1 / Average HEC

**Table 25. Summary of URF estimates and calculated air concentration corresponding to 1 in 100,000 excess cancer risk**

| Tumor | Exposure groups modeled | Dose metric | Average HEC (ppm) | URF estimate $(ppb)^{-1}$ | Air Concentration (ppb) |
|---|---|---|---|---|---|
| Female rat combined liver tumors | 0, 5, 25, 125 ppm | MRAMKL | 10.77 (67.78 $mg/m^3$) | $9.3 \times 10^{-6}$ ($1.5 \times 10^{-6}$ $[\mu g/m^3]^{-1}$) | 1.1 (6.8 $\mu g/m^3$) |
| Male mouse combined liver tumors | 0, 5, 25, 125 ppm | MRAMKL | 9.67 (60.86 $mg/m^3$) | $1.0 \times 10^{-5}$ ($1.6 \times 10^{-6}$ $[\mu g/m^3]^{-1}$) | 0.97 (6.1 $\mu g/m^3$) |
| Female mouse combined liver tumors | 0, 5, 25, 125 ppm | MRAMKL | 4.89 (30.78 $mg/m^3$) | $2.04 \times 10^{-5}$ ($3.2 \times 10^{-6}$ $[\mu g/m^3]^{-1}$) | 0.49 (3.1 $\mu g/m^3$) |
| Male mouse combined liver tumors | 0, 5, 25 ppm | MRAMKL | 9.04 (56.88 $mg/m^3$) | $1.1 \times 10^{-5}$ ($1.8 \times 10^{-6}$ $[\mu g/m^3]^{-1}$) | 0.90 (5.7 $\mu g/m^3$) |
| Female mouse combined liver tumors | 0, 5, 25 ppm | MRAMKL | 5.18 (32.56 $mg/m^3$) | $1.9 \times 10^{-5}$ ($3.1 \times 10^{-6}$ $[\mu g/m^3]^{-1}$) | 0.52 (3.3 $\mu g/m^3$) |
| Male mouse pheochromocytomas | 0, 5, 25, 125 ppm | MCA | 4.40 (27.69 $mg/m^3$) | $2.3 \times 10^{-5}$ ($3.6 \times 10^{-6}$ $[\mu g/m^3]^{-1}$) | 0.44 (2.8 $\mu g/m^3$) |
| Female mouse pheochromocytomas | 0, 5, 25, 125 ppm | MCA | 20.85 (131 $mg/m^3$) | $4.8 \times 10^{-6}$ ($7.6 \times 10^{-7}$ $[\mu g/m^3]^{-1}$) | 2.1 (13 $\mu g/m^3$) |

MCA: time-averaged arterial blood concentration of $CCl_4$ (µmol/L blood)
MRAMKL: time-averaged rate of metabolism of $CCl_4$ (µmol/h/kg liver weight)

As can be seen from Table 25, the estimated inhalation URF for the tumor responses range from $7.6 \times 10^{-7}$ to $3.6 \times 10^{-6}$ $(\mu g/m^3)^{-1}$. The highest estimated inhalation URF of $3.6 \times 10^{-6}$ $(\mu g/m^3)^{-1}$ is based on pheochromocytomas in the male mouse. This data set was judged to be applicable, scientifically sound, and yielded the highest estimate of excess risk and thus, this URF was used to calculate the $^{chronic}ESL_{nonthreshold(c)}$. The URF is similar to USEPA's inhalation unit risk (IUR, same as the URF) of $6 \times 10^{-6}$ $(\mu g/m^3)^{-1}$, which is also based on pheochromocytomas in the male mouse.

## 4.2.7 Calculation of an Air Concentration at $1x10^{-5}$ Excess Cancer Risk

The $10^{-5}$ excess risk air concentrations in last column of Table 25 were calculated based on the URFs using the following equation:

$10^{-5}$ risk air concentration = $1 \times 10^{-5}$ / URF

The calculated air concentrations at $10^{-5}$ excess risk range from 2.8 to 13 $\mu g/m^3$ based on the estimated inhalation URFs for the tumor responses, which range from $7.6 \times 10^{-7}$ to $3.6 \times 10^{-6}$ $(\mu g/m^3)^{-1}$ (Table 25). As indicated above, the highest URF (based on pheochromocytomas in male mice) was conservatively selected for calculation of the air concentration corresponding to an excess risk of $10^{-5}$.

## 4.2.8 Comparison of Cancer Potency Factors

As shown in Table 25, the calculated air concentration at $10^{-5}$ excess risk for combined liver tumors in female rats is 6.8 $\mu g/m^3$, and in male mice and female mice with all doses used in benchmark dose modeling the $10^{-5}$ excess risk air concentrations are 6.1 and 3.1 $\mu g/m^3$, respectively. With the highest dose dropped in benchmark dose modeling, the calculated air concentration at $10^{-5}$ risk for combined liver tumors in male and female mice are 5.7 and 3.3 $\mu g/m^3$, respectively. For adrenal pheochromocytomas, the calculated air concentrations at $10^{-5}$ excess risk are 2.8 and 13 $\mu g/m^3$ in male and female mice, respectively.

The lowest calculated air concentration at the $10^{-5}$ excess risk of 2.8 $\mu g/m^3$ was based on a URF of $3.6 \times 10^{-6}$ $(\mu g/m^3)^{-1}$ for adrenal gland pheochromocytomas in the male mouse. Therefore, the TCEQ conservatively used the level of 2.8 $\mu g/m^3$ (0.44 ppb) as the $^{chronic}ESL_{nonthreshold(c)}$ with an inhalation URF = $3.6 \times 10^{-6}$ $(\mu g/m^3)^{-1}$.

As described in Section 4.2.3, consideration was given to a threshold for liver tumors. If the liver tumors form via a threshold MOA, then because the MOA for fatty liver changes and carcinogenesis both involve generation of reactive $CCl_4$ metabolites and covalent binding to macromolecules and that fatty changes in liver, consistent with liver toxicity, observed in the 13-wk studies in rats and mice precedes development of liver tumors, the $^{chronic}ReV_{threshold(nc)}$ of 160 $\mu g/m^3$ based on fatty liver changes in rats should also protect against liver cancer. If one

applies a hazard quotient of 0.3, the chronic $ESL_{threshold(c)}$ would be 48 µg/m$^3$ (7.5 ppb). This toxicity factor is higher than the ambient air concentration of 2.8 µg/m$^3$ associated with TCEQ's inhalation unit risk factor based on adrenal pheochromocytomas in male mice, with an excess cancer risk of 1 in 100,000. Because the MOA for $CCl_4$-induced tumors is not entirely clear and may include a genotoxic component (which is conservatively considered to have a linear dose-response), the $^{chronic}ESL_{nonthreshold(c)}$ of 2.8 µg/m$^3$ (0.44 ppb) will be used for evaluation of long-term ambient air data and for air permit reviews.

## 4.2.9 Evaluating Susceptibility from Early-Life Exposures

There is no direct evidence for increased or decreased susceptibility to $CCl_4$ in children. Vieira et al. (1996, as cited in USEPA 2010) indicated that hepatic concentrations of CYP2E1 do not achieve adult levels until sometime between 1 and 10 yrs of age, although large increases in hepatic CYP2E1 protein occur postnatally between 1 and 3 months in humans. To the extent that hepatic CYP2E1 levels are lower, USEPA (2010, 2020) suggests that infants and children would be less susceptible than adults to free radical-induced liver injury from $CCl_4$. As the MOA for carcinogenicity has not been demonstrated to be mutagenicity (Section 4.2.3), age-dependent default adjustment factors (i.e., EPA default ADAFs) will not be applied at this time (TCEQ 2015a).

## 4.2.10 Chronic Carcinogenic IOAEL

Estimated HEC values, based on a $V_{maxC(H)}$ = 1.49 mg/h/kg BW$^{0.70}$, corresponding to $BMD_{10}$ values ($HECs_{BMD10}$) (Table 21) for adrenal pheochromocytomas in the mouse as reported in the 2-yr rat inhalation bioassay (Nagano et al. 2007a) are selected as candidate POD values for derivation of a chronic carcinogenic IOAEL. As described in Section 4.2.5.3, human PBPK models were used to estimate HEC ($HEC_{BMD10}$) values. Based on Equation 2 (for MCA values 0.01-1) and Equation 3 (for MCA values 1-5), the calculated $HEC_{BMD10}$ values for adrenal pheochromocytomas using MCA as the dose metric in male and female mice are 52.40 and 162 mg/m$^3$, respectively (Table 26).

**Table 26. Summary of $BMD_{10}$ values and corresponding $HEC_{BMD10}$ values for adrenal pheochromocytomas in male and female mice**

| | **$BMD_{10}$ $V_{MAX(R)}$ by Fisher et al.** | **$BMD_{10}$ $V_{MAX}$ by Thrall et al.** | **$HEC_{BMD10}$ (mg/m$^3$) $V_{MAX}$ by Fisher et al.** | **$HEC_{BMD10}$ (mg/m$^3$) $V_{MAX}$ by Thrall et al.** | **$HEC_{BMD10}$ (mg/m$^3$) Average** |
|---|---|---|---|---|---|
| MCA male | 0.292 | 1.04 | 23.13 | 81.67 | 52.40 |
| MCA female | 1.43 | 2.95 | 109 | 215 | 162 |

The average $HEC_{BMD10}$ based on male rat data (52.40 mg/m$^3$) is the lower of the two values and thus was selected as the POD to derive the chronic carcinogenic IOAEL. The $HEC_{BMD10}$ of

52 mg/$m^3$ (8.3 ppm) (rounded to 2 significant figures) is then used as the chronic carcinogenic IOAEL. The chronic carcinogenic IOAEL is provided for informational purposes only (TCEQ 2015a).

The margin of exposure between the estimated chronic carcinogenic IOAEL (8,300 ppb or 52,000 µg/$m^3$) and the $^{chronic}ESL_{nonthreshold(c)}$ (0.44 ppb or 2.8 µg/$m^3$) for $CCl_4$ is a factor of approximately 18,570.

## *4.3 Welfare-Based Chronic Evaluation*

No data were found regarding adverse effects observed in plants due to chronic air exposure to $CCl_4$. The only information found was in regard to adverse plant effects not being observed (see Section 3.2.2). Therefore, no chronic vegetation-based ESL ($^{chronic}ESL_{veg}$) was derived.

## *4.4 Summary of the Chronic Values*

The chronic evaluation resulted in the derivation of the following values:

- Chronic ReV = 160 µg/$m^3$ (25 ppb)
- $^{chronic}ESL_{threshold(nc)}$ = 48 µg/$m^3$ (7.5 ppb)
- URF = $3.6 \times 10^{-6}$ per µg/$m^3$
- $^{chronic}ESL_{nonthreshold(c)}$ = 2.8 µg/$m^3$ (0.44 ppb)

The $^{chronic}ESL_{nonthreshold(c)}$ of 2.8 µg/$m^3$ (0.44 ppb) is the critical long-term health-based AMCV for the evaluation of long-term ambient air data as this value is lower than the chronic ReV (Table 2). The long-term ESL for air permit reviews is the $^{chronic}ESL_{nonthreshold(c)}$ of 2.8 µg/$m^3$ (0.44 ppb).

## Chapter 5 References

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# Appendix A Benchmark Dose Modeling for Deriving the Chronic Reference Value

**Table 27. Male F344 rat using MCA dose metric**

| **Model Restricted** | **AIC VmaxC = 0.40** [a] | **p-Value VmaxC = 0.40** [a] | **$BMD_{10}$ VmaxC = 0.40** [a] | **$BMDL_{10}$ VmaxC = 0.40** [a] | **AIC VmaxC = 0.65** [b] | **p-Value VmaxC = 0.65** [b] | **$BMD_{10}$ VmaxC = 0.65** [b] | **$BMDL_{10}$ VmaxC = 0.65** [b] |
|---|---|---|---|---|---|---|---|---|
| Dichotomous Hill (Restricted) | 138.87 | NA | 0.157 | 0.084 | 138.87 | NA | 0.143 | 0.076 |
| Gamma (Restricted) | 144.34 | 0.00068 | 0.079 | 0.055 | 144.77 | 0.00045 | 0.069 | 0.051 |
| **Log-Logistic** (Restricted) | **137.40** | **0.436** | **0.137** | **0.079** | **137.46** | **0.409** | **0.123** | **0.071** |
| Log-Probit (Restricted) | 138.41 | 0.176 | 0.125 | 0.088 | 138.53 | 0.158 | 0.112 | 0.080 |
| Multistage Degree 3 (Restricted) | 142.39 | 0.0074 | 0.071 | 0.055 | 142.78 | 0.0031 | 0.067 | 0.051 |
| Multistage Degree 2 (Restricted) | 142.39 | 0.0074 | 0.071 | 0.055 | 142.78 | 0.0031 | 0.067 | 0.051 |
| Multistage Degree 1 (Restricted) | 142.39 | 0.0074 | 0.071 | 0.055 | 142.78 | 0.0031 | 0.067 | 0.051 |
| Weibull (Restricted) | 142.39 | 0.0074 | 0.071 | 0.055 | 142.78 | 0.0031 | 0.067 | 0.051 |
| Logistic (Unrestricted) | 155.10 | <0.0001 | 0.171 | 0.137 | 156.51 | <0.0001 | 0.158 | 0.127 |
| Log-Probit (Unrestricted) | 138.41 | 0.176 | 0.125 | 0.076 | 138.53 | 0.158 | 0.112 | 0.067 |
| Probit (Unrestricted) | 161.96 | 0.975 | 0.153 | 0.123 | 171.23 | <0.0001 | 0.215 | 0.199 |
| Quantal Linear (Unrestricted) | 142.39 | 0.0074 | 0.071 | 0.055 | 142.78 | 0.0031 | 0.067 | 0.051 |

[a] Exposure using the internal dose metric MCA: 0, 0.128, 0.708, 3.892 µmol/L blood
[b] Exposure using the internal dose metric MCA: 0, 0.116, 0.653, 3.775 µmol/L blood

**Table 28. Male F344 rat using MRAMKL dose metric**

| Model | AIC VmaxC = 0.40 [a] | p-Value VmaxC = 0.40 [a] | $BMD_{10}$ VmaxC = 0.40 [a] | $BMDL_{10}$ VmaxC = 0.40 [a] | AIC VmaxC = 0.65 [b] | p-Value VmaxC = 0.65 [b] | $BMD_{10}$ VmaxC = 0.65 [b] | $BMDL_{10}$ VmaxC = 0.65 [b] |
|---|---|---|---|---|---|---|---|---|
| Dichotomous Hill (Restricted) | 136.93 | 0.801 | 4.567 | 3.085 | 137.00 | 0.725 | 6.204 | 4.003 |
| Gamma (Restricted) | 137.47 | 0.418 | 3.987 | 2.634 | 137.34 | 0.476 | 5.311 | 3.357 |
| Log-Logistic (Restricted) | 136.93 | 0.801 | 4.567 | 3.085 | 137.00 | 0.725 | 6.204 | 4.003 |
| Log-Probit (Restricted) | 136.87 | 0.954 | 4.272 | 3.065 | 136.87 | 0.947 | 5.736 | 3.978 |
| Multistage Degree 3 (Restricted) | 137.07 | 0.270 | 3.552 | 2.062 | 139.00 | 0.094 | 4.997 | 2.502 |
| Multistage Degree 2 (Restricted) | 137.07 | 0.270 | 3.552 | 2.062 | 139.00 | 0.094 | 4.997 | 2.502 |
| Multistage Degree 1 (Restricted) | 151.67 | 0.0008 | 1.019 | 0.831 | 148.90 | 0.0025 | 1.455 | 1.184 |
| Weibull (Restricted) | 139.00 | 0.132 | 3.348 | 2.183 | 138.60 | 0.175 | 4.478 | 2.819 |
| **Logistic (Unrestricted)** | **136.75** | **0.344** | **3.257** | **2.586** | **136.51** | **0.367** | **4.601** | **3.653** |
| Log-Probit (Unrestricted) | 136.87 | 0.953 | 4.272 | 3.065 | 136.87 | 0.947 | 5.736 | 3.978 |
| Probit (Unrestricted) | 138.89 | 0.083 | 2.978 | 2.416 | 138.71 | 0.073 | 4.238 | 3.443 |
| Quantal Linear (Unrestricted) | 151.67 | 0.0008 | 1.019 | 0.831 | 149.00 | 0.0025 | 1.455 | 1.184 |

[a] Exposure using the internal dose metric MRAMKL: 0, 3.813, 12.092, 24.32 µmol/h/kg liver
[b] Exposure using the internal dose metric MRAMKL: 0, 4.991, 17.626, 36.266 µmol/h/kg liver

**Figure 1. Male rat using MCA dose metric: Vmax = 0.4 mg/hour/kg $BW^{0.07}$, log-logistic model**

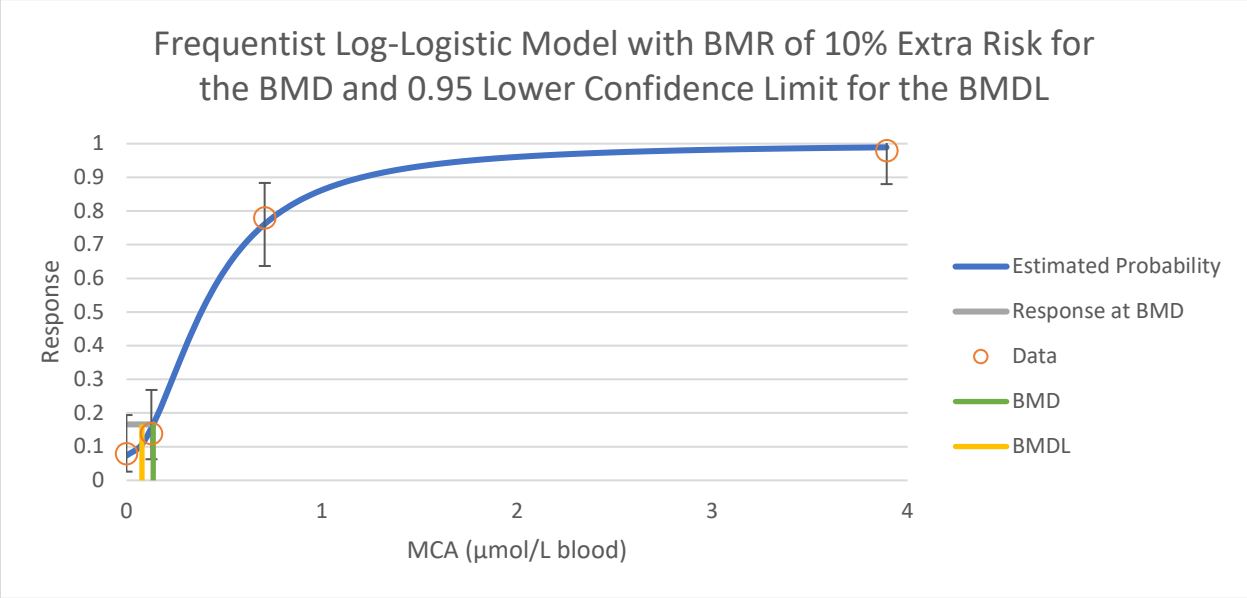



**Figure 2. Male rat using MCA dose metric: Vmax = 0.65 mg/hour/kg $BW^{0.07}$, log-logistic model**

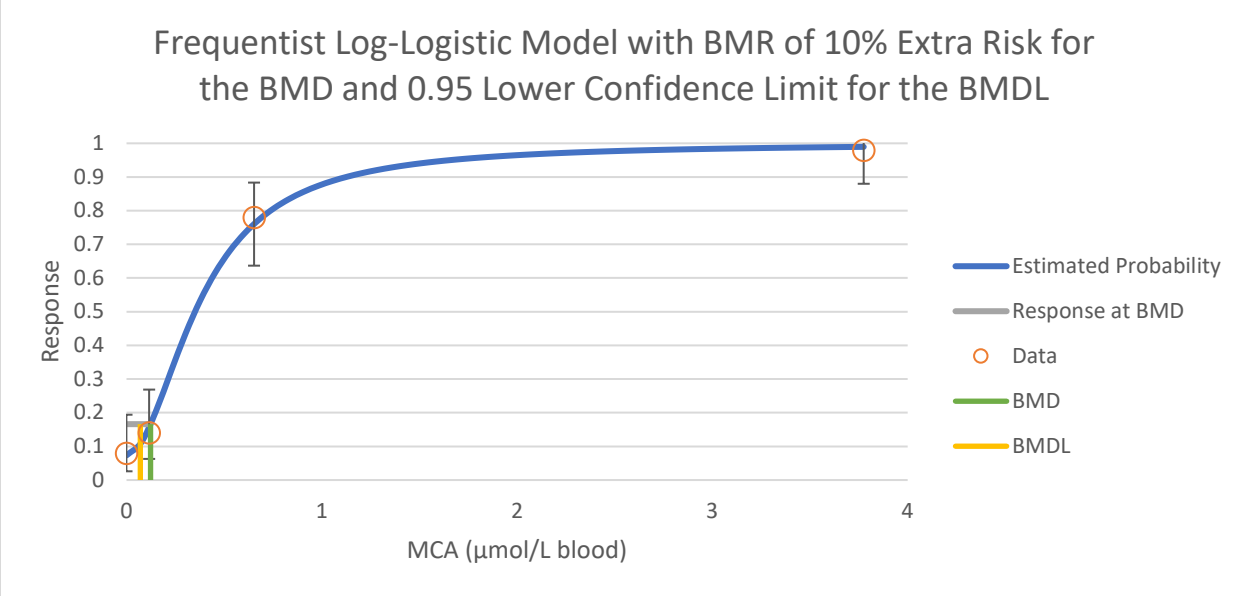


**Figure 3. Male rat using MRAMKL dose metric: Vmax = 0.4 mg/hour/kg $BW^{0.07}$, logistic model**

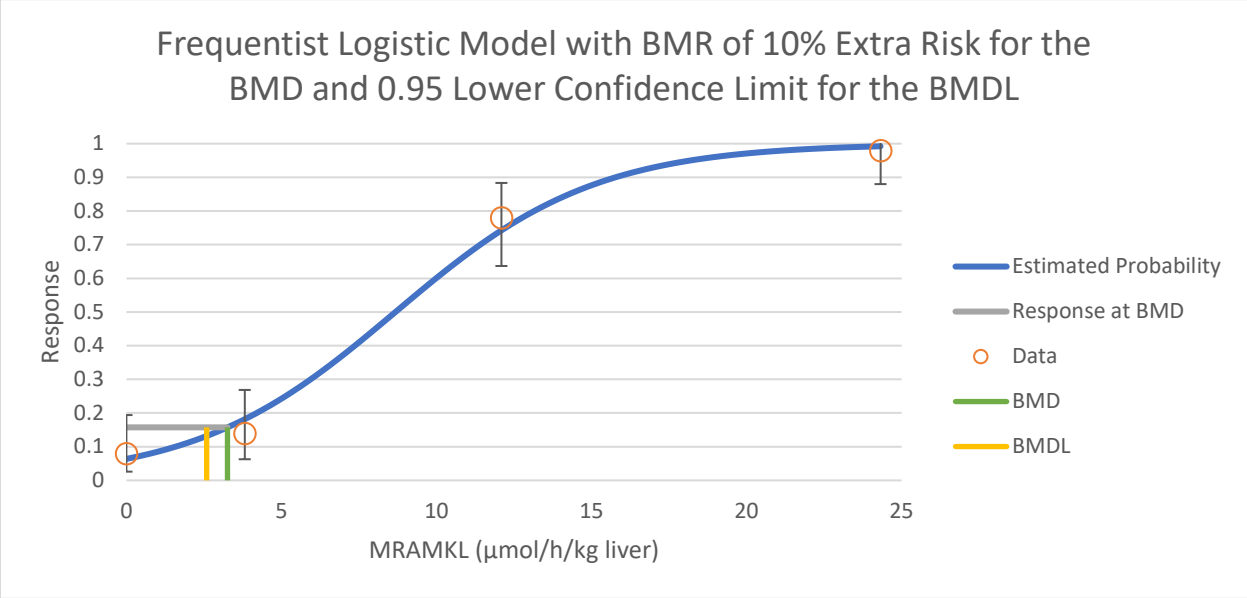



**Figure 4. Male rat using MRAMKL dose metric: Vmax = 0.65 mg/hour/kg $BW^{0.07}$, logistic model**

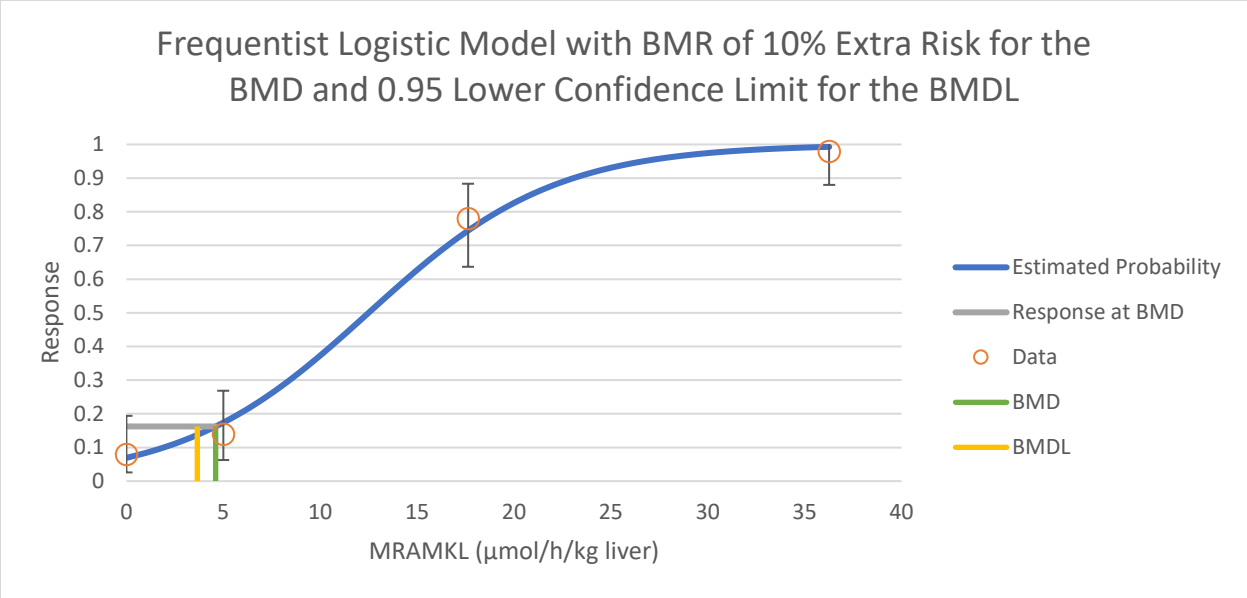


**Table 29. Female F344 rat using MCA dose metric**

| Model | AIC VmaxC = 0.40 [a] | p-Value VmaxC = 0.40 [a] | $BMD_{10}$ VmaxC = 0.40 [a] | $BMDL_{10}$ VmaxC = 0.40 [a] | AIC VmaxC = 0.65 [b] | p-Value VmaxC = 0.65 [b] | $BMD_{10}$ VmaxC = 0.65 [b] | $BMDL_{10}$ VmaxC = 0.65 [b] |
|---|---|---|---|---|---|---|---|---|
| Dichotomous Hill (Restricted) | 124.93 | NA | 0.162 | 0.116 | 124.94 | NA | 0.147 | 0.105 |
| Gamma (Restricted) | 171.53 | <0.0001 | 0.0793 | 0.0615 | 173.38 | <0.0001 | 0.076 | 0.058 |
| Log-Logistic (Restricted) | 146.71 | <0.0001 | 0.0925 | 0.0552 | 147.13 | <0.0001 | 0.083 | 0.049 |
| Log-Probit (Restricted) | 151.66 | <0.0001 | 0.0963 | 0.0739 | 152.48 | <0.0001 | 0.089 | 0.068 |
| Multistage Degree 3 (Restricted) | 171.53 | <0.0001 | 0.0793 | 0.0615 | 173.38 | <0.0001 | 0.076 | 0.058 |
| Multistage Degree 2 (Restricted) | 171.53 | <0.0001 | 0.0793 | 0.0615 | 173.38 | <0.0001 | 0.076 | 0.058 |
| Multistage Degree 1 (Restricted) | 171.53 | <0.0001 | 0.0793 | 0.0615 | 173.38 | <0.0001 | 0.076 | 0.058 |
| Weibull (Restricted) | 171.53 | <0.0001 | 0.0793 | 0.0615 | 173.38 | <0.0001 | 0.076 | 0.058 |
| Logistic (Unrestricted) | 200.96 | <0.0001 | 0.215 | 0.158 | 202.99 | <0.0001 | 0.212 | 0.154 |
| Log-Probit (Unrestricted) | 152.85 | <0.0001 | 0.0781 | 0.0435 | 153.33 | <0.001 | 0.069 | 0.038 |
| Probit (Unrestricted) | 206.43 | <0.0001 | 0.295 | 0.238 | 207.97 | <0.0001 | 0.287 | 0.232 |
| Quantal Linear (Unrestricted) | 171.53 | <0.0001 | 0.0793 | 0.0615 | 173.38 | <0.0001 | 0.076 | 0.058 |

[a] Exposure using internal dose metric MCA: 0, 0.128, 0.708, 3.892 µmol/L blood
[b] Exposure using internal dose metric MCA: 0, 0.116, 0.653, 3.775 µmol/L blood

**Table 30. Female F344 rat using MRAMKL dose metric**

| Model | AIC VmaxC = 0.40 [a] | p-Value VmaxC = 0.40 [a] | $BMD_{10}$ VmaxC = 0.40 [a] | $BMDL_{10}$ VmaxC = 0.40 [a] | AIC VmaxC = 0.65 [b] | p-Value VmaxC = 0.65 [b] | $BMD_{10}$ VmaxC = 0.65 [b] | $BMDL_{10}$ VmaxC = 0.65 [b] |
|---|---|---|---|---|---|---|---|---|
| Dichotomous Hill (Restricted) | 124.89 | NA | 4.358 | 3.582 | 124.89 | NA | 5.759 | 4.661 |
| Gamma (Restricted) | 148.29 | <0.0001 | 2.853 | 1.743 | 147.45 | <0.0001 | 3.700 | 2.156 |
| Log-Logistic (Restricted) | 137.53 | <0.0001 | 3.551 | 2.635 | 136.70 | <0.0001 | 4.683 | 3.390 |
| Log-Probit (Restricted) | 141.60 | <0.0001 | 3.386 | 2.489 | 140.54 | <0.0001 | 4.467 | 3.210 |
| Multistage Degree 3 (Restricted) | 155.67 | <0.0001 | 1.539 | 0.829 | 154.23 | <0.0001 | 1.741 | 1.086 |
| Multistage Degree 2 (Restricted) | 155.67 | <0.0001 | 1.539 | 0.829 | 154.23 | <0.0001 | 1.741 | 1.086 |
| Multistage Degree 1 (Restricted) | 155.89 | <0.0001 | 0.906 | 0.737 | 153.11 | <0.0001 | 1.291 | 1.047 |
| Weibull (Restricted) | 152.02 | <0.0001 | 2.025 | 1.193 | 150.70 | <0.0001 | 2.628 | 1.500 |
| Logistic (Unrestricted) | 151.17 | <0.0001 | 2.470 | 1.982 | 151.00 | <0.0001 | 3.494 | 2.802 |
| Log-Probit (Unrestricted) | 141.60 | <0.0001 | 3.386 | 2.489 | 140.54 | <0.0001 | 4.467 | 3.210 |
| Probit (Unrestricted) | 158.91 | <0.0001 | 2.436 | 2.019 | 159.11 | <0.0001 | 3.509 | 2.911 |
| Quantal Linear (Unrestricted) | 155.89 | <0.0001 | 0.906 | 0.737 | 153.11 | <0.0001 | 1.291 | 1.047 |

[a] Exposure using internal dose metric MRAMKL: 0, 3.813, 12.092, 24.32 µmol/h/kg liver
[b] Exposure using internal dose metric MRAMKL: 0, 4.991, 17.626, 36.266 µmol/h/kg liver

**Table 31. Female F344 rat using MCA dose metric (highest dose dropped)**

| Model | AIC VmaxC = 0.40 [a] | p-Value VmaxC = 0.40 [a] | $BMD_{10}$ VmaxC = 0.40 [a] | $BMDL_{10}$ VmaxC = 0.40 [a] | AIC VmaxC = 0.40 [a] | p-Value VmaxC = 0.40 [a] | $BMD_{10}$ VmaxC = 0.40 [a] | $BMDL_{10}$ VmaxC = 0.40 [a] |
|---|---|---|---|---|---|---|---|---|
| Dichotomous Hill (Restricted) | 94.99 | NA | 0.179 | 0.112 | 94.99 | NA | 0.164 | 0.101 |
| Gamma (Restricted) | 92.99 | NA | 0.188 | 0.107 | 92.99 | NA | 0.171 | 0.097 |
| Log-Logistic (Restricted) | 92.99 | NA | 0.183 | 0.112 | 92.99 | NA | 0.166 | 0.101 |
| Log-Probit (Restricted) | 92.99 | NA | 0.174 | 0.113 | 92.99 | NA | 0.158 | 0.102 |
| **Multistage Degree 2 (Restricted)** | **92.41** | **0.244** | **0.124** | **0.085** | **92.30** | **0.262** | **0.114** | **0.078** |
| Multistage Degree 1 (Restricted) | 111.42 | <0.0001 | 0.036 | 0.028 | 111.02 | <0.0001 | 0.033 | 0.025 |
| Weibull (Restricted) | 92.99 | NA | 0.213 | 0.103 | 92.99 | NA | 0.194 | 0.093 |
| Logistic (Unrestricted) | 93.42 | 0.112 | 0.107 | 0.080 | 93.32 | 0.120 | 0.098 | 0.073 |
| Log-Probit (Unrestricted) | 92.99 | NA | 0.174 | 0.113 | 92.99 | NA | 0.158 | 0.102 |
| Probit (Unrestricted) | 93.68 | 0.097 | 0.100 | 0.078 | 93.57 | 0.104 | 0.092 | 0.071 |
| Quantal Linear (Unrestricted) | 111.42 | <0.0001 | 0.036 | 0.028 | 111.02 | <0.0001 | 0.033 | 0.025 |

[a] Exposure using internal dose metric MCA: 0, 0.128, 0.708 µmol/L blood
[b] Exposure using internal dose metric MCA: 0, 0.116, 0.653 µmol/L blood

**Table 32. Female F344 rat using MRAMKL dose metric (highest dose dropped)**

| Model | AIC VmaxC = 0.40 [a] | p-Value VmaxC = 0.40 [a] | $BMD_{10}$ VmaxC = 0.40 [a] | $BMDL_{10}$ VmaxC = 0.40 [a] | AIC VmaxC = 0.65 [b] | p-Value VmaxC = 0.65 [b] | $BMD_{10}$ VmaxC = 0.65 [b] | $BMDL_{10}$ VmaxC = 0.65 [b] |
|---|---|---|---|---|---|---|---|---|
| Dichotomous Hill (Restricted) | 94.99 | NA | 4.780 | 3.485 | 94.99 | NA | 6.488 | 4.518 |
| Gamma (Restricted) | 92.99 | NA | 4.855 | 3.426 | 92.99 | NA | 6.523 | 4.430 |
| Log-Logistic (Restricted) | 92.99 | NA | 4.847 | 3.481 | 92.99 | NA | 6.488 | 4.518 |
| Log-Probit (Restricted) | 92.99 | NA | 4.692 | 3.497 | 92.99 | NA | 6.261 | 4.540 |
| Multistage Degree 2 (Restricted) | 100.7 | 0.0039 | 2.433 | 1.994 | 98.11 | 0.0125 | 3.423 | 2.756 |
| Multistage Degree 1 (Restricted) | 127.03 | <0.0001 | 0.817 | 0.634 | 123.55 | <0.0001 | 1.125 | 0.871 |
| Weibull (Restricted) | 92.99 | NA | 5.380 | 3.291 | 92.99 | NA | 7.272 | 4.249 |
| Logistic (Unrestricted) | 99.73 | 0.0020 | 2.458 | 1.904 | 97.87 | 0.0064 | 3.345 | 2.583 |
| Log-Probit (Unrestricted) | 92.99 | NA | 4.692 | 3.497 | 92.99 | NA | 6.261 | 4.540 |
| Probit (Unrestricted) | 100.99 | 0.0013 | 2.161 | 1.701 | 98.81 | 0.0044 | 2.984 | 2.347 |
| Quantal Linear (Unrestricted) | 127.03 | <0.0001 | 0.817 | 0.634 | 123.55 | <0.0001 | 1.125 | 0.871 |

[a] Exposure using internal dose metric MRAMKL: 0, 3.813, 12.092 µmol/h/kg liver
[b] Exposure using internal dose metric MRAMKL: 0, 4.991, 17.626 µmol/h/kg liver

**Figure 5. Female rat using MCA dose metric: Vmax = 0.4 mg/hour/kg $BW^{0.07}$ (highest dose dropped), multistage degree 2 model**

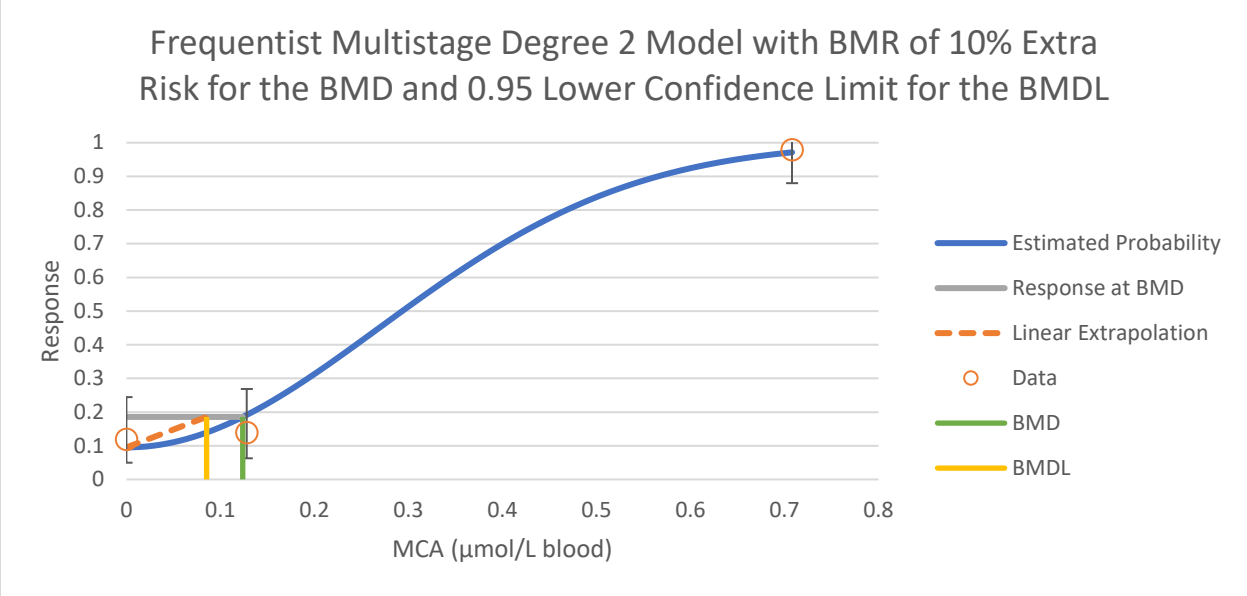
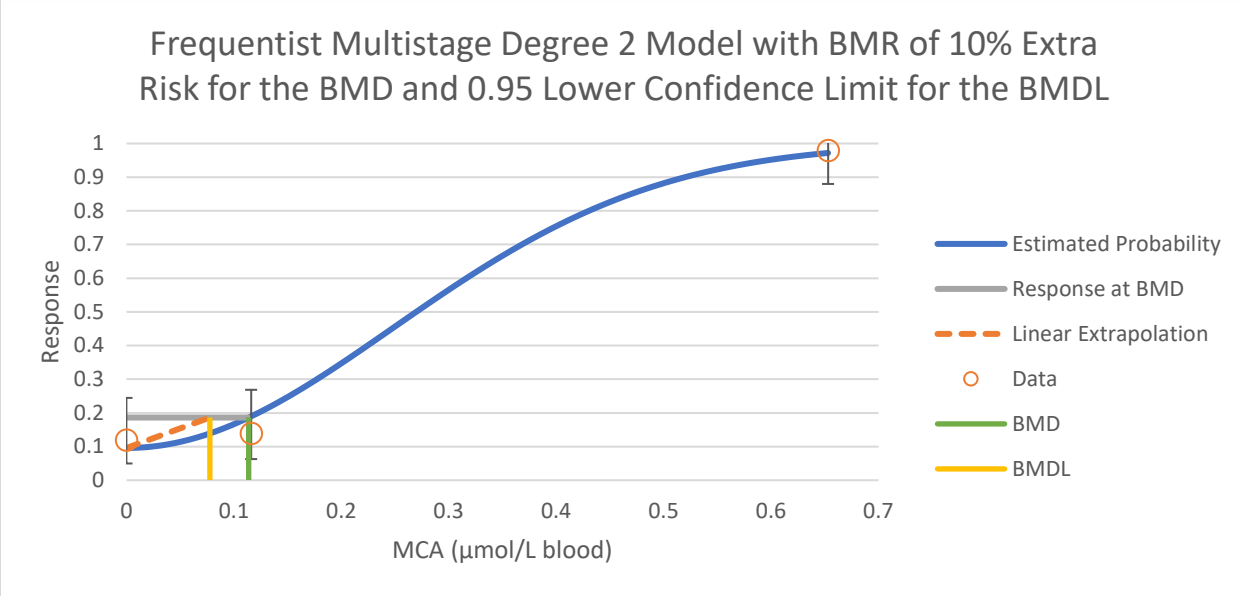



**Figure 6. Female rat using MCA dose metric: Vmax = 0.65 mg/hour/kg $BW^{0.07}$ (highest dose dropped), multistage degree 2 model**



# Appendix B Benchmark Dose Modeling Outputs For Low-Dose Linear Extrapolation Approach

**Table 33. Female rat combined liver tumors using MRAMKL dose metric**

| Model | AIC VmaxC = 0.40 [a] | p-Value VmaxC = 0.40 [a] | $BMD_{10}$ VmaxC = 0.40 [a] | $BMDL_{10}$ VmaxC = 0.40 [a] | AIC VmaxC = 0.65 [b] | p-Value VmaxC = 0.65 [b] | $BMD_{10}$ VmaxC = 0.65 [b] | $BMDL_{10}$ VmaxC = 0.65 [b] |
|---|---|---|---|---|---|---|---|---|
| Dichotomous Hill (Restricted) | 65.39 | 0.979 | 12.514 | 11.429 | 65.39 | 0.999 | 18.302 | 16.649 |
| Gamma (Restricted) | 63.39 | 1.000 | 13.066 | 11.441 | 63.39 | 1.000 | 19.100 | 16.643 |
| Log-Logistic (Restricted) | 63.39 | 0.999 | 13.123 | 11.449 | 63.39 | 1.000 | 19.178 | 16.653 |
| Log-Probit (Restricted) | 63.39 | 1.000 | 12.968 | 11.514 | 63.39 | 1.000 | 18.946 | 16.754 |
| Multistage Degree 3 (Restricted) | 70.44 | 0.070 | 9.703 | 8.817 | 69.32 | 0.100 | 14.372 | 13.014 |
| Multistage Degree 2 (Restricted) | 86.75 | 0.0003 | 6.968 | 6.140 | 84.90 | 0.0005 | 10.258 | 9.027 |
| Multistage Degree 1 (Restricted) | 120.68 | <0.0001 | 3.103 | 2.446 | 118.3 | <0.0001 | 4.498 | 3.544 |
| **Weibull (Restricted)** | **61.40** | **1.000** | **13.45** | **11.28** | **61.4** | **1.000** | **19.70** | **16.39** |
| Logistic (Unrestricted) | 63.68 | 0.925 | 13.718 | 11.621 | 63.69 | 0.922 | 20.119 | 16.948 |
| Log-Probit (Unrestricted) | 63.39 | 1.000 | 12.968 | 11.514 | 63.39 | 1.000 | 18.946 | 16.754 |
| Probit (Unrestricted) | 63.42 | 0.991 | 13.348 | 11.388 | 63.42 | 0.991 | 19.541 | 16.563 |
| Quantal Linear (Unrestricted) | 120.68 | <0.0001 | 3.103 | 2.446 | 118.3 | <0.001 | 4.498 | 3.545 |

[a] Exposure internal dose MRAMKL: 0, 3.813, 12.092, 24.32 µmol/h/kg liver
[b] Exposure internal dose MRAMKL: 0, 4.991, 17.626, 36.266 µmol/h/kg liver

**Figure 7. Female rat combined liver tumors using MRAMKL dose metric: Vmax = 0.4 mg/hour/kg $BW^{0.07}$, Weibull model**

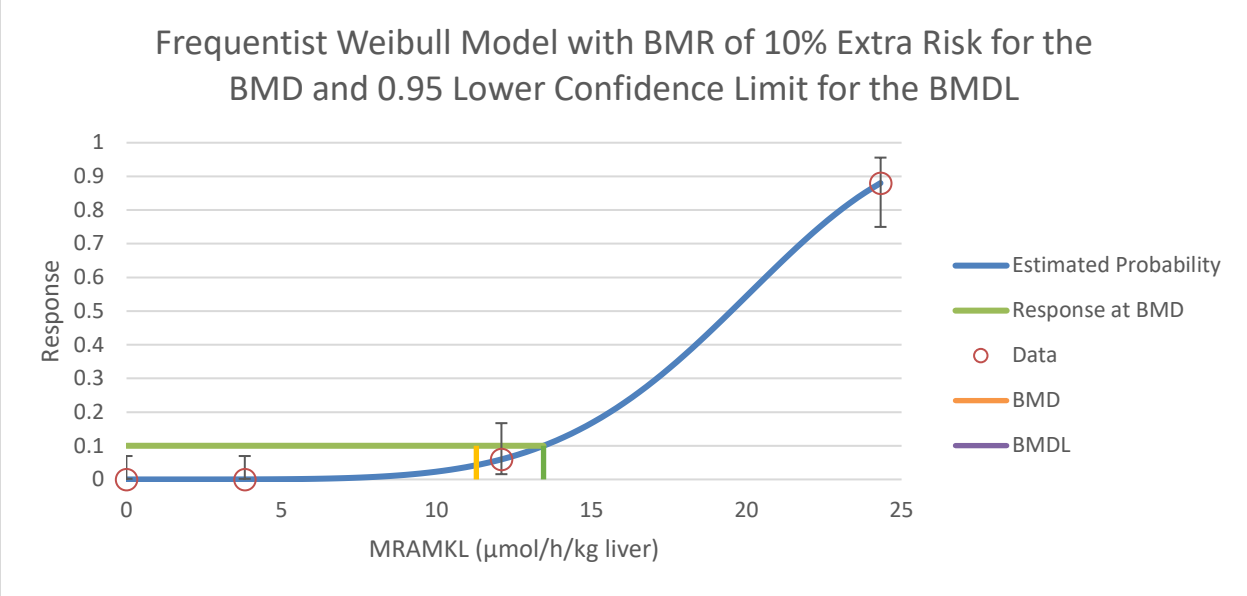



**Figure 8. Female rat combined liver tumors using MRAMKL dose metric: Vmax = 0.65 mg/hour/kg $BW^{0.07}$, Weibull model**

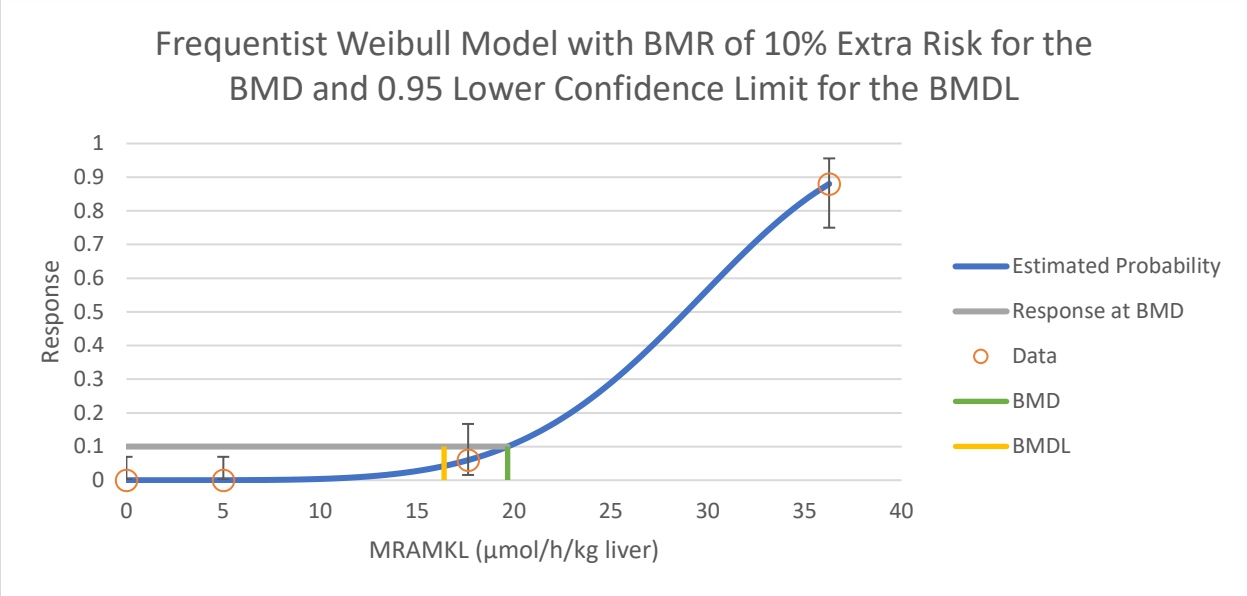


**Table 34. Male mice combined liver tumors using MRAMKL dose metric**

| Model | AIC Vmax C by Fisher et al. 2004 [a] | p-Value VmaxC by Fisher et al. 2004 [a] | $BMD_{10}$ Vmax C by Fisher et al. 2004 [a] | $BMDL_{10}$ VmaxC by Fisher et al. 2004 [a] | AIC Vmax C by Thrall et al. 2000 [b] | p-Value VmaxC by Thrall et al. 2000 [b] | $BMD_{10}$ VmaxC by Thrall et al. 2000 [b] | $BMDL_{10}$ VmaxC by Thrall et al. 2000 [b] |
|---|---|---|---|---|---|---|---|---|
| **Dichotomous Hill (Restricted)** | **162.79** | **0.420** | **23.36** | **11.23** | **162.79** | **0.420** | **25.21** | **13.90** |
| Gamma (Restricted) | 169.36 | 0.0007 | 12.74 | 7.21 | 167.41 | 0.007 | 16.71 | 10.55 |
| Log-Logistic (Restricted) | 165.81 | 0.026 | 14.63 | 9.35 | 165.06 | 0.058 | 18.21 | 12.20 |
| Log-Probit (Restricted) | 167.31 | 0.008 | 13.69 | 8.96 | 166.14 | 0.026 | 17.28 | 11.92 |
| Multistage Degree 3 (Restricted) | 170.13 | 0.001 | 9.78 | 4.69 | 170.67 | 0.001 | 12.55 | 6.61 |
| Multistage Degree 2 (Restricted) | 170.13 | 0.001 | 9.78 | 4.69 | 169.07 | 0.014 | 9.80 | 6.35 |
| Multistage Degree 1 (Restricted) | 179.66 | 0.0001 | 2.48 | 1.90 | 183.97 | <0.0001 | 2.62 | 2.03 |
| Weibull (Restricted) | 172.10 | 0.0004 | 9.21 | 4.95 | 170.08 | 0.0018 | 13.03 | 7.73 |
| Logistic (Unrestricted) | 173.79 | 0.002 | 3.84 | 3.08 | 176.42 | 0.0004 | 4.06 | 3.30 |
| Log-Probit (Unrestricted) | 167.31 | 0.008 | 13.69 | 8.96 | 166.14 | 0.026 | 17.28 | 11.92 |
| Probit (Unrestricted) | 173.83 | 0.002 | 4.124 | 3.387 | 175.22 | 0.0008 | 4.22 | 3.51 |
| Quantal Linear (Unrestricted) | 179.66 | 0.0001 | 2.481 | 1.899 | 183.97 | <0.0001 | 2.62 | 2.03 |

[a] Exposure internal dose for MRAMKL: 0, 12.666, 41.675, 71.589 µmol/h/kg liver
[b] Exposure internal dose for MRAMKL: 0, 15.456, 43.599, 63.596 µmol/h/kg liver

**Table 35. Male mice combined liver tumors using MRAMKL dose metric, highest dose dropped**

| Model | AIC Vmax C by Fisher et al. 2004 [a] | p-Value VmaxC by Fisher et al. 2004 [a] | $BMD_{10}$ Vmax C by Fisher et al. 2004 [a] | $BMDL_{10}$ VmaxC by Fisher et al. 2004 [a] | AIC Vmax C by Thrall et al. 2000 [b] | p-Value VmaxC by Thrall et al. 2000 [b] | $BMD_{10}$ VmaxC by Thrall et al. 2000 [b] | $BMDL_{10}$ VmaxC by Thrall et al. 2000 [b] |
|---|---|---|---|---|---|---|---|---|
| Dichotomous Hill (Restricted) | 152.99 | NA | 30.07 | 10.95 | 154.99 | NA | 31.93 | 13.63 |
| Gamma (Restricted) | 151.00 | 0.415 | 20.29 | 10.64 | 151.06 | 0.396 | 21.27 | 13.28 |
| Log-Logistic (Restricted) | **150.99** | **0.420** | **30.71** | **10.95** | 150.99 | 0.420 | 32.13 | 13.61 |
| Log-Probit (Restricted) | 152.99 | NA | 33.38 | 11.04 | 152.99 | NA | 33.22 | 13.71 |
| Multistage Degree 2 (Restricted) | 155.81 | 0.021 | 8.26 | 5.60 | 158.01 | 0.006 | 9.03 | 6.43 |
| Multistage Degree 1 (Restricted) | 169.58 | <0.0001 | 2.65 | 1.91 | 172.54 | <0.0001 | 3.04 | 2.19 |
| **Weibull (Restricted)** | 152.99 | NA | 34.39 | 10.08 | **150.99** | **0.420** | **35.99** | **12.67** |
| Logistic (Unrestricted) | 163.63 | 0.0004 | 3.67 | 2.88 | 166.56 | <0.0001 | 4.13 | 3.27 |
| Log-Probit (Unrestricted) | 152.99 | NA | 32.43 | 11.04 | 152.99 | NA | 33.80 | 13.71 |
| Probit (Unrestricted) | 162.38 | 0.0006 | 3.69 | 2.96 | 165.29 | 0.0001 | 4.10 | 3.31 |
| Quantal Linear (Unrestricted) | 169.58 | <0.0001 | 2.65 | 1.91 | 172.54 | <0.0001 | 3.04 | 2.19 |

[a] Exposure internal dose for MRAMKL: 0, 12.666, 41.675 µmol/h/kg liver
[b] Exposure internal dose for MRAMKL: 0, 15.456, 43.599 µmol/h/kg liver

**Figure 9. Male mice combined liver tumors using MRAMKL dose metric: VmaxC by Fisher et al., dichotomous Hill model**

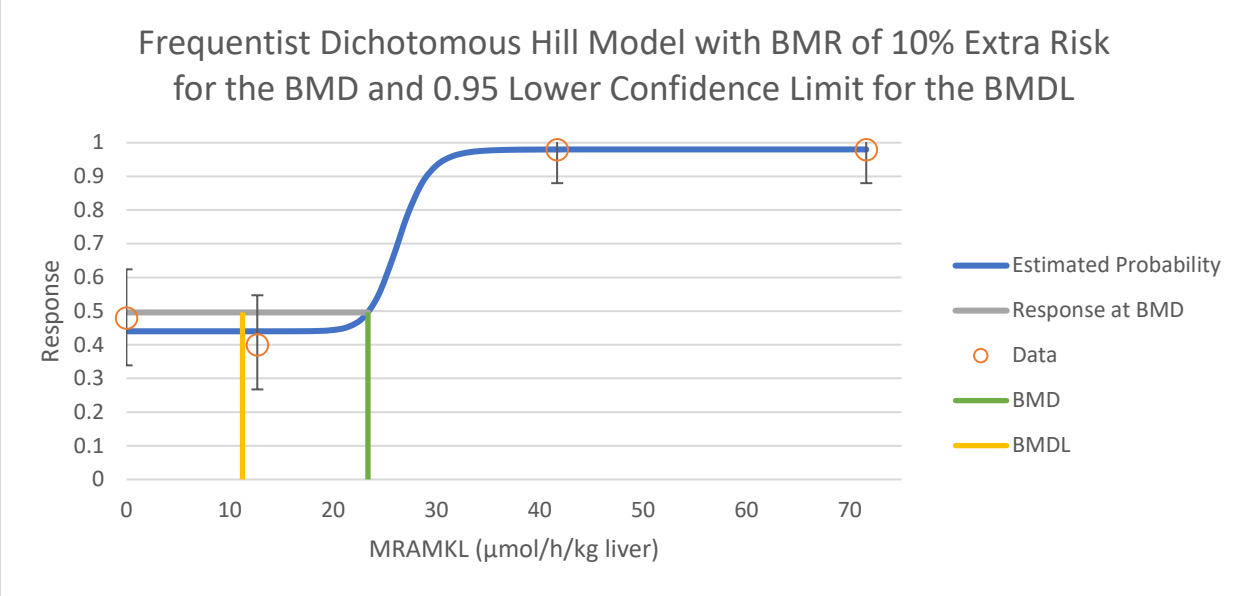



**Figure 10. Male mice combined liver tumors using MRAMKL dose metric: VmaxC by Thrall et al., dichotomous Hill model**

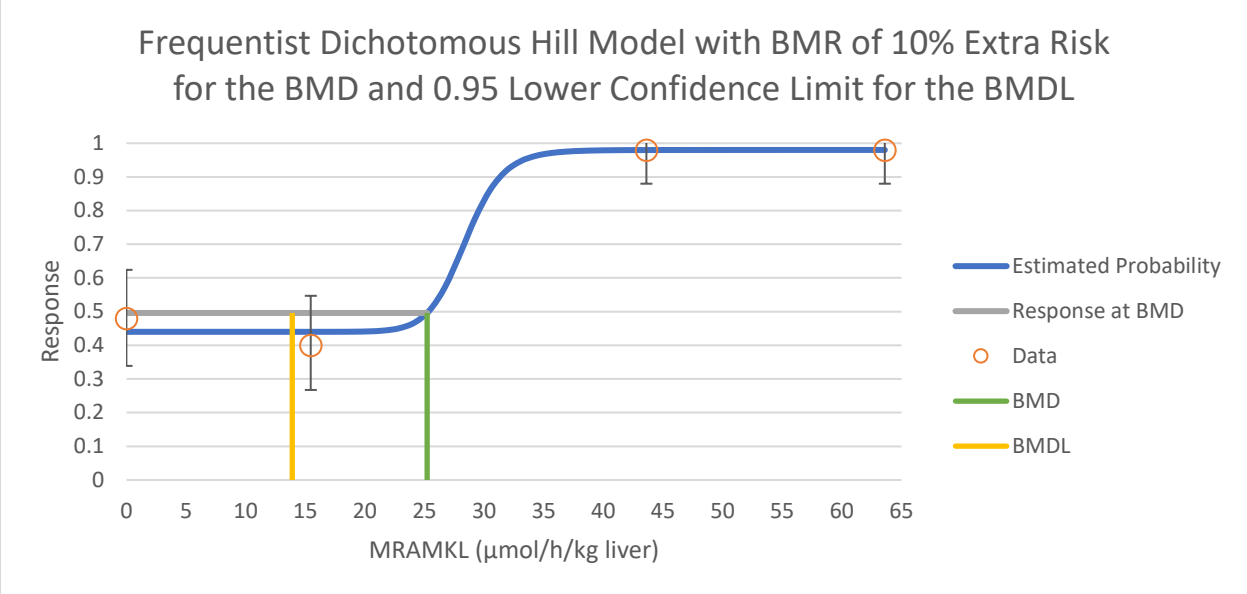


**Figure 11. Male mice combined liver tumors using MRAMKL dose metric: VmaxC by Fisher et al. (highest dose dropped), log-logistic model**

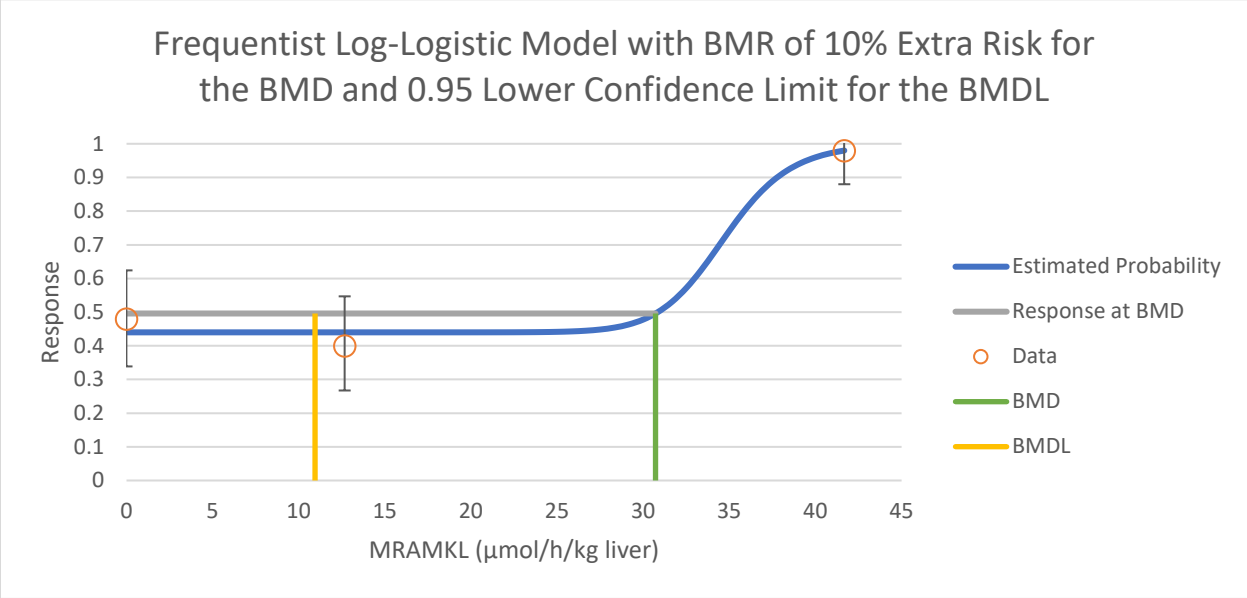



**Figure 12. Male mice combined liver tumors using MRAMKL dose metric: VmaxC by Thrall et al. (highest dose dropped), Weibull model**

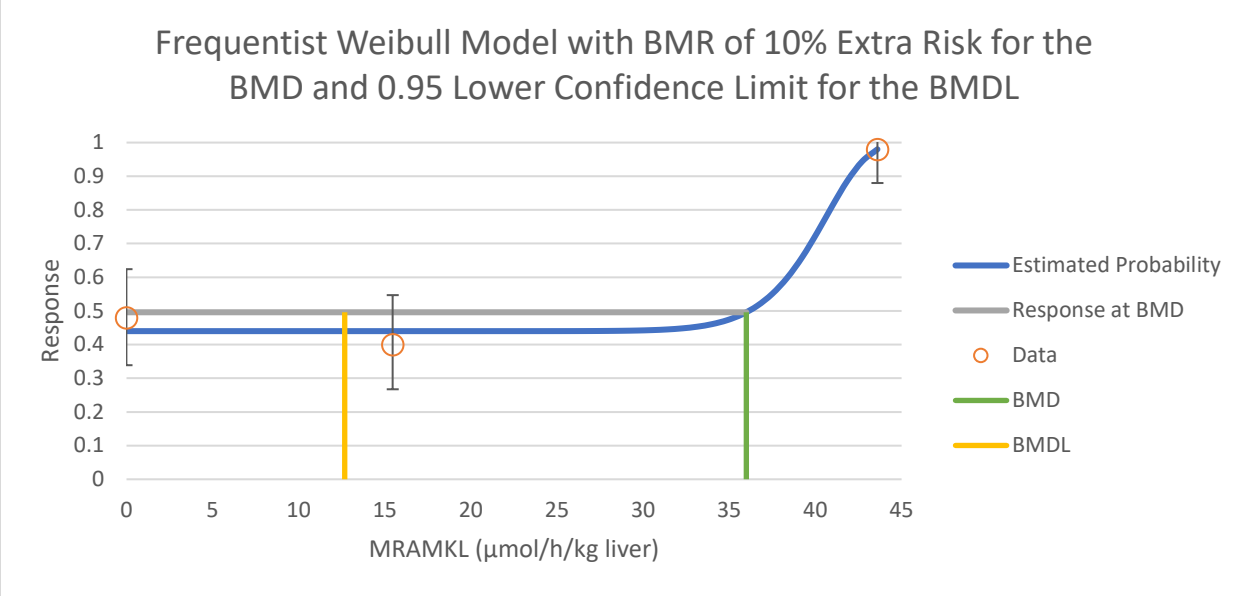


**Table 36. Female mice combined liver tumors frequentist multistage model using MRAMKL dose metric**

| Dose Metric | Model | AIC VmaxC by Fisher et al.[a] | p-Value VmaxC by Fisher et al.[a] | $BMD_{10}$ VmaxC by Fisher et al.[a] | $BMDL_{10}$ VmaxC by Fisher et al.[a] | AIC VmaxC by Thrall et al.[b] | p-Value VmaxC by Thrall et al.[b] | $BMD_{10}$ VmaxC by Thrall et al.[b] | $BMDL_{10}$ VmaxC by Thrall et al.[b] |
|---|---|---|---|---|---|---|---|---|---|
| MRAMKL | Multistage Degree 2 | 127.23 | 0.242 | 10.30 | 5.57 | 127.11 | 0.381 | 10.49 | 7.59 |

a Exposure internal dose for MRAMKL: 0, 12.666, 41.675, 71.589 µmol/h/kg liver
b Exposure internal dose for MRAMKL: 0, 15.456, 43.599, 63.596 µmol/h/kg liver

**Table 37. Female mice combined liver tumors frequentist multistage model using MRAMKL dose metric (highest dose dropped)**

| Dose Metric | Model | AIC VmaxC by Fisher et al.[a] | p-Value VmaxC by Fisher et al.[a] | $BMD_{10}$ VmaxC by Fisher et al.[a] | $BMDL_{10}$ VmaxC by Fisher et al.[a] | AIC VmaxC by Thrall et al.[b] | p-Value VmaxC by Thrall et al.[b] | $BMD_{10}$ VmaxC by Thrall et al.[b] | $BMDL_{10}$ VmaxC by Thrall et al.[b] |
|---|---|---|---|---|---|---|---|---|---|
| MRAMKL | Multistage Degree 2 | 115.9 | 0.444 | 9.71 | 6.32 | 117.34 | 0.165 | 10.46 | 7.59 |

[a] Exposure internal dose for MRAMKL: 0, 12.666, 41.675 µmol/h/kg liver
[b] Exposure internal dose for MRAMKL: 0, 15.456, 43.599 µmol/h/kg liver

**Figure 13. Female mice combined liver tumors using MRAMKL dose metric: VmaxC by Fisher et al., multistage degree 2 model**

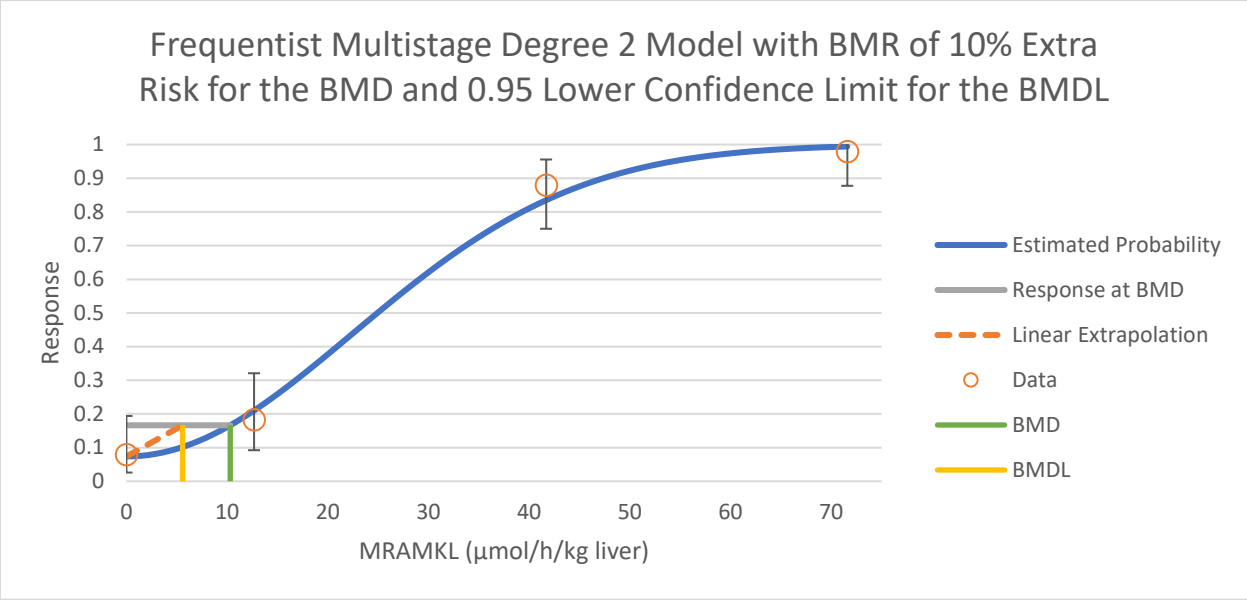



**Figure 14. Female mice combined liver tumors using MRAMKL dose metric: VmaxC by Thrall et. al., multistage degree 2 model**

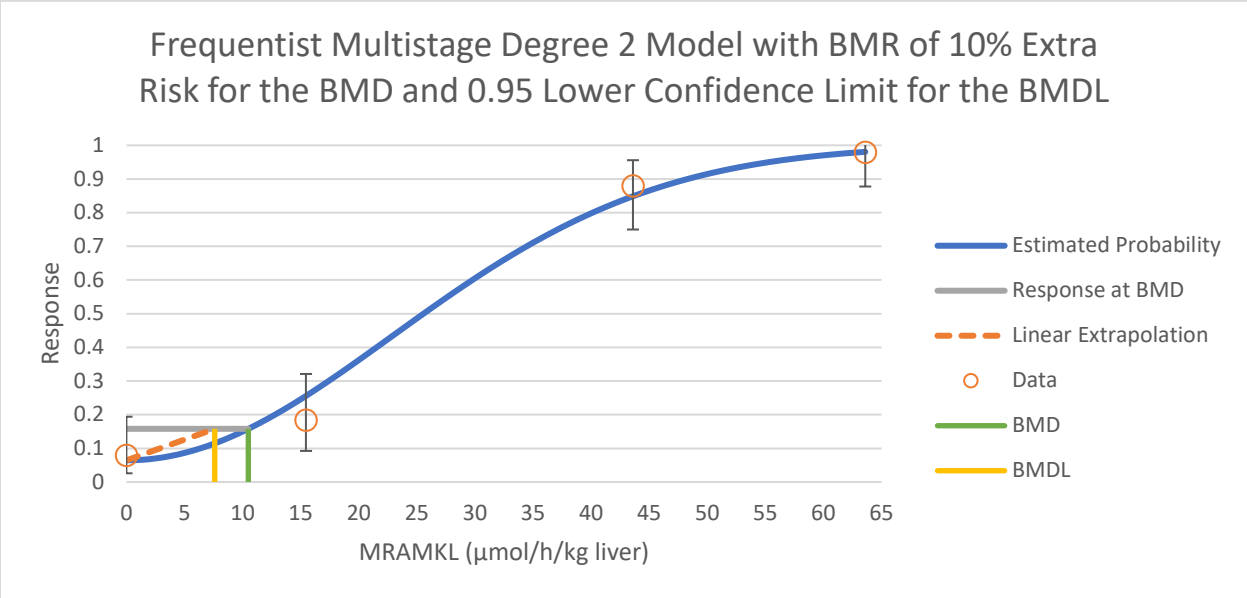



**Figure 15. Female mice combined liver tumors using MRAMKL dose metric: VmaxC by Fisher et al. (highest dose dropped), multistage degree 2 model**

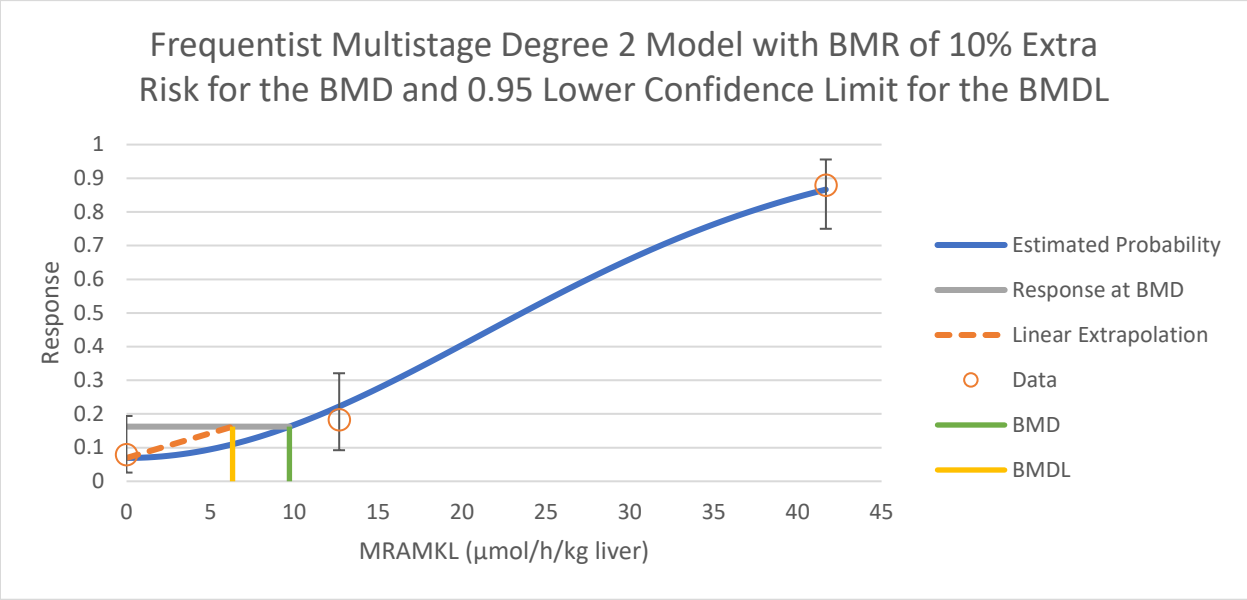



**Figure 16. Female mice combined liver tumors using MRAMKL dose metric: VmaxC by Thrall et al. (highest dose dropped), multistage degree 2 model**

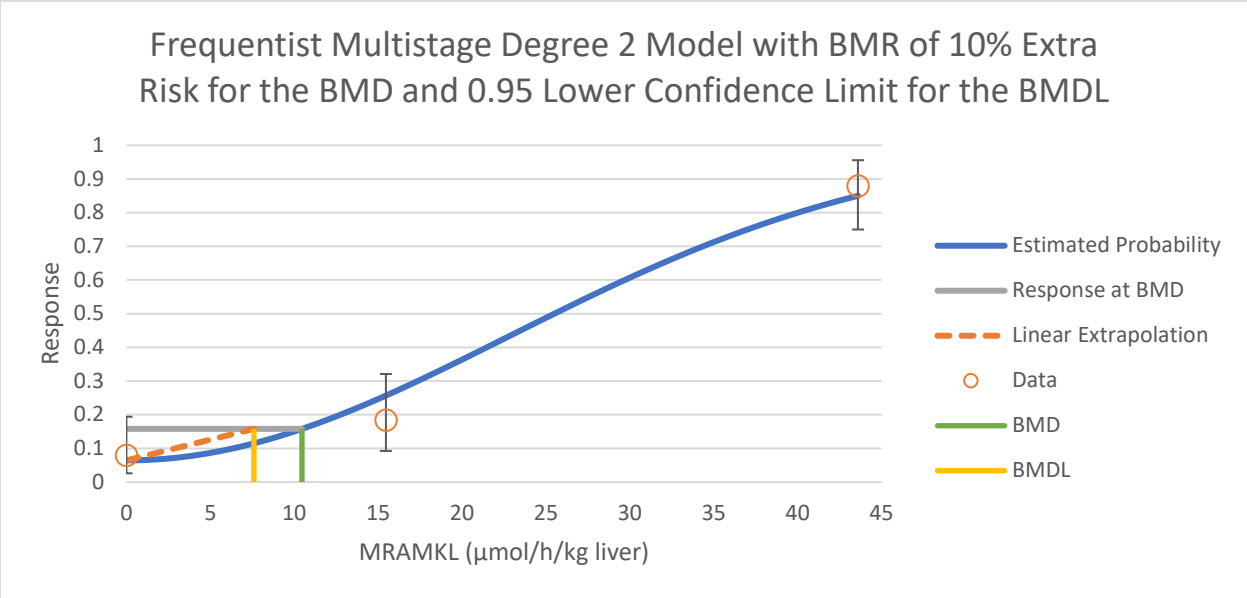


**Table 38. Male and female mice adrenal pheochromocytomas frequentist multistage model using MCA dose metric**

| Dose Metric | Model | AIC Vmax C by Fisher et al.[a] | p-Value VmaxC by Fisher et al.[a] | $BMD_{10}$ VmaxC by Fisher et al.[a] | $BMDL_{10}$ VmaxC by Fisher et al.[a] | AIC Vmax C by Thrall et al.[b] | p-Value VmaxC by Thrall et al.[b] | $BMD_{10}$ VmaxC by Thrall et al.[b] | $BMDL_{10}$ VmaxC by Thrall et al.[b] |
|---|---|---|---|---|---|---|---|---|---|
| MCA (male) | Multistage Degree 1 | 139.13 | 0.051 | 0.292 | 0.230 | --- | --- | --- | --- |
| MCA (female) | Multistage Degree 2 | 71.41 | 0.795 | 1.43 | 1.14 | 71.33 | 0.804 | 2.95 | 2.34 |

[a] Exposure internal dose for MCA: 0, 0.111, 0.603, 3.315 µmol/L blood
[b] Exposure internal dose for MCA: 0, 0.213, 1.226, 6.856 µmol/L blood

**Table 39. Male mice adrenal pheochromocytomas using MCA dose metric and VmaxC by Thrall et al. 2000**

| Model | AIC<br>VmaxC by Thrall et al. 2000 [a] | p-Value<br>VmaxC by Thrall et al. 2000 [a] | $BMD_{10}$<br>VmaxC by Thrall et al. 2000 [a] | $BMDL_{10}$<br>VmaxC by Thrall et al. 2000 [a] |
|---|---|---|---|---|
| **Dichotomous Hill (Restricted)** | **134.03** | **0.999** | **1.04** | **0.470** |
| Gamma (Restricted) | 139.08 | 0.049 | 0.600 | 0.473 |
| Log-Logistic (Restricted) | 138.47 | 0.105 | 0.493 | 0.297 |
| Log-Probit (Restricted) | 141.99 | 0.003 | 0.868 | 0.696 |
| Multistage Degree 3 (Restricted) | 139.08 | 0.049 | 0.600 | 0.473 |
| Multistage Degree 2 (Restricted) | 139.08 | 0.049 | 0.600 | 0.473 |
| Multistage Degree 1 (Restricted) | 139.08 | 0.049 | 0.600 | 0.473 |
| Weibull (Restricted) | 139.08 | 0.049 | 0.600 | 0.473 |
| Logistic (Unrestricted) | 161.35 | <0.0001 | 1.92 | 1.56 |
| Log-Probit (Unrestricted) | 136.94 | 0.165 | 0.528 | 0.297 |
| Probit (Unrestricted) | 159.95 | <0.0001 | 1.76 | 1.45 |
| Quantal Linear (Unrestricted) | 139.08 | 0.049 | 0.600 | 0.473 |

[a] Exposure internal dose for MCA: 0, 0.213, 1.226, 6.856 µmol/L blood

**Figure 17. Male mice adrenal pheochromocytomas using MCA dose metric: VmaxC by Fisher et al., multistage degree 1 model**

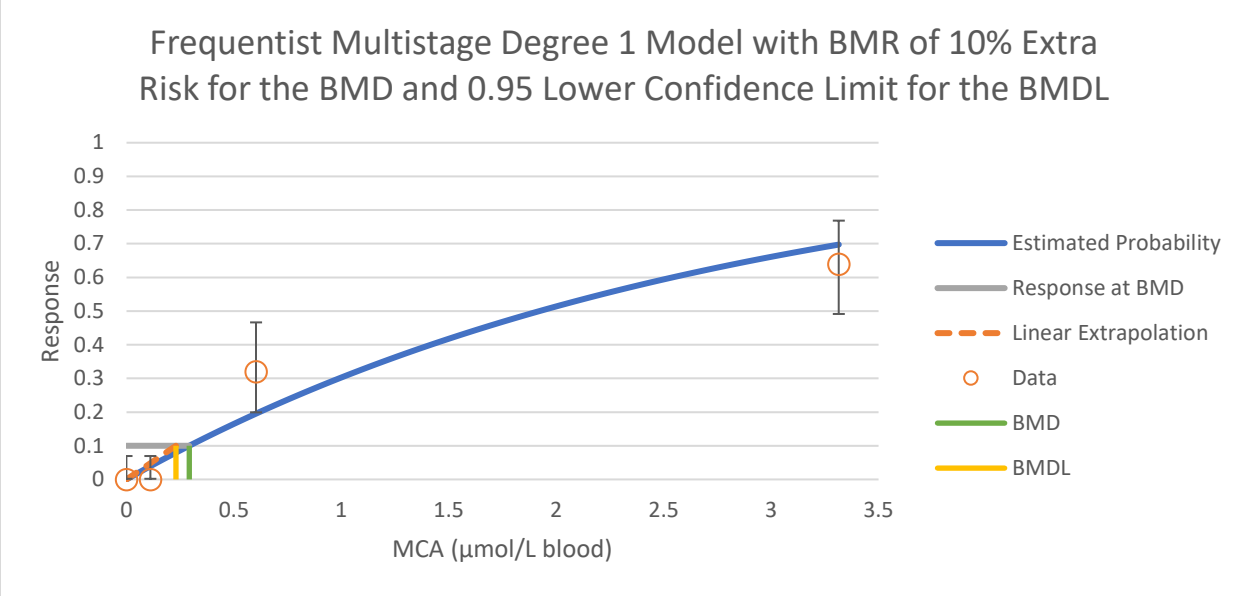



**Figure 18. Male mice adrenal pheochromocytomas using MCA dose metric: VmaxC by Thrall et al., dichotomous Hill model**

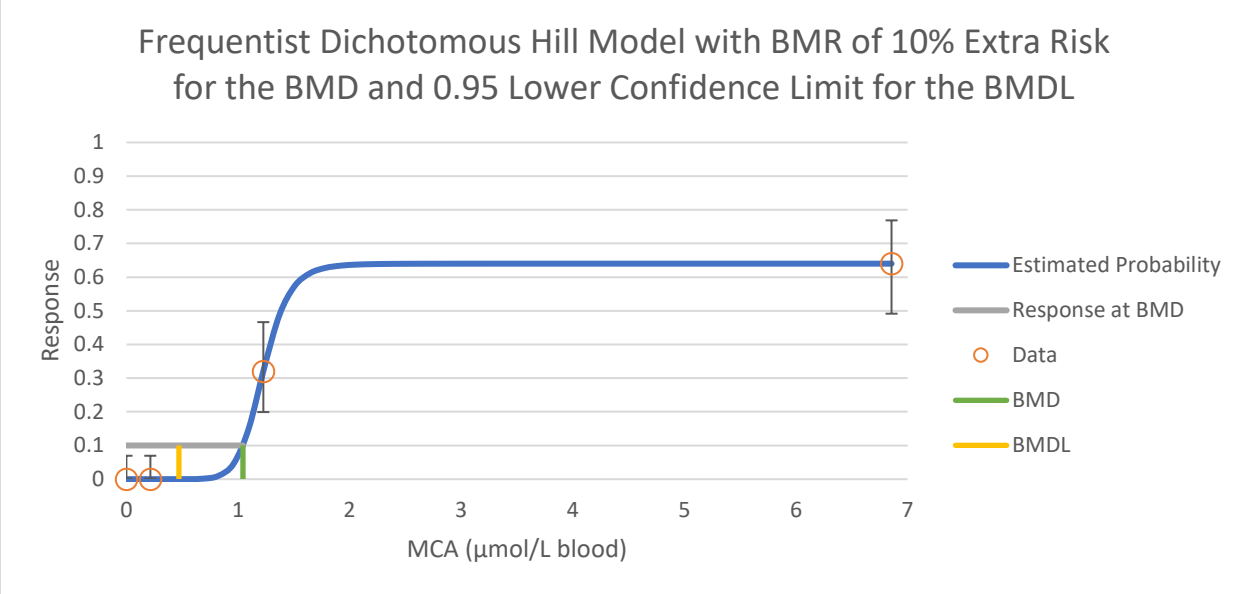
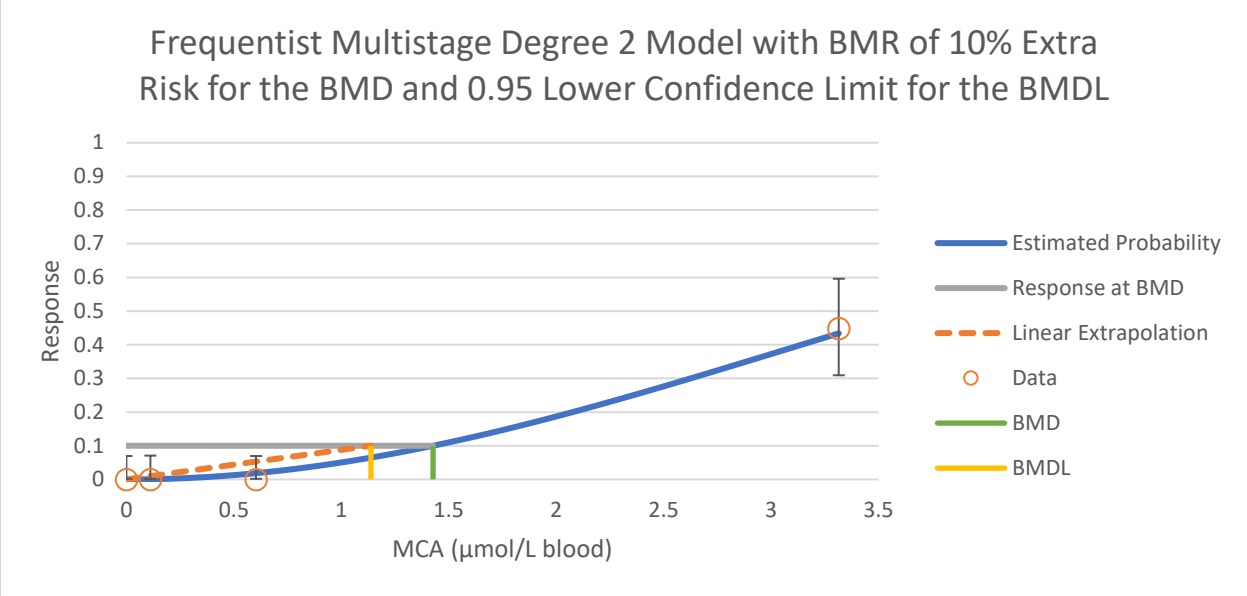


**Figure 19. Female mice adrenal pheochromocytomas using MCA dose metric: VmaxC by Fisher et al., multistage degree 2 model**



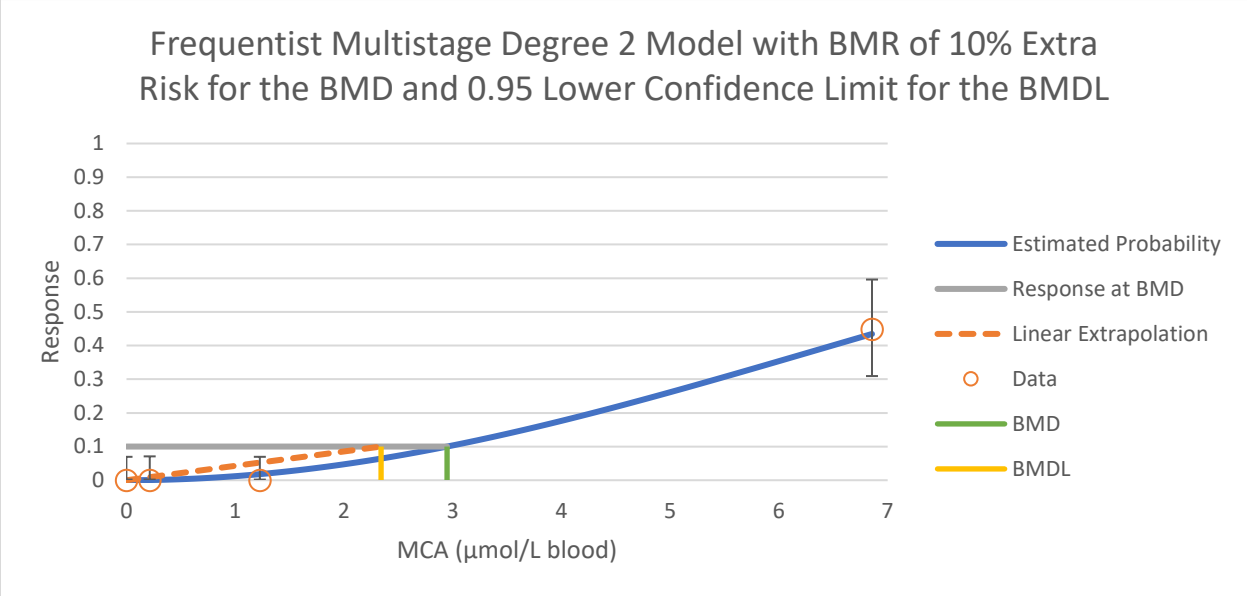


**Figure 20. Female mice adrenal pheochromocytomas using MCA dose metric: VmaxC by Thrall et al., multistage degree 2 model**