# CHAPTER 2. HEALTH EFFECTS

## 2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of ethylene oxide. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to ethylene oxide, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to ethylene oxide was also conducted; the results of this review are presented in Appendix C.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2, and animal oral studies are presented in Table 2-2 and Figure 2-3; limited dermal data were identified for ethylene oxide.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death,

or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of ethylene oxide are indicated in Tables 2-1 and 2-2 and Figures 2-2 and 2-3.

A User's Guide has been provided at the end of this profile (see Appendix D). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

The health effects of ethylene oxide have been evaluated in a number of occupational cohorts and a variety of animal studies. As illustrated in Figure 2-1, the inhalation exposure route was employed in the majority of animal studies; inhalation was assumed to be the predominant exposure route in the occupational cohort studies. The most examined endpoints in animal studies were body weight and neurotoxicity. Cancer was the most examined endpoint in epidemiological studies.

Human and/or animal studies suggest that relatively sensitive noncancer targets of ethylene oxide include respiratory, hematological, endocrine, neurological, reproductive, and developmental endpoints (see Appendix C for more information on the systematic review and for definitions of evidence levels). Human and animal studies have also reported carcinogenic effects.

- **Respiratory Endpoints:** Respiratory effects are a presumed health effect for humans based on a moderate level of evidence in workers and a high level of evidence in experimental animal studies. Compromised respiratory function has been reported in workers exposed to high levels of ethylene oxide. Inhalation studies in experimental animals have reported several respiratory effects including labored breathing, nasal discharge, pulmonary lesions, rhinitis, and pulmonary edema.

- **Hematological Endpoints:** Hematological effects are a suspected health effect for humans based on a moderate level of evidence in animal studies. Repeated exposure of experimental animals to ethylene oxide vapor has resulted in hematological effects such as decreases in hemoglobin,

hematocrit, erythrocyte count, packed cell volume, and/or reticulocytes, and splenic extramedullary hematopoiesis.

- **Endocrine Endpoints:** Adrenal gland effects are a suspected health effect in humans based on a moderate level of evidence in animal studies. Adverse effects (gross and/or histopathologic changes in the adrenal gland) have been observed in rats and guinea pigs following acute- and/or chronic-duration exposure to ethylene oxide vapor.

- **Neurological Endpoints:** Neurological effects are a presumed health effect in humans based on a low level of evidence in occupational exposure studies and a high level of evidence in animal studies. Clinical signs of neurotoxicity (e.g., neuropathy, weakness in extremities, impaired hand-eye coordination, cognitive dysfunction, memory loss, headache, lethargy) were reported among workers exposed to ethylene oxide for various durations. Sural nerve biopsies revealed axonal degeneration and regeneration in two studies. Neurological effects such as ataxia, impaired sensory reflexes, hindlimb paralysis, and/or degenerative histopathologic lesions have been observed among laboratory animals exposed to ethylene oxide by inhalation.

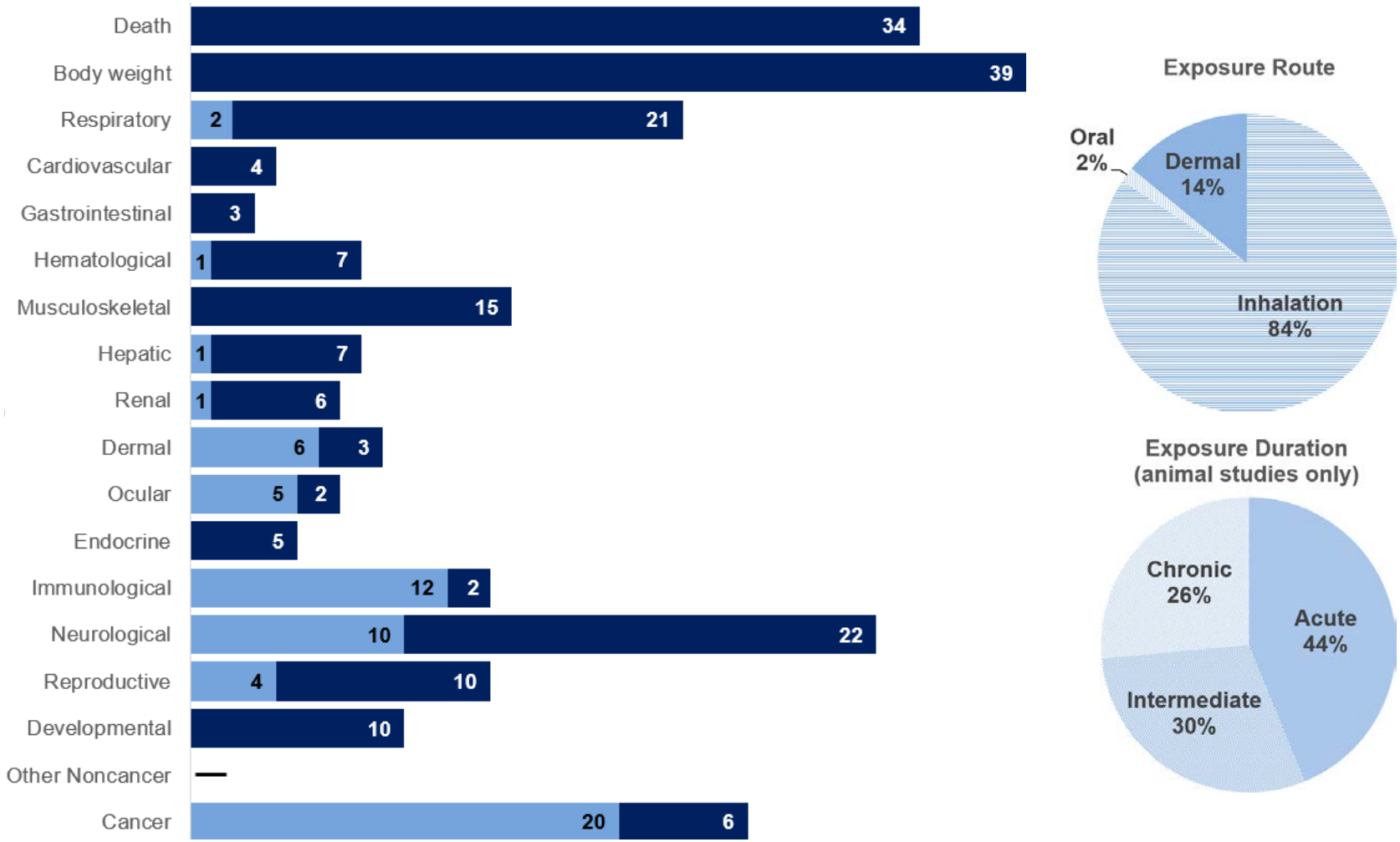
- **Reproductive Endpoints:** Male reproductive effects are a presumed health effect in humans based on a moderate level of evidence in occupational exposure studies and a high level of evidence in animal studies. Limited human data suggest possible ethylene oxide-related effects on sperm production. Animal studies provide convincing evidence of ethylene oxide-induced effects on the male reproductive system (e.g., decreases in male reproductive organ weights, germ cell survival, and sperm count; histopathologic lesions).

- **Developmental Endpoints:** Ethylene oxide is a presumed developmental toxicant in humans based on animal studies that demonstrated ethylene oxide-induced developmental effects such as depressed fetal weight, delayed ossification, fetal fluid retention, and ocular defects.

- **Cancer:** The carcinogenicity of ethylene oxide has been evaluated in a number of cohorts involved in ethylene oxide production and/or uses in sterilization. Results from some cohort studies suggest that exposure to ethylene oxide may increase the risk of selected cancer types (e.g., lymphohematopoietic cancer, leukemia, breast cancer). In laboratory animals exposed by inhalation, ethylene oxide was associated with a variety of cancer types (leukemia, mesotheliomas, lymphomas, tumors of lungs, Harderian gland, and female mammary gland and reproductive organs). Forestomach cancer was reported in rats treated by the oral exposure route.

## Figure 2-1. Overview of the Number of Studies Examining Ethylene Oxide Health Effects

**Most studies examined the potential body weight, neurological, and cancer effects of ethylene oxide**
Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)




*Includes studies discussed in Chapter 2. A total of 124 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints. Chronic exposure was considered the most prevalent exposure duration for human occupational studies.

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | | | |
| 1 | Rat, mouse, guinea pig, rabbit, monkey (NS) 1–10 (NS) | Up to 10 days Up to 8 exposures 7 hours/exposure | 0, 841 | GN, HP, LE | Death | | | 841 | 100% mortality for each species |
| **Hollingsworth et al. 1956** | | | | | | | | | |
| 2 | Rat, guinea pig (NS) 5/sex/species | 2 or 3 exposures 7 hours/exposure | 0, 841 | HP, LE | Resp | | 841 | | Slight to moderate edema; slight hemorrhage and congestion in lungs |
| | | | | | Hepatic | | 841 | | Light coloration and slight fatty degeneration |
| | | | | | Renal | | 841 | | Enlarged kidney; slight congestion and cloudy swelling of convoluted tubules |
| | | | | | Endocr | | 841 | | Pale coloration and enlargement in adrenals; numerous fat vacuoles in adrenal cortex |
| **Hollingsworth et al. 1956** | | | | | | | | | |
| 3 | Rat (NS) 10/sex Mouse (NS) 10 F | 9 days 7 exposures 7 hours/exposure | 0, 357 | BW, GN, LE | Death | | | 357 | Death of 2/20 rats and 4/20 mice |
| | | | | | Bd wt | | 357 | | Moderate body weight loss (not otherwise described) |
| | | | | | Resp | | | 357 | Severe lung injury (not otherwise described) |
| **Hollingsworth et al. 1956** | | | | | | | | | |
| 4 | Rat (White) 10 M | Once for 4 hours | 882, 1,343, 1,648, 1,843, 1,992, 2,298 | CS, LE | Death | | | 1,460 | 4-hour $LC_{50}$ |
| **Jacobson et al. 1956** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| 5 | Rat (CD) 25 F | GDs 6–15 6 hours/day | 0, 50, 125, 225 | BW, CS, DX, FI, FX, LE, MX, OF, OW | Bd wt | 225 | | | |
| | | | | | Develop | 50 | 125 | | 5% depressed mean fetal weight/litter |
| **Neeper-Bradley and Kubena 1993** | | | | | | | | | |
| 6 | Rat (Sprague-Dawley) 39 or 41 F | GDs 7–16 7 hours/day | 0, 150 | BW, DX, HP, OW | Bd wt | 150 | | | |
| | | | | | Develop | | 150 | | 5–6% depressed fetal body weight; decreased crown-rump length; delayed ossification (skull, sternebrae) |
| **NIOSH 1982** | | | | | | | | | |
| 7 | Rat (Sprague-Dawley) 20–21 F | GDs 6–15 1 time/day 30 minutes | 0, 400, 800, 1,200 | BW, DX, FX, LE, MX | Bd wt | 1,200 | | | |
| | | | | | Develop | 800 | 1,200 | | Increased incidence of dilation in renal pelvis and ureter |
| **Saillenfait et al. 1996** | | | | | | | | | |
| 8 | Rat (Sprague-Dawley) 18 F | GDs 5–16 3 times/day 30 minutes each | 0, 200, 400 | BW, DX, FX, LE, MX | Bd wt | 400 | | | NOAEL is for three 30-minute exposures at 400 ppm per day |
| | | | | | Develop | 400 | | | NOAEL is for three 30-minute exposures at 400 ppm per day |
| **Saillenfait et al. 1996** | | | | | | | | | |
| 9 | Rat (Sprague-Dawley) 21 F | GDs 6–15 3 times/day 30 minutes each | 0, 800, 1,200 | BW, DX, FX, LE, MX | Bd wt | 800 | | 1,200 | 70% depressed mean maternal body weight gain for GDs 6–16 |
| | | | | | Develop | | 800 | | 4–7% depressed mean fetal weight |
| **Saillenfait et al. 1996** | | | | | | | | | |
| 10 | Rat (Fischer- 344) 22 F | GDs 6–15 6 hours/day | 0, 0, 10, 33, 100 | CS, DX | Develop | 33[b] | 100 | | 3–9% depressed fetal body weight; $BMCL_{05}$ of 45.12 ppm |
| **Snellings et al. 1982a** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| 11 | Rat (Sprague-Dawley) 5 M or 5 F | Once for 1 hour | M: 4,827, 5,546, 6,161; F: 3,966, 4202, 4,827 | CS, LE | Death | | | 5,748 M 4,439 F | 1-hour $LC_{50}$ |
| **Snellings et al. 2011** | | | | | | | | | |
| 12 | Rat (Sprague-Dawley) 5 M or 5 F | Once for 4 hours | M: 1,850, 2,026, 2,182; F: 1,443, 1,637, 1,850 | CS, LE | Death | | | 1,972 M 1,537 F | 4-hour $LC_{50}$ |
| **Snellings et al. 2011** | | | | | | | | | |
| 13 | Mouse (White) 10 F | Once for 4 hours | 533, 860, 882, 960, 1,343, 1,365 | CS, LE | Death | | | 835 | 4-hour $LC_{50}$ |
| **Jacobson et al. 1956** | | | | | | | | | |
| 14 | Mouse (B6C3F1) 5 M, 5 F | Once for 4 hours | 100, 200, 400, 800, 1,600 | CS, LE | Death | | | 800 | 5/5 males and 4/5 females died at 800 ppm and 5/5 males and 5/5 females died at 1,600 ppm |
| | | | | | Resp | | 800 | 1,600 | LOAEL: Dyspnea observed after 4 hours of exposure to 800 ppm Serious LOAEL: Dyspnea observed after 3 hours of exposure to 1,600 ppm; dyspnea graded as severe in the 1,600 ppm after 3.5 hours of exposure. |
| | | | | | Neuro | | | 1,600 | Incoordination after 3 hours and semi-consciousness after 3.5 hours of exposure to 1,600 ppm |
| **NTP 1987** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| 15 | Mouse (B6C3F1) 10 M, 10 F | Up to 2 weeks during a 14-week study 5 days/week 6 hours/day | 0, 50, 100, 200, 400, 600 | BW, CS, GN, HP, LE | Death | | | 400 | 100% mortality at 400 and 600 ppm, deaths occurred during first 2 weeks of exposure |
| | | | | | Resp | | 400 | | Rhinitis |
| | | | | | Renal | | | 400 | Renal tubular degeneration and necrosis |
| | | | | | Immuno | | | 600 | Lymphocyte necrosis in thymus |
| **NTP 1987** | | | | | | | | | |
| 16 | Mouse (B6C3F1) 5 M, 5 F | 2 weeks 5 days/week 6 hours/day | 0, 50, 100, 200, 400, 800 | BW, CS, GN, HP, LE | Death | | | 800 | 100% mortality |
| | | | | | Bd wt | 400 | | | |
| **NTP 1987** | | | | | | | | | |
| 17 | Mouse (Hybrid) 22–55 F | Once for 1.5 hours 1, 6, 9, or 25 hours postmating | 0, 1,200 | DX, FX | Develop | | | 1,200 | Fetal defects (predominantly hydrops and eye defects) |
| **Rutledge and Generoso 1989** | | | | | | | | | |
| 18 | Dog (Beagle) 3 M | Once for 4 hours | 327, 710, 1,393, 2,830 | CS, LE | Death | | | 960 | 4-hour $LC_{50}$ |
| **Jacobson et al. 1956** | | | | | | | | | |
| 19 | Rabbit (New Zealand) 20 or 21 F | GDs 7–19 7 hours/day | 0, 150 | BW, DX, HP, OW | Bd wt | 150 | | | |
| | | | | | Develop | 150 | | | |
| **NIOSH 1982** | | | | | | | | | |
| **INTERMEDIATE EXPOSURE** | | | | | | | | | |
| 20 | Monkey (NS) 3 M, 1 F | 60 or 140 days 38–41 or 94 exposures 7 hours/exposure | 0, 357 | BW, CS, HP, LE | Musc/skel | | | 357 | Muscular atrophy in hindlimbs |
| | | | | | Neuro | | | 357 | Impaired sensory reflexes, paralysis of hindlimbs |
| **Hollingsworth et al. 1956** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| 21 | Rat (CD) 28 M, 28 F | 6 hours/day, 5 days/week for 10 weeks pre-mating; 6 hours/day, 5 days/week during mating, GDs 0–20, and LDs 5–28 | 0, 10, 33, 100 | CS, BW, OW, HP, DX, RX | Bd wt | 10 | 33 | | Decreased body weight (7–11%) in F1 males |
| | | | | | Resp | 100 | | | |
| | | | | | Hepatic | 100 | | | |
| | | | | | Neuro | 100 | | | |
| | | | | | Repro | 10 | | 33 | Post implantation loss in F0 rats; decreased number of live pups per litter in F1 and F2 generations at 100 ppm |
| | | | | | Develop | 10 | 33 | | Decreased pup body weight in F1 and F2 generations |
| EPA 1994 | | | | | | | | | |
| 22 | Rat (Wistar) 8 M | 13 weeks 3 days/week 6 hours/day | 0, 500 | BW, EA, HE, OF, OW, UR | Bd wt | 500 | | | |
| | | | | | Hemato | | 500 | | Decreases in hemoglobin, hematocrit, erythrocyte count; increased reticulocytes |
| **Fujishiro et al. 1990** | | | | | | | | | |
| 23 | Rat (White) 20 M | 6 weeks 5 days/week 6 hours/day | 0, 406 | BW, CS, GN, LE | Death | | | 406 | 13/20 rats died during the 6-week exposure period |
| | | | | | Resp | | | 406 | Labored breathing, nasal discharge |
| | | | | | Neuro | | | 406 | Loss of hindquarter usefulness |
| **Jacobson et al. 1956** | | | | | | | | | |
| 24 | Rat (White) 20 M | 6 months 5 days/week 6 hours/day | 0, 102 | BW, CS, GN, LE | Resp | 102 | | | |
| | | | | | Neuro | 102 | | | |
| **Jacobson et al. 1956** | | | | | | | | | |
| 25 | Rat (Wistar) 6 or 8 M (28 M controls) | Up to 13 weeks 3 days/week 6 hours/day | 0, 500 | BW, CS, OF, OW | Neuro | | 500 | | Awkward gait |
| | | | | | Repro | | | 500 | Decreased testicular weight, decreased germ cell survival, degenerative effects on germ cells |
| **Kaido et al. 1992** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| 26 | Rat (Wistar) Up to 9 M | 12 weeks 3 days/week 6 hours/day | 0, 500 | BW, CS, EA | Bd wt | 500 | | | |
| | | | | | Neuro | | 500 | | Ataxic gait |
| **Matsuoka et al. 1990** | | | | | | | | | |
| 27 | Rat (Wistar) 6 or 12 M | 13 weeks 5 days/week 6 hours/day | 0, 50, 100, 250 | BW, EA, OF, OW | Bd wt | 250 | | | |
| | | | | | Repro | 100 | | 250 | 20% decreased epididymal weight, 73% decreased epididymal sperm count, histopathologic lesions in seminiferous tubules |
| **Mori et al. 1991a** | | | | | | | | | |
| 28 | Rat (Wistar) 8 M | 6 weeks 3 days/week 6 hours/day | 0, 500 | BW, EA, OF, OW | Bd wt | 500 | | | |
| | | | | | Repro | | | 500 | 26% decreased testicular weight, 32% decreased epididymal weight, 87% decrease in sperm counts, increased sperm head abnormalities |
| **Mori et al. 1991b** | | | | | | | | | |
| 29 | Rat (Sprague-Dawley) 39 or 41 F | GDs 1–16 7 hours/day | 0, 150 | BW, DX, FX, HP, OW | Bd wt | 150 | | | |
| | | | | | Resp | 150 | | | |
| | | | | | Hepatic | 150 | | | |
| | | | | | Renal | 150 | | | |
| | | | | | Repro | | 150 | | Increased incidence of resorptions |
| | | | | | Develop | | 150 | | 7–9% decreased fetal body weight; delayed ossification (skull, sternebrae) |
| **NIOSH 1982** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| 30 | Rat (Sprague-Dawley) 32–45 F | 3 weeks premating and GDs 1–16 7 hours/day | 0, 150 | BW, DX, FX, OW | Bd wt | 150 | | | |
| | | | | | Resp | 150 | | | |
| | | | | | Hepatic | 150 | | | |
| | | | | | Renal | 150 | | | |
| | | | | | Repro | | 150 | | Increased incidence of resorptions |
| | | | | | Develop | | 150 | | 10–12% decreased fetal weight, decreased crown-rump length, delayed ossification (skull, sternebrae) |
| **NIOSH 1982** | | | | | | | | | |
| 31 | Rat (Wistar) 5 M | 13 weeks 3 days/week 6 hours/day | 0, 500 | CS, HP, OF | Bd wt | 500 | | | |
| | | | | | Neuro | | | 500 | Peripheral neuropathy |
| **Ohnishi et al. 1985** | | | | | | | | | |
| 32 | Rat (Wistar) 7 M | 9 months 5 days/week 6 hours/day | 0, 250 | CS, HP, OF | Neuro | | 250 | | Retarded growth and maturation of myelinated fibers in hindleg nerves and mild axonal degeneration in absence of clinical signs of neuropathy |
| **Ohnishi et al. 1986** | | | | | | | | | |
| 33 | Rat (Fischer- 344) 30 M, 20 F | 12 weeks premating, 2 weeks mating, and throughout gestation and lactation 5 or 7 days/week 6 hours/day | 0, 0, 10, 33, 100 | BW, DX, FX | Bd wt | 100 | | | |
| | | | | | Repro | 33 | 100 | | Decreases in the number of pups/litter and the ratio of fetuses born to implantation sites |
| | | | | | Develop | 100 | | | |
| **Snellings et al. 1982b** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| 34 | Rat, mouse, rabbit, monkey (NS) 1–10 (NS) | 48–85 days 33–59 exposures 7 hours/exposure | 0, 357 | CS, LE | Death | | | 357 | Death of 18/20 rats, 10/10 mice,1/2 rabbits |
| | | | | | Bd wt | | | 357 | Markedly subnormal growth of each species (not otherwise described) |
| | | | | | Musc/skel | | | 357 | Muscular atrophy in hindlimbs of rats, rabbits, and monkey |
| | | | | | Neuro | | | 357 | Impaired sensory and motor function; paralysis in hindlimbs of rats, rabbits, monkey |
| **Hollingsworth et al. 1956** | | | | | | | | | |
| 35 | Rat, mouse, monkey, guinea pig (NS) 2–20 (NS) | 176–226 days 122–157 exposures 7 hours/exposure | 0, 113 | BW, CS, HP, LE, OF, OW | Bd wt | 113 F | 113 M | | 13% depressed final body weight in male rats |
| | | | | | Resp | | 113 | | 20–22% increased relative lung weight in male and female rats |
| **Hollingsworth et al. 1956** | | | | | | | | | |
| 36 | Rat, mouse, rabbit, monkey, guinea pig (NS) 2–20 (NS) | 176–226 days 122–157 exposures 7 hours/exposure | 0, 204 | BW, CS, HP, LE, OF, OW | Death | | | 204 | Death of 14/20 male and 8/20 female rats |
| | | | | | Bd wt | | 204 | | 20, 13, and 10% depressed final body weight among male and female rats and female guinea pigs, respectively |
| | | | | | Resp | | 204 | | 18–31% increased relative lung weight among male and female rats and female guinea pigs |
| | | | | | Musc/skel | | | 204 | Muscular atrophy in hindlimbs of monkeys |
| | | | | | Neuro | | | 204 | Impaired sensory reflexes and partial paralysis in hind quarters and back of monkeys; slight to marked paralysis in hindlegs of rabbits |
| **Hollingsworth et al. 1956** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| 37 | Mouse (A/J) 30 F | 6 months 5 days/week 6 hours/day | 0, 70, 200 | HP, LE | Cancer | | | 70 | CEL: Lung tumors |
| **Adkins et al. 1986** | | | | | | | | | |
| 38 | Mouse (White) 30 F | 6 weeks 5 days/week 6 hours/day | 0, 406 | BW, CS, GN, LE | Death | | | 406 | 24/30 mice died |
| **Jacobson et al. 1956** | | | | | | | | | |
| 39 | Mouse (White) 30 F | 6 months 5 days/week 6 hours/day | 0, 102 | BW, CS, GN, LE | Bd wt | 102 | | | |
| **Jacobson et al. 1956** | | | | | | | | | |
| 40 | Mouse (B6C3F1) 10 M, 10 F | Up to 14 weeks 5 days/week 6 hours/day | 0, 50, 100, 200, 400, 600 | BW, CS, GN, HP, LE | Bd wt | 200 | | | |
| | | | | | Resp | 100 | 200 | | Rhinitis in males and females |
| | | | | | Renal | 100 F | 100 M 200 F | | Renal tubular degeneration (5/10 M; 8/10 F) |
| **NTP 1987** | | | | | | | | | |
| 41 | Mouse (B6C3F1) 30 M, 30 F | 10–11 weeks 5 days/week 6 hours/day | 0, 10, 50, 100, 250 | BC, BW, HE, HP, MX | Bd wt | 250 | | | |
| | | | | | Hemato | 100 | 250 | | Males: decreases in hemoglobin Females: decreases in RBC count, hemoglobin, packed cell volume, mean corpuscular hemoglobin concentration |
| | | | | | Musc/skel | 250 | | | |
| | | | | | Hepatic | 250 | | | |
| | | | | | Neuro | 10[c] | 50 | | Males: reduced locomotor activity Females: hunched posture during gait |
| | | | | | Repro | 250 M | | | |
| **Snellings et al. 1984a** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| 42 | Dog (Beagle) 3 M | 6 months 5 days/week 6 hours/day | 0, 102 | BC, BW, CS, HE, LE | Bd wt | 102 | | | |
| | | | | | Hemato | | 102 | | Signs indicative of normochromic anemia in 2/3 dogs |
| **Jacobson et al. 1956** | | | | | | | | | |
| 43 | Dog (Beagle) 3 M | 6 weeks 5 days/week 6 hours/day | 0, 292 | BC, BW, CS, GN, HE | Bd wt | 292 | | | |
| | | | | | Resp | | 292 | | Pulmonary congestion, moderate alveolar collapse; consistent with mild irritation of lung parenchyma |
| | | | | | Hemato | | 292 | | Decreases in erythrocyte count, hemoglobin, hematocrit |
| | | | | | Musc/skel | | | 292 | Fatty changes consistent with muscular atrophy |
| | | | | | Neuro | | | 292 | Slight tremors, hindleg weakness |
| **Jacobson et al. 1956** | | | | | | | | | |
| 44 | Rabbit (New Zealand) 21 or 23 F | GDs 1–19 7 hours/day | 0, 150 | BW, DX, HP, OW | Bd wt | 150 | | | |
| | | | | | Resp | 150 | | | |
| | | | | | Hepatic | 150 | | | |
| | | | | | Renal | 150 | | | |
| | | | | | Develop | 150 | | | |
| **NIOSH 1982** | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **CHRONIC EXPOSURE** | | | | | | | | | |
| 45 | Monkey (Cynomolgus) 12 M | Up to 2 years 5 days/week 7 hours/day | 0, 50, 100 | BC, BW, GN, HE, HP, LE, OF, OP, UR | Cardio | 100 | | | |
| | | | | | Hemato | 100 | | | |
| | | | | | Repro | | 50 | | Decreased sperm count and motility |
| **Lynch et al. 1984a** | | | | | | | | | |
| 46 | Rat (Fischer-344) 120 M, 120 F | Up to 2 years 5 days/week 6 hours/day | 0, 0, 10, 33, 100 | BW, HP, LE | Death | | | 100 | Increase in mortality in months 22–23 |
| | | | | | Bd wt | 33 | 100 | | M: Up to 12% depressed body weight gain<br>F: 12–18% depressed body weight gain |
| | | | | | Cancer | | | 33 | CEL: Mononuclear leukemia in females at ≥33 ppm; peritoneal mesothelioma in males at 100 ppm; subcutis fibroma in males at 100 ppm; brain tumors in males and females at ≥33 ppm |
| **Garman et al. 1986; Snellings et al. 1984b** | | | | | | | | | |
| 47 | Rat (Fischer- 344) 80 M | Up to 2 years 5 days/week 7 hours/day | 0, 50, 100 | BC, BW, GN, HE, HP, UR | Death | | | 100 | Decreased survival |
| | | | | | Bd wt | 50 | 100 | | 16% decrease in body weight gain |
| | | | | | Cardio | 100 | | | |
| | | | | | Gastro | 100 | | | |
| | | | | | Hemato | | 50 | | Splenic extramedullary hematopoiesis at ≥50 ppm and splenic focal fibrosis at 100 ppm; no alterations in hematological parameters |
| | | | | | Musc/skel | 50 | 100 | | Multifocal myopathy in skeletal muscle |
| | | | | | Hepatic | 100 | | | |
| | | | | | Renal | 100 | | | |
| | | | | | Ocular | 100 | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Endocr | | 50 | | Multifocal cortical vacuolation and hyperplasia in the adrenal gland |
| | | | | | Neuro | 100 | | | |
| | | | | | Repro | 100 | | | |
| | | | | | Cancer | | | 50 | CEL: Mononuclear cell leukemia at ≥50 ppm; peritoneal mesotheliomas and brain gliomas at 100 ppm |
| **Lynch et al. 1984a, 1984b** | | | | | | | | | |
| 48 | Mouse (B6C3F1) 50 M, 50 F | 102 weeks 5 days/week 6 hours/day | 0, 50, 100 | BW, CS, GN, HP, LE | Bd wt | 100 | | | |
| | | | | | Resp | 100 | | | |
| | | | | | Cardio | 100 | | | |
| | | | | | Gastro | 100 | | | |
| | | | | | Musc/skel | 100 | | | |
| | | | | | Hepatic | 100 | | | |
| | | | | | Renal | 100 | | | |
| | | | | | Dermal | 100 | | | |
| | | | | | Endocr | 100 | | | |
| | | | | | Neuro | 100 | | | |
| | | | | | Repro | 100 | | | |

**Table 2-1. Levels of Significant Exposure to Ethylene Oxide – Inhalation**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (ppm) | Parameters monitored | Endpoint | NOAEL (ppm) | Less serious LOAEL (ppm) | Serious LOAEL (ppm) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Cancer | | | 50 M | CEL: Harderian gland papillary cystadenoma in males at ≥50 ppm and females at 100 ppm; mammary gland tumors in females at 50 ppm; lung alveolar/bronchiolar adenoma or carcinoma, malignant lymphomas (females only), and uterine adenocarcinomas at 100 ppm |
| **NTP 1987** | | | | | | | | | |

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive a provisional acute-duration inhalation minimal risk level (MRL) of 0.4 ppm for ethylene oxide; based on a rat $BMCL_{HEC}$ of 11.28 ppm ($BMCL_{05}$ of 45.12 ppm adjusted for intermittent exposure and converted to a human equivalent concentration) and an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability); see Appendix A for more detailed information regarding the provisional MRL.
[c]Used to derive a provisional intermediate-duration inhalation MRL of 0.02 ppm for ethylene oxide; based on a $NOAEL_{HEC}$ of 1.8 ppm (NOAEL of 0.02 ppm adjusted for intermittent exposure and converted to a human equivalent concentration), an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability), and a modifying factor of 3 for lack of neurotoxicity data; see Appendix A for more detailed information regarding the provisional MRL.

Principal studies for the MRLs.

Bd wt or BW = body weight; BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; F = female(s); FI = food intake; FX = fetal toxicity; Gastro = gastrointestinal; GD = gestation day(s); GN = gross necropsy; HE = hematology; HEC = human equivalent concentration; Hemato = hematological; HP = histopathology; Immuno = immunological; $LC_{50}$ = lethal concentration, 50% kill; LD = lactation day; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive effects; UR = urinalysis

**Figure 2-2. Levels of Significant Exposure to Ethylene Oxide – Inhalation**
Acute (≤14 days)

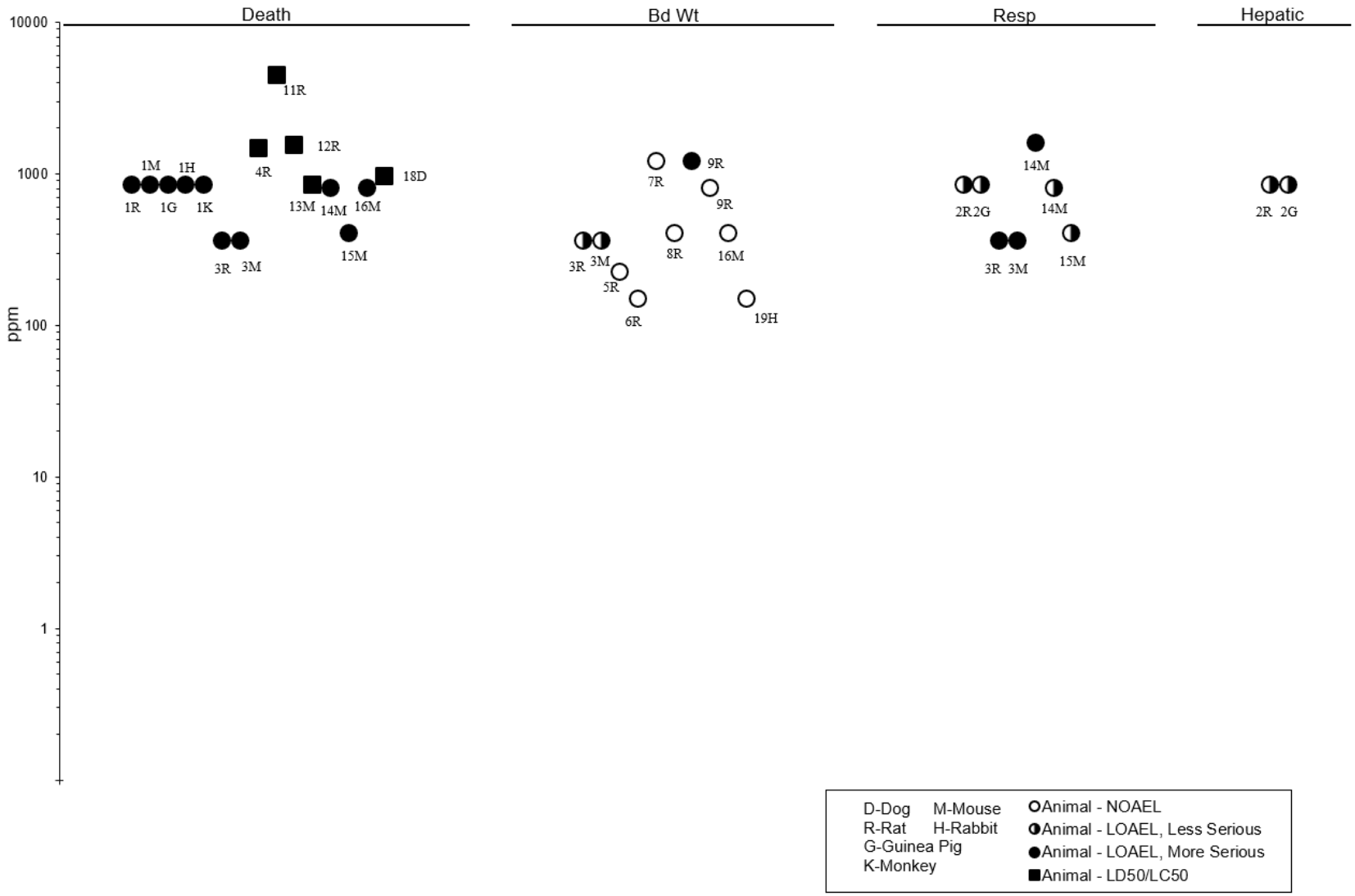


**Figure 2-2. Levels of Significant Exposure to Ethylene Oxide – Inhalation**
Acute (≤14 days)

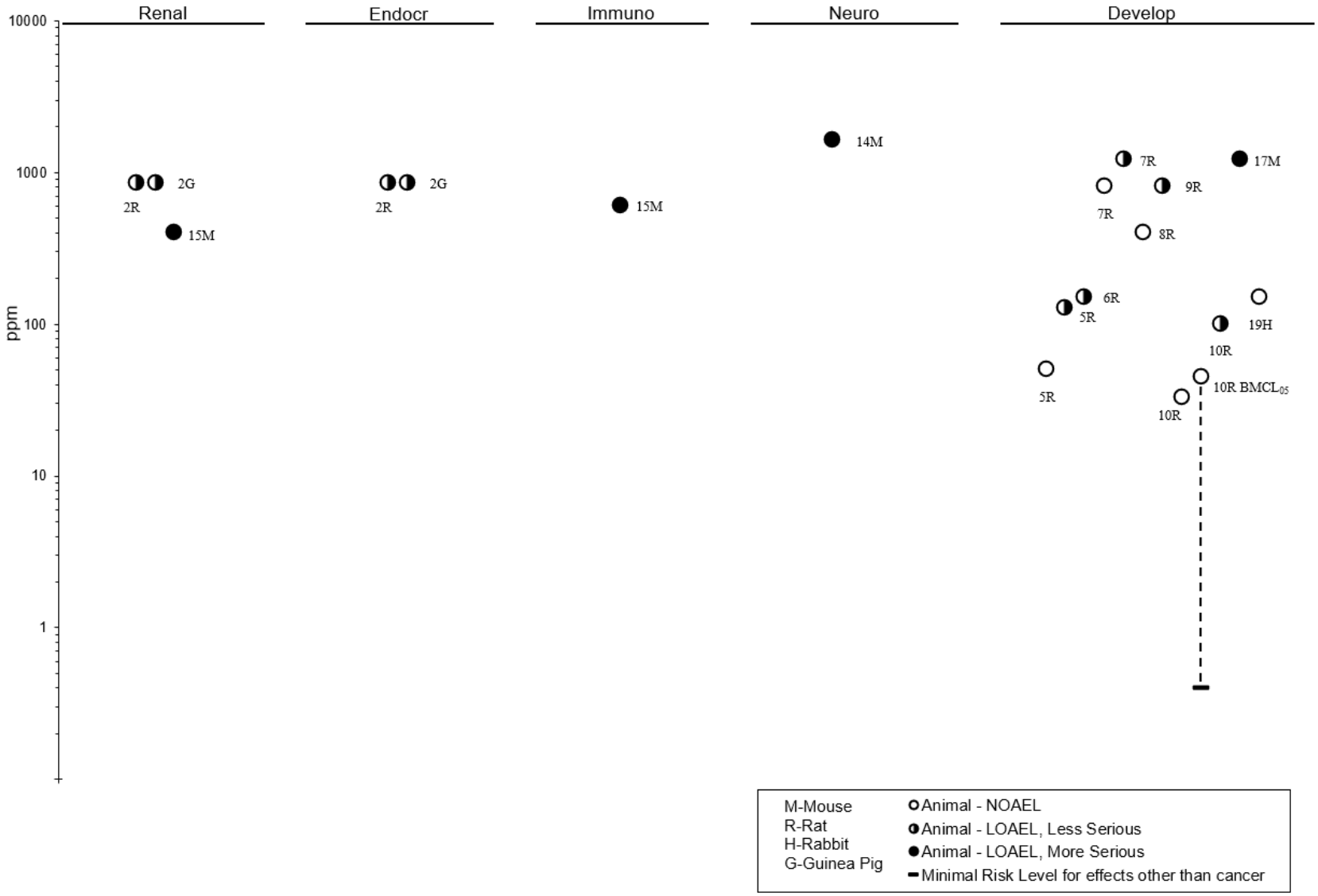


**Figure 2-2. Levels of Significant Exposure to Ethylene Oxide – Inhalation**
Intermediate (15-364 days)

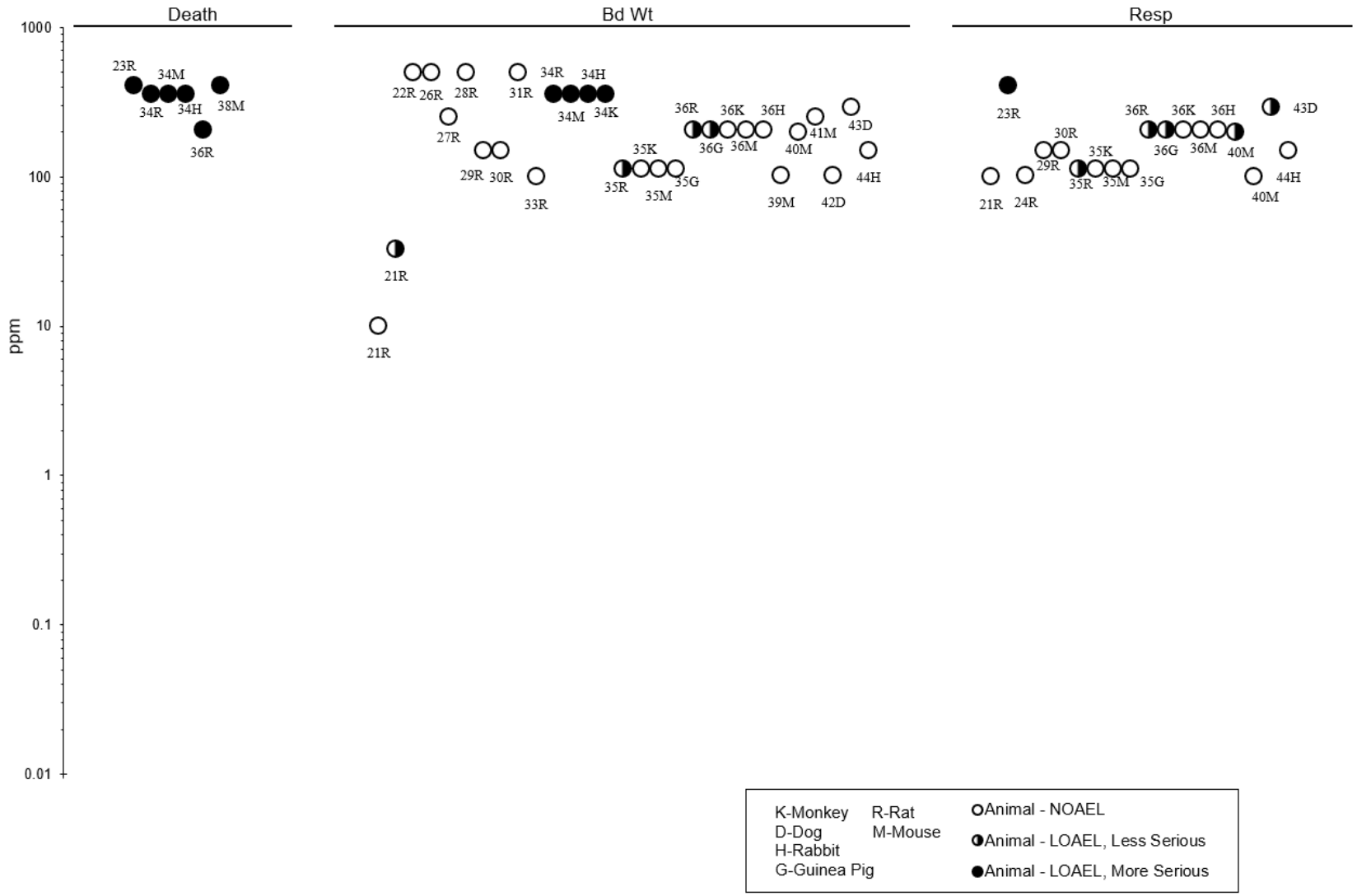


**Figure 2-2. Levels of Significant Exposure to Ethylene Oxide – Inhalation**
Intermediate (15-364 days)

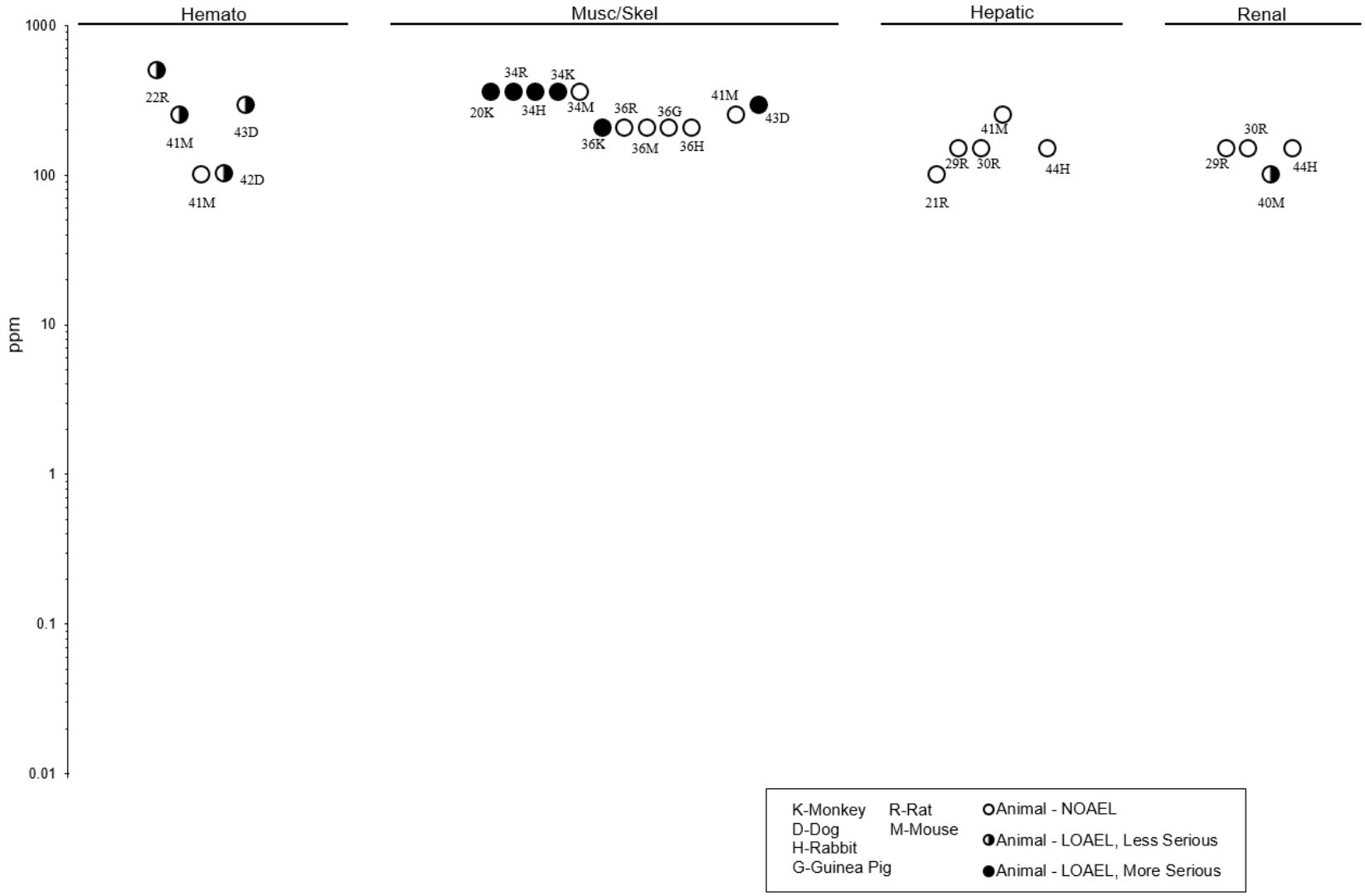


**Figure 2-2. Levels of Significant Exposure to Ethylene Oxide – Inhalation**
Intermediate (15-364 days)

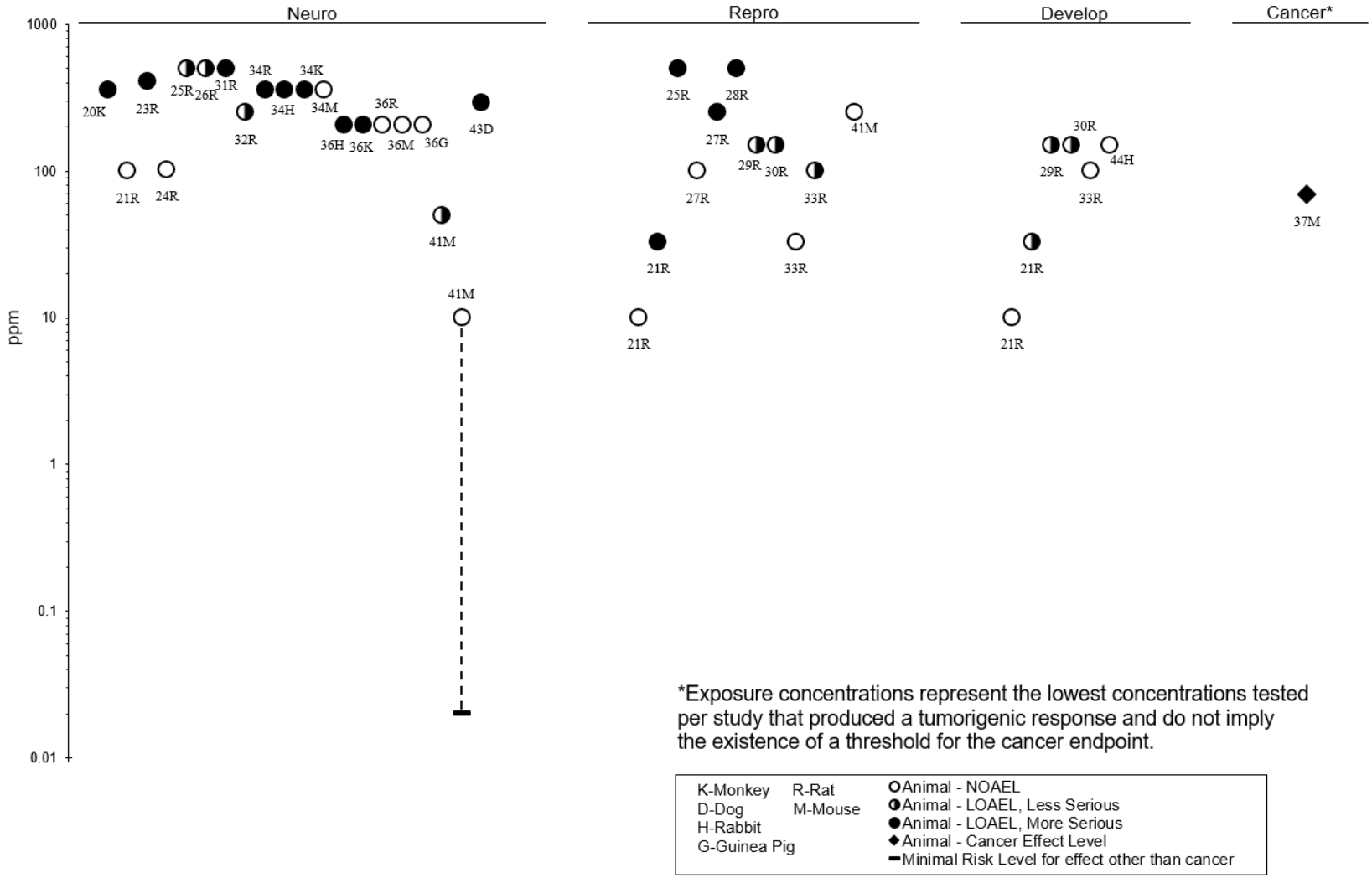


**Figure 2-2. Levels of Significant Exposure to Ethylene Oxide – Inhalation**
Chronic (≥365 days)

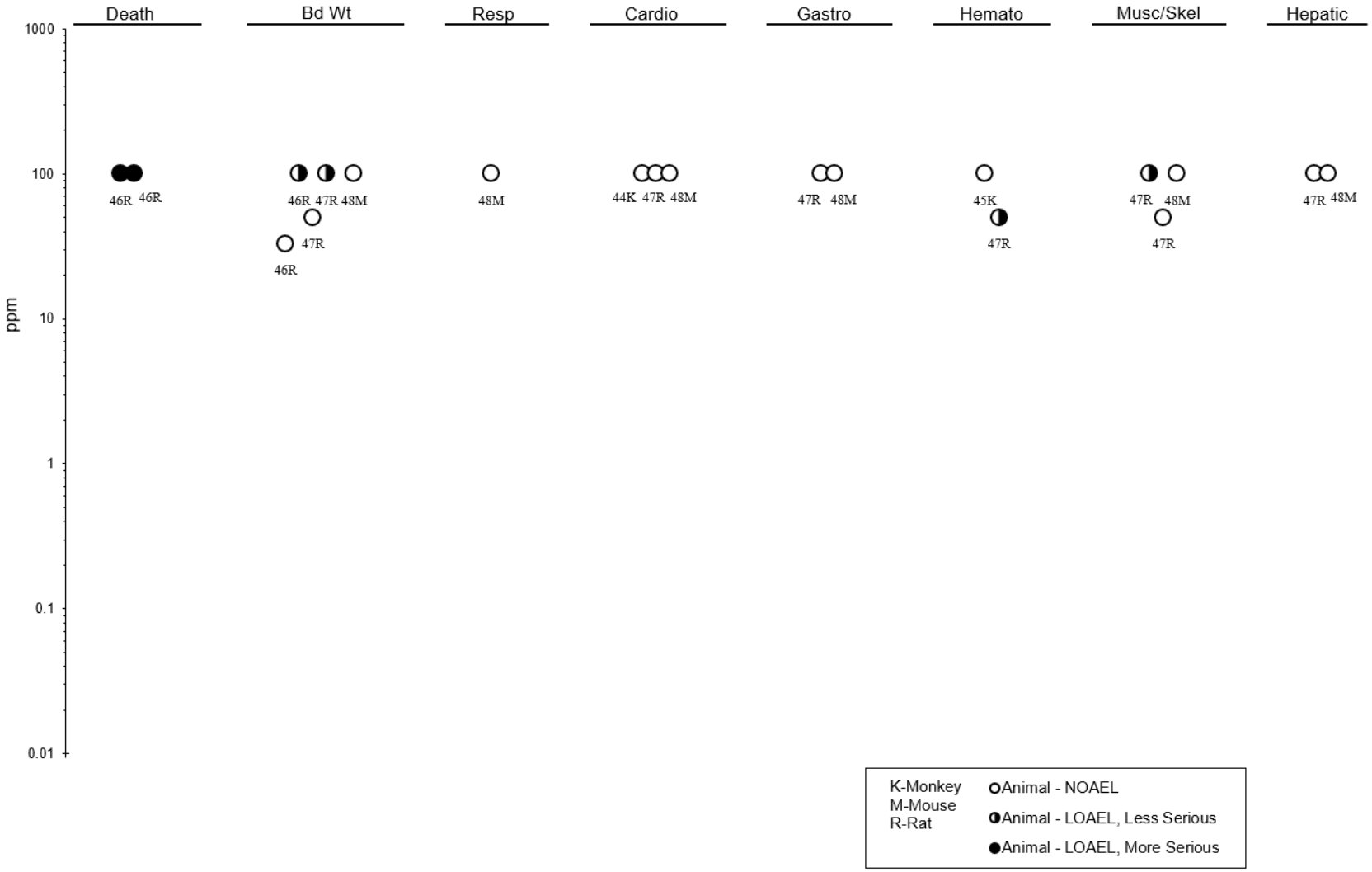
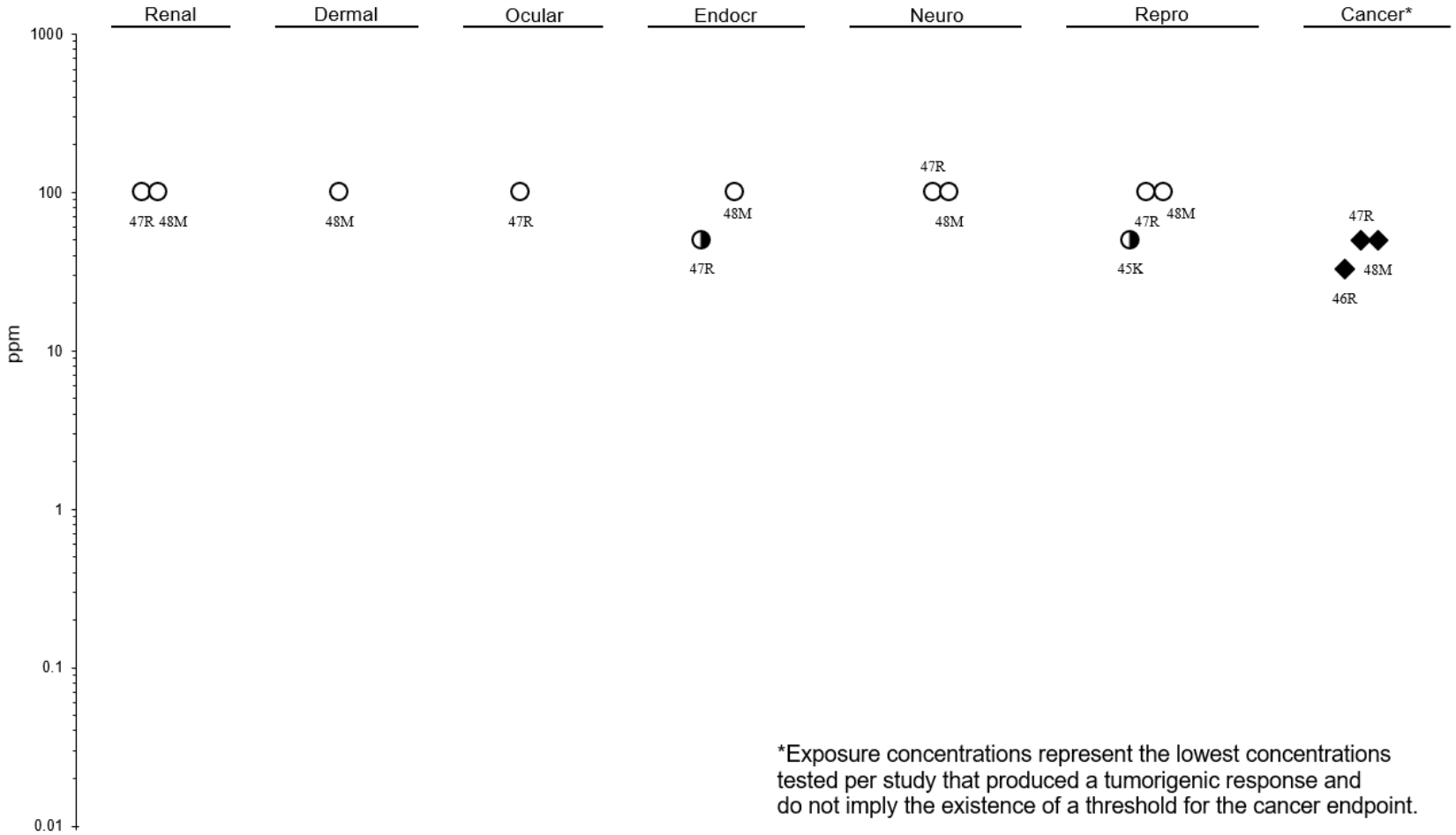


**Figure 2-2. Levels of Significant Exposure to Ethylene Oxide – Inhalation**
Chronic (≥365 days)




*Exposure concentrations represent the lowest concentrations tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

| | |
|---|---|
| K-Monkey<br>M-Mouse<br>R-Rat | ○ Animal - NOAEL<br>◑ Animal - LOAEL, Less Serious<br>◆ Animal - Cancer Effect Level |

## Table 2-2. Levels of Significant Exposure to Ethylene Oxide – Oral

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses (mg/kg/day) | Parameters monitored | Endpoint | NOAEL (mg/kg/day) | Less serious LOAEL (mg/kg/day) | Serious LOAEL (mg/kg/day) | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | | | |
| 1 | Rat (NS) 5 M, 5 F | Once (GO) | 100, 200 | BW, LE | Death | | | 200 | 100% mortality |
| | | | | | Bd wt | 100 | | | |
| **Hollingsworth et al. 1956** | | | | | | | | | |
| 2 | Rat (Wistar) 10 M | Once (GW) | NS | LE | Death | | | 330 | $LD_{50}$ |
| **Smyth et al. 1941** | | | | | | | | | |
| 3 | Guinea pig (NS) NS M, NS F | Once (GW) | NS | LE | Death | | | 270 | $LD_{50}$ |
| **Smyth et al. 1941** | | | | | | | | | |
| **INTERMEDIATE EXPOSURE** | | | | | | | | | |
| 4 | Rat (NS) 5 F | 15 times in 21 days (100 mg/kg/day) 22 times in 30 days (3, 10, 30 mg/kg/day) (GO) | 0, 3, 10, 30, 100 | BW, GN, HE, HP, OW | Bd wt | 30 | | 100 | Weight loss, magnitude not reported |
| | | | | | Gastro | 30 | 100 | | Gastric irritation; no additional information |
| | | | | | Hemato | 30 | | | |
| | | | | | Hepatic | 30 | 100 | | Slight liver damage; no additional information |
| **Hollingsworth et al. 1956** | | | | | | | | | |
| **CHRONIC EXPOSURE** | | | | | | | | | |
| 5 | Rat (Sprague-Dawley) 50 F | 150 weeks 2 times/week (GO) | 0, 7.5, 30 | HP, LE | Death | | | 30 | Decreased survival |
| | | | | | Cancer | | | 7.5 | CEL: Forestomach squamous cell carcinoma |
| **Dunkelberg 1982** | | | | | | | | | |

[a]The number corresponds to entries in Figure 2-3.

Bd wt or BW = body weight; CEL = cancer effect level; F = female(s); (GO) = gavage in oil; (GW) = gavage in water; Gastro = gastrointestinal; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; $LD_{50}$ = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified

**Figure 2-3. Levels of Significant Exposure to Ethylene Oxide – Oral**
Acute (≤14 days)

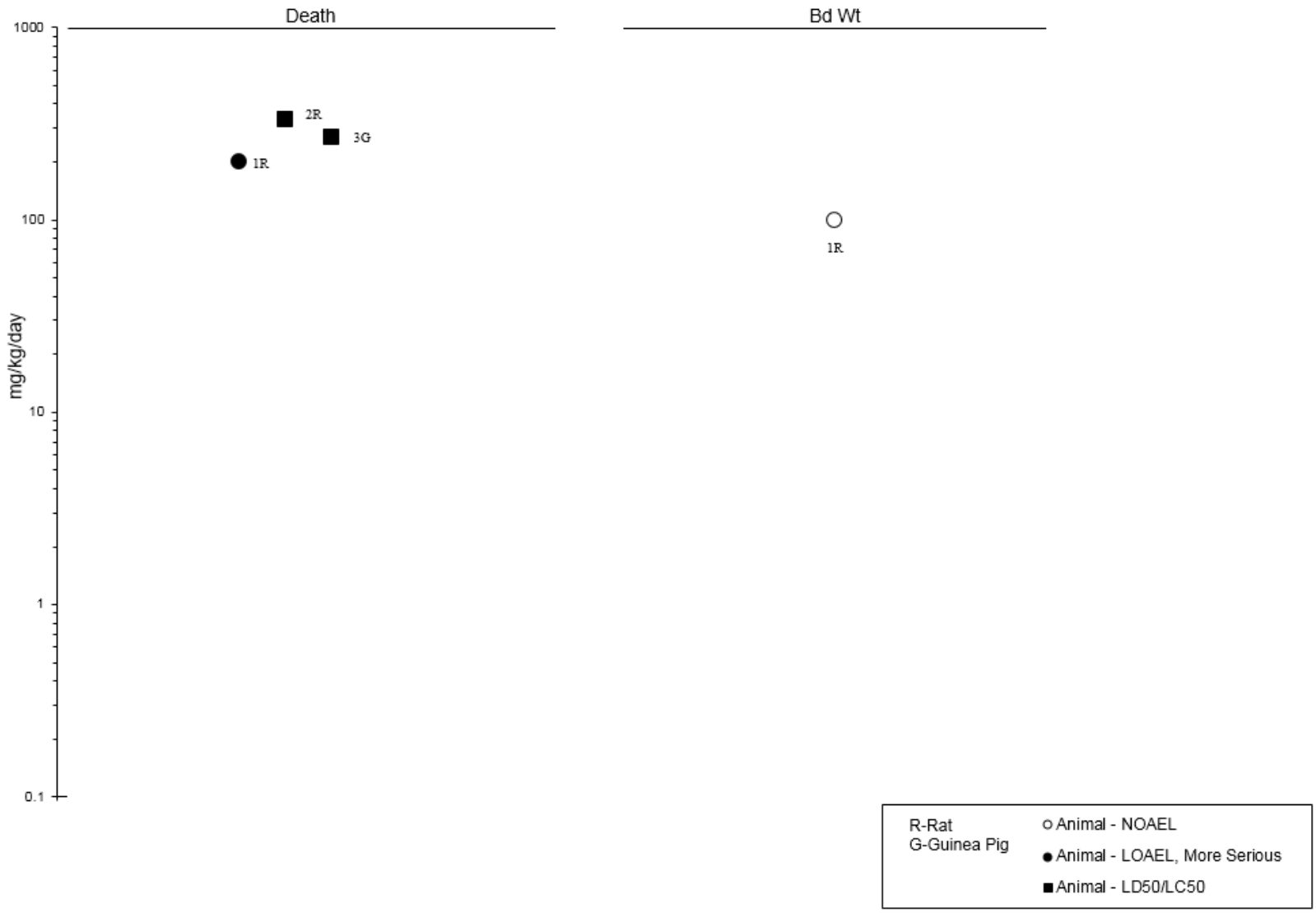


**Figure 2-3. Levels of Significant Exposure to Ethylene Oxide – Oral**
Intermediate (15-364 days)

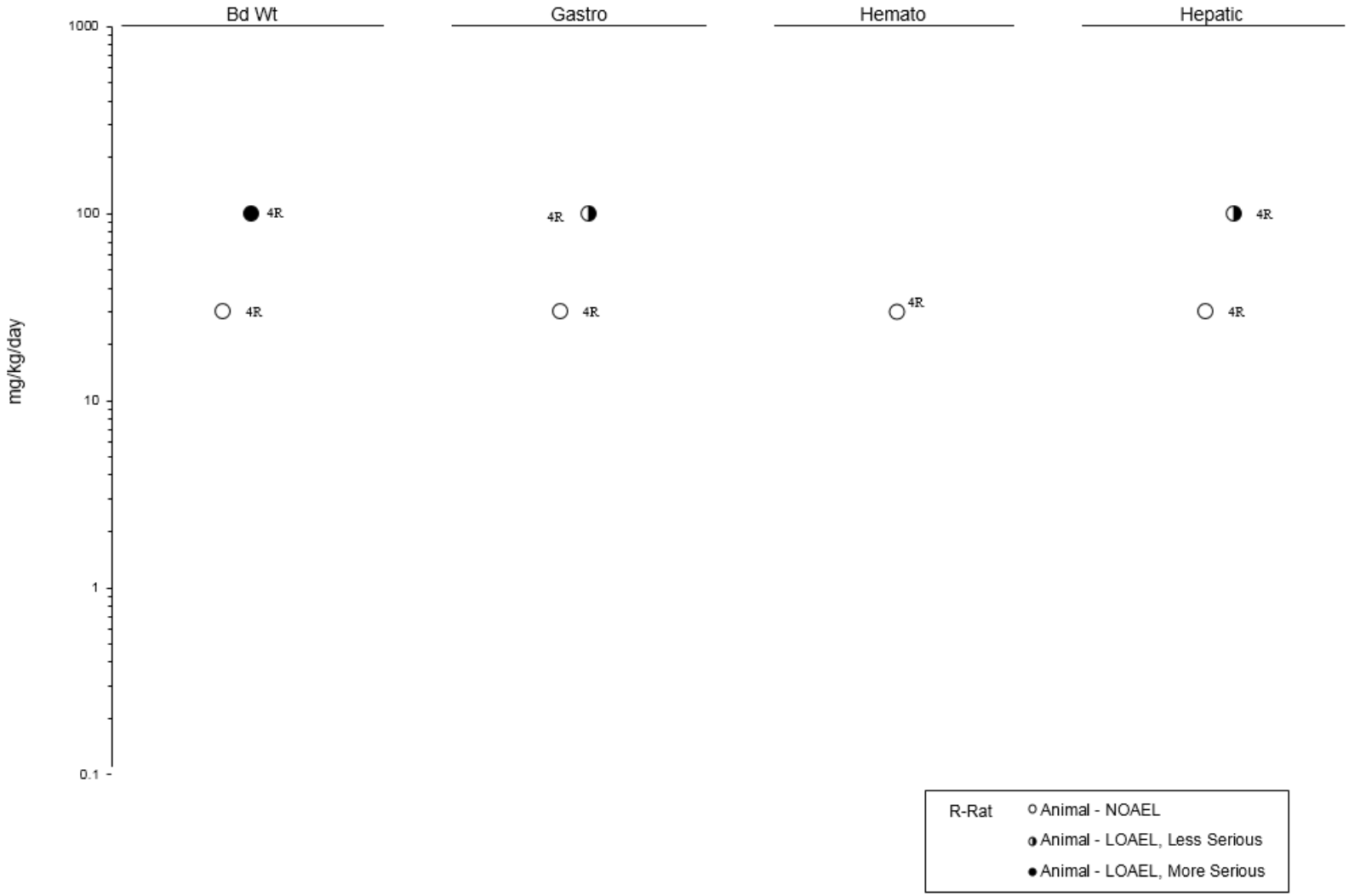
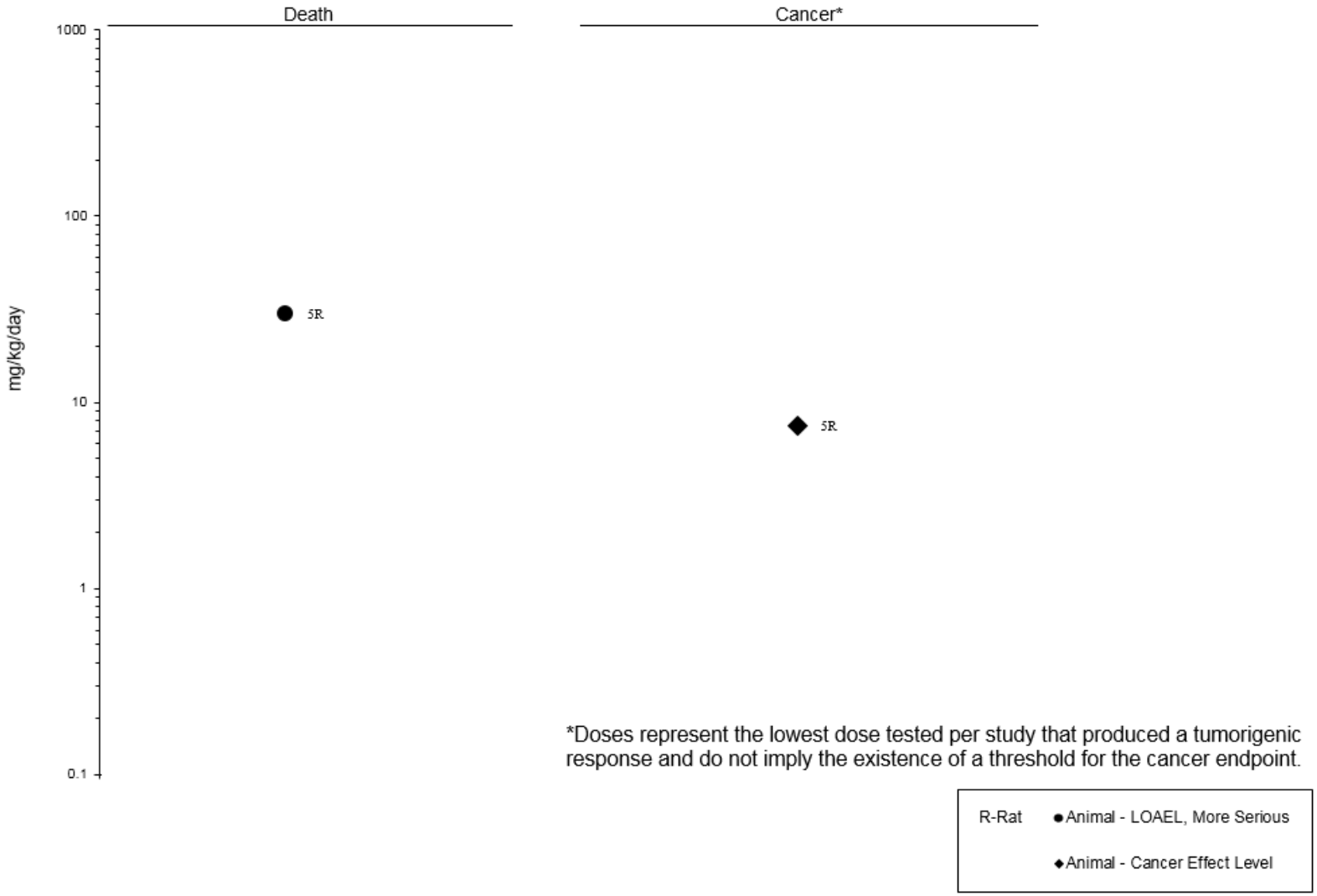


**Figure 2-3. Levels of Significant Exposure to Ethylene Oxide – Oral**
Chronic (≥365 days)



## 2.2 DEATH

Results from a number of epidemiological studies involving occupational exposure to ethylene oxide and risk of death from various cancer types (particularly all cancers, lymphohematopoietic cancer, leukemia, stomach cancer, pancreatic cancer, and brain and nervous system cancer) are summarized in Section 2.19.

Information regarding death in experimental animals following inhalation exposure to ethylene oxide is available. Limited information is available for the oral exposure route. No information was located for the dermal exposure route.

For rats exposed once to ethylene oxide vapor, 1-hour $LC_{50}$ values were 5,748 and 4,439 ppm for males and females, respectively; 4-hour $LC_{50}$ values were 1,972 and 1,537, respectively (Snellings et al. 2011). A 4-hour $LC_{50}$ of 1,460 ppm was reported for male rats (Jacobson et al. 1956). Mice and dogs were more sensitive to acute lethality than rats; reported 4-hour $LC_{50}$ values were 835 and 960 ppm, respectively (Jacobson et al. 1956). Lethality occurred in rats, mice, rabbits, guinea pigs, and monkeys following single or repeated inhalation exposure at 204–841 ppm for periods up to 6 weeks (Hollingsworth et al. 1956; Jacobson et al. 1956; NTP 1987). Studies that employed repeated exposure of rats for up to 2 years reported increased mortality at exposures as low as 100 ppm (Garman et al. 1986; Lynch et al. 1984a, 1984b; Snellings et al. 1984b).

Hollingsworth et al. (1956) reported 100% mortality among rats (5/sex) gavaged once at 200 mg/kg. Dunkelberg (1982) reported decreased length of survival among 50 female rats gavaged at 30 mg/kg/day, 2 times/week, for up to 150 weeks.

## 2.3 BODY WEIGHT

No studies were located regarding body weight effects in humans following inhalation, oral, or dermal exposure to ethylene oxide.

Information for the inhalation exposure route is available for body weight effects in experimental animals. Limited information is available for the oral exposure route. No information was located for the dermal exposure route.

Depressed body weight was reported in a few acute-duration studies of rats or mice repeatedly exposed to ethylene oxide vapor at levels in the range of 357–1,200 ppm (Hollingsworth et al. 1956; Saillenfait et al. 1996) and in mature F1 males exposed to 33 ppm during a 10-week mating period (EPA 1994). Decreases in final body weights (10–20%) were observed in rats and guinea pigs exposed to 113–204 ppm ethylene oxide for up to 226 days (Hollingsworth et al. 1956). Hollingsworth et al. (1956) also reported "markedly subnormal growth" (magnitude not reported) among rats, mice, rabbits, guinea pigs, and/or monkeys intermittently exposed to ethylene oxide vapor at 357 ppm for up to 85 days. Other acute- and intermediate-duration inhalation studies of rats, mice, and/or rabbits found no body weight effects at exposure levels in the range of 100–500 ppm (Jacobson et al. 1956; Matsuoka et al. 1990; Mori et al. 1991a, 1991b; Neeper-Bradley and Kubena 1993; NTP 1987; Ohnishi et al. 1985; Saillenfait et al. 1996; Snellings et al. 1982a, 1982b). In 2-year rat studies that employed repeated exposure to ethylene oxide vapor, 12–18% depressed body weight gain was reported at an exposure level of 100 ppm (Garman et al. 1986; Lynch et al. 1984a, 1984b). No body weight effects were observed for mice repeatedly exposed at up to 100 ppm for 102 weeks (NTP 1987).

Hollingsworth et al. (1956) found no body weight effects among rats gavaged once at 100 mg/kg/day. Repeated dosing of female rats at 100 mg/kg/day resulted in an unspecified magnitude of weight loss; the NOAEL was 30 mg/kg/day.

## 2.4 RESPIRATORY

Limited data are available regarding ethylene oxide-related respiratory effects in humans. Inhalation of ethylene oxide is irritating to mucous membranes including those associated with the respiratory system. Inhalation exposure of workers to high concentrations of ethylene oxide for brief periods has resulted in bronchitis, pulmonary edema, and emphysema (Thiess 1963). Deschamps et al. (1992) reported a case of persistent asthma in a 35-year-old male who was highly exposed to ethylene oxide from a leaky railroad tank. Pulmonary function remained compromised when tested 1 and 3 years after the accidental exposure. There was no evidence of increased risk of death from non-malignant respiratory disease within various cohorts of workers involved in production or use of ethylene oxide (Bisanti et al. 1993; Coggon et al. 2004; Hogstedt 1988; Morgan et al. 1981; Steenland et al. 1991; Swaen et al. 2009; Wong and Trent 1993).

Information is available regarding respiratory effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

Dyspnea was observed after 4 hours of exposure of mice to ethylene oxide vapor at a lethal exposure level of 800 ppm (NTP 1987). Adverse respiratory effects (e.g., dyspnea, pulmonary edema, pulmonary hemorrhage and congestion, "severe lung injury") were reported for experimental animals (rats, mice, and/or guinea pigs) exposed to 357–841 ppm ethylene oxide vapor for acute durations (Hollingsworth et al. 1956; NTP 1987). Rhinitis was also observed in mice exposed to 400 ppm ethylene oxide for up to 2 weeks (NTP 1987). Intermediate-duration studies reported labored breathing and nasal discharge in rats exposed to 406 ppm for 6 weeks (Jacobsen et al. 1956), an increase in relative lung weight in rats and guinea pigs exposed to 113–204 ppm for up to 226 days (Hollingsworth et al. 1956), rhinitis in mice exposed to 200 ppm for up to 14 weeks (NTP 1987), and pulmonary congestion and alveolar collapse in dogs exposed to 292 ppm for 6 weeks (Jacobsen et al. 1956). Acute bronchopneumonia, chronic pneumonia, pulmonary edema, and suppurative rhinitis were observed in rats exposed at 50 ppm for 104 weeks (Lynch et al. 1984a, 1984b). However, all groups of rats in this study (including controls) experienced a pulmonary bacterial infection as early as 8 months into the treatment period and were treated at months 8, 16, and 20. The infection likely played a significant role in the reported respiratory effects. There were no indications of exposure-related respiratory effects in rats exposed to 100 ppm as part of a 2-generation reproduction study (EPA 1994) or in mice repeatedly exposed at up to 100 ppm for 102 weeks (NTP 1987).

## 2.5 CARDIOVASCULAR

Limited data are available regarding ethylene oxide-related cardiovascular effects in humans. There was no evidence of increased risk of death from cardiovascular or cerebrovascular diseases within various cohorts of workers involved in production or use of ethylene oxide (Bisanti et al. 1993; Coggon et al. 2004; Hagmar et al. 1991; Hogstedt 1988; Kiesselbach et al. 1990; Morgan et al. 1981; Olsen et al. 1997; Steenland et al. 2004; Swaen et al. 2009; Wong and Trent 1993).

Limited information is available regarding cardiovascular endpoints in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

Lynch et al. (1984b) found no effects on electrocardiograms of male monkeys during repeated exposure to ethylene oxide vapor at 50 or 100 ppm for up to 2 years. There was no histopathological evidence of ethylene oxide-induced cardiovascular effects in mice repeatedly exposed at 100 ppm for 102–104 weeks (Lynch et al. 1984a, 1984b; NTP 1987).

## 2.6 GASTROINTESTINAL

Limited information is available regarding the potential for ethylene oxide-induced gastrointestinal effects in humans. Nausea and vomiting have been reported, but these are considered to be secondary effects due to neurotoxicity rather than a primary effect of inhaled ethylene oxide on the gastrointestinal tract. There was no evidence of increased risk of death from gastrointestinal effects within cohorts of workers with potential for ethylene oxide exposure (Bisanti et al. 1993; Morgan et al. 1981; Steenland et al. 1991; Wong and Trent 1993).

Limited information is available regarding gastrointestinal effects in experimental animals following inhalation or oral exposure to ethylene oxide. No information was located for the dermal exposure route.

There was no histopathological evidence of gastrointestinal effects in rats or mice repeatedly exposed to ethylene oxide vapor at 100 ppm for 102–104 weeks (Lynch et al. 1984a, 1984b; NTP 1987). Hollingsworth et al. (1956) reported gastric irritation in female rats following repeated gavage exposure at 100 mg/kg/day; the NOAEL was 30 mg/kg/day.

## 2.7 HEMATOLOGICAL

Limited human data are available. Joyner (1964) reported no effects on hemoglobin levels or red blood cell (RBC) or white blood cell (WBC) counts in workers exposed to ethylene oxide at about 5–10 ppm for approximately 10 years.

Information is available regarding hematological effects in experimental animals following inhalation or oral exposure to ethylene oxide. No information was located for the dermal exposure route.

Decreases in hemoglobin, hematocrit, erythrocyte count, packed cell volume, and/or increased reticulocytes were reported in experimental animals (rats, mice, dogs) repeatedly exposed to ethylene oxide vapor at 250–500 ppm for 6–13 weeks (Fujishiro et al. 1990; Jacobson et al. 1956; Snellings et al. 1984a). Hematological alterations indicative of normochromatic anemia were also observed in dogs exposed to 102 ppm for 6 months (Jacobson et al. 1956). Splenic extramedullary hematopoiesis was reported in rats repeatedly exposed at 50 or 100 ppm for 104 weeks and splenic focal fibrosis was noted at 100 ppm (Lynch et al. 1984a, 1984b). There was no histopathological evidence of ethylene oxide-

induced hematological effects in mice intermittently exposed at 100 ppm for up to 102 weeks (NTP 1987).

Hollingsworth et al. (1956) found no evidence of exposure-related hematological effects in female rats repeatedly gavaged with ethylene oxide at up to 30 mg/kg/day.

## 2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans exposed to ethylene oxide.

Information is available regarding musculoskeletal effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

Histopathologic indicators of muscular atrophy were reported for experimental animals (rats, rabbits, monkeys, dogs) repeatedly exposed to ethylene oxide vapor for 6 weeks to 226 days at exposure levels in the range of 204–357 ppm (Hollingsworth et al. 1956; Jacobson et al. 1956). There was no indication of musculoskeletal effects in mice repeatedly exposed to ethylene oxide vapor for up to 14 weeks at 250 ppm (Snellings et al. 1984a). However, multifocal myopathy in skeletal muscle was reported in rats following inhalation exposure at 100 ppm for 104 weeks; the NOAEL was 50 ppm (Lynch et al. 1984a, 1984b). See Section 2.15 for a discussion of neuromuscular alterations.

## 2.9 HEPATIC

Information regarding hepatic effects in humans after inhalation exposure to ethylene oxide is limited to a report by Joyner (1964). The results suggested that workers exposed to about 5–10 ppm for 10.7 years did not have major signs of hepatic toxicity such as jaundice or palpable liver.

Limited information is available regarding hepatic effects in experimental animals following inhalation or oral exposure to ethylene oxide. No information was located for the dermal exposure route.

Hollingsworth et al. (1956) reported slight discoloration and fatty degeneration in livers from rats and guinea pigs exposed to ethylene oxide vapor at 841 ppm for two or three 7-hour exposures. Snellings et al. (1984a) reported an elevation in the liver to body weight ratio in female mice exposed to ethylene oxide at 250 ppm for 11 weeks; however, histological examination showed that the livers were normal at

this and all other lower exposure levels for both sexes in this study. No evidence for hepatic effects were seen among rats or mice repeatedly exposed at 100 ppm during a 10-week premating period (EPA 1994) or for 50 or 100 ppm for up to 102–104 weeks (Lynch et al. 1984a, 1984b; NTP 1987).

Slight liver damage (no further details) was reported in rats following repeated gavage exposure to ethylene oxide at 100 mg/kg/day, but not in animals dosed at ≤30 mg/kg/day (Hollingsworth et al. 1956).

## 2.10 RENAL

Information regarding renal effects in humans after inhalation exposure to ethylene oxide is limited to a report by Joyner (1964) which indicates that there was no evidence of nephritis or other parenchymal disease among workers exposed to ethylene oxide at 5–10 ppm for a mean exposure time of 10.7 years.

Information is available regarding renal effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

Hollingsworth et al. (1956) reported enlargement, slight congestion, and cloudy swelling of convoluted tubules in kidneys from rats and guinea pigs exposed to ethylene oxide vapor at 841 ppm for two or three 7-hour exposures. In a 14-week study of mice repeatedly exposed to ethylene oxide vapor, renal tubular degeneration was observed in 5/10 and 6/10 males exposed at 100 and 200 ppm, respectively, and in 8/10 females exposed at 200 ppm (NTP 1987). Renal tubular necrosis was observed in 1/10 males of the 100 and 200 ppm exposure groups. Lethal exposure levels (400 and 600 ppm) resulted in increased incidences of necrosis in both sexes exposed for up to 2 weeks. There were no indications of renal effects in rats or mice exposed at 50 or 100 ppm for up to 102–104 weeks (Lynch et al. 1984a, 1984b; NTP 1987).

## 2.11 DERMAL

Data related to human dermal exposure to ethylene oxide are generally associated with case reports of industrial accidents, some of which occurred in the 1930s and 1940s. Concentrated ethylene oxide evaporates rapidly from the skin and produces a freezing effect, often compared to frostbite, leaving burns ranging from first- to third-degree severity (Taylor 1977). Workers drenched with a 1% solution developed large vesiculated blisters (Sexton and Henson 1949).

A study using volunteers by Sexton and Henson (1950) showed that the magnitude of skin injury was related to the concentration of ethylene oxide in solution but peaked at about 50%. This was attributed to the rapid evaporation of the more concentrated solutions, which prevented more prolonged skin contact.

Case reports of patients whose intact skin or wounds had contact with gauze or other hospital supplies that had been sterilized with ethylene oxide indicated that the observed skin reactions included erythema, blister formation, scaling, crusted ulcerations, and second-degree burns (Alomar et al. 1981; Hanifin 1971; Karacalar and Karacalar 2000).

Shupack et al. (1981) demonstrated that human skin reactions to ethylene oxide in patch materials were directly related to the total dose.

Limited information is available regarding dermal effects in experimental animals following inhalation or dermal exposure to ethylene oxide. No information was located for the oral exposure route.

NTP (1987) found no evidence of dermal effects in mice repeatedly exposed to ethylene oxide vapor for up to 102 weeks at up to 100 ppm. Dermal application of ethylene oxide (10 and 50% aqueous solutions) to rabbits for ≥6 minutes resulted in hyperemia and edema (Hollingsworth et al. 1956). Dermal application of undiluted ethylene oxide to the back of rabbits (0.5 mL for 4 hours under occluded conditions) resulted in severe erythema and edema, subdermal hemorrhages, and chemical burns during 72 hours postapplication; the chemical was considered corrosive (Celanese Chem Co. 1972).

## 2.12 OCULAR

There is some evidence that occupational exposure to high levels of ethylene oxide can result in cataracts and corneal burns (McLaughlin 1946; Thiess 1963). For example, cataracts developed in four sterilizer operators who were exposed to ethylene oxide from a leaking sterilizer for up to 2 months (Gross et al. 1979; Jay et al. 1982). Because these persons could intermittently smell the fumes, a level of ≥700 ppm was estimated by the authors in retrospect. Although none of the patients were examined before this accidental exposure, the occurrence of cataracts was viewed as unlikely to be a chance occurrence in all four persons in this age range (31–35 years old) who had no systemic or ocular disease that might be associated with cataract formation.

Ocular instillation of a 1% solution of ethylene oxide-to eyes of rabbits resulted in slight corneal cloudiness (severity score 0.8–1.9; maximum possible score of 4.0) during 48 hours postinstillation (McDonald et al. 1977). Lynch et al. (1984a, 1984b) reported cataracts in monkeys and rats intermittently exposed to ethylene oxide vapor for 2 years. Incidences in 50 and 100 ppm groups of monkeys were 2/11 and 3/12, respectively, compared to 0/11 among controls. Incidences in 50 and 100 ppm groups of rats were 3/79 and 9/78, respectively, compared to 2/77 among controls.

## 2.13 ENDOCRINE

No information was located regarding endocrine effects in humans associated with ethylene oxide exposure.

Information is available regarding endocrine effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

Hollingsworth et al. (1956) reported pale coloration and enlargement of adrenals, and numerous fat vacuoles in adrenal cortex from rats and guinea pigs exposed two or three times to ethylene oxide vapor at 841 ppm for 7 hours per exposure. Lynch et al. (1984a, 1984b) reported multifocal cortical vacuolation and hyperplasia in adrenal glands from rats intermittently exposed to ethylene oxide vapor at 50 ppm for up to 104 weeks. There was no histopathological evidence of ethylene oxide-induced effects on thyroid, parathyroid, adrenals, or pituitary of mice intermittently exposed at 100 ppm for up to 102 weeks (NTP 1987).

## 2.14 IMMUNOLOGICAL

The immunological effects of human inhalation exposure to ethylene oxide were studied in workers employed for up to 14 years in an ethylene oxide manufacturing plant. Workplace concentrations were generally <0.05 ppm (the detection limit of the analytical method) with occasional peaks of 8 ppm during the 4 years that the air was monitored. There was no effect on any of the blood parameters relating to immune function that were investigated, including T and B lymphocyte counts, lymphocyte activation, and serum IgG, IgM, and IgA levels (Van Sittert et al. 1985).

Theiss (1963) did not observe skin sensitization in ethylene oxide plant workers (average exposure: 10.4 years) who were challenged with a single dermal application of 1% ethylene oxide. However,

ethylene oxide was implicated as a skin sensitizer in studies of volunteers following dermal exposure (Sexton and Henson 1950; Shupack et al. 1981). Contact dermatitis and delayed-type hypersensitivity dermatitis have been observed in case reports of ethylene oxide-exposed health care workers and patients (Alomar et al. 1981; Belen and Polat 2015; Brashear et al. 1996; Caroli et al. 2005; Dagregorio and Guillet 2004; Kerre and Goossens 2009; Lerman et al. 1995; Romaguera and Vilaplana 1998).

Limited information is available regarding immunological effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

Thymic lymphocytic hypoplasia was reported for male and female mice intermittently exposed to ethylene oxide vapor at 400 ppm for up to 2 weeks; at 600 ppm, necrosis was observed in the thymus (males and females) and spleen (males) (NTP 1987).

## 2.15 NEUROLOGICAL

Neurological effects have frequently been reported in association with human and animal exposure to ethylene oxide via inhalation at a wide range of concentrations and exposure durations.

In humans exposed to high levels of ethylene oxide in occupational settings, headache, nausea, and vomiting were reported (Blackwood and Erskine 1938; Sexton and Henson 1949; von Oettingen 1939). Exposure levels were not measured or estimated in these situations.

Neuropathy, impaired hand-eye coordination, cognitive dysfunction, memory loss, headache, and hand numbness were reported in case studies of workers exposed to ethylene oxide for various durations (Brashear et al. 1996; Crystal et al. 1988; Dretchen et al. 1992; Estrin et al. 1987; Finelli et al. 1983; Kuzuhara et al. 1983; Salinas et al. 1981; Schröder et al. 1985; Zampollo et al. 1984). These effects were seen at estimated average exposure levels as low as 3 ppm; however, short-term exposures may have been as high as 700 ppm for some of these workers. Sural nerve biopsies revealed axonal degeneration and regeneration in two studies (Kuzuhara et al. 1983; Schröder et al. 1985).

Information on the neurological effects of inhalation exposure to ethylene oxide has also been derived from case studies of longer-term occupational exposure. Headaches, nausea, vomiting, clumsiness, blunting of the senses, lethargy, numbness, and weakness in the extremities were reported among four sterilizer operators exposed to ethylene oxide for up to 2 months on an intermittent basis at levels of

approximately 700 ppm (estimated by the authors based on the fact that the exposed workers could smell the vapors emitted from a leaking apparatus) (Gross et al. 1979). One of the operators experienced recurrent major motor seizures at 20–30-minute intervals near the end of the work shift; nerve conduction testing indicated sensorimotor neuropathy.

Information is available regarding neurological effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

Incoordination and semiconsciousness were reported during a 4-hour exposure of mice to ethylene oxide vapor at 1,600 ppm (NTP 1987). Repeated inhalation exposures of experimental animals resulted in neurological effects at lower exposure levels. Effects including impaired sensory and motor function (particularly in hindlimbs), altered gait, slight tremors, various degrees of hindlimb paralysis, and peripheral neuropathy have been reported in experimental animals intermittently exposed to ethylene oxide vapor at 100–500 ppm for periods in the range of 48–226 days (Hollingsworth et al. 1956; Jacobson et al. 1956; Kaido et al. 1992; Matsuoka et al. 1990; Ohnishi et al. 1985; Snellings et al. 1984a).

In a 9-month study of rats exposed to ethylene oxide at 250 ppm, retarded growth and maturation of myelinated fibers and mild axonal degeneration in hindleg nerves were observed in the absence of clinical signs of neuropathy (Ohnishi et al. 1986). Snellings et al. (1984a) reported clinical signs of neurotoxicity (hunched posture during gait, reduced locomotor activity) in mice intermittently exposed to ethylene oxide vapor for 10–11 weeks at an exposure level as low as 50 ppm. There were no indications of ethylene oxide exposure-related neurological effects in a 2-generation study of rats exposed to concentrations as high as 100 ppm (EPA 1994) or in a 2-year study of mice intermittently exposed at airborne concentrations as high as 100 ppm (NTP 1987). Lynch et al. (1984a) reported axonal dystrophy in the brain of 1/2 control monkeys and 2/2 and 1/2 monkeys exposed at 50 and 100 ppm, respectively. Demyelination was reported in 1/2 monkeys at the 50 and 100 ppm exposure levels. However, the results could not be specifically attributed to ethylene oxide exposure due to the small number of animals evaluated and the reported histopathologic brain lesion in a control animal.

Nagata et al. (1992) designed a study to investigate potential mechanisms of ethylene oxide neurotoxicity in the rat. Groups of male Wistar rats (5/group) were exposed to ethylene oxide vapor at 0 or 500 ppm for 6 hours/exposure, 3 days/week, for 15 weeks. Following the final exposure period, $^{35}$S-methionine was injected into the right dorsal root ganglion to evaluate rapid anterograde axonal transport. The velocity in the ethylene oxide-exposed rats was 33% slower than that of controls. Morphometric analysis of selected

portions of sural and peroneal nerve preparations revealed significantly greater incidental degeneration of myelinated fibers from the ethylene oxide-exposed rats than from controls. The study authors suggested that the morphological changes and decreased axonal transport velocity might play a causative role in the development of peripheral neuropathy from chronic ethylene oxide exposure.

## 2.16 REPRODUCTIVE

Limited information is available regarding reproductive effects in humans associated with ethylene oxide exposure. In an epidemiological study by Hemminki et al. (1982), the spontaneous abortion rates in ethylene oxide sterilizer workers in hospitals in Finland were higher than those of non-exposed workers. Although exposure levels were not measured, the authors estimated that 8-hour weighted mean concentrations ranging from 0.1 to 0.5 ppm with peaks to 250 ppm were associated with adverse outcomes. Limitations in the design and implementation of this study include recall bias, prior knowledge of the questionnaires, and analysis based on too few pregnancies. Decreased sperm counts in ethylene oxide workers were reported by Abrahams (1980). However, based on the small number of sperm samples obtained, the author viewed the results as inconclusive.

Rowland et al. (1996) evaluated possible associations between ethylene oxide exposure and the risk of spontaneous abortion, preterm birth, and postterm birth in a cross-sectional study of 1,320 female dental assistants (32 with self-reported ethylene oxide exposure and 1,288 unexposed). After adjusting for age, relative risks (RRs) were 2.5 (95% confidence interval [CI] 1.0–6.3) for spontaneous abortion, 2.7 (95% CI 0.8–8.8) for preterm birth, and 2.1 (95% CI 0.7–5.9) for postterm birth. Gresie-Brusin et al. (2007) evaluated risks of spontaneous abortion and pregnancy loss within 98 singleton pregnancies among women with ethylene oxide exposure in sterilizing units of 22 hospitals in South Africa. The study subjects were grouped according to “high” exposure (sterilizer operators) and “low” exposure (not directly involved in ethylene oxide sterilization). Prevalence odds ratios (PORs) were 20.8 (95% CI 2.1–199) for risk of spontaneous abortion and 8.6 (95% CI 1.8–43.7) for pregnancy loss.

Information is available regarding reproductive effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

Increased incidence of resorptions, decreased numbers of pups per litter, and decreased numbers of fetuses born relative to numbers of implantation sites were reported in a study of female rats intermittently exposed to ethylene oxide vapor at 150 ppm for 3 weeks premating and during gestation

days 1–16 (NIOSH 1982). In a 2-generation study, increased post-implantation loss was observed in F0 rats exposed to 33 ppm (EPA 1994) and decreased numbers of live pups per litter were observed at 100 ppm in the F1 and F2 generations (EPA 1994). Exposure-related effects on male reproductive organs (decreases in testicular and epididymal weights, germ cell survival, sperm count; histopathologic lesions in seminiferous tubules) have been reported for rats following intermittent inhalation exposure to ethylene oxide vapor for 6–13 weeks at exposure levels in the range of 250–500 ppm (Kaido et al. 1992; Mori et al. 1991a, 1991b). Intermittent inhalation exposure of monkeys to ethylene oxide vapor at 50 ppm (the lowest exposure level tested) for 24 months resulted in decreases in sperm count (28% less than controls) and motility (32% less than controls); however, reproductive function was not tested (Lynch et al. 1984a). There was no evidence of histopathological lesions in reproductive organs of rats or mice intermittently exposed for 102–104 weeks at 100 ppm (highest exposure level tested) (Lynch et al. 1984a, 1984b; NTP 1987).

## 2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans after inhalation exposure to ethylene oxide.

Information is available regarding developmental effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

Decreases in fetal body weight and crown-rump length and increased incidence of reduced ossification were reported following intermittent exposure of maternal rats to ethylene oxide vapor at 150 ppm (the only exposure level tested) for 3 weeks premating and during gestation days 1–16 (NIOSH 1982). Depressed fetal weight (3–9% less than controls) was noted in other studies of maternal rats intermittently exposed to ethylene oxide vapor in the range of 100–800 ppm during gestation (Neeper-Bradley and Kubena 1993; NIOSH 1982; Saillenfait et al. 1996; Snellings et al. 1982a). Decreases in the number of pups/litter and the ratio of fetuses born to implantation sites were reported in a study of rats intermittently exposed to ethylene oxide vapor at 100 ppm for 12 weeks prior to mating and throughout gestation and lactation periods (Snellings et al. 1982b). In a 2-generation study, decreased pup body weight was observed in the F1 and F2 generations at 33 and 100 ppm (EPA 1994). Saillenfait et al. (1996) reported increased incidence of dilation in renal pelvis and ureter of rat fetuses following intermittent maternal exposure at 1,200 ppm during gestation days 6–15. Fetal defects (predominantly hydrops and ocular defects) were reported following maternal exposure of mice to ethylene oxide vapor at 1,200 ppm for a

single 1.5-hour exposure at timepoints between 1 and 25 hours postmating (Rutledge and Generoso 1989). There were no indications of ethylene oxide exposure-related developmental effects in rabbit fetuses following intermittent inhalation exposure of their mothers at 150 ppm during gestation days 7–19 or 1–19 (NIOSH 1982).

## 2.18 OTHER NONCANCER

No information was located regarding other noncancer effects in humans or animals following exposure to ethylene oxide.

## 2.19 CANCER

A number of epidemiological studies have examined possible associations between occupational exposure to ethylene oxide and risk of cancer. Epidemiological data for ethylene oxide include limitations such as lack of personal estimations of ethylene oxide exposure levels, concomitant exposure to other toxic substances, small numbers of subjects, and/or small numbers of selected cancer deaths.

Ethylene oxide was first produced using a chlorohydrin process (reaction of ethylene gas with hypochlorous acid to produce ethylene chlorohydrin, which was reacted with calcium oxide to produce ethylene oxide). Workers involved with this process were exposed to a variety of chemicals, including organochlorine byproducts. The chlorohydrin process was inefficient and was gradually replaced with a direct vapor phase oxidation process (oxidation of ethylene gas in the presence of oxygen and silver catalyst at 10–30 atmospheres of pressure). Workers involved in the oxidation process did not experience exposures to the various chemicals used or produced during the chlorohydrin process. Available epidemiological studies include evaluation of cancer risk among cohorts of production workers involved in the chlorohydrin process and/or the ethylene oxidation process. Sterilization processes using ethylene oxide do not include exposures to the variety of chemicals encountered in the chlorohydrin process of ethylene oxide production.

IARC and EPA have evaluated ethylene oxide for carcinogenicity (EPA 2016a; IARC 2008, 2012). Both agencies concluded that the most convincing evidence for increased risk among workers exposed to ethylene oxide is for lymphohematopoietic cancers and female breast cancer. The discussion of the epidemiological database in this toxicological profile focuses on evaluations of ethylene oxide and risk of lymphohematopoietic cancers, leukemia, myeloma/multiple myeloma, non-Hodgkin’s lymphoma,

lymphosarcoma/reticulosarcoma, and breast cancer. Results from a few epidemiological studies and animal studies suggested associations between ethylene oxide exposure and cancer at other sites (e.g., stomach, pancreas, nervous system).

Selected study details for cohorts evaluated for possible associations between exposure to ethylene oxide and selected cancer endpoints are presented in Table 2-3. Study results for leukemia, non-Hodgkin's lymphoma, breast cancer, lymphohematopoietic cancer, myeloma/multiple myeloma, and lymphosarcoma/reticulosarcoma are summarized in Figures 2-4, 2-5, 2-6, 2-7, 2-8, and 2-9, respectively.

Figures 2-4, 2-5, 2-6, 2-7, 2-8, and 2-9 include information on cohort size, exposure via production or use in sterilization processes, number of observed cancers/expected number, and the plotted risk estimates with 95% CIs. The footnote ([1]) is used to identify cohorts exposed during ethylene oxide production via the chlorohydrin process. The footnote ([2]) is used to identify cohorts exposed during ethylene oxide production via the nonchlorohydrin process. Some studies included cohorts that may have been exposed during production of ethylene oxide by the chlorohydrin process in earlier years and by direct oxidation during later years; therefore, these cohorts were not assigned a footnote. The study of Kiesselbach et al. (1990) did not specify a method of ethylene oxide production.

***Case Reports.*** Hogstedt et al. (1979a) reported 3 cases of leukemia within a group of 230 workers at a Swedish facility where hospital equipment was sterilized using ethylene oxide. The study authors estimated an 8-hour time-weighted average (TWA) ethylene oxide level of 20±10 ppm in a storage hallway, which was considered higher than levels in the sterilization area. According to national statistics, 0.2 leukemia cases would have been expected. In a 5-year update of the study, 4 cases of lymphohematopoietic cancer (0.3 expected) were observed among 203 of the workers employed ≥1 year (Hogstedt et al. 1986).

Tompa et al. (1999) reported 8 cases of breast cancer within a group of 98 nurses exposed to ethylene oxide for 5–15 years in a hospital in Hungary. Reported ethylene oxide concentrations in the working area ranged from 5 to 150 mg/m$^3$ (2.75–82.5 ppm). However, the study did not account for natural low dose radioactivity from radon in local drinking water, genetic predisposition, or effects of potential environmental, occupational, and/or lifestyle confounders.

**Table 2-3. Study Design Details for Selected Cohorts of Workers Exposed to Ethylene Oxide**

| Reference/cohort description | Comments |
|---|---|
| **Hogstedt et al. 1979b** | |
| 241 male workers at a Swedish ethylene oxide production facility using the chlorohydrin method of production | Workers employed ≥1 year; 10-year latency applied; study included years 1961–1977; expected deaths based on Swedish national rates: |
| 66 unexposed workers (955 person-years) | O/E ratio 0/0 for leukemia; no reported SMR |
| 89 full-time production workers (1,324 person-years) | O/E ratio 2/0.14 for leukemia; no reported SMR |
| 86 maintenance workers (1,211 person-years) | O/E ratio 1/0.13 for leukemia; no reported SMR |
| **Hogstedt et al. 1986 (5-year update of Hogstedt et al. 1979b subcohort)** | |
| 89 full-time production workers (chlorohydrin method) employed ≥1 year with 10-year latency | Results for leukemia and lymphohematopoietic cancers are summarized in Figures 2-4 and 2-7, respectively; update included the years 1961–1982; expected deaths based on Swedish national rates; the study authors noted that excess mortality was most pronounced among workers with ≥10 years of exposure. |
| **Hogstedt 1988 (8-year update of Hogstedt et al. 1979b cohort)** | |
| 233 male workers at a Swedish ethylene oxide production facility using the chlorohydrin method of production | Update included the years 1962–1985; restricted to male production workers at chlorohydrin unit and employed ≥1 year: |
| 66 unexposed workers | O/E ratio 0/0.1 for leukemia; no reported SMR |
| 89 exposed operators | O/E ratio 2/0.2 for leukemia; no reported SMR |
| 78 exposed repairmen | O/E ratio 1/0.2 for leukemia; no reported SMR |
| 167 exposed operators and repairmen | O/E ratio 3/0.4 for leukemia reported SMR 7.03 (no reported 95% CI) |
| **Hogstedt 1988 (follow-up of combined cohorts of Hogstedt et al. 1979a, 1979b, 1986)** | |
| 709 workers (539 men, 170 women) at production facility using the chlorohydrin process, a production facility using ethylene oxidation process, and an ethylene oxide sterilization unit | Update performed through 1985; most excess mortality attributed to the facility using chlorohydrin method to produce ethylene oxide.<br>O/E ratio for leukemia 7/0.8; SMR 9.21 (no 95% CI)<br>O/E ratio for blood/lymphatic cancers 9/2; SMR 4.59 (no 95% CI) |
| 539 male workers only | Results for leukemia and lymphohematopoietic cancers are summarized in Figures 2-4 and 2-7, respectively. |

**Table 2-3. Study Design Details for Selected Cohorts of Workers Exposed to Ethylene Oxide**

| Reference/cohort description | Comments |
|---|---|
| **Hagmar et al. 1991** | |
| 2,170 ethylene oxide-exposed workers (58,220 person-years) at two Swedish plants producing disposable medical equipment; 1,151 workers (594 men, 557 women) at plant A employed ≥12 months during 1964–1985; 1,019 workers (267 men, 752 women) at plant B employed ≥12 months during 1964–1985 | Results for leukemia, female breast cancer, and lymphohematopoietic cancers are summarized in Figures 2-4, 2-6, and 2-7, respectively.<br>Expected cancer incidences based on rates in the surrounding county; exposure estimates up to 10–40 ppm during 1970–1981 and ≤3 ppm during 1982–1986 in plant A and up to 10–75 ppm during 1964–1975 and ≤4 ppm during 1976–1986 in plant B |
| **Hagmar et al. 1995 (4-year update of Hagmar et al. 1991 cohort)** | |
| 2,170 (1,309 women and 861 men) ethylene oxide-exposed workers (24,851 person-years) at plants A and B with no induction latency<br>1,649 ethylene oxide-exposed workers (7,326 person-years) at plants A and B with ≥10-year induction latency | Results for leukemia, female breast cancer, lymphohematopoietic cancers, and multiple myeloma are summarized in Figures 2-4, 2-6, 2-7, and 2-8, respectively.<br>2 leukemia cases (0.28 expected) among 930 workers with at least 0.14 ppm-years of cumulative exposure to ethylene oxide and ≥10-year induction latency considered “minor evidence” of association (SIR 7.14; 95% CI 0.87–25.8). |
| **Mikoczy et al. 2011 (20-year update of Hagmar et al. 1991 cohort)** | |
| 2,171 ethylene oxide-exposed workers (58,220 person-years); no induction latency period | Results for leukemia, non-Hodgkin’s lymphoma, female breast cancer, lymphohematopoietic cancers, and multiple myeloma are summarized in Figures 2-4, 2-5, 2-6, 2-7, and 2-8, respectively. |
| 2,046 ethylene oxide-exposed workers (58,220 person-years; induction latency ≥15 years | Results for leukemia, non-Hodgkin’s lymphoma, female breast cancer, lymphohematopoietic cancers, and multiple myeloma are summarized in Figures 2-4, 2-5, 2-6, 2-7, and 2-8, respectively. |
| **Gardner et al. 1989** | |
| 1,471 workers (1,470 men, 1 woman) in England and Wales employed at four companies that produced/used ethylene oxide | Result for leukemia is summarized in Figure 2-4.<br>O/E ratio 2/1.04 for non-Hodgkin’s lymphoma; no reported 95% Cis.<br>O/E ratio 0/0.06 for breast cancer; no reported 95% Cis. |
| 1,405 workers (394 men, 1,011 women) at hospital sterilization units in England and Wales | No leukemia deaths; 0.76 expected.<br>O/E ratio 2/0.57 for non-Hodgkin’s lymphoma; no reported 95% Cis.<br>O/E ratio 4/5.91 for breast cancer; no reported 95% Cis.<br>Study spanned the years 1956–1987; expected deaths based on local and national rates; industrial hygiene data not available before 1977, but subsequent TWA |

**Table 2-3. Study Design Details for Selected Cohorts of Workers Exposed to Ethylene Oxide**

| Reference/cohort description | Comments |
|---|---|
| | exposures considered <5 ppm in most jobs and <1 ppm in many of the jobs; previous exposures likely somewhat higher. |
| **Coggon et al. 2004 (13-year update of Gardner et al. 1989 cohort)** | |
| 1,471 workers (1,470 men, 1 woman at 4 four companies that produced/used ethylene oxide) | Results for leukemia, non-Hodgkin's lymphoma, and multiple myeloma are summarized in Figures 2-4, 2-5, and 2-8, respectively. |
| 1,405 workers (394 men, 1,011 women) at hospital sterilization units in England and Wales | Results for leukemia, non-Hodgkin's lymphoma, female breast cancer, and multiple myeloma are summarized in Figures 2-4, 2-5, 2-6, and 2-8, respectively. |
| Combined cohort of 2,876 workers (1,864 men, 1,012 women) at ethylene oxide production facilities and hospital sterilization units | Results for leukemia, non-Hodgkin's lymphoma, female breast cancer, and multiple myeloma are summarized in Figures 2-4, 2-5, 2-6, and 2-8, respectively. |
| **Greenberg et al. 1990** | |
| Cohort included 2,174 ethylene oxide-exposed workers at Union Carbide Corporation chemical production facilities in West Virginia (278 workers ever assigned to chlorohydrin units; 1,896 workers never assigned to chlorohydrin units) | Study spanned the years 1940–1978; expected deaths based on U.S. general population, regional population, and 26,965 unexposed men from the same facilities. |
| Subcohort of male workers (number not specified) assigned ≥2 years to chlorohydrin units | Results for leukemia, non-Hodgkin's lymphoma, lymphohematopoietic cancers, and multiple myeloma are summarized in Figures 2-4, 2-5, 2-7, and 2-8, respectively. |
| Subcohort of male workers (number not specified) assigned ≥2 years to ethylene oxide areas, but never to chlorohydrin units | Results for leukemia, non-Hodgkin's lymphoma, lymphohematopoietic cancers, and multiple myeloma are summarized in Figures 2-4, 2-5, 2-7, and 2-8, respectively. |
| Combined cohort of 2,174 male workers ever assigned to ethylene oxide using/producing departments | Results for leukemia, non-Hodgkin's lymphoma, lymphohematopoietic cancers, and multiple myeloma are summarized in Figures 2-4, 2-5, 2-7, and 2-8, respectively. |
| **Benson and Teta 1993 (10-year update of Greenberg et al. 1990 subcohort ever assigned to chlorohydrin units)** | |
| 278 male workers ever assigned to chlorohydrin units | Results for lymphohematopoietic cancers are summarized in Figure 2-7. SMRs not calculated for leukemia or lymphosarcoma/reticulosarcoma (observed and/or expected deaths <5). |

**Table 2-3. Study Design Details for Selected Cohorts of Workers Exposed to Ethylene Oxide**

| Reference/cohort description | Comments |
|---|---|
| **Teta et al. 1993 (10-year update of a subcohort from the cohort of Greenberg et al. 1990)** | |
| 1,896 male workers (excludes 278 workers with “low” potential for exposure to ethylene oxide) | Results for leukemia and lymphohematopoietic cancers are summarized in Figures 2-4 and 2-7, respectively.<br>SMR not calculated for lymphosarcoma/reticulosarcoma (observed and expected deaths <5). |
| **Swaen et al. 2009 (25-year update of subcohort of male workers within original cohort of Greenberg et al. 1990)** | |
| Subcohort of 2,063 male workers from the original cohort of 2,174 workers | Results for leukemia, non-Hodgkin’s lymphoma, and lymphohematopoietic cancers are summarized in Figures 2-4, 2-5, and 2-7, respectively.<br>Update included years 1940–2003; original cohort of 2,174 workers from Greenberg et al. 1990 redefined, proportional hazards modeling for leukemia, lymphoid malignancies revealed no trends or associations with cumulative exposure; estimated 8-hour TWA exposure levels 0.3–21 ppm during 1940–1988 and up to 70 ppm during 1925–1939. |
| **Steenland et al. 1991 (NIOSH-based cohort)** | |
| 18,254 workers (8,214 men, 10,040 women) at 14 facilities producing sterilized medical supplies and spices; workers had at least 90 days of exposure to ethylene oxide | Results for leukemia, non-Hodgkin’s lymphoma, breast cancer, hematopoietic cancers, myeloma/multiple myeloma, and lymphosarcoma/reticulosarcoma are summarized in Figures 2-4, 2-5, 2-6, 2-7, 2-8, and 2-9, respectively.<br>Mortality evaluated through 1987 and compared to that of U.S. general population; estimated 8-hour TWA exposure levels 4.3 ppm for sterilizer operators and 2.0 ppm for other workers after 1977, likely several times higher in earlier years. |
| **Stayner et al. 1993 (evaluation includes 13 of the 14 facilities from the NIOSH-based cohort of Steenland et al. 1991)** | |
| Workers at 13 facilities producing sterilized medical supplies and spices | Results for leukemia, non-Hodgkin’s lymphoma, and hematopoietic cancers are summarized in Figures 2-4, 2-5, and 2-7, respectively.<br>One of the original 14 facilities excluded due to inadequate data for historical exposure estimation. |

**Table 2-3. Study Design Details for Selected Cohorts of Workers Exposed to Ethylene Oxide**

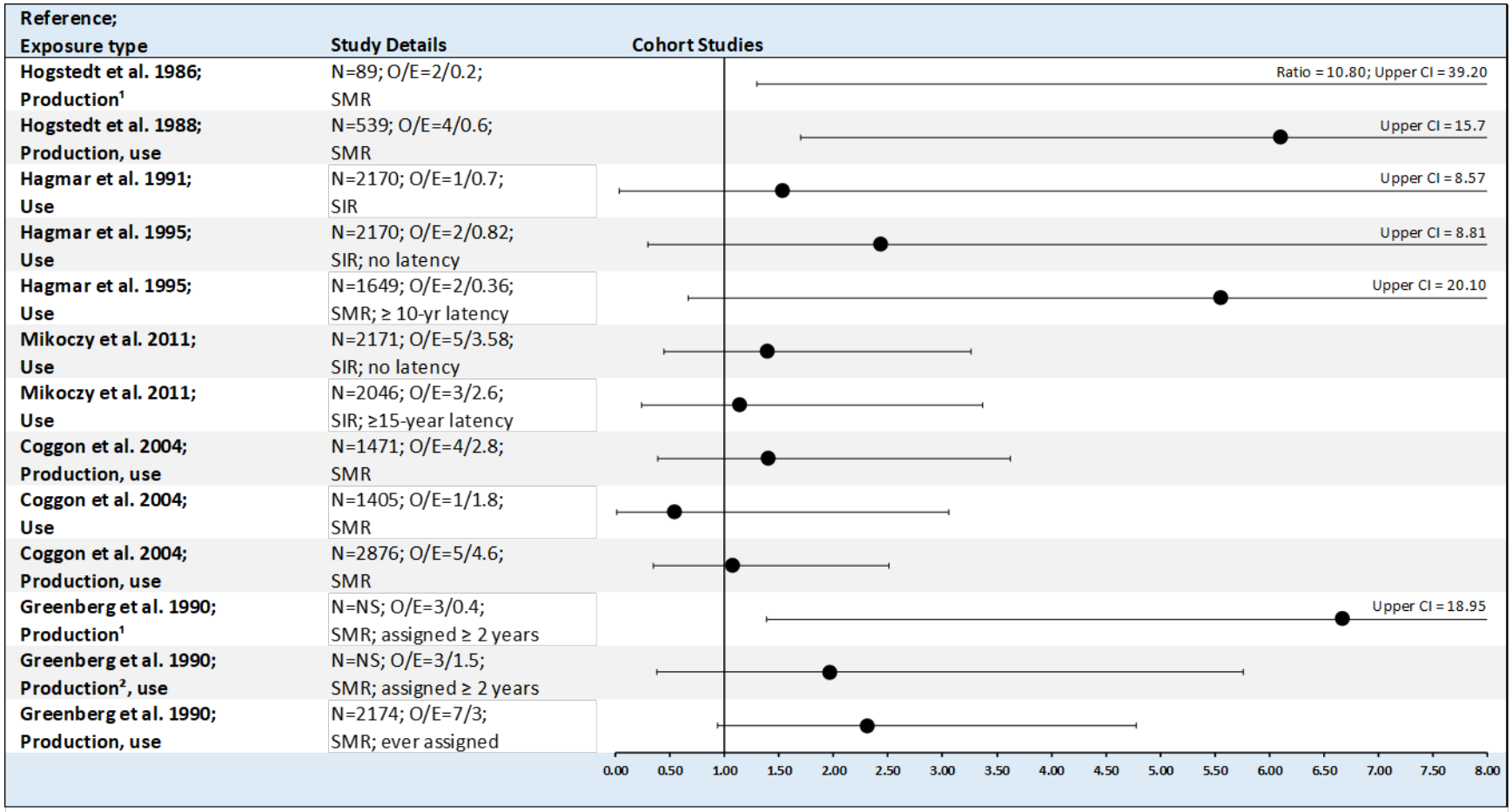
| Reference/cohort description | Comments |
|---|---|
| **Wong and Trent 1993 (evaluation includes the 14 facilities from the NIOSH-based cohort of Steenland et al. 1991)** | |
| 18,728 workers (8,709 men, 10,019 women) at 14 facilities producing sterilized medical supplies and spices | Results for leukemia, female breast cancer, lymphopoietic cancers, and lymphosarcoma/reticulosarcoma are summarized in Figures 2-4, 2-6, 2-7, and 2-9, respectively.<br>Mortality evaluated through 1988; 8-hour TWA exposure estimates for sterilizer workers (20% of cohort) were 4–5 ppm after 1978 and 16 ppm for earlier times; exposure estimates for the rest of the cohort were 2 ppm after 1978 and 5 ppm for earlier times; possibly higher short-term exposures. |
| **Steenland et al. 2004 (11-year update of the cohort of Steenland et al. 1991)** | |
| 18,235 workers (8,214 men, 10,040 women) at 14 facilities using ethylene oxide for sterilization | Results for leukemia, non-Hodgkin's lymphoma, female breast cancer, hematopoietic cancers, and myeloma/multiple myeloma are summarized in Figures 2-4, 2-5, 2-6, 2-7, and 2-8, respectively.<br>Positive trend for hematopoietic cancers in males with a 15-year lag time (driven by lymphoid tumors); positive trend for breast cancer using log of cumulative exposure and 20-year lag time. |
| **Steenland et al. 2003 (NIOSH-based cohort)** | |
| 7,576 female workers employed for at least 1 year at 14 facilities using ethylene oxide for sterilization | Results for breast cancer are summarized in Figure 2-6.<br>Significant trend (p=0.002) for increased breast cancer incidence with increasing cumulative ethylene oxide exposure using a 15-year lag period; breast cancer incidences evaluated through 1998; referent rates from non-exposed population in NCI's SEER Program. |
| **Bisanti et al. 1993** | |
| 1,971 male chemical workers licensed to handle ethylene oxide for ≥1 year in northern Italy | Results for leukemia, hematopoietic cancers, and lymphosarcoma/reticulosarcoma are summarized in Figures 2-4, 2-7, and 2-9, respectively. |
| Subcohort of 637 workers licensed to handle ethylene oxide only | Results for leukemia, hematopoietic cancers, and lymphosarcoma/reticulosarcoma are summarized in Figures 2-4, 2-7, and 2-9, respectively.<br>Evaluation included the years 1938–1984; expected deaths based on rates within the regional general population. |

**Table 2-3. Study Design Details for Selected Cohorts of Workers Exposed to Ethylene Oxide**

| Reference/cohort description | Comments |
|---|---|
| **Kiesselbach et al. 1990** | |
| 2,658 employees exposed to ethylene oxide for ≥1 year at German chemical companies | Results for leukemia and lymphohematopoietic cancers are summarized in Figures 2-4 and 2-7, respectively.<br>Evaluation included the years 1928–1981; expected deaths based on rates within the German general population. |
| **Morgan et al. 1981** | |
| 767 male workers employed for ≥5 years at ethylene oxide production facility (production method not specified) | Evaluation included the years 1955–1977.<br>No leukemia deaths versus 0.70 expected deaths based on U.S. vital statistics. |
| **Norman et al. 1995** | |
| 928 female workers employed for any time from July 1, 1974 through September 30, 1980 at a plant with potential for ethylene oxide exposure | Results for breast cancer are summarized in Figure 2-6; evaluated through December, 1987; expected incidences based on SEER rates. |
| **Olsen et al. 1997** | |
| 1,361 male workers employed at Dow Chemical facilities for ≥1 year and potentially engaged for ≥1 month in ethylene chlorohydrin and/or propylene chlorohydrin production | Results for leukemia, lymphohematopoietic cancers, and lymphosarcoma/ reticulosarcoma are summarized in Figures 2-4, 2-7, and 2-9, respectively.<br>Evaluation included the years 1940–1992; expected death rates based on U.S. white males; analyses examining location, production process, duration of employment, and 25-year induction latency did not result in significant findings. |

95% CI = 95% confidence interval; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; O/E = observed deaths or incidences/expected deaths or incidences; RR = rate ratio; SEER = Surveillance Epidemiology and End Results; SIR = standardized incidence ratio; SMR = standardized mortality ratio; TWA = time-weighted average

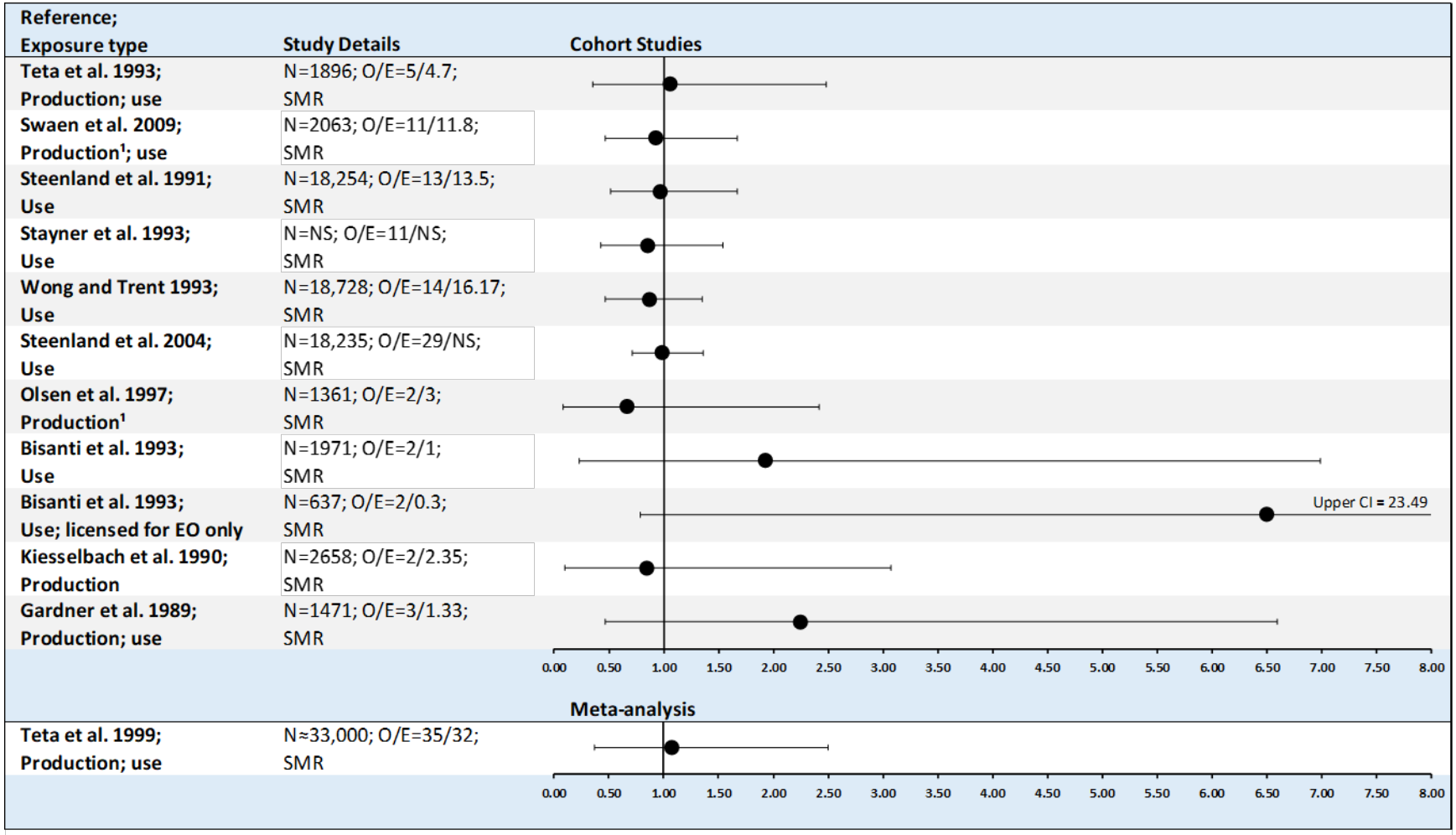
## Figure 2-4. Summary of Epidemiological Studies Evaluating Associations between Inhaled Ethylene Oxide and Leukemia




= risk estimate and 95% CI

1 = production via chlorohydrin process; 2 = production via non-chlorohydrin process; N = cohort size; NS = not specified; O/E = observed deaths (incidences)/expected; SIR = standardized incidence ratio; SMR = standardized mortality ratio; Production = workers involved in ethylene oxide production; Use = workers exposed via ethylene oxide sterilization process

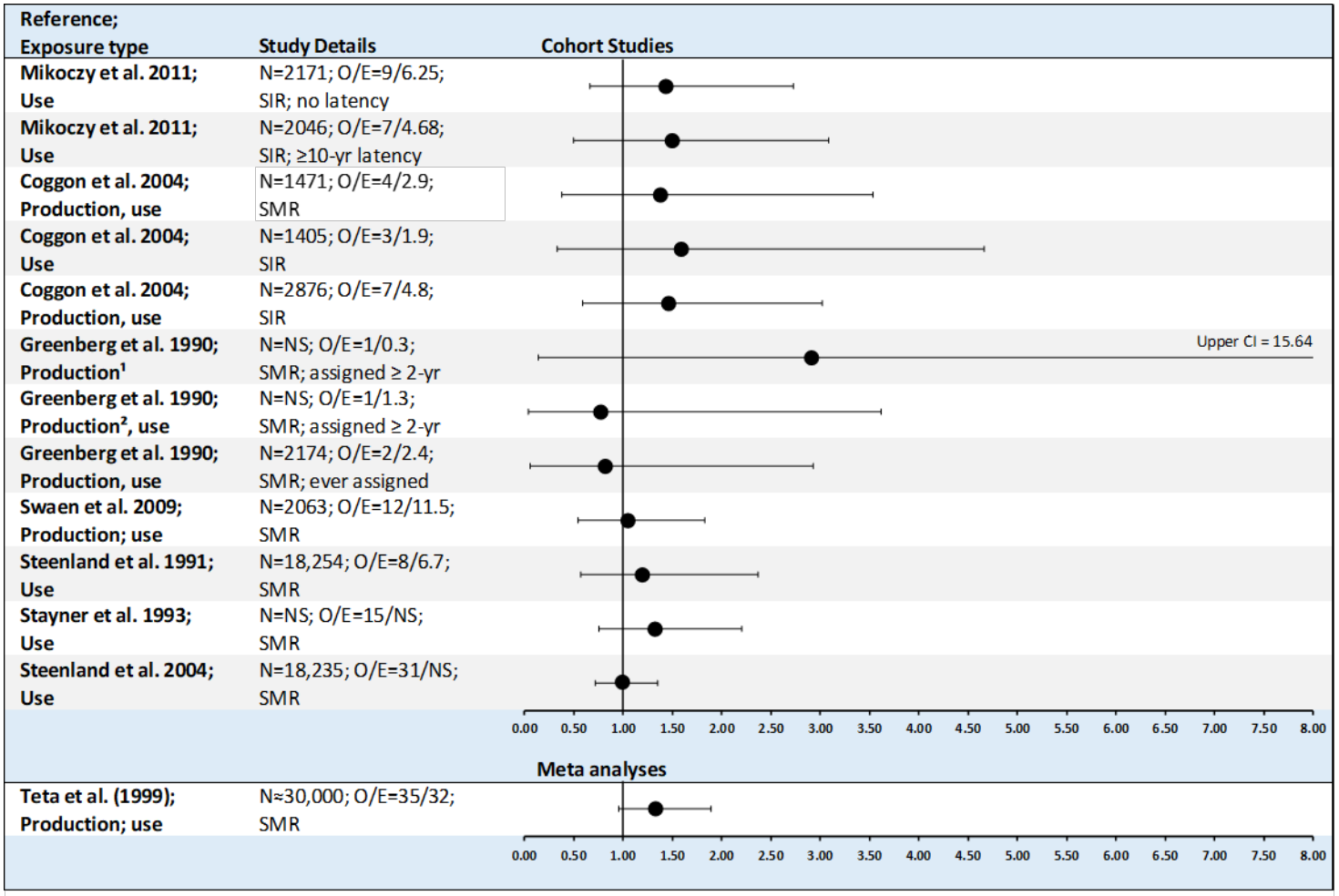
## Figure 2-4. Summary of Epidemiological Studies Evaluating Associations between Inhaled Ethylene Oxide and Leukemia (continued)




= risk estimate and 95% CI

1 = production via chlorohydrin process; CI = confidence interval; N = cohort size; NS = not specified; O/E = observed deaths (incidences)/expected; SMR = standardized mortality ratio; Production = workers involved in ethylene oxide production; Use = workers exposed via ethylene oxide sterilization process

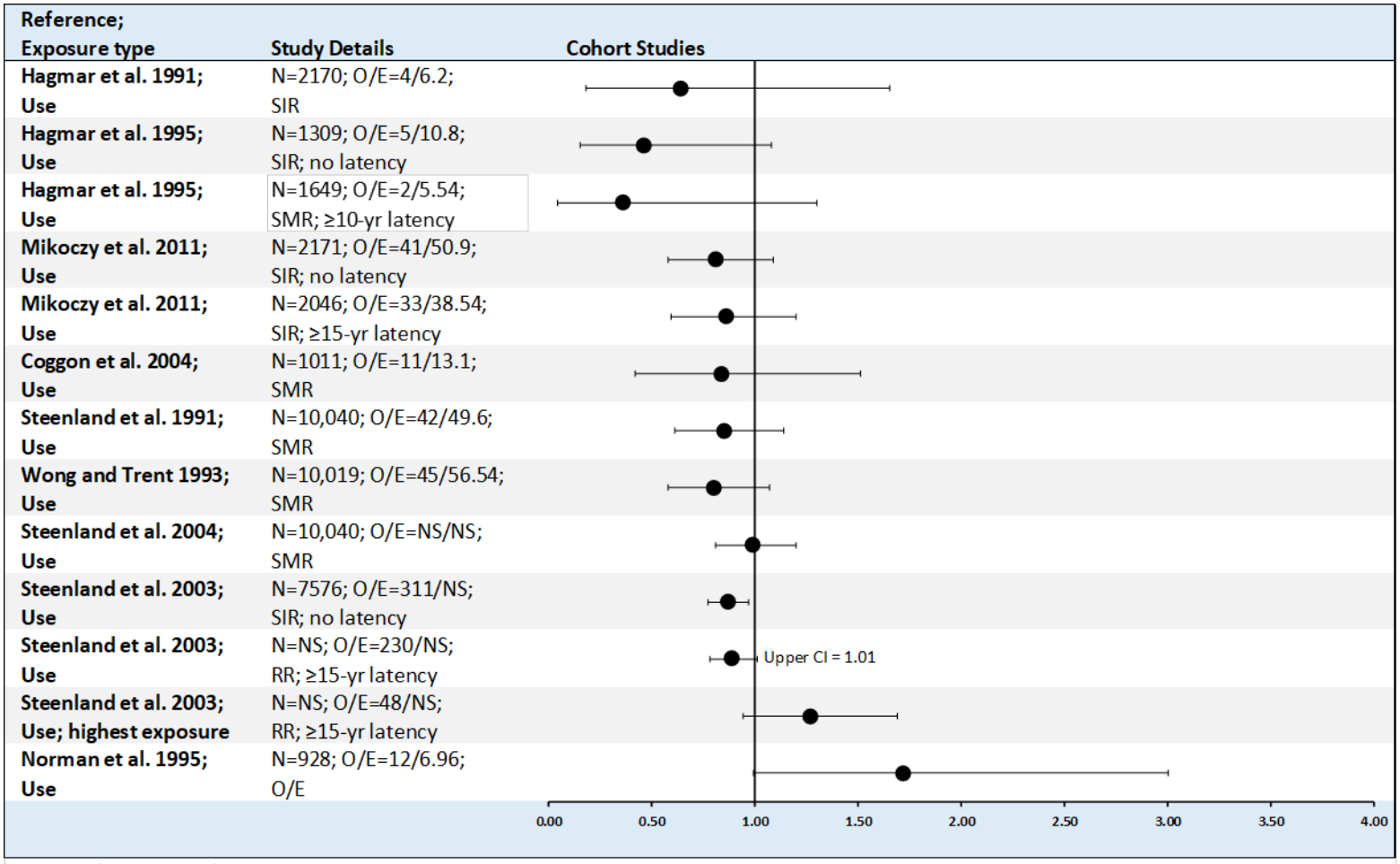
## Figure 2-5. Summary of Epidemiological Studies Evaluating Associations between Inhaled Ethylene Oxide and Non-Hodgkin's Lymphoma




= risk estimate and 95% CI

1 = production via chlorohydrin process; 2 = production via non-chlorohydrin process; N = cohort size; NS = not specified; O/E = observed deaths (incidences)/expected; SIR = standardized incidence ratio; SMR = standardized mortality ratio; Production = workers involved in ethylene oxide production; Use = workers exposed via ethylene oxide sterilization process

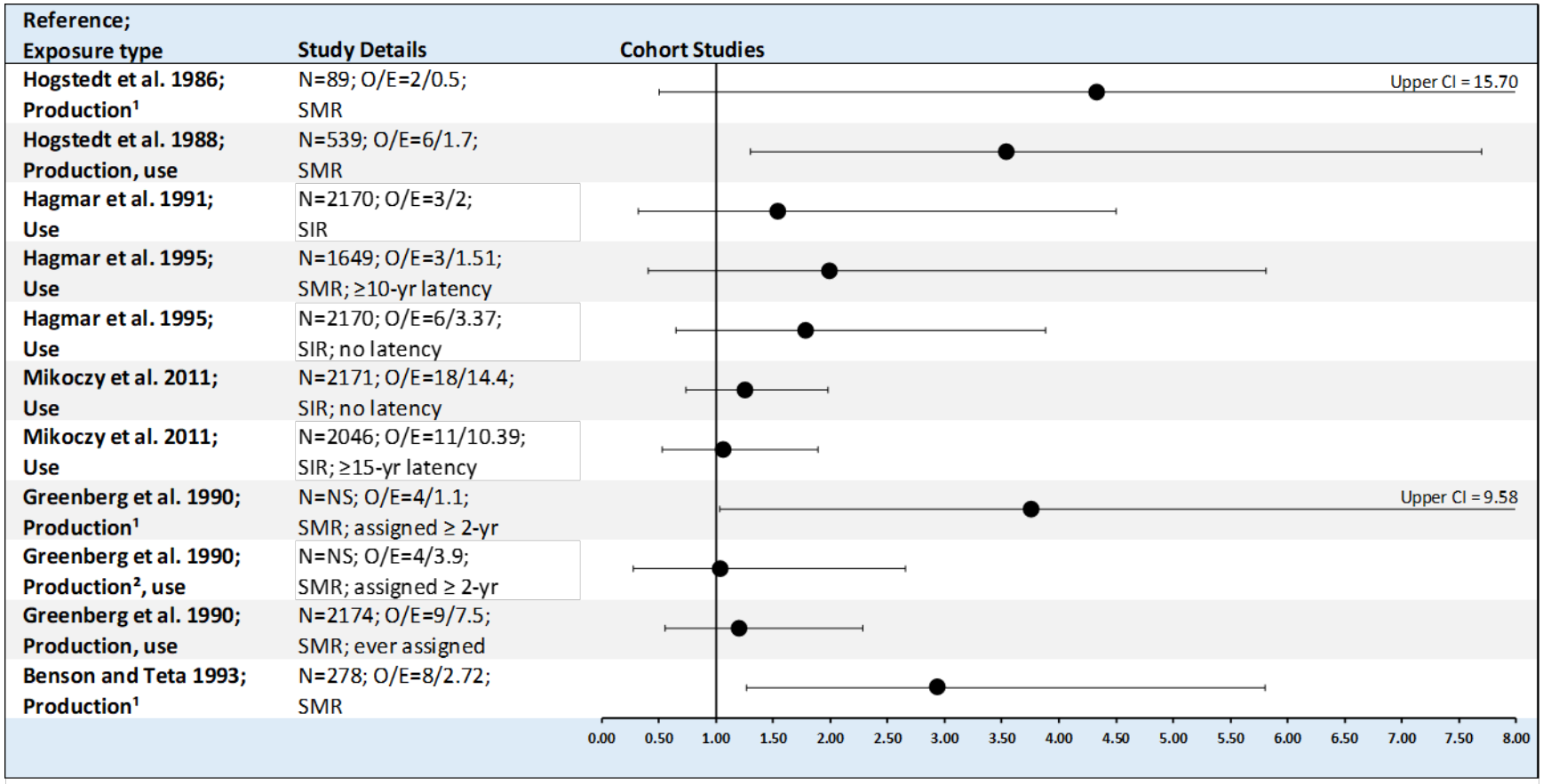
## Figure 2-6. Summary of Epidemiological Studies Evaluating Associations between Inhaled Ethylene Oxide and Breast Cancer




= risk estimate and 95% CI

CI = confidence interval; N = cohort size; NS = not specified; O/E = observed deaths (incidences)/expected; RR = rate ratio; SIR = standardized incidence ratio; SMR = standardized mortality ratio; Production = workers involved in ethylene oxide production; Use = workers exposed via ethylene oxide sterilization process

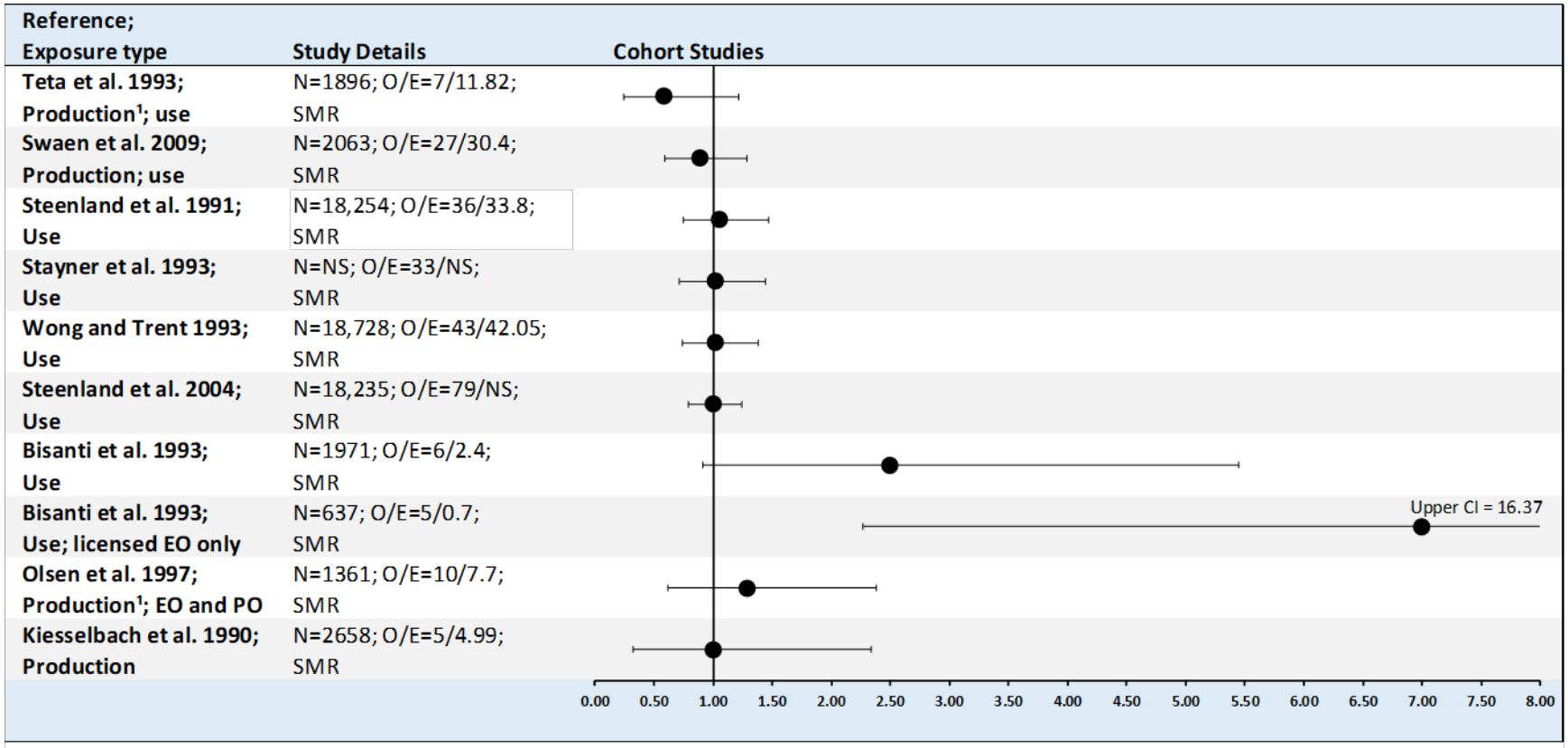
**Figure 2-7. Summary of Epidemiological Studies Evaluating Associations between Inhaled Ethylene Oxide and Lympho-Hematopoietic Cancer**




= risk estimate and 95% CI

1 = production via chlorohydrin process; 2 = production via non-chlorohydrin process; CI = confidence interval; N = cohort size; NS = not specified; O/E = observed deaths (incidences)/expected; SIR = standardized incidence ratio; SMR = standardized mortality ratio; Production = workers involved in ethylene oxide production; Use = workers exposed via ethylene oxide sterilization process

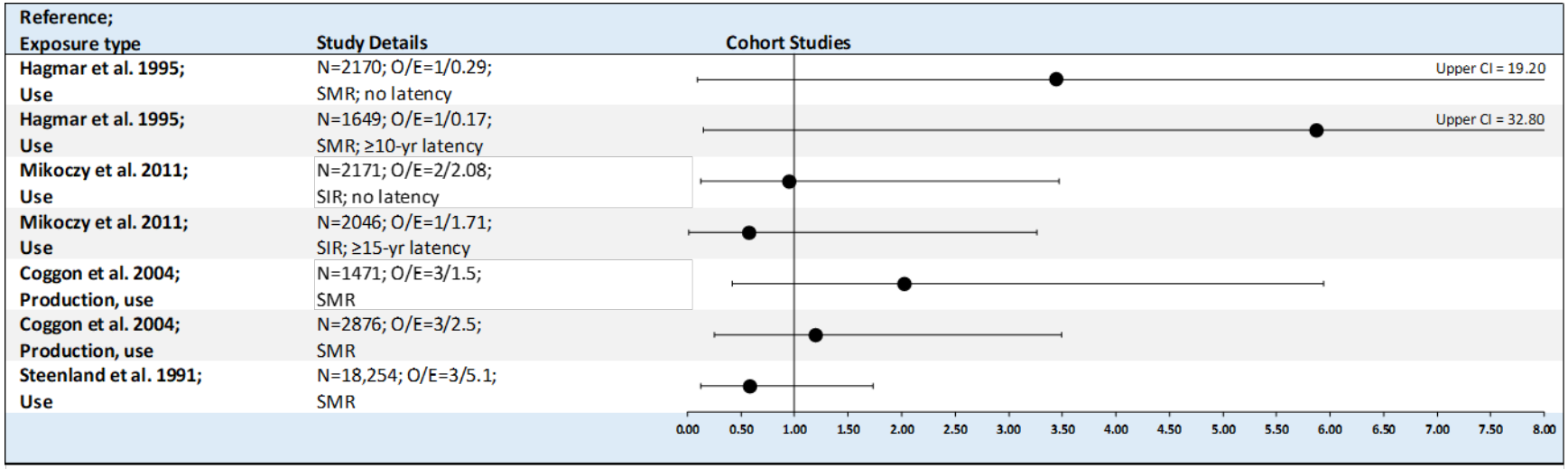
**Figure 2-7. Summary of Epidemiological Studies Evaluating Associations between Inhaled Ethylene Oxide and Lympho-Hematopoietic Cancer (continued)**




= risk estimate and 95% CI

1 = production via chlorohydrin process; CI = confidence interval; EO = Ethylene oxide; N = cohort size; NS = not specified; O/E = observed deaths (incidences)/expected; PO = Propylene oxide; SMR = standardized mortality ratio; Production = workers involved in ethylene oxide production; Use = workers exposed via ethylene oxide sterilization process

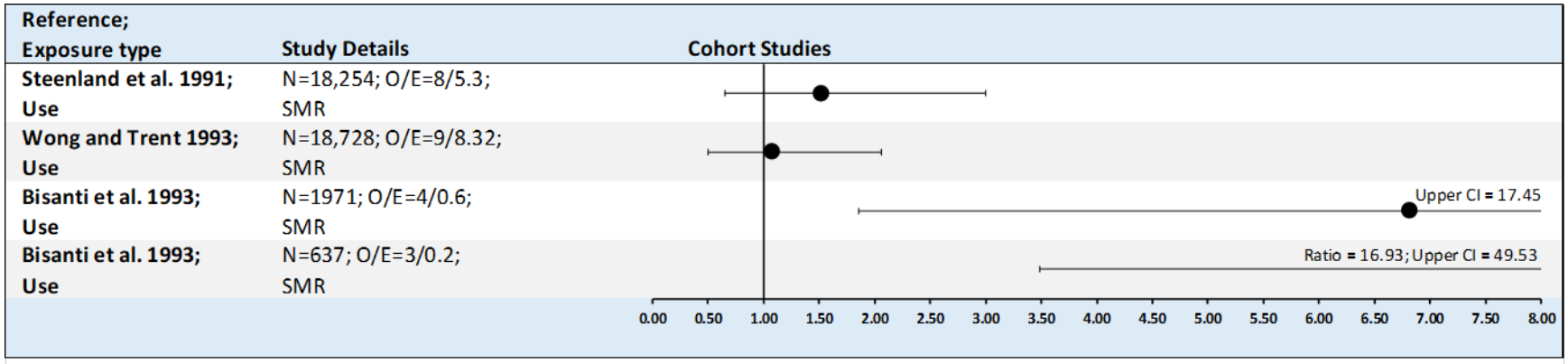
## Figure 2-8. Summary of Epidemiological Studies Evaluating Associations between Inhaled Ethylene Oxide and Myeloma-Multiple Myeloma




= risk estimate and 95% CI

CI = confidence interval; N = cohort size; O/E = observed deaths (incidences)/expected; SIR = standardized incidence ratio; SMR = standardized mortality ratio;
Production = workers involved in ethylene oxide production; Use = workers exposed via ethylene oxide sterilization process

## Figure 2-9. Summary of Epidemiological Studies Evaluating Associations between Inhaled Ethylene Oxide and Lymphosarcoma/Reticulosarcoma Cancer




= risk estimate and 95% CI

CI = confidence interval; N = cohort size; O/E = observed deaths (incidences)/expected; SMR = standardized mortality ratio;

Use = workers exposed via ethylene oxide sterilization process

Swaen et al. (1996) investigated a cluster of 10 cases of Hodgkin's lymphoma within a large chemical manufacturing complex in Belgium. Among 214 different chemical substances evaluated, 5 chemicals exhibited elevated odds ratios (ORs) (ammonia, benzene, ethylene oxide, sodium hydroxide, and oleum). The cluster of cases could not be specifically associated with ethylene oxide exposure.

***Meta-Analyses of Epidemiological Studies.*** Shore et al. (1993) conducted a review and meta-analysis on cohorts from previously-published studies. The meta-analysis for leukemia was conducted using data from Bisanti et al. (1993), Gardner et al. (1989), Hagmar et al. (1991), Hogstedt (1988), Kiesselbach et al. (1990), Teta et al. (1993), Wong and Trent (1993), and an unpublished study (update to Greenberg et al. 1990). The reported O/E ratio was 1.06 (95% CI 0.73–1.48) based on 31 incident cases or deaths versus 29.31 expected. The meta-analysis for non-Hodgkin's lymphoma was conducted using data from Bisanti et al. (1993), Gardner et al. (1989), Hagmar et al. (1991), Hogstedt (1988), Teta et al. (1993), Thiess et al. (1981), Wong and Trent (1993), and an unpublished study (update to Greenberg et al. 1990). The reported O/E ratio was 1.35 (95% CI 0.93–1.90) based on 31 incident cases or deaths versus 22.93 expected.

Teta et al. (1999) updated the meta-analysis of Shore et al. (1993) by including results from Hagmar et al. (1995) and Olsen et al. (1997) and excluding results from Greenberg et al. (1990), Morgan et al. (1981), and Thiess et al. (1981). The updated meta-analysis included nearly 33,000 workers and more than 800 cancers. For leukemia and non-Hodgkin's lymphoma risks, reported standardized mortality ratios (SMRs) were 1.08 (95% CI 0.61–1.93) based on 35 deaths versus 32 expected, and 1.34 (95% CI 0.96–1.89) based on 33 deaths versus 25 expected, respectively; neither leukemia nor non-Hodgkin's lymphoma results exhibited positive trends with duration, intensity, or latency. The study authors considered the results inconclusive for leukemia and for non-Hodgkin's lymphoma.

Marsh et al. (2019) conducted a systematic literature review of occupational exposure to ethylene oxide and risk of lymphohematopoietic cancer and breast cancer and identified 13 studies that were included in a meta-analysis. Overall meta-relative risks (meta-RRs) were 1.48 (95% CI 1.07–2.05) for lymphohematopoietic cancer and 0.97 (95% CI 0.80–1.18) for breast cancer. For lymphohematopoietic cancer, the study authors reported meta-RRs of 1.46 (95% CI 0.85–2.50) among ethylene oxide production workers and 1.07 (95% CI 0.87–1.30) among ethylene oxide sterilization workers. Higher risks of lymphohematopoietic cancer were noted for earlier published studies, compared to more recent studies.

***Animal Studies.*** Animal studies examining the carcinogenicity of ethylene oxide have reported increased incidences of several tumor types. Significantly increased incidence of brain gliomas (5/79 versus 0/76 controls; $p<0.05$) and peritoneal mesotheliomas (21/79 versus 3/78 controls; $p<0.01$) were reported among male Fischer 344 rats intermittently exposed by inhalation for up to 2 years at 100 ppm (Lynch et al. 1984a, 1984b). An increase in the incidence of mononuclear cell leukemia was also observed at 50 ppm (38/79 versus 24/77 controls, $p<0.01$).

Significantly increased incidence of spleen mononuclear cell leukemia was observed in female rats exposed to ethylene oxide vapor for up to 2 years at 100 ppm (58% versus 8 or 11% controls; $p<0.01$) (Garman et al. 1985, 1986; Snellings et al. 1984b). Although the incidence was only statistically significantly elevated at 100 ppm, increased incidences were also observed at 10 ppm (11/54 versus 11/115 in controls) and 33 ppm (14/48). When incidences among rats dying early or killed in moribund condition were included, there were significant mortality-adjusted trends for mononuclear cell leukemia in males and females at ≥33 ppm (incidence data not reported). Male rats of the 100 ppm exposure group exhibited significant increases in peritoneal mesothelioma when rats that died or were killed in moribund condition were included (no incidence reported) and subcutis fibroma (36% versus 2 and 4% control; $0.01>p>0.001$). A significant trend for primary brain neoplasms was observed in males ($p<0.01$) and females ($p<0.05$).

In a 2-year inhalation study of male and female B6C3 F1 mice, ethylene oxide exposure at 100 ppm resulted in significantly increased incidence of alveolar/bronchiolar carcinoma (16/50 versus 6/50 controls, $p=0.048$ according to life table analysis) in males, and significantly increased incidences of alveolar/bronchiolar adenoma (17/49 versus 2/49 controls; $p=0.001$) and alveolar/bronchiolar carcinoma (7/49 versus 0/49 controls, $p=0.019$) in female mice (NTP 1987). Incidences of Harderian gland papillary cystadenoma were increased at 50 ppm in males (9/44, $p=0.014$) and females (6/46, $p=0.052$) and at 100 ppm in males (8/42, $p=0.021$) and females (8/47, $p=0.039$). The 100 ppm group of female mice also exhibited marginally significantly increased incidence of malignant lymphoma in the hematopoietic system (22/49 versus 9/49 controls; $p=0.049$) and uterine adenocarcinoma (5/49 versus 0/49 controls; $p=0.058$). The 50 ppm (but not 100 ppm) group of female mice also exhibited increased incidences of hepatocellular adenoma (8/48 versus 1/49; $p=0.021$) and mammary gland adenocarcinoma or adenosquamous carcinoma combined (8/48 versus 1/49 controls; $p=0.020$). Picut et al. (2003) re-evaluated the pathology and tumor incidence data from NTP (1987) and confirmed the results.

Significantly increased incidences of lung adenomas were reported among female A/J mice intermittently exposed to ethylene oxide vapor for 6 months at 70 or 200 ppm; lung adenoma incidences were 16/28 and 25/29, respectively, compared to 8/30 controls (Adkins et al. 1986). Incidences were statistically significantly increased in both ethylene oxide-exposed groups. In a replicate study that included controls and 200 ppm groups, incidences of lung adenomas in surviving mice were 9/29 (29%) and 12/28 (42%), respectively.

Dose-related increased incidence of malignant tumors of the forestomach was reported among female rats administered ethylene oxide by gavage at 7.5 or 30 mg/kg/day for 2 days/week for 150 weeks; incidences were 8/50 and 31/50, respectively, compared to no stomach tumors among 50 vehicle and 50 untreated controls (Dunkelberg 1982). A total of 37 of the 39 tumors were squamous cell carcinomas.

In a lifetime skin painting study, application of a 10% solution of ethylene oxide to the backs of mice did not result in skin tumors (Van Duuren et al. 1965).

***Carcinogenicity Assessments.*** The HHS has classified ethylene oxide as *known to be a human carcinogen* (NTP 2016) based on sufficient evidence of carcinogenicity from studies in humans (increased risk of cancer in workers exposed to ethylene oxide during its synthesis, production, and use), and evidence for a common mechanism of carcinogenesis in humans and experimental animals (similar genetic damage in cells of animals and workers exposed to ethylene oxide).

EPA (2016a) characterized ethylene oxide as “carcinogenic to humans” by the inhalation exposure route, based on the total weight of evidence including epidemiological evidence of lymphohematopoietic cancers and breast cancer in ethylene oxide-exposed workers, lymphohematopoietic cancers in rats and mice and mammary carcinomas in mice, clear evidence that ethylene oxide is genotoxic and sufficient evidence to support a mutagenic mode of action for ethylene oxide carcinogenicity, and strong evidence for occurrence of key precursor events and progression to tumors in humans. EPA derived unit risk estimates were based on results from the National Institute for Occupational Safety and Health (NIOSH) study (Steenland et al. 2003, 2004) that evaluated cancer risk in a cohort of workers exposed to ethylene oxide. The authors found positive exposure responses for breast cancer mortality in females for log cumulative exposure and a 20-year lag. There was also a positive exposure response for lymphoid tumors (non-Hodgkin’s lymphoma, myeloma, and lymphocytic leukemia) in both sexes for cumulative exposure. The adult-based unit risk estimates were $2.6x10^{-3}$per µg/m$^3$ $4.8x10^{-3}$ per ppb) for lymphoid cancer, $7.0x10^{-4}$ per µg/m$^3$ ($1.3x10^{-3}$ per ppb) for female breast cancer, and $3.0x10^{-3}$ per µg/m$^3$ ($5.5x10^{-3}$ per ppb)

for both cancer types combined. Application of standard age-dependent adjustment factors yields a full lifetime total cancer unit risk estimate of $5.0 \times 10^{-3}$ per µg/m$^3$ ($9.1 \times 10^{-3}$ per ppb) (EPA 2016a). The commensurate lifetime chronic (lower bound) exposure level of $2 \times 10^{-4}$ µg/m$^3$ ($1 \times 10^{-4}$ ppb) corresponds to an increased cancer risk of $10^{-6}$ (1 in 1,000,000). The unit risk estimate was developed for environmental ethylene oxide exposures up to about 40 µg/m$^3$ (20 ppb) and is not applicable to higher exposure levels that may occur in occupational exposure scenarios. Maximum likelihood estimates of extra risk of lymphoid cancer and breast cancer (combined) occupational exposure scenarios in the range of 0.1–1 ppm for an 8-hour TWA for 35 years range from 0.037 to 0.11 (upper bound estimates 0.081–0.22).

IARC has produced several reports on the carcinogenicity of ethylene oxide (IARC 1976, 1985, 1987, 1994, 2008, 2012). IARC has designated ethylene oxide as *carcinogenic to humans (Group 1)* (IARC 1987, 2012) based on limited evidence for a causal association between exposure to ethylene oxide and lymphatic and hematopoietic cancers and breast cancer in humans, sufficient evidence for carcinogenicity in animals, and strong evidence for a genotoxic mechanism of action for ethylene oxide carcinogenicity.

## 2.20 GENOTOXICITY

Ethylene oxide has been demonstrated to be genotoxic in human and animal studies *in vivo* and a wide variety of test systems *in vitro*. Extensive reviews are available regarding the genotoxicity of ethylene oxide (see EPA 2016a; IARC 1994, 2008, 2012). Studies evaluating the genotoxicity of ethylene oxide in humans and *in vivo* studies using experimental test species are summarized in Tables 2-4 and 2-5, respectively. Results from selected *in vitro* assays are summarized in Table 2-6. Available data collectively demonstrate the mutagenicity and clastogenicity of ethylene oxide both *in vitro* and *in vivo*. Ethylene oxide induced gene mutation, chromosomal aberrations, sister chromatid exchange, micronucleus formation, deoxyribonucleic acid (DNA) strand breaks, unscheduled DNA synthesis, and cell transformation *in vitro*. Ethylene oxide induced gene mutation, specific locus mutation, chromosomal aberrations, sister chromatid exchange, micronucleus formation, dominant lethal mutation, and heritable translocation in test species and/or occupationally-exposed humans.

In addition to these genotoxic effects, *in vitro* studies in mammal tissues, *in vivo* studies in rats and mice, and studies in humans have demonstrated the formation of DNA adducts. Ethylene oxide is an alkylating agent that forms adducts with DNA, ribonucleic acid (RNA), and proteins. The primary DNA adduct formed is N7-(2-hydroxyethyl)guanine (7-HEG). Other DNA adducts have been found in lesser amounts, these include N3-(2-hydroxyethyl)adenine and $O^6$-(2-hydroxyethyl)guanine (EPA 2016a; IARC 2012).

7-HEG has been detected in various tissues of rats exposed via inhalation to ethylene oxide for up to 4 weeks (Walker et al. 1990). Duration-related increases were observed in the brain, lungs, spleen, kidneys, leukocytes, liver, and testes.

**Table 2-4. Genotoxicity of Ethylene Oxide in Humans**

| Ethylene oxide exposure group | Test system | Endpoint | Result | Reference |
|---|---|---|---|---|
| Factory sterilization workers | Blood DNA | Gene mutation | + | Kamel et al. 2011 |
| Hospital, factory sterilization workers | Lymphocytes | Gene mutation | – | Tates et al. 1991 |
| Chemical manufacturing workers | Lymphocytes | Gene mutation | – | Tates et al. 1995 |
| Occupationally-exposed workers | Lymphocytes | Chromosomal aberrations | + | Abrahams 1980 |
| Sterilization plant workers | Lymphocytes | Chromosomal aberrations | + | Galloway et al. 1986 |
| Production workers | Lymphocytes | Chromosomal aberrations | + | Högstedt et al. 1990 |
| Production, sterilization workers | Lymphocytes | Chromosomal aberrations | + | Karelová et al. 1987 |
| Hospital sterilization workers | Lymphocytes | Chromosomal aberrations | + | Lerda and Rizzi 1992 |
| Sterilization workers | Lymphocytes | Chromosomal aberrations | + | Richmond et al. 1985 |
| Hospital, factory sterilization workers | Lymphocytes | Chromosomal aberrations | + | Tates et al. 1991 |
| Chemical industry workers | Lymphocytes | Chromosomal aberrations | + | Thiess et al. 1981 |
| Sterilization plant workers | Lymphocytes | Chromosomal aberrations | (+) | Pero et al. 1981 |
| Hospital sterilization workers | Lymphocytes | Chromosomal aberrations | (+) | Sarto et al. 1984 |
| Chemical industry workers | Lymphocytes | Chromosomal aberrations | – | Clare et al. 1985 |
| Hospital sterilization workers | Lymphocytes | Chromosomal aberrations | – | Högstedt et al. 1983 |
| Hospital sterilization workers | Lymphocytes | Chromosomal aberrations | – | Mayer et al. 1991 |
| Chemical production workers | Lymphocytes | Chromosomal aberrations | – | Ribeiro et al. 1994 |
| Chemical industry workers | Lymphocytes | Chromosomal aberrations | – | van Sittert et al. 1985 |
| Occupationally-exposed workers | Lymphocytes | Sister chromatid exchange | + | Abrahams 1980 |
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | + | Garry et al. 1979 |
| Highly-exposed sterilization workers | Lymphocytes | Sister chromatid exchange | + | Laurent 1988 |
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | + | Laurent et al. 1984 |
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | + | Lerda and Rizzi 1992 |
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | + | Mayer et al. 1991 |
| Hospital workers | Lymphocytes | Sister chromatid exchange | + | Richmond et al. 1985 |

**Table 2-4. Genotoxicity of Ethylene Oxide in Humans**

| Ethylene oxide exposure group | Test system | Endpoint | Result | Reference |
|---|---|---|---|---|
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | + | Sarto et al. 1984 |
| Sanitary workers | Lymphocytes | Sister chromatid exchange | + | Sarto et al. 1987 |
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | + | Schulte et al. 1992 |
| Sterilization plant workers | Lymphocytes | Sister chromatid exchange | + | Stolley et al. 1984 |
| Hospital, factory sterilization workers | Lymphocytes | Sister chromatid exchange | + | Tates et al. 1991 |
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | + | Yager et al. 1983 |
| Sterilization plant workers | Lymphocytes | Sister chromatid exchange | (+) | Lambert and Lindblad 1980 |
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | ± | Sarto et al. 1991 |
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | – | Hansen et al. 1984 |
| Hospital sterilization workers | Lymphocytes | Sister chromatid exchange | – | Högstedt et al. 1983 |
| Production workers | Lymphocytes | Sister chromatid exchange | – | Tates et al. 1995 |
| Hospital workers | Lymphocytes | Sister chromatid exchange | – | Tomkins et al. 1993 |
| Production workers | Lymphocytes | Micronucleus formation | + | Högstedt et al. 1990 |
| Hospital, factory sterilization workers | Lymphocytes | Micronucleus formation | + | Tates et al. 1991 |
| Hospital sterilization workers | Lymphocytes | Micronucleus formation | – | Mayer et al. 1991 |
| Hospital sterilization workers | Lymphocytes | Micronucleus formation | – | Schulte et al. 1992 |
| Hospital sterilization, preparation workers | Lymphocytes | Micronucleus formation | – | Sarto et al. 1991 |
| Production workers | Lymphocytes | Micronucleus formation | – | Tates et al. 1995 |
| Hospital sterilization workers | Bone marrow cells | Micronucleus formation | + | Högstedt et al. 1983 |
| Chemical production workers | Buccal cells | Micronucleus formation | – | Ribeiro et al. 1994 |
| Factory sterilization workers | Buccal cells | Micronucleus formation | – | Sarto et al. 1990 |
| Hospital sterilization, preparation workers | Buccal cells | Micronucleus formation | – | Sarto et al. 1991 |
| Factory sterilization workers | Nasal cells | Micronucleus formation | + | Sarto et al. 1990 |
| Hospital sterilization workers | Lymphocytes | DNA adducts | (+) | Yong et al. 2007 |
| Hospital sterilization workers | | DNA strand breaks | – | Mayer et al. 1991 |
| Hospital sterilization workers | | DNA cross-links | + | Popp et al. 1992 |

**Table 2-4. Genotoxicity of Ethylene Oxide in Humans**

| Ethylene oxide exposure group | Test system | Endpoint | Result | Reference |
|---|---|---|---|---|
| Commercial, hospital sterilization workers | Peripheral blood mononuclear cells | DNA damage | + | Fuchs et al. 1994 |

– = negative result; + = positive result; (+) = weakly positive result; ± = inconclusive

**Table 2-5. Genotoxicity of Ethylene Oxide in Experimental Test Species *In Vivo***

| Species (test system) | Endpoint | Results | Reference |
|---|---|---|---|
| *Drosophila melanogaster* | Sex-linked recessive lethal mutation | + | Bird 1952; Fahmy and Fahmy 1956; Nakao and Auerbach 1961, as cited in IARC 1994; Vogel and Nivard 1997, 1998; Watson 1966; Zijlstra and Vogel 1988 |
| *D. melanogaster* | Somatic mutation | + | Fahmy and Fahmy 1970 |
| *D. melanogaster* | Heritable translocation | + | Nakao and Auerbach 1961, as cited in IARC 1994 |
| *D. melanogaster* | Heritable translocation | + | Watson 1966 |
| *D. melanogaster* | DNA adducts | + | Nivard et al. 2003 |
| Rat splenic, thymic T-lymphocytes | Gene mutation | + | Walker et al. 1997 |
| Rat splenic T-lymphocytes | Gene mutation | (+) | Tates et al. 1999 |
| Mouse splenic T-lymphocytes | Gene mutation | + | Walker and Skopek 1993 |
| Mouse lung lymphocytes | Gene mutation | + | Sisk et al. 1997 |
| Mouse bone marrow, germ cells | Gene mutation | + | Recio et al. 2004 |
| Mouse bone marrow, splenic lymphocytes | Gene mutation | – | Sisk et al. 1997 |
| Mouse germ cells | Gene mutation | – | Sisk et al. 1997 |
| Mouse germ cells | Specific locus gene mutation | –<br>+ | Russell et al. 1984<br>Lewis et al. 1986, 1990 |
| Rat lymphocytes | Chromosomal aberrations | – | Kligerman et al. 1983 |
| Rat lymphocytes | Chromosomal aberrations | – | van Sittert et al. 2000 |
| Mouse lymphocytes | Chromosomal aberrations | + | Donner et al. 2010 |
| Rat bone marrow cells | Chromosomal aberrations | – | Union Carbide Corp. 1980 |
| Mouse bone marrow cells | Chromosomal aberrations | + | Ribeiro et al. 1987a; Farooqi et al. 1993 |
| Mouse spermatocytes | Chromosomal aberrations | + | Ribeiro et al. 1987a |
| Monkey lymphocytes | Chromosomal aberrations | + | Lynch et al. 1984c |
| Rat lymphocytes | Sister chromatid exchange | + | Kligerman et al. 1983 |
| Rat bone marrow, splenic cells | Sister chromatid exchange | + | Lorenti Garcia et al. 2001; Ong et al. 1993 |

**Table 2-5. Genotoxicity of Ethylene Oxide in Experimental Test Species *In Vivo***

| Species (test system) | Endpoint | Results | Reference |
|---|---|---|---|
| Mouse bone marrow cells | Sister chromatid exchange | + | Farooqi et al. 1993 |
| Rabbit lymphocytes | Sister chromatid exchange | + | Yager 1987; Yager and Benz 1982 |
| Monkey lymphocytes | Sister chromatid exchange | + | Kelsey et al. 1988; Lynch et al. 1984c |
| Rat bone marrow cells | Micronucleus formation | + | Appelgren et al. 1978, as cited in IARC 1994 |
| Rat bone marrow, splenic cells | Micronucleus formation | + | Hochberg et al. 1990; Lorenti Garcia et al. 2001 |
| Mouse bone marrow cells | Micronucleus formation | + | Appelgren et al. 1978, as cited in IARC 1994; Farooqi et al. 1993; Jensen and Ramel 1980 |
| Rat splenic T-lymphocytes | Micronucleus formation | (+) | Tates et al. 1999 |
| Rat lymphocytes | Micronucleus formation | – | van Sittert et al. 2000 |
| Mouse sperm | Single strand breaks | + | Sega and Generoso 1988 |
| Mouse spermatids | Single strand breaks | + | Sega et al. 1988 |
| Mouse lymphocytes | Reciprocal translocation | (+) | Donner et al. 2010 |
| Mouse (germ cells) | Heritable translocation | + | Generoso et al. 1980, 1990 |
| Chinese hamster V79 cells | Heritable translocation | + | Generoso et al. 1980, 1990 |
| Rat | Dominant lethal mutation | + | Embree et al. 1977 |
| Mouse | Dominant lethal mutation | + | Generoso et al. 1980, 1983, 1986, 1990 |
| Mouse | Dominant lethal mutation | – | Appelgren et al. 1977 |
| Rat liver, testis DNA | DNA adducts | + | Osterman-Golkar et al. 1983 |
| Rat DNA | DNA adducts | + | Potter et al. 1989 |
| Rat brain, spleen, liver DNA | DNA adducts | + | Rusyn et al. 2005 |
| Rat brain, lung, spleen, kidney, liver, testis DNA | DNA adducts | + | Walker et al. 1992b |
| Rat lymphocyte DNA | DNA adducts | (+) | van Sittert et al. 2000 |
| Mouse DNA | DNA adducts | + | Ehrenberg et al. 1974 |
| Rat brain, lung, spleen, kidney, liver, testis DNA | DNA adducts | + | Walker et al. 1990 |
| Mouse germ cell DNA | DNA adducts | + | Sega et al. 1991 |
| Mouse DNA | DNA adducts | + | Segerbäck 1983 |

– = negative result; + = positive result; (+) = weakly positive result

**Table 2-6. Genotoxicity of Ethylene Oxide *In Vitro***

| Species (test system) | Endpoint | Results: Activation: With | Results: Activation: Without | Reference |
|---|---|---|---|---|
| Prokaryotic organisms: | | | | |
| *Salmonella typhimurium* | | | | |
| TA100, TA1535 | Gene mutation | | + | Agurell et al. 1991 |
| TA100, TA1535 | Gene mutation | + | + | De Flora 1981 |
| TA98, TA1537, TA1538 | Gene mutation | – | – | De Flora 1981 |
| TA100, TA102 | Gene mutation | + | | Hughes et al. 1987 |
| TA98, TA100, TA1535, TA1537 | Gene mutation | | + | Pfeiffer and Dunkelberg 1980 |
| TA1535 | Gene mutation | | + | Rannug et al. 1976 |
| TA100, TA1535 | Gene mutation | | + | Simmon 1981 |
| TA100 | Gene mutation | + | + | Victorin and Ståhlberg 1988 |
| *Escherichia coli* | | | | |
| WU36-10, WU36-10-89 | Gene mutation | | + | Kolman 1984; Kolman et al. 1989a |
| KMBL 3835 | Gene mutation | | + | Kolman 1985 |
| WP2, WP2 uvrA, WP6 | Gene mutation | | + | Kolman and Näslund 1987 |
| *Bacillus subtilis* | | | | |
| HA101, TKJ5211, TKJ8201 | Gene mutation | | + | Tanooka 1979 |
| Eukaryotic organisms: | | | | |
| *Saccharomyces cerevisiae* RS112 | Gene mutation | | + | Agurell et al. 1991 |
| *Schizosaccaromyces pombe* | Gene mutation | + | + | Migliore et al. 1982 |
| *Streptomyces griseoflavus* | Gene mutation | | – | Mashima and Ikeda 1958 |
| *Aspergillus nidulans* | Gene mutation | | (+) | Morpurgo 1963 |
| *Neurospora crassa* | Gene mutation | | + | de Serres and Brockman 1995; Kilbey and Kolmark 1968; Kolmark and Kilbey 1968; Kolmark and Westergaard 1953 |
| *Aspergillus nidulans* | Somatic crossing-over | – | | Morpurgo 1963 |
| Mammalian cells: | | | | |
| Chinese hamster | | | | |
| V79 cells | Gene mutation | | + | Hatch et al. 1986 |
| SP5/V79 cells | Gene mutation | | – | Zhang and Jenssen 1994 |
| K1-BH4 ovary cells | Gene mutation | + | + | Tan et al. 1981 |
| Mouse L5178Y TK cells | Gene mutation | | + | Brown et al. 1979; Krell et al. 1979 |
| Human fibroblasts | Gene mutation | | + | Bastlová et al. 1993; Kolman et al. 1992; Lambert et al. 1994 |

**Table 2-6. Genotoxicity of Ethylene Oxide *In Vitro***

| Species (test system) | Endpoint | Results: Activation: With | Results: Activation: Without | Reference |
|---|---|---|---|---|
| Chinese hamster V79 cells | Chromosomal aberrations | | + | Zhong et al. 1991 |
| Monkey lymphocytes | Chromosomal aberrations | | + | Lynch et al. 1984c |
| Human transformed amniotic cells | Chromosomal aberrations | | + | Poirier and Papadopoulo 1982 |
| Monkey lymphocytes | Sister chromatid exchange | | + | Kelsey et al. 1989; Lynch et al. 1984c |
| Human lymphocytes | Sister chromatid exchange | | + | Agurell et al. 1991; Garry et al. 1982; Hallier et al. 1993; Tucker et al. 1986 |
| Chinese hamster V79 cells | Micronucleus formation | | + | Zhong et al. 1991 |
| Mouse C3H10T1/2 cells | Cell transformation | | + | Kolman et al. 1989b, 1990 |
| SA7/Syrian hamster embryo cells | Cell transformation | | + | Hatch et al. 1986 |
| Chinese hamster V79 cells | DNA single strand breaks | | + | Herrero et al. 1997 |
| Human fibroblasts | DNA single strand breaks | | + | Nygren et al. 1994 |
| Human fibroblasts | DNA double strand breaks | | + | Nygren et al. 1994 |
| Human lymphocytes | DNA double strand breaks | | + | Hengstler et al. 1997 |
| Human lymphocytes | Unscheduled DNA synthesis | | + | Pero et al. 1981 |
| Human peripheral blood mononuclear cells | DNA damage | | + | Godderis et al. 2006 |
| Calf thymus DNA | DNA adducts | | + | Li et al. 1992 |
| Calf thymus DNA | DNA adducts | | + | Segerbäck 1990 |

– = negative result; + = positive result; (+) = weakly positive result