Overview information for

Acrolein

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
HUMAN HEALTH ASSESSMENT BRANCH

SUMMARY OF TOXICOLOGY DATA

Acrolein

Chemical Code # 3**, Document Processing Number (DPN)** 50032
SB 950 # 4
August 7, 1986
December 12, 1986, February 8, 1988, November 23, 1988, November 7, 1990, August 18, 1994, August 3, 2015

DATA GAP STATUS

Chronic toxicity, rat:	Data gap, no adverse effect indicated
Chronic toxicity, dog:	No data gap, possible adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	Data gap, no adverse effect indicated
Reproduction, rat:	No data gap, possible adverse effect
Developmental toxicity, rat:	No data gap, no adverse effect indicated
Developmental toxicity, rabbit:	No data gap, no adverse effect indicated
Developmental toxicity, mouse:	No data gap, possible adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effect indicated[1]
DNA damage:	No data gap, no adverse effect indicated[1]
Neurotoxicity:	Data gap

[1]Studies in the open literature but not on file at DPR indicate a possible adverse effect under these categories.

Toxicology one-liners are attached.

All record numbers for the above study types through 283479 (Document No. 50032-0077) were examined. This includes all relevant studies indexed by DPR as of 8/3/15.

File name: T150803

Revised by T. Moore, 8/3/15

NOTE: The following symbols may be used in the Table of Contents which follows:
* = data adequately address FIFRA requirement
† = study(ies) flagged as “possible adverse effect”
N/A = study type not currently required

This record contains summaries of studies. Individual worksheets may be useful for detailed assessment.

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METABOLISM AND PHARMACOKINETICS

Study not submitted.

GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat

50032-0072 283474; Acute oral toxicity; 811; Rat: Sprague-Dawley; Microbiological Associates Inc. (MBA), Bethesda, MD; Study # G-7230.220, MRID # 41257001; David, R. M.; 9/19/89; Acrolein, Inhibited (Lot Number 4035) (Acrolein, 96.58%); 50 animals, 5/sex/dose level; administered by oral gavage to assess acute oral toxicity; Dose: 10, 15, 20, 25, 30 mg/kg; Mortality (M/F): 10 (2/5 M, 1/5 F), 15 (4/5 M/F), 20 (5/5 M/F), 25 (5/5 M/F), 30 mg/kg (5/5 M/F); Clinical observations: lethargy, and/or hypothermia, and/or gasping, and/or rapid and shallow respiration, or no clinical signs of toxicity prior to death within 1 hour or 1 day of dosing; no clinical signs of toxicity were noted for any of the surviving animals at a dose level of 10 mg/kg; one male and female animal survived oral exposure to 15 mg/kg, and exhibited hypothermia and lethargy at 3 hours after dosing, with recovery by day 2; the male also showed rales during days 8-14; all the surviving animals gained weight by day 8, but lost weight by day 15; Gross necropsy: no visible lesions in the decedents, following terminal sacrifice, no visible lesions were noted for the surviving animals, with the exception of two male animals at 10 mg/kg level exhibiting mottled kidneys and petechial hemorrhage in the lungs, respectively; Reported LD (F): 11.8 (7.9-17.6) mg/kg, LD50 (M): 10.3 (6.4-16.7) mg/kg. **Toxicity Category: I; Study Acceptable.** (Guo, 4/16/15)

50032-0073 283475; Acute oral toxicity; 811; Rat: Sprague-Dawley; Bioassay Systems Corporation (BSC), Woburn, MA; BSC Project No.: 10258, MRID # 00141027; Muni, I. A.; 5/06/81; Acrolein (Lot No. SFSL-5893) (Acrolein, 95%); 50/sex animals, 10/sex/dose level; administered by oral gavage to assess acute oral toxicity; Dose: 0, 20, 25, 30, 40 mg/kg; Mortality (M/F): 0 (0/10 M/F), 20 (2/10 M, 0/10 F), 25 (0/10 M/F), 30 (8/10 M, 4/10 F) and 40 mg/kg (10/10 M, 7/10 F); Clinical observations: immediately following dose administration, Level III (30 mg/kg) and Level IV (40 mg/kg) animals showed signs of lethargy, respiratory distress and squinted eyes until death within 15 hours of dosing (18/M, 10/F) or by day 4 (1/F); the surviving animals exhibited similar signs for varying lengths of time through the observation period; Levels I (20 mg/kg) and II (25 mg/kg) animals showed similar signs but in most cases,

the conditions were less severe and did not persist as long in the survivors, except for 2 Level I male animals that died within 15 hours of dosing; control animals remained healthy throughout the observation period; the body weight data for the surviving animals were not reported; Gross necropsy: overall reddening of the lungs, hemorrhagic stomach and intestines (blood-filled), and dilation of blood vessels on the brain's surface in the decedents with deaths within 4 hours of dosing; darkening of the medulla of the kidneys was evident in most animals which died greater than 5 hours after dosing; all levels of the surviving animals sacrificed at study termination showed few, non-specific lesions (several pin-point black spots on lungs); Reported LD50 (F): 33.3 (30.0-36.9) mg/kg, LD (M): 25.0 (22.9-27.5) mg/kg, or LD50 (M/F): 29.0 (26.6-31.6) mg/kg. **Toxicity Category: I; Study Acceptable.** (Guo, 4/14/15)

50032-0074 283476; Acute oral toxicity; 811; Mouse: CD-1; Bioassay Systems Corporation (BSC), Woburn, MA; BSC Project No.: 11479, MRID #: NA; Mansur, C. A.; 3/17/83; Acrolein (Acrolein, > 96%); 10 males/dose level; administered by oral gavage to assess acute oral toxicity; Dose: 0.0, 11.0, 13.2, 15.84, 19.0 mg/kg; Mortality (F): 0.0 (0/10), 11.0 (4/10), 13.2 (4/10), 15.84 (8/10), 19.0 mg/kg (6/10); Clinical observations: a total of 22 animals died within two days after dosing; immediately following dose administration, through day 3 post-dosing, the majority of animals in Levels I to IV (11.0-19.0 mg/kg) showed signs of lethargy, squinted eyes, rough coats, hunching, and piloerection; the surviving animals in Levels I-IV showed rough coats for varying lengths of time through most of the observation period; blackening, followed by necrosis and breaking of the tip of the tail, was noted for several of the survivors with the exception of the controls; control animals remained healthy throughout the observation period; all the surviving animals exhibited weekly weight gains during the study, with the exception of one animal in the 13.2 mg/kg group losing weight by day 14; however, the surviving animals at all levels still showed reduced weight gains of -26.8% to -53.7% by day 7 and -11.6% to -28.6% by day 14, when compared to controls; Gross necropsy: reddening of the lungs, and hemorrhagic stomach and intestines in most decedents, one Level II (13.2 mg/kg) animal exhibited reddening of the lungs at terminal sacrifice; all other animals sacrificed at the termination of the study showed minimal, non-specific lesions; Reported LD50 (M): 13.9 (12.8-15.1) mg/kg. **Toxicity Category: I; Study Acceptable.** (Guo, 4/10/15)

50032-0075 283477; Acute oral toxicity; 811; Mouse: CD-1; Bioassay Systems Corporation (BSC), Woburn, MA; Study #10258, MRID # 00141031; Muni, I. A.; 12/30/81; Acrolein (Acrolein, > 96%); 10 females/dose level; administered by oral gavage to assess acute oral toxicity; Dose: 0.0, 11.0, 13.2, 15.8, 19.0 mg/kg; Mortality (F): 0.0 (0/10), 11.0 (0/10), 13.2 (0/10), 15.8 (3/10), 19.0 mg/kg (6/10); Clinical observations: immediately following dose administration, Level III (15.8 mg/kg) and Level IV (19.0 mg/kg) animals exhibited signs of lethargy, respiratory distress and squinted eyes until death occurred on day 1, 2 or 3; the surviving animals exhibited similar signs for varying lengths of time through most of the observation period; blackening, followed by necrosis and breaking of the tip of the tail, was noted for several surviving animals in the 15.8 and 19.0 mg/kg groups; the animals receiving 11.0 or 13.2 mg/kg all survived and showed similar signs except for no evidence of evident respiratory distress, but their conditions were less severe and did not persist as long as that in the other surviving animals; control animals remained healthy throughout the observation period; all the surviving animals gained weight during the study; there were no obvious differences in mean body weight change between the control group and the acrolein-treated groups (surviving animals only); Gross necropsy: reddening of the lungs, hemorrhagic stomach and intestines (blood-filled), dilation of blood vessels on the brain's surface and darkening of the medulla of the kidneys in the decedents, all the surviving animals sacrificed at the termination of the study exhibited minimal, non-specific lesions, with no evident differences from those in control animals; Reported LD50 (F): 17.7 (16.3-19.2) mg/kg. **Toxicity Category: I; Study Acceptable.** (Guo, 4/08/15)

Acute dermal toxicity

50032-0070 283472; Acute dermal toxicity; 812; Rabbit: New Zealand White; Bioassay Systems Corporation (BSC), Woburn, MA; BSC Study # 10258, MRID # 00141028; Muni, I. A.; 9/17/81; Acrolein (Lot No. SFSL-5993) (Acrolein, purity: 95%); 10/sex animals/dose level; topical, dermal application; Dose: 200, 240, 288 mg/kg (Levels I to III) or 0 mg/kg (untreated control and vehicle control); ; Mortality (M/F): 0 (0/10, M/F), 200 (3/10 M, 4/10 F), 240 (7/10 M/F), 288 mg/kg (5/10 M, 8/10 F); Clinical observations: approximately 1 to 2 minutes after dose administration, all acrolein-treated animals exhibited signs of severe pain including screaming and severe hyperactive behavior; these conditions lasted for 10-20 minutes following dose application; thereafter, the animals became lethargic, and had squinted eyes and evidence of respiratory distress; cyanosis became evident in varying intensity on the day of dosing in acrolein-treated rabbits; evidence of lethargy, mild respiratory problems and mild nasal discharge continued for varying lengths of time throughout the study period for both males and females; a total of 11 males and 18 females died within 24 hours after dosing, and all the other deaths occurred by days 3 (1 male each at 200 and 288 mg/kg), 6 (1 male at 240 mg/kg) and 9 (1 male and 1 female at 200 mg/kg); skin lesions of varying severity were noted throughout the observation period for acrolein-treated rabbits; control animals (vehicle-treated and untreated) remained healthy throughout the study period; all the surviving animals gained body weight during the study; Gross necropsy: macroscopic postmortem findings for the decedents were not included in the report; as to microscopic findings in cutaneous structure of the dose site skin, hemorrhage and edema within the hypodermis and dermis, and/or necrosis and mineralization of skeletal muscle, and/or vascular necrosis and thrombosis, and/or the overlying epidermis and dermis, or more advanced tissue injury (ulcerated epidermis, interstitial infiltration of necrotic heterophils and macrophages, a heterophil-laden fibrin-rich zone of edema and extravasated erythrocytes within the hypodermis, and fragmentation, loss of cross striations, mineralization and proliferation of sarcolemmal nuclei of individual myofibers within the panniculus carnosus), or no significant lesions were reported for the decedents or the acrolein-treated surviving animals; other than acrolein-associated dermatopathy (discoloration (yellow-brown) of the dose site skin, an intimately adherent red-brown scab for the ulcerated skin, or the firm, wrinkled, non-pliable and overlying skin when not ulcerated, edematous and hemorrhagic hypodermis, and the glistening and gelatinous appearance of the cubcutis extending to the axillary and inguinal regions (dependent edema)), the only gross lesions occurring consistently at all treatment levels are pulmonary petechiae, atelectasis and discoloration at scheduled sacrifice and necropsy; no gross lesions or only minimal, nonspecific lesions were reported for the untreated and vehicle-treated control animals; LD50 (95% confidence limit): (M) 240.0 (217.3-265.1) mg/kg, (F) 223.1 (200.1-248.7) mg/kg, or (M/F) 231.4 (216.5-247.4) mg/kg. **Toxicity Category: II; Study Acceptable.** (Guo, 4/22/15)

Acute inhalation toxicity, rat

Study not submitted.

Primary eye irritation, rabbit

50032-0068 283470; Primary eye irritation; 814; Rabbit: New Zealand White; Bioassay Systems Corporation (BSC), Woburn, MA; BSC Study # 10258, MRID # 00141025; Dunn, G. R. and Goodband, J.; 7/20/81; DOT Acrolein Inhibited (Acrolein, 95%); 9 males; Dose: 0.1 mL into an eye of each rabbit; Mortality (M): 1/9; Clinical observations: one test animal died at 4 days after instillation; score 4 corneal opacity and score 2 iritis were noted for 9/9 treated eyes at 24 through 72 hours and for 8/9 treated eyes at 4 and 7 days after instillation; score 2 to 3 conjunctival redness (score 2, 8/9; score 3, 1/9) was reported for the treated eyes at 24 through 72 hours; score 2 conjunctival redness was reported for 8/9 treated eyes at 4 and 7 days; score 4 conjunctival chemosis was noted for 9/9 treated eyes at 24 through 72 hours, and for 8/9

treated eyes at 4 and 7 days after instillation; all the surviving animals gained or maintained body weight by 72 hours post-treatment, with the exception of three animals losing weight. **Toxicity Category: I; Study Acceptable.** (Guo, 4/20/15)

Primary dermal irritation

50032-0069 283471; Primary dermal irritation; 815; Rabbit: New Zealand White; Bioassay Systems Corporation (BSC), Woburn, MA; BSC Study # 10258, MRID # 00141026; Dunn, G. R. and Goodband, J.; 3/21/81; DOT Acrolein inhibited (DOT E8249) (Acrolein, 95%); 6 males; topical, dermal application; Dose: 0.5 mL/site, one intact site and one abraded site per animal; 24-hour exposure; Mortality (M): 2/6; Clinical observations: two test animals died with 24 hours of test substance application; score 1 to 2 erythema was noted for the abraded test sites (score 1, 1/4; score 2, 2/4) and unabraded test sites (score 2, 2/4) at 24 hours after application; score 1 to 3 erythema was reported for the abraded test sites (score 1, 2/4; score 3, 1/4) and unabraded test sites (score 2, 2/4) at 72 hours; score 1 to 2 erythema was noted for the abraded test sites (score 2, 2/4) and unabraded test sites (score 1, 1/4; score 2, 1/4) at 4 days after application; score 1 to 4 erythema was reported for the ubabraded test sites at 8 and 9 (score 1, 1/4), 10 through 12 (score 1, 2/4), and 13 and 14 days (score 2, 2/4, score 4, 1/4), with injuries in depth, bleeding and open sore observed at one unabraded test site on days 13 and 14; score 1 to 3 erythema was noted for the abraded test sites at 7 (score 1, 1/4; score 3, 1/4), 8 and 9 (score 1, 1/4, score 2, 1/4; score 3, 1/4), 10 and 11 (score 1, 1/4; score 2, 2/4), 12 (score 1, 2/4; score 2, 1/4), and 13 and 14 days (score 1, 1/4; score 2, 2/4); score 1 to 4 edema was reported for both the abraded and unabraded test sites at 24 (score 1, 1/4; score 3, 1/4; score 4, 2/4) and 72 hours (score 2, 2/4; score 3, 1/4; score 4, 1/4) and at 4 days (score 3, 3/4; score 4, 1/4) after application; score 2 to 3 edema was noted for the unabraded test sites at 7 (score 2, 2/4; score 3, 1/4), 8 and 9 days (score 2, 1/4; score 3, 2/4), and for the abraded test sites at 7 and 8 (score 2, 3/4; score 3, 1/4), and 9 days (score 2, 2/4; score 3, 2/4); score 1 to 4 edema was reported for the unabraded test sites at 10 (score 1, 1/4; score 2, 1/4; score 4, 2/4), 11 (score 1, 1/4; score 3, 1/4; score 4, 2/4), and 12 through 14 days (score 2, 1/4; score 4, 3/4); score 2 to 4 edema was noted for the abraded test sites at 10 (score 2, 2/4; score 4, 2/4), 11 (score 2, 1/4; score 3, 1/4; score 4, 2/4), 12 and 13 (score 2, 1/4; score 4, 3/4), and 14 days (score 3, 1/4; score 4, 3/4); by day 7, the sample test sites exhibited yellow discoloration (4/4), and/or swelling (2/4), and/or minimal flaking of skin (1/4), whereas the control sites showed normal appearance (2/4), and/or no swelling plus moderate flaking of skin (1/4), and/or discoloration (slightly red and yellow) plus swelling (1/4, for abraded control sites only); yellow discoloration, large area of necrotic skin, dark patches and some open sores were noted for the test sites at 14 days after application, with one animal showing a large open sore at the test site and scabs and flaking skin at the control site; all the surviving animals gained body weight during the study. **Toxicity Category: I; Study Acceptable.** (Guo, 4/22/15)

Dermal sensitization

Study not submitted.

SUBCHRONIC STUDIES

Rat Subchronic Oral Toxicity Study

50032-0076 283478; “Subchronic Oral Toxicity of Acrolein (Lot No. SFSL-5893) in Rats”; (Muni, I. A.; Bioassay Systems Corporation (BSC), Woburn, MA; BSC Project No.: 10258, MRID # 001413029; 9/11/81). Thirty Sprague-Dawley rats/sex/dose level were dosed orally by gavage with 0.0 (vehicle: deionized water), 0.05, 0.5 or 5.0 mg/kg/day (5 days per week) of Acrolein (Lot No. SFSL-5893, a.i.: 95%, diluted with deionized water) for 13 consecutive weeks. One male

animal in the 0.5 mg/kg group died by week 5 due to gavage accident; all other animals survived to their designated sacrifice dates. No obvious clinical signs were consistently seen in acrolein-treated rats. Several non-specific clinical signs were noted sporadically over the course of the study. No obvious numerical differences in body weight means or weekly mean food consumption were present between control and treated groups (males and females). Hematology, clinical chemistry and urinalysis evaluations revealed that overall, there were no differences of significance between dose levels or between treatment and control groups. No treatment-related effect was noted in the mean absolute or relative organ weights. No treatment-related gross abnormalities were noted. The gross lesions observed were characterized by their apparent random nature, low frequency, and lack of consistency in type, and most likely they are incidental findings unrelated to test substance administration. The histopathological examination did not reveal any treatment-related lesions. Rat Subchronic Dietary NOEL: 5.0 mg/kg; based on the data collected over the course of the 90-day study, no significant toxicological effects were found in rats dosed with 0.05, 0.5 and 5.0 mg/mL of acrolein. **Study Acceptable.** (Guo, 5/25/15)

Mouse 14-Day Oral Toxicity Study

50032-0077 283479; "14-Day Oral Toxicity Test in Mice"; (Mansur, C. A.; Bioassay Systems Corporation (BSC), Woburn, MA; BSC Project No.: 11496; 6/20/83); Acrolein (Acrolein, > 96%); 10 animals/sex/dose level; administered by oral gavage to assess acute oral toxicity of acrolein in mice over a 14-day period; Dose: 0.0, 4.6, 5.8, 7.2, 9.0 mg/kg; Mortality (M/F): 0.0 (0/10 M/F), 4.6 (0/10 M/F), 5.8 (0/10 M, 1/10 F), 7.2 (2/10 M, 0/10 F), 9.0 mg/kg (1/10 M, 0/10 F); Clinical observations: two males dosed at 7.2 mg/kg and one male at 9.0 mg/kg died by day 3 or 4, and one female dosed at 5.8 mg/kg died on day 6; lethargy, and/or rough coat, and/or hunched posture, and/or squinted eyes, and/or exudate around eyes, and/or extreme, very shallow breathing were noted for the above animals prior to their death; all ten males receiving 9.0 mg/kg showed rough coat during days 2-12, and other signs in some of these males were piloerection (1/10, on day 8), reddening of the tip of the tail (2/10, during days 10-14), bite wounds on back (3/10, during days 11-14), closed eyes (1/10, during days 4-8 and 10-14), and orange stain around left eye (1/10, during days 4-8); reddening of the tip of the tail was noted for 2 females dosed at 9.0 mg/kg during days 10-14, and for one female at 7.2 mg/kg during days 10-12; one male receiving 7.2 mg/kg exhibited lethargy, rough coat, and squinted eyes by day 3; another male in the 7.2 mg/kg group showed blood around the mouth after dosing on day 14, and the injury might be an artifact of gavage; all other animals appeared healthy during the study; all the surviving animals and the control animals gained weight during the study period; Gross necropsy: the most common findings at necropsy were in the squamous portion of the gastric mucosa; one male dosed at 5.8 mg/kg, 2 males at 7.2 mg/kg, 9 males and 6 females receiving 9.0 mg/kg showed white and thickened gastric mucosa in the squamous portion of the stomach; five males dosed at 7.2 mg/kg and 2 females at 9.0 mg/kg had pinpoint raised foci or nodules in the squamous portion of the stomach; other observations include hemorrhagic lungs and tail darkened near the tip. Mouse Subchronic Dietary NOEL: Not established at this time, due to the limited time frame of dosing. Based on the mortality and necropsy observations seen in the 14-day toxicity study, the levels recommended for the chronic study are 5.8, 4.6 and 2.3 mg/kg in male mice and 7.2, 5.8 and 4.6 mg/kg in female mice. **Study Acceptable.** (Guo, 4/27/15)

Oral toxicity, non-rodent:

Study not submitted.

Rabbit 21-Day Repeated Dosing Dermal Toxicity Study

50032-0071 283473; "21-Day Dermal Test of Acrolein in Rabbits"; (Muni, I. A.; Bioassay Systems Corporation (BSC), Woburn, MA; BSC Project No.: 10258, MRID # 00141030; 7/28/82). The skin of 10 New Zealand White rabbits/sex/group was treated with 0.0, 7.0, 21.0 or 63.0 mg/kg/day of Acrolein (Lot Number SFSL-5993) (Acrolein, > 96%) 6 hours per day for 21 days (5 days per week). Prior to application, the test substance was diluted with a 50:50 (v/v) solution of deionized water and absolute ethanol. Two females dosed at 21.0 or 63.0 mg/kg died by day 5 or 4, and two females receiving 7.0 or 63.0 mg/kg were sacrificed moribund (likely due to broken backs attributable to hyperactivity behavior following dosing) at 5 days after the first application. Slight to moderate conditions of nasal mucus (discharge) and lethargy were seen more frequently in the acrolein-treated animals than in the controls. Several acrolein-treated animals also showed slight to moderate diarrhea, and/or apparent weight loss. After the first dose application, slight to moderate erythema and edema at the dose site were noted for almost all rabbits treated with acrolein at a level of 7.0 or 21.0 mg/kg; the animals receiving 63.0 mg/kg had similar severity of erythema, along with a more prominent edema formation. In general, for both male and female rabbits as the study progressed, the erythema and edema conditions became more pronounced in all acrolein-treated groups. Overall, the animals dosed at 63.0 mg/kg showed more pronounced skin damage (higher erythema/edema scores) than those in the 7.0 and 21.0 mg/kg groups. Skin damage was similar between animals with abraded skin and those with unabraded skin. By contrast, no notable redness or swelling was seen on the skin of the untreated or vehicle control animals. No significant differences were observed between the control and acrolein-treated groups for body weights, although acrolein-treated males exhibited lower (not significantly lower) final body weights than the controls. However, there were significantly smaller body weight changes (final minus initial body weights) ($p < 0.01$) in both sexes among acrolein-treated groups when compared with controls. A significant effect ($p < 0.01$) of the test substance on food consumption was found among male animals, but not among females. No treatment-related effects were noted in the hematological and blood chemistry evaluations, organ weights, organ-to-body weight ratios and organ-to-brain weight ratios. No visible lesions were observed in the untreated or vehicle control animals, other than some liver and lung abnormalities. Epidermal application of acrolein caused firm, thickened, reddened epidermis and dermis, skin discoloration, hemorrhage, edema, necrosis, and ulceration, or firm, thickened, corrugated and folded epidermis or epidermal crusts overlying the epidermis, with the severity of skin damage in a dose-related pattern. As to the animals that died or were sacrificed moribund during the first week of dosing, the gross abnormalities include vertebral column-subluxated at the T12-L1 region, swollen, edematous, discolored epidermis (brown or reddened) at dose site, the presence of blood in urine, liver with multiple white depressed spots and congested lungs, and gastrointestinal tract with liquid-like contents. The histological examination revealed that at higher dose levels, chronic topical administration of acrolein produces severe necrotizing and ulcerative dermatitis, resulting in healing with marked dermal fibrosis, hyperkeratosis and occasionally pseudoepitheliomatous hyperplasia. These are expected reactions of the integument to severe but reversible injury. There were significant skin lesions in the animals dosed at 7.0 mg/kg: epidermal necrosis, loss of adnexa, marked infiltrate of heterophilic cells and dermal edema. Mild pulmonary lesions (lymphoid hyperplasia and multifocal nonsuppurative interstitial pneumonia) were observed at this dose level, with mild bilateral nonsuppurative interstitial nephritis seen in 5/20 animals. At 21.0 mg/kg, necrotizing dermatitis was accentuated, and also observed were early reparative changes: hyperkeratosis, acanthosis, dermal fibrosis at the periphery of the ulcerated epidermis, and marked fibrosis in the area of the ulcer. Pulmonary lesions were intensified and a more diffuse nonsuppurative interstitial pneumonia was observed in 13/20 animals. Nonsuppurative interstitial nephritis was seen in 7/10 animals, with mesangioproliferative glomerulopathy noted for 3/20 animals. At 63.0 mg/kg, severe necrotizing dermatitis was observed, along with extensive hyperkeratosis,

acanthosis and parakeratosis, which was accompanied by dermal fibrosis. Severe diffuse nonsuppurative interstitial pneumonia was observed in 16/20 animals. Renal lesions in this group were the same as those observed in the 21.0 mg/kg dose group. No significant histopathological lesions of the skin were noted for the untreated or vehicle control animals. Overall, fewer histopathological lesions in the kidneys, eyes, liver and trachea were found among the control animals, with interstitial pneumonia in the lungs noted for 7/20 control animals. Of note, cholangiohepatitis secondary to Eimeria steidiae infestation was prominent in all dosage groups. Therefore, it was impossible to evaluate the toxicity of acrolein on the liver in this study. 21-Day Repeated Dose Dermal Toxicity NOEL: Not established because of the compelling evidence of toxicity at all doses tested; Dermal Irritation NOEL: Not established due to the compelling evidence of toxicity at all doses tested; **Study Acceptable.** (Guo, 5/18/15)

CHRONIC STUDIES

Chronic, rat

50032-028 085062; "24-Month Chronic Toxicity and Oncogenicity Study in the Rat with Acrolein," (Long, J. E. & Johnson, J. A., Tegeris Laboratories, Inc., Laboratory Project No. TL 85047; 9/6/89). Acrolein, purity ~96%, was administered by gavage at the nominal concentrations of 0 (water), 0.05, 0.5 and 2.5 mg/kg to 70-75 Sprague-Dawley rats/sex/group, for 102 weeks. Analytical testing of dosing solutions was done only 0-4 times/month for the first 18 months of the study. Dose levels were based on a short (6-week) pilot study, which was also included in the report. At 13 weeks and at 1 y, 5 high-dose rats (both sexes) and 10 rats/sex/group were sacrificed, respectively. Also, gavaging-induced deaths and loss of tissues due to autolysis effectively reduced the number of rats/sex/treatment level, depending on the tissue in question. There were no obviously treatment-related effects on the following: survival, bodyweights, organ weights, clinical signs, serum chemistry, hematology, urinalysis, and gross findings. The ophthalmology portion of this study was inadequate for interpretation. Two possible histological findings were increased incidences of hepatocellular adenomas and acinar-cell adenomas of the pancreas in the males; both tumor types exhibited dose responses and maximum percentages affected that were clearly greater than the values observed in the supplied historical control data. No histological findings were noted in the obvious target organ, the forestomach. This study was considered unacceptable when first reviewed (Rinkus, 8/13/90); and upgrading would require the submission of supplemental information that addressed the many concerns that DPR MT had about the study. The Registrant responded by submitting records 097781, 124586 and 126430. These records are discussed in worksheet W085062.S01. Based on these submissions, record 085062 is considered marginally **ACCEPTABLE** only as an oncogenicity study. Hepatocellular adenomas in males have been dropped as an adverse-effect finding but pancreatic acinar cell tumors (adenoma and carcinoma) remain as adverse effects, with a **LOAEL of 0.05 mg/kg.** Record 085062 is **NOT ACCEPTABLE** and **NOT UPGRADABLE** as a chronic-toxicity study (inadequate hematology). (Rinkus, 3/18/94)

011 059502; Status at 30 weeks in terms of survivors. Doses of 0, 0.05, 0.5, and 2.5 mg/kg are being given by oral gavage. Doses were selected based on a 6-week range-finding study. Document is dated December 23, 1986. No review sheet. (Gee, 2/5/88)

50032-044 097781; This record uses a question-and-answer format to address issues raised in worksheet W085062.835. **Supplemental information**. (Rinkus, 5/18/94)

50032-047 124586; This record is the pilot study on which the selection of the high dose for the rat reproduction study (record 092721) was based. **Supplemental information.** (Rinkus, 5/18/94)

50032-048 126430; This record contains three mailings of explanatory information regarding the statistical analyses contained in record 097881 for hepatic and pancreatic tumors in males. **Supplemental information.** (Rinkus, 5/18/94)

Chronic, dog

50032-026 073320; "Acrolein--Chronic (12 Month) Oral Toxicity Study in the Dog," (Long, J. E., Tegeris Laboratories Inc., Project I.D. no. TL #85016; 10/23/87). Acrolein, purity of > 94%, was administered by gelatin capsule at concentrations of 0, 0.1, 0.5 or 2.0 mg/kg/day to 6 beagle dogs/sex/group for 12 months. Dose levels were based on a range-finding study, which was included in the report. The only effects identified in the first review (9/11/90) as related to treatments were: vomiting in the mid and high-dose groups (both sexes), with the frequency being greater in the high-dose groups, and decreases in the serum levels for calcium and albumin (consequently total protein, too) for the high-dose male and female groups, with both reductions being seen at each of the 3-month testing intervals. When first reviewed, this study was considered unacceptable, but upgradable upon submission of the ophthalmology raw data, a complete list of protocol deviations and resolution of matters concerning the route of exposure, preparation of dosing solutions, controlling of mites, frequency of dosing per week, and assaying for serum gamma-glutamyl transpeptidase. The Registrant responded by submitting records 097215 and 128724. These records are discussed in worksheet W073320.S01. A decrease in the activated partial thromboplastin time (hypercoagulation) is now being identified as a possible adverse effect (**NOAEL = 0.1 mg/kg**), for reasons discussed in worksheet W073320.S01. This study is now considered **ACCEPTABLE.** (Rinkus, 5/13/94)

Oncogenicity, rat

See Rat Chronic Toxicity above.

Oncogenicity, mouse

50032-029 090136; "18-Month Oncogenicity Study in the Mouse with Acrolein," (Long, J. E. & Johnson, J. A., Tegeris Laboratories, Inc., Laboratory Project No. TL 86057; 10/16/89). Acrolein, purity ~96%, was administered by gavage at concentrations of 0 (water), 0.5, 2.0, and 4.5 mg/kg to CD-1 Swiss Albino mice for 18 months. Dose levels were based on a short (6 week) pilot study, which was also included in the report. Although the original study design called for 70-75 mice/sex/group, gavaging-induced deaths during the first few weeks of the study reduced the "effective number" of mice/sex/group to 37-67; and autolysis prevented the histological examination of some tissues, thereby reducing further the "effective number" for some organs. Excluding the gavaging-induced deaths occurring before week 3, there was no evidence that survival was affected in this study. Also, there was no evidence of a treatment-related effect on the following: bodyweights; clinical signs; wet organ weights (terminal sacrifice) for the brain, liver, kidneys, and testes; WBC differentials; and ophthalmology. Neither gross nor microscopic examinations identified any treatment-related effects, including no effects on the obvious target organ for this direct-acting agent, the forestomach. **NOEL > 4.5 mg/kg.** This study was considered **UNACCEPTABLE** when first reviewed (Kishiyama and Rinkus, 7/5/90) and upgrading would require the submission of supplemental information that would address the following: whether the highest dose tested was sufficiently close to the maximum tolerated dose; mice brought late into the study; the choice of the oral route of exposure over inhalation; amendments and deviations to the protocol; and discontinued QA inspections. The Registrant responded by submitting record 097780 and its contents are discussed in worksheet

W090136.S01. This study is considered **UNACCEPTABLE** and **NOT UPGRADABLE (MTD not tested, poor study conduct, data for a parallel study not submitted)**. (Rinkus, 9/7/93)

011 059216; Status report at week 5 for mouse study with survivors. Doses of 0, 0.5, 2.0, and 4.5 mg/kg are being given by oral gavage. A cover letter, dated July 10, 1987, discusses the problems Tegeris Laboratories is having with the dosing, resulting in numerous deaths. Additional mice will be added to the original 70/sex/group at the same doses so that an adequate number should be available at termination of the study. Status report is dated December 23, 1986. No review sheet. (Gee, 2/5/88). Inspection of the data for the full study (record 090136) indicates that the water-gavaged mice also were dying as much as the acrolein-gavaged mice were. Therefore, the dying in this study appears to have been due to the gavaging technique and not the fact that three of the four dosing solutions contained acrolein. (Rinkus, 9/28/90)

GENOTOXICITY

Gene mutation

Summary. The data requirement for gene mutation studies (842) is considered filled, with an adverse effect indicated. The results from bacterial testing included positive results (records 061604 & 061607), borderline results (records 061603, 061605, 061606 & 061609), and negative results (records 061602 & 061603). The two studies indicating a reproducible mutagenic effect also have provided a rationale for why other studies may have failed to demonstrate mutagenicity and this has persuaded CDFA to treat acrolein as a potential mutagen. The fact that acrolein is very reactive with sulfhydryl groups makes it very cytotoxic; as a result, "nonstandard" test procedures are needed to demonstrate mutagenicity. In record 061607, the new *Salmonella* tester strain TA104 was used with a liquid preincubation method of exposure; also, it was found that the presence of the plasmid pKM101 and deletion of the uvrB gene facilitated the detection of acrolein and that adding glutathione after the liquid preincubation period reduced the cytotoxicity but not the mutagenicity of acrolein. In record 061604, TA100 was used with a liquid preincubation method; afterwards, the cells were isolated by centrifugation and resuspended into fresh phosphate buffer for plating. Elsewhere one of the authors of record 061604 has reported that the liquid preincubation method using an increased cell density is necessary for demonstrating the mutagenicity of acrolein (Naunyn-Schmied. *Arch. Pharmacol.* 316 (Supplement), Abstract 54, 1981); these same conditions also are critical for detecting 3-methylacrolein (*Environ. Mol. Mutagen.* 14:146-148, 1989). (Rinkus, 9/28/90)

50032-027 075135; "CHO/HGPRT Mutation Assay with Confirmation," (J.W. Harbell, Microbiological Associates Inc., Laboratory Study No. T8403.332001; 5/25/89). Acrolein, 96% purity, was tested as dimethyl sulfoxide solutions for mutagenicity at the HGPRT locus (6-thioguanine resistance) using Chinese hamster ovary K_1-BH_4 cells. Test concentrations ranged from 0.0002 to 0.008 µl/ml and testing was done in the absence and presence of a Aroclor-induced rat liver S-9 activation system; in both cases, cells were exposed to acrolein for 5 h. Testing also involved the routine use of two negative controls (no treatment & DMSO at 1% v/v) and two positive controls (ethyl methanesulfonate and benzo(a)pyrene), which in all cases gave satisfactory results. Three trials, each involving testing up to concentrations that produced significant cytotoxicity, were conducted. In the first two trials, testing in the absence and presence of an S-9 mix was done, while in the third trial, no metabolic activation system was used. Slightly increased mutant frequencies were observed with concentrations of 0.0060 µl/ml in the presence of S-9 in the first trial and 0.0008 µl/ml in the absence of S-9 in the second trial.

However, in both cases, these increased mutant frequencies were not observed again in re-testing that either included the suspect concentration or testing up to high cytotoxicity. Therefore, **no reproducible mutagenicity was demonstrated. ACCEPTABLE.** (Kishiyama & Rinkus, 9/21/90)

006 042949; "*In vitro* Gene Mutation Assay (HGPRT Locus) in Cultured Chinese Hamster Ovary (CHO) Cells on Acrolein," (Bioassay System Corp., 4-28-82). Acrolein (>95%) at 0, 0.04, 0.06, 0.08, 0.1, 0.2, and 0.3 µg/ml with S9 rat liver activation, and 0, 0.1, 0.2, 0.3, 0.4, and 0.5 µg/ml - S9 on Chinese Hamster cells (CHO); HGPRT system; single trial, duplicate cultures; No evidence of increased mutation frequency; **UNACCEPTABLE.** (No repeat trial). (Gee, 8-4-86)

013 061602; "Salmonella Liquid Suspension Mutant Fraction Assay on Acrolein", (Bioassay Systems, Project no. 10258, 12-30-80). Acrolein, > 99%, was assayed with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 at concentrations of 1, 3, 10, 20, or 40 µg/ml in DMSO with and without S9 activation, duplicate samples, repeat trials with three strains. Suspensions were plated for viability and revertants. At 40 µg/ml, survival was less than 5%. No increase in revertants was noted. **ACCEPTABLE.** (Shimer, 1-13-88 and Gee, 2-1-88)

Note: Although this report conforms with guidelines with minor variations, the negative results are in contrast to the positive findings of other investigators also using *Salmonella* strains. The overall conclusion for gene mutation is that acrolein is a mutagen. See Summary above. (Gee, 2/5/88; Rinkus, 9/28/90)

The following one-liners are literature citations which contain gene mutation assays on acrolein.

013 061603; "Mutagenicity of Vinyl Compounds in *Salmonella typhimurium*", (Frederick Cancer Research Center, in *Teratogenesis, Carcinogenesis, and Mutagenesis* (1980) 1:259-267). Eighteen compounds structurally related to vinyl chloride were tested for mutagenicity in five strains of Salmonella typhimurium – TA1535, TA1537, TA1538, TA98 and TA100. All chemicals were greater than 95% pure, activation was provided with rat and hamster S9 mix. Acrolein was mutagenic without activation only at concentrations from 0.02 to 0.07 µl in strain TA98 in the plate incorporation assay. **UNACCEPTABLE.** No individual plate counts. Publication indicates repeat trials were run for mutagenic compounds but no data for repeat trial. (Shimer, 1/13/88 and Gee, 2/1/88)

013 061604; "Structure Mutagenicity Relationship in α,β-Unsaturated Carbonylic Compounds and Their Corresponding Allylic Alcohols", (*Mutation Research* (1982) 93:305-315). Several compounds were tested with strain TA100 of *Salmonella typhimurium* for mutagenic activity in a modified suspension assay, incubated for 90 minutes and plated in duplicate. Two independent trials were run. Acrolein, 99.9% was found to be highly mutagenic with 2400 revertants per mmole reported without activation. It was nonmutagenic and less cytotoxic with S9 activation. Apparently tested at 0 to 0.15 mmoles/2 mls incubation volume. Publication contains the statement that acrolein was negative in the plate incorporation assay. **UNACCEPTABLE.** One strain, no individual plate counts – data presented in graphic form only. (Shimer, 1/13/88 and Gee, 2/1/88)

013 061605; "Comparison of the Mutagenicity and Teratogenicity of Cyclophosphamide and Its Active Metabolites, 4-Hydroxycyclophosphamide, Phosphoramide Mustard, and Acrolein",

(McGill University, Montreal, *Cancer Research* (1982) 42:3016-3021). See under Rat Teratology above for one-liner.

013 061606; "Salmonella Mutagenicity Test Results for 250 Chemicals", (EG&G Mason Research Institute, *Environmental Mutagenesis* Supplement (1983) 1:3-142). Four strains of *Salmonella*, TA1535, TA1537, TA98 and TA100, were used to test 250 coded chemicals, at three different labs – Case Western Reserve, Microbiological Associates and SRI. Male Sprague-Dawley rats and male Syrian hamsters were used for liver activation. Acrolein, practical grade, was 82% and was tested at 0 to 100 µg/plate in triplicate with a repeat trial. The preincubation procedure for 20 minutes was used. The summary table indicates a positive effect (+) with acrolein but the data do not substantiate this conclusion. TA100 shows a less than 2-fold increase in revertant colonies with rat liver activation and less of an increase with hamster activation. There is, however, a concentration-dependent trend. Acrolein was tested to the limit of cytoxicity. **UNACCEPTABLE.** Data presented as mean ± SEM, practical grade was used. (Shimer, 1/13/88 and Gee, 2/2/88)

013 061607; "Naturally Occurring Carbonyl Compounds are Mutagens in *Salmonella* Tester Strain TA104", (U.C. Berkeley, *Mutation Research* (1985), 148:25-34). Acrolein, purity not stated; a new *Salmonella* tester strain, TA104 (nonsense mutation - TAA - at site of reversion in a single copy), was tested with carbonyl compounds to see how it compared with other strains in assaying for mutagenicity. In the liquid preincubation procedure, 20 minutes with shaking, in duplicate, acrolein was mutagenic in TA104 but not TA102 (no data). The maximum non-toxic concentration was 0.9 umoles with 1080 revertants/µmole with TA104. The text contains the statement that acrolein was much less mutagenic in TA2638 (uvr+) than in TA104. The marked toxicity of acrolein limits its detectability as a mutagen. A reference is made to the formation of adducts by acrolein with deoxyguanosine. **UNACCEPTABLE.** Data in graphic form only, one strain only. **Possible adverse effect with increase in revertants**. (Shimer, 1/1/3/88 and Gee, 2/2/88)

013 061608; "Mutagenic Activity of Major Mammalian Metabolites of Cyclophosphamide Toward Several Genes of *Escherichia coli*", (National Institute of Environmental Health Sciences, *J. of Toxicology and Environmental Health*, (1977) 3:637-650). Acrolein, 98%, along with other compounds was tested with *E. coli* 343/113 for its ability to induce mutations. After treatment for 180 minutes, *E. coli* was plated on selective medium for detection of back mutations to gal+ and arg+ MTR (from 5-methyltryptophan sensitivity to resistance) forward mutations. Acrolein did not cause an increase in mutation frequency – tested only without activation. It was quite cytotoxic – data inactivation of *E. coli* are presented. **UNACCEPTABLE.** No data for mutation frequency, no activation included. (Shimer, 1/13/88 and Gee, 2/2/88)

013 061609; "Comparison of Alkylation Rates and Mutagenicity of Directly Acting Industrial and Laboratory Chemicals", (Institute of Occupational Health, Finland, *Arch. Toxicol.* (1980) 46:277-285). The alkylation activity was compared with mutagenicity of industrial and laboratory chemicals to *E. coli* WP2 uvrA without metabolic activation, incubated for 18 hours, then plated for revertants. Acrolein, analytical grade, no purity stated, was considered as showing "weak" mutagenicity and alkylation activity with 4-(p-nitrobenzyl)-pyridine and deoxyguanosine. The mutagenicity is expressed as the change in reversion frequency X 10^{-11}/µM (4) or as % in relation to epichlorohydrin (2) so the significance is difficult to evaluate. **UNACCEPTABLE.** No activation, no plate counts, no concentrations tested, other missing information. (Shimer, 1/19/88 and Gee, 2/2/88)

013 061610; "Chemical Mutagenesis Testing in Drosophila. II. Results of 20 Coded Compounds Tested for the National Toxicology Program", (*Environmental Mutagensis* (1985) 7:87-100). Results are presented from mutagenesis testing in the sex-linked recessive lethal test in *Drosophila* of 20 coded compounds. Acrolein, 96.9%, considered negative. In feeding studies, males were placed in vials containing glass filters saturated with the test material in 5% sucrose at 0 or 3000 ppm and exposed for 3 days, then mated. For injection studies, males were mated 24 hours after injection with 0 or 200 ppm (volume not stated). Canton-S wild-type males were each mated to three Basc females to produce three broods over 7 days. Two experiments were run for a total of >5800 total tests. An earlier study is cited in which acrolein reportedly gave positive effects after larval feeding – no data. **UNACCEPTABLE.** No justifications of the concentrations used, unclear if two trials with both feeding and injection. (Shimer, 1/19/88 and Gee, 2/2/88)

Chromosome damage

Note: CDFA is aware of the following reports in the open literature that indicate positive and equivocal effects under this category: the induction of sister-chromatid exchanges in CHO cells (Au et al., *Cytogenet. Cell Genet.* 26:108-116, 1980; Galloway et al., *Environ. Mol. Mutagen.* 10 (Suppl. 10):1-175, 1987).

006 042950; "Effects of Acrolein on the *In Vivo* Induction of Chromosomal Aberrations in Rat Bone Marrow Cells", (Bioassay Systems Corp., 11/17/82). Acrolein (>95%) given at 0, 1, 2.1, and 4.1 mg/kg in a single i.p. injection to Sprague-Dawley rats, 3 to 5 males/group; sampling times of 6, 12, and 24 hours; no evidence of increased chromosomal aberrations; **UNACCEPTABLE** (males only). (Gee, 8-7-86)

006 042951; "Effects of Acrolein on the *In Vitro* Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells", (Bioassay Systems Corp., 7/23/82). Acrolein (>95%) tested at 0, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0 µg/ml +S9 on CHO cells, 2 hours, and 0, 0.2, 0.4, 0.6, 0.8, 1.0, and 2.0 µg/ml -S9 for 6 hours; single time of harvest, duplicate cultures; No adverse effects reported; **ACCEPTABLE** with variances (single time of harvest and varying number of cells analyzed). (Gee, 8-4-86)

006 042952; "Effects of Acrolein on the *In Vitro* Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells", (Bioassay Systems Corp., 5/1/82). Acrolein (>95%) tested 0, 0.01, 0.3, 0.5, and 0.75 µg/ml on CHO cells, +S9, 4 hours; at 0, 0.3, 0.5, and 0.75 µg/ml -S9, for 30 hours. No evidence for SCE formation; **ACCEPTABLE.** (Gee, 8-4-86)
(Shimer, 1/19/88 and Gee, 2/2/88)

DNA damage or miscellaneous effects

Note: CDFA is aware of the following reports in the open literature that indicate a positive effect under this category: the induction of DNA single-strand breaks, as measured by the alkaline elution assay (Erickson et al., *Cancer Research* 40:4216-4220, 1980); and the formation of DNA adducts (Chung et al., *Cancer Research* 44:990-995, 1984; Wilson et al., *Proceedings of the American Association for Cancer Research* 31:95 (Abstract No. 563), 1990).

006 042953; "Effect of Acrolein on the Incidence of C3H/10T1/2 transformed cells *In Vitro*", (Bioassay System Corp., 4/28/82). Acrolein (>95%) tested at 0, 0.04, 0.06, 0.08, and 0.1 µg/ml on mouse fibroblasts (C3H/10T1/2), exposed for 3 days; 20 dishes/concentration; scored at 6-7 weeks; No increased incidence of type II - III foci reported; **ACCEPTABLE.** (Gee, 8-4-86)

REPRODUCTIVE TOXICITY, RAT

007-8 50058-9; "Two Generation Reproductive Study of Acrolein in Albino Rats", (Bioassay Systems Corporation, 12-7-84). Acrolein by gavage at 0, 4.0, 5.4 and 7.2 mg/kg/day. Reproductive NOEL > 7.2 mg/kg. Chronic toxicity NOEL < 4.0 mg/kg (decreased body weight F1 males, stomach lesions F0 and F1). **UNACCEPTABLE;** cannot be upgraded (limited histopathology and necropsy, poor study performance, gestation period ranged from 11 to 27 days). No adverse effect. (Parker, 12/16/86). Rebuttal by Baker Performance Chemicals and supplemental data (Record 067445) led to no change in evaluation. (Davis, 10/5/88)

021 067445; "Report Amendment: Two Generation Reproductive Study of Acrolein in Albino Rats." Supplemental material (revisions and corrections for the report; dosing solution analyses; dosing records; breeding observations) in support of the rebuttal in volume 021, part 1. (Davis, 10/5/88)

025 072525; Audit of the study prepared at Argus Research Laboratories. No worksheet. (Gee, 2/16/89)

50032-035 092721; "Reproductive Effects of Acrolein Administered Orally via Gavage to Crl:CD*(SD)BR Rats for Two Generations, with One Litter per Generation," (Alan M. Hoberman, Argus Research Laboratories, Inc., Horsham, PA; Report # 603-003, 4/12/91). Acrolein, >96% purity, was administered by gavage once daily at 0 (water), 1, 3, and 6 mg/kg, to 30 Crl:CD*(SD)BR rats/sex/dose in the F0 generation and to 40 rats/sex/dose in the F1 generation. F0 rats and F1 rats, derived from F1a litters, were exposed for ~10 weeks before their single mating trials and were exposed for a total of 93-149 daily dosings before they were sacrificed. No effects on the mating, fertility, or gestation indices were observed in either mating trial. Forestomach hyperplasia and (or) hyperkeratosis was seen at > 80% incidence in the 6 mg/kg groups (both sexes, both generations); also, an incipient effect was present at the 3 mg/kg level (females only, both generations). The incidences of mortalities and respiratory complications (e.g., rales, gasping) were increased significantly in the 6 mg/kg groups (both sexes, both generations). The composite picture that emerges from considering these mortalities and their respective clinical, necropsy and histology data is that aspiration of the dosing solution was commonplace, it resulted in lung damage, and in many instances this resulted in deaths. That is, these aspiration-associated lung effects and deaths may not represent a systemic or gastrointestinal effect by acrolein; rather, they are directly dependent on the use of gavage to accomplish oral dosing with a known pulmonary toxicant. **Parental NOAEL = 1 mg/kg (hyperplasia/hyperkeratosis).** The only progeny effect was a significant reduction in pup bodyweights, starting on lactation day 7 for the 6 mg/kg group (F1a offspring only). For the F1 males (but not the F1 females) derived from the F1a 6 mg/kg pups, this reduction in bodyweight (relative to the controls) persisted till their sacrifice at the end of the study. **Progeny NOAEL = 3 mg/kg (reduced F1a pup bodyweights during lactation).** This study is considered **ACCEPTABLE.** (Rinkus, 4/12/94)

50032-047 124586; This record is the pilot study on which the selection of the high dose for the rat reproduction study (record 092721) was based. **Supplemental information.** (Rinkus, 5/18/94)

50032-046 124351; This record is the protocol for the rat reproduction study (record 092721). **Supplemental information.** (Rinkus, 5/18/94)

50032-046 124343; This record is the protocol for the pilot rat reproduction study (record 124586). **Supplemental information.** (Rinkus, 5/18/94)

DEVELOPMENTAL TOXICITY

Rat

004 021617; "Teratology study of Acrolein in Rats", (Bioassay Systems Corp., 11-12-82, Project No. 10258). Acrolein, Batch 6151, 96.48%; analysis in 063285; given by oral gavage to Sprague-Dawley CD rats, days 7-19 (day of mating = day 1) at 0, 3.6, 6.0, or 10 mg/kg/day; **maternal NOEL = 3.6 mg/kg/day** (decreased weight gain, increased mortality and clinical signs), **developmental NOEL = 6.0 mg/kg/day** (decreased fetal weight and increased skeletal variants). Initially reviewed by J. Christopher, 3/27/85. Re-reviewed by J. Parker, 8/6/86, as not showing an adverse developmental effect. Submission of supplemental data in 063285 provides sufficient additional information to upgrade the study to **ACCEPTABLE** status with minor deficiencies. No adverse effect. (Gee, 2/5/88, with Parker)

> EPA 1-liner: Minimum. Maternal NOEL = 3.6 mg/kg (decreased body weight gain and increased mortality), fetotoxic NOEL = 6.0 mg/kg (decreased fetal weights, delayed ossifications), teratogenic NOEL = 6.0 mg/kg (fetal runts).

014 063285; "Report Amendment--Teratology Study of Acrolein in Rats," (MacRill, G. E.; Baker Performance Chemicals, Inc.; "revised" 8/14/87). This is also entitled "Appendix F" (to record 021617). It contains supplementary data to address the concerns raised by the then CDFA toxicologists that reviewed record 021617. These concerns are summarized in a question-and-answer format in a four-page letter dated October 23, 1987 from the Registrant to then CDFA that accompanied this submission; this letter (no record number) appears in the front of 50032-014. **Supplementary information.** (Rinkus, 5/18/94)

50032-003 920144; This record is a two-page summary of the results for the rat teratogenicity study (record 021617) and the mouse teratogenicity study (record 021618). A letter dated November 2, 1982 from the Registrant to then CDFA accompanied this submission; this letter (no record number) appears in 50032-003 just before record 920144. **Supplemental information.** (Rinkus, 5/18/94)

Rabbit

015 063594; "Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Acrolein Administered Orally (Stomach Tube) to New Zealand White Rabbits", (Argus Research Laboratories, Inc., Report no. 603-001, 5-20-87). Acrolein, 96.15% was administered to inseminated New Zealand White rabbits on days 7-19 of gestation at 0, 0.1, 0.75, and 2 mg/kg/day, 20/group. Maternal NOEL = 0.75 mg/kg/day (initial reduction of body weight gain); Developmental NOEL = 0.75 mg/kg/day (increase in number of resorptions not statistically significant). No adverse effect. **ACCEPTABLE.** (Shimer, 1/12/88 and Gee, 2/3/88)

Mouse

003 021618; "Teratology Study of Acrolein in Mice", (Bioassay Systems Corp., 9-1-82). Acrolein, Batch 6104, 96%, by oral gavage on days 7-17 of gestation, at 0, 4.0, 6.3, and 10 mg/kg/day. Developmental NOEL less than 4.0 mg/kg (Resorptions at 10, increase in structural changes at all dose levels). Maternal NOEL = 6.3 mg/kg (decreased weight gain).

UNACCEPTABLE, not upgradeable and **possible adverse effect indicated**. (Christopher, 3-28-85, Parker, 8-6-86)

EPA 1-liner: Supplementary. Maternal NOEL ≤ 4 mg/kg (LDT) (decreased body weight gain); fetotoxic NOEL ≤ 4 mg/kg (generalized delayed ossification); teratogenic NOEL, not established: possible cleft palate at 6.3 and 10 mg/kg. Conclusion could not be reached due to lack of critical data in report.

NEUROTOXICITY

Acute neurotoxicity, rat
Study not submitted.

90-day neurotoxicity, rat
Study not submitted.

Developmental neurotoxicity, rat
Study not submitted.

Delayed neurotoxicity, hen
Study not submitted nor required at this time.

IMMUNOTOXICITY
Study not submitted.

ENDOCRINE DISRUPTOR STUDIES
Study not submitted nor required at this time.

SUPPLEMENTAL STUDIES
013 061605; "Comparison of the Mutagenicity and Teratogenicity of Cyclophosphamide and Its Active Metabolites, 4-Hydroxycyclophosphamide, Phosporamide Mustard, and Acrolein", (McGill University, Montreal, *Cancer Research* (1982) 42, 3016-3021). Acrolein (a metabolite of cyclophosphamide) was one of the 4 chemicals given to rats by an intra-amniotic injection to fetuses in one uterine horn on day 13 of gestation. At 100 µg/fetus, 98% of fetuses were dead or resorbed, at 10 µg/fetus, 100% of fetuses were dead or resorbed. At 1 µg/fetus, 85.7% of the fetuses had malformations very similar to those caused by cyclophosphamide (edema, hydrocephaly, open eyes, cleft palate, omphalocele and forelimb, hindlimb and tail defects.) At 0.1 µg, 0.002 µmol/fetus, acrolein had no effect on resorption or malformation rate compared with saline control. Fetal weight was decreased at both 0.1 and 1 µg/fetus. The chemicals were also tested in an Ames assay with strain TA1535 of *S. typhimurium* at 0.001 to 50 µg/plate. Acrolein was very bacteriotoxic, not mutagenic without S9 and very weakly mutagenic with S9 activation. Supplementary. Not a guideline type study. Possible adverse developmental effect in fetuses and weakly mutagenic in bacteria. (Shimer, 11/13/87 and Gee, 2/2/88)

Publications Regarding Acrolein

Anon. (1985) Acrolein. In: *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Volume 36, pp. 133-161. World Health Organization, Lyon, France.

Beauchamp, Jr., et al. (1985) A Critical Review of the Literature on Acrolein Toxicity. *CRC Critical Reviews in Toxicology* 14:309-380.

Witz, G. (1989) Biological Interactions of α,β-Unsaturated Aldehydes. *Free Radical Biology & Medicine* 7:333-349.

Selley et al. (1990) Effects of Acrolein on Human Platelet Aggregation. *Chem.-Biol. Interactions* 76:101-109.

Lijinsky, W. and Reuber, M.D. (1987) Chronic Carcinogenesis Studies of Acrolein and Related Compounds. *Toxicology and Industrial Health* 3:337-345.

Published Versions of Some of the Studies Submitted Under SB950

Parent et al. (1992) Two-Year Toxicity and Carcinogenicity Study of Acrolein in Rats. *J. Appl. Toxicol.* 12:131-139.

Parent et al. (1992) One Year Toxicity of Orally Administered Acrolein to the Beagle Dog. *J. Appl. Toxicol.* 12:311-316.

Parent et al. (1992) Oncogenicity Study of Acrolein in Mice. *J. Am. Coll. Toxicol.* 10:647-659.

Parent et al. (1992) Reproductive Study of Acrolein on Two Generations of Rats. *Fundam. Appl. Toxicol*. 19:228-237.

United States
Environmental Protection
Agency

EPA/690/R-02/001F
Final
4-30-2002

Provisional Peer Reviewed Toxicity Values for

Acrolein
(CASRN 107-02-8)

Derivation of an Oral Slope Factor

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR ACROLEIN (CASRN 107-02-8) Derivation of an Oral Slope Factor

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional

Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The CRAVE workgroup (U.S. EPA, 1992) assigned acrolein to weight-of-evidence Group C, possible human carcinogen, on the basis of no evidence in humans and limited evidence in animals (increased incidence of adrenal cortical adenomas in female rats in an oral study, but no increased tumors in inadequate inhalation, skin painting, and subcutaneous injection studies). Supporting evidence included the carcinogenic potential of an acrolein metabolite, the mutagenicity of acrolein in bacteria, and the structural relationship of acrolein to probable or known human carcinogens. This assessment is listed on IRIS (U.S. EPA, 2001). No oral slope factor for acrolein is listed on IRIS (U.S. EPA, 2001), in the HEAST (U.S. EPA, 1997), or in the Drinking Water and Health Advisories list (U.S. EPA, 2000). Source documents for the IRIS assessment were a Health Assessment Document (HAD) (U.S. EPA, 1986) and a Health Effects Assessment (HEA) for acrolein (U.S. EPA, 1987). The CARA list (U.S. EPA, 1991, 1994) also includes a Health and Environmental Effects Profile (HEEP) on acrolein (U.S. EPA, 1985). IARC (1979, 1985, 1995) assigned acrolein to Group 3, not classifiable as to human carcinogenicity because of inadequate evidence in humans and animals. A Toxicological Profile for acrolein (ATSDR, 1990), an Environmental Health Criteria document on acrolein (WHO, 1992), a carcinogenicity review of low-molecular-weight aldehydes (NIOSH, 1991), and a toxicity review on aldehydes (Morandi and Maberti, 2001) were consulted for relevant information. The NTP (2001) health and safety report for acrolein was also examined. These resources contained no additional studies of acrolein itself. However, a metabolite of acrolein,

glycidaldehyde, yielded positive results for carcinogenicity in skin painting assays in mice and subcutaneous injection assays in mice and rats. In addition, the reviews note that acrolein is a metabolite of cyclophosphamide, an immunosuppressive drug that is associated with an increase in bladder cancer in humans. The reviews report both positive and negative results for acrolein in genotoxicity tests. Literature searches were conducted from 1988 to April 2001 for studies relevant to the derivation of an oral slope factor for acrolein. The databases searched were: TOXLINE, MEDLINE, CANCERLIT, RTECS, GENETOX, HSDB, CCRIS, TSCATS, EMIC/EMICBACK, and DART/ETICBACK.

REVIEW OF THE PERTINENT LITERATURE

Human Studies

Reviews by the U.S. EPA (1985, 1986, 1987) and other agencies (ATSDR, 1990; NIOSH, 1991; WHO, 1992; IARC, 1979, 1985, 1995) reported that no relevant data were available regarding carcinogenicity of acrolein in humans following oral exposure. No relevant human studies were located in the literature search.

Animal Studies

Reviews by the U.S. EPA (1985, 1986, 1987) and other agencies (ATSDR, 1990; WHO, 1992; IARC, 1979, 1985, 1995) reported that the data regarding carcinogenicity in animals following oral exposure to acrolein were limited. The cancer assessment on IRIS (U.S. EPA, 2001) is based on the increased incidence of adrenal cortical adenomas (5/20 vs 0/20 controls) observed in female rats exposed to 625 ppm of acrolein in drinking water for 100 weeks (Lijinsky and Reuber, 1987). The literature search located two additional oral carcinogenicity assays for acrolein in rodents.

No increased tumor incidence was reported in rats exposed to acrolein by gavage for 2 years, but the complete tumor incidence data were not available for evaluation (Parent et al., 1992). Groups of Sprague-Dawley rats (70 per sex per group) were gavaged with acrolein (94.9-98.5% pure, stabilized with 0.25% hydroquinone) at doses of 0, 0.05, 0.5 or 2.5 mg/kg-day for 2 years. Rats were checked twice daily for signs of toxicity, morbidity and mortality. Detailed physical examinations were carried out daily for the first 4 weeks and weekly thereafter; animals were palpated weekly for masses. Body weight and food consumption were recorded weekly for the first 14 weeks and once every 4 weeks thereafter. At 13 weeks, 5 animals of each sex in the high-dose group were sacrificed and necropsied; only the stomach was examined for histopathology. Ten rats of each group sacrificed at 1 year and all surviving rats at termination were necropsied and organ weights were recorded. In the control and high-dose groups, 42 tissues and any gross lesions were examined by histopathological examination. In the low- and mid-dose groups, the lungs, liver, kidneys and any gross lesions were examined microscopically; additional organs were examined if lesions occurred in the high-dose rats. The frequency of clinical signs (including masses; data not shown) was elevated in a dose-related manner in mid- and high-dose rats. Treatment caused no significant effect on body weight. There were

significant dose-related trends for increased mortality in high-dose males during the first year and in mid- and high-dose females throughout the study. Nevertheless, survival was adequate to allow for late-developing tumors in all groups. The incidence of tumors in the adrenal gland did not exhibit any dose-relationship. Since no tumor incidence data were reported for any other organ, there is no basis for evaluating the authors' statements that tumor incidences were within historical control values and occurred independently of dose. One source of uncertainty is that several of the references for historical control data are several decades out-of-date and are, therefore, not an appropriate basis for evaluating background levels for tumor incidences. Another source of uncertainty centers on the authors' definition of 'dose-related effects.' In the context of the frequency of clinical signs, the authors stated that no dose-related effects were observed, despite finding a dose-effect at the mid- and high-doses; in this instance, low-dose animals showed fewer clinical signs than controls. Thus, it is not clear whether the authors may have discounted significant tumor frequencies at higher doses if the incidences in the control group were higher than in the low exposure group.

No increase in tumor incidence was observed in CD-1 mice that were gavaged daily with acrolein at doses of $\leq$4.5 mg/kg-day for 18 months (Parent et al., 1991). Groups of CD-1 mice (70-75 per sex per group) were gavaged with 0, 0.5, 2.0, or 4.5 mg/kg-day of acrolein (94.9-98.5% pure, stabilized with 0.25% hydroquinone) daily for 18 months. Mice were checked twice daily for signs of toxicity, morbidity and mortality. Detailed physical examinations were carried out daily for the first 4 weeks and weekly thereafter; animals were palpated weekly for masses. Body weight and food consumption were recorded weekly for the first 14 weeks and once every 4 weeks thereafter. At termination, all mice were subjected to gross necropsy, during which absolute and relative organ weights of liver, kidneys, brain, and testes were recorded. Gross lesions from all animals were examined for histopathology; in addition, 44 tissues in the control and high dose groups, and the lungs, liver, and kidneys of low and mid-dose groups, were also examined microscopically. Survival was significantly reduced in high-dose males throughout the study due to an excess of mortality during the first 50 days of exposure. Nevertheless, survival was adequate to allow for late-developing tumors in all groups. Body weights were significantly reduced in high-dose males after week 20 and in high- and mid-dose females after week 30. There was no increase in the incidence of neoplastic lesions in the liver or lung in mice treated with acrolein compared to controls; no tumor incidence data were presented for other organs.

Other Studies

Recent studies by other routes provide negative or only suggestive evidence for the carcinogenicity of acrolein in animals. When doses of 1-2 mg/kg were administered by i.p. injection into male F344 rats once or twice a week for 6 weeks, acrolein initiated urinary bladder carcinogenesis promoted by dietary uracil, doubling the incidence of papilloma compared to uracil treatment alone (Cohen et al., 1992). Papillary/nodular hyperplasia of the bladder developed in a few rats treated with acrolein alone for 26 weeks, but no tumors developed. In an acute study by Roemer et al. (1993), groups of 3-5 male Sprague Dawley rats were exposed (head only) by inhalation to 0, 0.2 or 0.6 ppm of acrolein vapor for 6 hours/day for 1 or 3 successive days. Exposure to acrolein significantly increased cell proliferation in the trachea and

lung at ≥0.2 ppm and in the nose at 0.6 ppm. However, the effect of 3 days of exposure was less than in rats exposed a single time, which the authors considered an adaptive response.

The review documents cited above reported positive and negative results for acrolein in genotoxicity assays. Varied results were also reported in the additional genotoxicity studies located in the literature search. With or without metabolic activation with S9, acrolein was mutagenic in *Salmonella typhimurium* strains TA100, TA2638, and TA98, and was not mutagenic in strains TA102, TA104, TA1535, TA1537, or TA1538 (Parent et al., 1996; Eder et al., 1990; Jung et al., 1992; Kato et al., 1989; Müller et al., 1993; Watanabe et al., 1998). Acrolein was mutagenic in the *Bacillus subtilis* rec-assay without, but not with, S9 activation (Matsui et al., 1989), was not mutagenic in *Escherichia coli* WP2/pKM101 or WP2 *uvrA*/pKM101 without activation (Watanabe et al., 1998), but was marginally mutagenic to strain WP2 *uvrA*, with or without activation (Parent et al., 1996). Mutagenicity of acrolein to *E. coli* was increased in a strain that was deficient in glutathione (Nunoshiba and Yamamoto, 1999). Acrolein did not induce the expression of SOS-regulated genes in *S. typhimurium* TA1535/pSK1002 (Benamira and Marnett, 1992) and *E. coli* strain PQ37 (Eder et al., 1993).

The formation of acrolein-DNA adducts has been reviewed (Marnett, 1994; Chung et al., 1999). Endogenous acrolein-derived exocyclic adducts (1,N^2-propanodeoxyguanosine adducts) have been identified as common DNA lesions in human and rat liver (Nath and Chung, 1994; Nath et al., 1996), and human lung and colon (Yang et al., 1999). Acrolein-DNA adducts have been generated following reactions with deoxynucleotides (Chenna et al., 1992; Chenna and Iden, 1993), purified eukaryotic DNA (Maccubbin et al., 1990, 1992; Kuchenmeister et al., 1998), or bacterial cells (Hoffman et al., 1989). Acrolein has induced DNA cross-links in plasmids (Kawanishi et al., 1998), and DNA-protein cross-links in cultured human lymphoma cells (Costa et al., 1997), in mixtures of plasmid DNA and calf thymus histone (Kuykendall and Bogdanffy, 1992), and in SV40 virus (Permana and Snapka, 1994). Acrolein-modified DNA was identified in peripheral blood leukocytes of 6/12 cancer patients who were treated with cyclophosphamide, compared to 0/15 patients not treated with the drug (McDiarmid et al., 1991).

In studies on cultured human bronchial cells (reviewed in Grafström, 1990), acrolein reduced colony forming efficiency, clonal growth rate, and cellular levels of glutathione, and increased the frequency of DNA single-strand breaks, DNA-protein cross-links, and the percent of cells synthesizing cross-linked envelopes. Acrolein induced single-strand breaks in DNA in human skin fibroblasts (Dypbukt et al., 1993), in a human lymphoblastoid cell line (Eisenbrand et al., 1995), and, at high cytotoxic doses (1 mmol), in *Salmonella typhimurium* (Eder et al., 1993).

DERIVATION OF A PROVISIONAL ORAL SLOPE FACTOR FOR ACROLEIN

The cancer bioassays by Parent et al. (1991, 1992) provide no evidence for increased tumor incidence in rats or mice following chronic oral exposure to acrolein. In both studies, an

adequate number of animals of both sexes was tested and evaluated comprehensively, and survival was long enough for tumors to have been detected. Based on survival and/or body weight effects at the highest doses, the dosing levels appear to have been adequate for both species. A limitation of both studies is that reporting of tumor incidence data was restricted to the adrenals for rats and the lung and liver for mice. Thus, the complete data sets were not available for evaluation. Older studies provided at best marginal evidence of acrolein carcinogenicity and were not considered suitable for derivation of an oral slope factor in prior assessments (U.S. EPA, 2001). On the basis of the available information, it is not possible to derive a provisional oral slope factor for acrolein.

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Provisional Peer Reviewed Toxicity Values for

Acrolein
(CASRN 107-02-8)

Derivation of an Inhalation Unit Risk

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR ACROLEIN (CASRN 107-02-8) Derivation of an Inhalation Unit Risk

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional

Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The CRAVE workgroup (U.S. EPA, 1992) assigned acrolein to weight-of-evidence Group C, possible human carcinogen, on the basis of no evidence in humans and limited evidence in animals (increased incidence of adrenal cortical adenomas in female rats in an oral study, but no increased tumors in inadequate inhalation, skin painting, and subcutaneous injection studies). Supporting evidence included the carcinogenic potential of an acrolein metabolite, the mutagenicity of acrolein in bacteria, and the structural relationship of acrolein to probable or known human carcinogens. This assessment is listed on IRIS (U.S. EPA, 2001). No inhalation unit risk factor for acrolein is listed on IRIS (U.S. EPA 2001) or in the HEAST (U.S. EPA, 1997). Source documents for the IRIS assessment were a Health Assessment Document (HAD) (U.S. EPA, 1986) and a Health Effects Assessment (HEA) for acrolein (U.S. EPA, 1987). The CARA list (U.S. EPA, 1991, 1994) also includes a Health and Environmental Effects Profile (HEEP) on acrolein (U.S. EPA, 1985). IARC (1979, 1985, 1995) assigned acrolein to Group 3, not classifiable as to human carcinogenicity because of inadequate evidence in humans and animals. ACGIH (1998, 2000) lists an A4 notation for acrolein, indicating its status as not classifiable as a human carcinogen. NIOSH (1991, 2001) notes that although carcinogenicity testing is not complete for acrolein, enough studies report chemical reactivity and mutagenicity to warrant efforts to reduce exposure. A Toxicological Profile for acrolein (ATSDR, 1990), an Environmental Health Criteria document on acrolein (WHO, 1992), a carcinogenicity review of low-molecular-weight aldehydes (NIOSH, 1991), and a toxicity review on aldehydes (Morandi

and Maberti, 2001) were consulted for relevant information. The NTP (2001) health and safety report for acrolein was also examined. These resources contained no additional studies of acrolein itself. However, a metabolite of acrolein, glycidaldehyde, yielded positive results for carcinogenicity in skin painting assays in mice and subcutaneous injection assays in mice and rats. In addition, the reviews note that acrolein is a metabolite of cyclophosphamide, an immunosuppressive drug that is associated with an increase in bladder cancer in humans. The reviews report both positive and negative results for acrolein in genotoxicity tests. Literature searches were conducted from 1988 to April 2001 for studies relevant to the derivation of an inhalation unit risk for acrolein. The databases searched were: TOXLINE, MEDLINE, CANCERLIT, RTECS, GENETOX, HSDB, CCRIS, TSCATS, EMIC/EMICBACK, and DART/ETICBACK.

REVIEW OF THE PERTINENT LITERATURE

Human Studies

Reviews by the U.S. EPA (1985, 1986, 1987) and other agencies (ATSDR, 1990; NIOSH, 1991; WHO, 1992; IARC, 1979, 1985, 1995) reported that no relevant data were available regarding carcinogenicity of acrolein in humans following inhalation exposure. The literature search uncovered a single case report in which acrolein was suggested as the cause of alveolar cell carcinoma in a non-smoking cook (Wardle, 1988). The author argued that the individual, who unavoidably inhaled the fumes of hot fat and oils in a confined space over many years, was likely to have been exposed to acrolein as a common constituent of smoke. However, exposure to acrolein was not established in this case, and in addition, the individual was likely to have been exposed to other potential carcinogens as well.

Animal Studies

Reviews by the U.S. EPA (1985, 1986, 1987) and other agencies (ATSDR, 1990; WHO, 1992; IARC, 1979, 1985, 1995) report that the data regarding carcinogenicity in animals following inhalation exposure to acrolein is limited. No tumors were found in hamsters intermittently exposed to acrolein for one year, but the duration of the experiment was too short to allow for latency (Feron and Kruysse, 1977). No additional studies were located in the literature search regarding carcinogenicity in animals following chronic or subchronic inhalation exposure to acrolein. The cancer assessment on IRIS (U.S. EPA, 2001) is based on the increased incidence of adrenal cortical adenomas in female rats exposed to 625 ppm of acrolein in drinking water for 100 weeks (Lijinsky and Reuber, 1987). However, in more recent studies, acrolein administered by gavage did not increase the incidence of tumors in mice dosed with $\leq$4.5 mg/kg-day for 18 months (Parent et al., 1991) or in rats dosed with $\leq$2.5 mg/kg-day for 2 years (Parent et al., 1992).

Other Studies

Recent short-term studies in animals provide negative or only suggestive evidence of the carcinogenic potential of acrolein. When doses of 1-2 mg/kg were administered by i.p. injection into male F344 rats once or twice a week for 6 weeks, acrolein initiated urinary bladder carcinogenesis promoted by dietary uracil, doubling the incidence of papilloma compared to uracil treatment alone (Cohen et al., 1992). Papillary/nodular hyperplasia of the bladder developed in a few rats treated with acrolein alone for 26 weeks, but no tumors developed. In an acute study by Roemer et al. (1993), groups of 3-5 male Sprague Dawley rats were exposed (head only) by inhalation to 0, 0.2 or 0.6 ppm of acrolein vapor for 6 hours/day for 1 or 3 successive days. Exposure to acrolein significantly increased cell proliferation in the trachea and lung at $\geq$0.2 ppm and in the nose at 0.6 ppm. However, the effect of 3 days of exposure was less than in rats exposed a single time, which the authors considered an adaptive response.

The review documents cited above reported positive and negative results for acrolein in genotoxicity assays. Varied results were also reported in the additional genotoxicity studies located in the literature search. With or without metabolic activation with S9, acrolein was mutagenic in *Salmonella typhimurium* strains TA100, TA2638, and TA98, and was not mutagenic in strains TA102, TA104, TA1535, TA1537, or TA1538 (Parent et al., 1996; Eder et al., 1990; Jung et al., 1992; Kato et al., 1989; Müller et al., 1993; Watanabe et al., 1998). Acrolein was mutagenic in the *Bacillus subtilis* rec-assay without, but not with, S9 activation (Matsui et al., 1989), was not mutagenic in *Escherichia coli* WP2/pKM101 or WP2 *uvrA*/pKM101 without activation (Watanabe et al., 1998), but was marginally mutagenic to strain WP2 *uvrA*, with or without activation (Parent et al., 1996). Mutagenicity of acrolein to *E. coli* was increased in a strain that was deficient in glutathione (Nunoshiba and Yamamoto, 1999). Acrolein did not induce the expression of SOS-regulated genes in *S. typhimurium* TA1535/pSK1002 (Benamira and Marnett, 1992) and *E. coli* strain PQ37 (Eder et al., 1993).

The formation of acrolein-DNA adducts has been reviewed (Marnett, 1994; Chung et al., 1999). Endogenous acrolein-derived exocyclic adducts (1,N^2-propanodeoxyguanosine adducts) have been identified as common DNA lesions in human and rat liver (Nath and Chung, 1994; Nath et al., 1996), and human lung and colon (Yang et al., 1999). Acrolein-DNA adducts have been generated following reactions with deoxynucleotides (Chenna et al., 1992; Chenna and Iden, 1993), purified eukaryotic DNA (Maccubbin et al., 1990, 1992; Kuchenmeister et al., 1998), or bacterial cells (Hoffman et al., 1989). Acrolein has induced DNA cross-links in plasmids (Kawanishi et al., 1998), and DNA-protein crosslinks in cultured human lymphoma cells (Costa et al., 1997), in mixtures of plasmid DNA and calf thymus histone (Kuykendall and Bogdanffy, 1992), and in SV40 virus (Permana and Snapka, 1994). Acrolein-modified DNA was identified in peripheral blood leukocytes of 6/12 cancer patients who were treated with cyclophosphamide, compared to 0/15 patients not treated with the drug (McDiarmid et al., 1991).

In studies on cultured human bronchial cells (reviewed in Grafström, 1990), acrolein reduced colony forming efficiency, clonal growth rate, and cellular levels of glutathione, and increased the frequency of DNA single-strand breaks, DNA-protein cross-links, and the percent

of cells synthesizing cross-linked envelopes. Acrolein induced single-strand breaks in DNA in human skin fibroblasts (Dypbukt et al., 1993), in a human lymphoblastoid cell line (Eisenbrand et al., 1995), and, at high cytotoxic doses (1 mmol), in *Salmonella typhimurium* (Eder et al., 1993).

FEASIBILITY OF DERIVING A PROVISIONAL INHALATION UNIT RISK FOR ACROLEIN

The literature search disclosed no new information regarding carcinogenicity of acrolein following inhalation exposure in humans or animals. Although acrolein is designated a possible human carcinogen (Group C) on IRIS (U.S. EPA, 2001), there are no inhalation data upon which to base an inhalation unit risk.

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United States
Environmental Protection
Agency

Prevention, Pesticides
And Toxic Substances
(7508P)

September 2008

Reregistration Eligibility Decision

Acrolein

Reregistration Eligibility Decision (RED) Document for Acrolein

List B

Case No. 2005

Approved by:

Steven Bradbury, Ph.D.
Director,
Special Review and Reregistration Division

Date: 9/24/08

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Glossary of Terms and Abbreviations

ai	Active Ingredient
CFR	Code of Federal Regulations
CSF	Confidential Statement of Formula
DCI	Data Call-In
EC	Emulsifiable Concentrate Formulation
EEC	Estimated Environmental Concentration
EPA	Environmental Protection Agency
EUP	End-Use Product
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FFDCA	Federal Food, Drug, and Cosmetic Act
FQPA	Food Quality Protection Act
G	Granular Formulation
GLN	Guideline Number
LOC	Level of Concern
LOD	Limit of Detection
LOAEL	Lowest Observed Adverse Effect Level
µg/g	Micrograms Per Gram
µg/L	Micrograms Per Liter
mg/kg/day	Milligram Per Kilogram Per Day
mg/L	Milligrams Per Liter
MOE	Margin of Exposure
MRID	Master Record Identification (number). EPA's system of recording and tracking studies submitted.
MUP	Manufacturing-Use Product
NA	Not Applicable
NPDES	National Pollutant Discharge Elimination System
NR	Not Required
NOAEL	No Observed Adverse Effect Level
OPP	EPA Office of Pesticide Programs
OPPTS	EPA Office of Prevention, Pesticides and Toxic Substances
PHED	Pesticide Handler's Exposure Data
PHI	Preharvest Interval
ppb	Parts Per Billion
PPE	Personal Protective Equipment
ppm	Parts Per Million
RED	Reregistration Eligibility Decision
REI	Restricted Entry Interval
RfD	Reference Dose
RQ	Risk Quotient
SAP	Science Advisory Panel
SF	Safety Factor
SLC	Single Layer Clothing
SLN	Special Local Need (Registrations Under Section 24(c) of FIFRA)
TGAI	Technical Grade Active Ingredient
USDA	United States Department of Agriculture
USGS	United States Geological Survey
UF	Uncertainty Factor
UV	Ultraviolet
WPS	Worker Protection Standard

I. Introduction

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was amended in 1988 to accelerate the reregistration of products with active ingredients registered prior to November 1, 1984, and amended again by the Food Quality Protection Act of 1996 (FQPA). FIFRA calls for the development and submission of data to support the reregistration of an active ingredient, as well as a review of all data submitted to the U.S. Environmental Protection Agency. Reregistration involves a thorough review of the scientific database underlying a pesticide's registration. The purpose of the Agency's review is to reassess the potential risks arising from the currently registered uses of a pesticide, to determine the need for additional data on health and environmental effects, and to determine whether or not the pesticide meets the "no unreasonable adverse effects" criteria of FIFRA.

This document summarizes EPA's human health and ecological risk assessments and reregistration eligibility decision (RED) for acrolein. The document consists of six sections. Section I contains the regulatory framework for reregistration; Section II provides an overview of the chemical and a profile of its use and usage; Section III gives an overview of the human health and environmental effects risk assessments; Section IV presents the Agency's decision on reregistration eligibility and risk management; and Section V summarizes the label changes necessary to implement the risk mitigation measures outlined in Section IV. Finally, the Appendices (Section VI) list related information, supporting documents, and studies evaluated for the reregistration decision. The risk assessments for acrolein and all other supporting documents are available in the Office of Pesticides Program (OPP) public docket at www.regulations.gov under docket number EPA-HQ-OPP-2007-0588.

II. Chemical Overview

There are 8 active acrolein registrations: two registered under section 3 of FIFRA, and six Special Local Need (SLN or 24c) registrations registered under section 24(c) of FIFRA. Acrolein has two use patterns: as an herbicide and biocide. The herbicidal use (EPA Reg# 10707-9) is for direct applications to water irrigation canal systems in the western U.S. Water from the irrigation system may be used on cropland immediately after application of the herbicide, but a specific "holding time" is required before irrigation water can be discharged to natural water systems. All six SLNs are associated with the herbicide registration. Three of these SLNs (WA0400017, ID900005, and NE030003) reduce the holding time specified on the Section 3 label for treated water. The other three SLNs (UT030001, OR910018 and CA780039) are for reservoir use. The reservoirs are irrigation water use only and are not used to store drinking water. The Section 3 registration (EPA Reg# 10707-10) is for application as a biocide for oil well drilling equipment. All registrations for use of acrolein in rodent burrows and burrow entrances have been cancelled. See Table 1 for a current product listing.

Table 1. Summary Report of Supported Registered Products

Reg #	Name	Company Name	%Active Ingredient
10707-9	MAGNACIDE H HERBICIDE	Baker Petrolite Corporation	95
10707-10	MAGNACIDE B MICROBIOCIDE		95
CA780039	MAGNACIDE H HERBICIDE		95

Table 1. Summary Report of Supported Registered Products

Reg #	Name	Company Name	%Active Ingredient
ID900005	MAGNACIDE H HERBICIDE		95
NE030003	MAGNACIDE H HERBICIDE		95
OR910018	MAGNACIDE H HERBICIDE		95
UT030001	MAGNACIDE H HERBICIDE		95
WA040017	MAGNACIDE H HERBICIDE		95

A. Regulatory History

The acrolein reregistration case (2005) contains only one active ingredient, acrolein, which was first registered as an herbicide by Baker Petrolite Corporation (BPC) in November 1975. Baker Petrolite Corporation is the sole technical registrant for both Magnicide H Herbicide and Magnicide B Microbicide. Prior to its registered use as an herbicide, acrolein was registered in 1959 as a biocide. Several Data Call-In (DCI) notices were previously issued in the late 1980s and early 1990s identifying outstanding data needs for acrolein. The DCIs included requests for plant and animal metabolism studies in order to determine the need for crop tolerances.

B. Chemical Identification

Acrolein is registered as a restricted use pesticide for control of submerged and floating aquatic weeds and algae in irrigation canals as well as irrigation reservoirs in some states. In addition, acrolein is used as a biocide to kill bacteria that accumulate within the pipes of petroleum producing systems. Acrolein forms several degradates (acrylic acid, allyl alcohol, propanol, propionic acid, oxalic acid, and ultimately carbon dioxide) in the environment. In addition, glycidol, a metabolite of acrolein, is considered a probable human carcinogen by the International Agency for Research on Cancer (part of the World Health Organization). The National Toxicology Program Annual Report concludes that glycidol is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. The chemical structures and properties of acrolein and its metabolite (glycidol) are presented in Tables 2-4.

Table 2: Acrolein Nomenclature

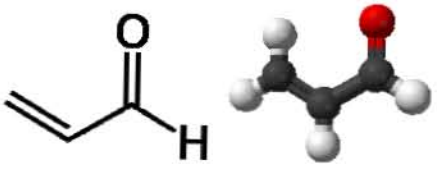
Chemical Structure	
Common Name	Acrolein
Synonyms	2-propenal, acrylaldehyde, acrylic aldehyde, allyl aldehyde, propenal, trans acrolein, acquinite, aqualin, biocide, crolean, ethylene aldehyde, Magnicide, Magnicide H, NSC 8819, prop-2-en-1-al, 2-propene-1-one, slimicide, prop-2-enal
Molecular Formula	C_3H_4O
PC Code	000701

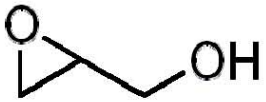
Table 2: Acrolein Nomenclature

IUPAC Name	2-propenal; Acrylaldehyde
CAS Registry Number	107-02-8

Table 3: Physiochemical Properties of Acrolein

Melting Point/Range	-88 °C (-126 °F)
Boiling Point	53 °C (127 °F)
Molecular Weight	56.1 g/mol
Specific Gravity	0.0839
Vapor Density	1.94 (air = 1)
Solubility	208 g/L at 20 °C soluble in water, alcohol, ether, and acetone
Vapor Pressure	220 torr at 20 °C
Flashpoint	-15 °F (-26.1 °C)
Octanol Water Partition Coefficient (Log k)	0.98-1.10
Description	Clear, colorless to yellow liquid

Table 4: Nomenclature and Physiochemical Properties of Glycidol

Chemical Structure	
Molecular Formula	$C_3H_6O_2$
IUPAC Name	Oxiranylmethanol
CAS Registry Number	556-52-5
Melting Point/Range	-54 °C
Boiling Point	167 °C (decomposes)
Molecular Weight	74.1 g/mol
Specific Gravity	0.0839
Vapor Density	2.15 (air = 1)
Solubility	Miscible
Vapor Pressure	120 Pa at 20 °C
Flashpoint	72 °C
Octanol Water Partition Coefficient (Log p)	- 0.95
Description	Clear, colorless liquid

C. Acrolein Use Profile

Type of Pesticide: Acrolein is an aquatic herbicide and biocide.

Summary of Use: Acrolein has two use patterns: as an herbicide for the control of vegetation in irrigation canals and as a biocide in water pumped into injection wells associated with petroleum production.

Mode of Action: Acrolein binds to organic material and degrades cellular structure by cross-linking proteins.

Formulation Type: Both section 3 acrolein products: Magnicide H (aquatic herbicide, EPA Registration #:10707-9) and Magnicide B (biocide, EPA Registration #: 10707-10) are packaged as liquids and stored under an inert gas blanket. Each contains 95% acrolein as the active ingredient.

Application Methods: As an herbicide, acrolein is injected directly below the surface of moving water and moves with the flow of water killing weeds on contact in irrigation canals and holding ponds. Acrolein is also used as a biocide in water pumped into injection wells associated with petroleum production. Acrolein is not directly applied to any crops. Both the herbicide and biocide products are applied through a closed system.

Application Rates: For herbicidal use in irrigation canals, the maximum single application concentration of acrolein is 15 ppm. The typical application rate is 8 ppm. For the biocide use, the maximum single application rate is 15 ppm. No maximum number of applications or minimum re-application intervals are specified on the labels.

Application Timing: Magnacide H and Magnacide B applications can occur multiple times during a year. Magnacide H may be applied up to 26 times per year in some irrigation systems with an application interval as short as every 7 days, but 6 applications per year is the most common, with a two to three week interval between applications. In some irrigation systems applications are more frequent but at lower concentrations to control the lower weed density. Detailed application information for Magnacide B is not currently available.

Registrant: Baker Petrolite Corporation

D. Estimated Usage

Based on available data, approximately one million pounds of acrolein is sold annually. Acrolein is a restricted use pesticide subject to strict use limitations. It can only be sold to and applied by trained and certified applicators or persons under their direct supervision, and can only be used for a use covered by the applicator's certification. There are no products available for residential application.

III. Summary of Acrolein Risk Assessments

The purpose of this summary is to assist the reader by identifying the key features and findings of the human health and environmental risk assessments, and to help the reader better understand the conclusions reached in the assessments. The assessments and supporting documents referenced in Appendix C were used to formulate the safety finding and regulatory decision for the pesticidal use of acrolein.

While the risk assessments and related addenda are not included in this document, they are available in the OPP Public Docket at www.regulations.gov, docket number EPA-HQ-OPP-2007-0588. In addition, the documents may be accessed through the Agency's website at http://www.epa.gov/pesticides.

- *Acrolein HED Risk Assessment for Reregistration Eligibility Decision (RED) Document (PC Code No. 000701)* (B. Daiss.; 3/25/08, D348777).
- *Environmental Fate and Ecological Risk Assessment Chapter in Support of Phase V of the Reregistration Eligibility Decision of Acrolein* (Jones, R.D., Ph.D., Garber, K. and Steeger, T., Ph.D.; 7/23/08, D354775).

A. Human Health Risk Assessment

Acrolein is a pesticide with two registered uses in the U.S. In agriculture, acrolein is registered for application in irrigation systems only in the western U.S. In petroleum production industries, acrolein is applied to injection wells to control slime-producing organisms in drilling muds. The human health risk assessment addresses potential exposure risks from all registered sources; however, exposures to acrolein from the biocide use in petroleum production (Magnicide B) are not expected since the current use pattern consists of application through closed systems and with no release of the fluids to the above ground environment. Therefore, only potential occupational and residential bystander exposures resulting from the use of the herbicide (Magnicide H) were assessed.

Acrolein exposure to handlers can occur in occupational environments. There are no registered food/feed uses for acrolein and thus no food-related dietary risk assessments were conducted based on the use pattern and available data on plant metabolism. Risks from drinking water exposures were not assessed because applications are made to irrigation canals and holding ponds. The Agency does not anticipate that the water released from these canal systems would contain acrolein residues that would reach drinking water sources.

Since there are no residential uses of acrolein, an assessment of residential handler and post-application exposure scenarios was not required. However, residential bystanders may be exposed due to the volatilization of acrolein from irrigation canals. For this reason, potential inhalation exposure for bystanders was assessed using available air monitoring data collected during and after the application of acrolein to canals.

In addition to the parent compound, acrolein, compounds of potential concern include glycidol, a metabolite of acrolein that has been found in fish, and 3-hydroxypropanal, a metabolite of acrolein that has been found in acrolein-treated water. While acrolein forms 3-hydroxypropanal spontaneously in solution, it is an equilibrium process and acrolein will be reformed from 3-hydroxypropanal as acrolein is dissipated by other processes. Therefore, 3-hydroxypropanal is not considered a metabolite of concern for risk assessment purposes.

An assessment of the dietary exposure of subsistence fishermen to glycidol was conducted because glycidol is a potential human carcinogen. Based on available data on acrolein concentrations in fishable waters, and EPA data on the location and fishing habits of

tribes living in areas proximate to treated canals, the Agency believes exposures to subsistence fishermen are possible. Therefore, the Agency conducted a cancer dietary risk assessment for glycidol and this assessment of dietary exposure of subsistence fishermen to glycidol indicates cancer risks do not exceed the Agency's level of concern. For the complete human health risk assessment, refer to the *Acrolein HED Risk Assessment for Reregistration Eligibility Decision (RED) Document,* dated March 25, 2008, which is available in the public docket.

1. Toxicity of Acrolein

The human health risk assessment utilized animal toxicity studies to estimate risk to humans exposed to acrolein. The toxicological database for acrolein is considered adequate for evaluating and characterizing acrolein toxicity and selecting endpoints for the purpose of a risk assessment.

Acrolein is acutely toxic by inhalation, oral, and dermal exposures (Toxicity Category I for all routes). It is a potent irritant to the mucous membranes. Direct contact with liquid acrolein causes rapid and severe eye and skin irritation or burns. Dermal exposure to acrolein liquids or vapors may cause stinging of the eyes, lacrimation, and reddening, ulceration, or necrosis of the skin. Table 5 describes the acute toxicity profile of acrolein.

Table 5: Acrolein Acute Toxicity Profile

Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral [rat]	41257001	LD_{50} = 11 mg/kg	I
870.1200	Acute dermal [rabbit]	00141028	LD_{50} = 231 mg/kg	I
870.1300	Acute inhalation [rat]	40945404	LC_{50} = 0.019mg/L	I
870.2400	Primary eye irritation [rabbit]	00141025	Severely irritating	I
870.2500	Acute dermal irritation [rabbit]	00141026	Severely irritating	I
870.2600	Skin sensitization	Sustin and Breienstein, 1990	Suggestive/limited data	N/A

Chronic Toxicity

Apart from rare cases of sensitization, no adverse effects in humans chronically exposed to low concentrations of acrolein have been reported. Animal studies indicate that the respiratory system is the major target organ for acrolein inhalation toxicity. Oral acrolein exposure may result in gastrointestinal discomfort, vomiting, and stomach ulceration and/or hemorrhage. Also, changes in body and organ weights, hematology, and serum biochemistry have been observed in animals exposed orally to acrolein, although some of these effects are believed to be secondary effects of gastrointestinal and/or respiratory tract irritation. In addition, the central nervous system does not appear to be a target of acrolein toxicity based on an Agency for Toxic Substances Disease Registry (ATSDR) 2005 review.

Developmental Toxicity

In a rat developmental toxicity study, the LOAEL was 10 mg/kg/day based on decreased fetal weights and litter weights and on incomplete ossification of the skeleton and general retarded development of the fetuses. The developmental NOAEL was 6 mg/kg/day. In a rabbit developmental toxicity study, the LOAEL was > 2 mg/kg/day and the developmental NOAEL was 2 mg/kg/day (the highest dose tested).

In a two-generation reproduction toxicity study for rats, the LOAEL for parental toxicity was 6 mg/kg/day, based on decreased body weights, body weight gains, and food consumption in both sexes and both generations during pre-mating and on gross and microscopic findings in the stomach. The NOAEL for this same study was 3 mg/kg/day.

Therefore, based on these developmental studies in rats and rabbits and reproductive toxicity study in rats, fetal or neonatal toxicity from the administration of acrolein does not occur at doses lower than doses causing effects in parental animals.

Carcinogenicity and Mutagenicity Toxicity

The evidence for the carcinogenicity of acrolein is equivocal, with a significant tumor incidence found in a single animal drinking water study. While the potential carcinogenicity of acrolein cannot be determined definitively due to insufficient data, the Agency does not believe cancer studies are required based on use patterns, anticipated exposure patterns, severe acute toxicity, and available data on mutagenicity and carcinogenicity. Oral exposures to acrolein via dietary and drinking exposure are not expected or assessed based on use patterns and physical/chemical property data. Continuous chronic exposures via inhalation and dermal pathways are not expected based on established use patterns. In vitro studies have shown acrolein to be weakly mutagenic.

Glycidol is a metabolite of acrolein reported in a fish metabolism study. Glycidol is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals (NTP 1990, IARC 2000). Two-year studies were conducted with mice and rats that were administered glycidol by gavage. Rats showed increased incidences of various effects such as tumors. To quantify the carcinogenic response of glycidol, a multistage model BMD analysis was performed to derive a slope factor of 0.16 $(mg/kg/day)^{-1}$. This method is explained further in the following section (Endpoint Selection) as well as in the *Acrolein HED Risk Assessment for Reregistration Eligibility Decision (RED) Document*.

Neurotoxicity Studies

The central nervous system does not appear to be a target of acrolein toxicity based on an Agency for Toxic Substances Disease Registry (ATSDR) 2005 review. Symptoms of central nervous system depression were observed in rodents after oral exposure to acrolein, but only after lethal concentrations (Sprince et al. 1979). No such effects were observed in animals after inhalation. In addition, no behavioral changes were observed in animals exposed to acrolein by any route. There were no studies addressing the neurotoxicity of acrolein

following dermal exposure. As such, the available data do not indicate that the central nervous system is the major target of acrolein toxicity.

2. Endpoint Selection

Acrolein

The inhalation endpoint was selected from a 1977 study in human volunteers (Weber-Tschopp et al. 1977; MRID 47060601). Acrolein is a component of cigarette smoke and the human study was conducted to determine the effects of different components of cigarette smoke on human volunteers. This study was subject to review by the Human Studies Review Board (HSRB). The HSRB reviewed the study at its June 2007 meeting and determined it to be ethically acceptable and sufficiently sound from a scientific perspective, to be used to estimate a safe level of acute inhalation exposure to acrolein. Because a human study is being used for the short-term intermittent inhalation exposure scenario for acrolein, an interspecies uncertainty factor is not necessary. To account for the individual variability, an intraspecies uncertainty factor of 10X applies. The endpoint selected was based on LOAELs for both (1) eye irritation and (2) nasal and throat irritation and decreased respiratory rate.

For eye irritation effects, the LOAEL was determined to be 0.09 ppm. Because a minimal (relatively non-severe) LOAEL threshold effect is used, a 3X uncertainty factor is sufficient along with the intraspecies factor. Therefore, a total of 30X uncertainty factor is applied to the endpoint.

For nasal and throat irritation and decreased respiratory rate, the LOAEL was determined to be 0.3 ppm. This LOAEL was divided by a factor of 100 (10X for using a LOAEL and 10X for human variability).

Therefore, based on both the LOAEL of 0.09 ppm for eye irritation and the LOAEL of 0.3 ppm for nasal and throat irritation, the concentration of concern for humans is determined to be 0.003 ppm when appropriate uncertainty factors are considered. Thus, the study provides the most comprehensive description available of acute/short-term effects in humans and provides the best information available for establishing a Point of Departure (PoD) for short-term intermittent inhalation worker and residential bystander exposure scenarios.

Glycidol

The Agency's Benchmark Dose (BMD) software (version 1.3.2) was used to fit a multistage model to the human lifetime average daily dose (LADDs). The benchmark response was randomly selected to be 10% (note that when calculating slope factors, the selection of benchmark response does not greatly affect the calculated slope factor). The BMD_{10} was calculated to be 0.79 mg/kg/day and the lower 95% confidence limit on the BMD_{10}, the Benchmark Dose Level $(BMDL)_{10,}$ was calculated to be 0.63 mg/kg/day. Thus, the slope factor is obtained by dividing the benchmark response level (0.1 or 10%) by the $BMDL_{10}$ of 0.63 mg/kg/day which equates to 0.16 $(mg/kg/day)^{-1}$.

Table 6 summarizes the toxicological doses and endpoints used in the human health risk assessment of acrolein.

Table 6: Summary of Toxicological Doses and Endpoints for Acrolein for Use in Human Health Risk Assessment

Exposure Scenario	Dose Used in Risk Assessment	Uncertainty/Safety Factor	Study and Toxicological Effects
Acute and Chronic Dietary – (All populations)	Acute and chronic oral (dietary and drinking water) exposures to acrolein are not expected based on use patterns, physical-chemical properties, and plant metabolism data. Therefore, RfDs are not required and were not selected for this assessment.		
Incidental Oral (all durations)	There are no residential uses for acrolein. Therefore, incidental oral exposure endpoints are not required and not selected for this assessment.		
Dermal (all durations)	Worker dermal exposures are not expected based on use patterns and personal protective equipment requirements. There are no residential uses for acrolein and dermal exposures to residential bystanders are not expected based on use patterns and physical-chemical properties. Therefore dermal exposure endpoints are not required and have not been selected for this assessment.		
Short –Term Inhalation (1-30 days)	LOAELs 0.09 ppm for eye irritation 0.3 ppm for nasal and throat irritation	Occupational LOC=30 Residential LOC=30 Eye irritation UF_H = 10x 3x lack of a NOAEL Nasal and throat irritation UF_H = 10x 10x lack of a NOAEL	Human volunteers (healthy male and female college students) exposed by inhalation for 60 minutes (Weber-Tschopp et al. 1977) based on a minimal effect LOAEL of 0.09 ppm for eye irritation. The LOAEL of 0.3 ppm for nasal and throat irritation and decreased respiratory rate is also considered for endpoint selection. (MRID 47060601)
Cancer (oral, dermal and inhalation)	"The potential carcinogenicity of acrolein is inconclusive; however, exposure to parent acrolein is not expected. Glycidol is a metabolite of acrolein in fish. Glycidol is anticipated to be a human carcinogen by NTP and IARC. To quantify the carcinogenic response of glycidol, a multistage model BMD analysis was performed to derive a cancer slope factor of 0.16 $mg^{-1}kg^{-1}day^{-1}$ at a 0.95 confidence level.		

UF_H = uncertainty factor for potential variation in sensitivity among members of the human population (intraspecies), NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, MOE = margin of exposure, LOC = level of concern, RfD = Reference Dose

3. Dietary Exposure and Risk (All Populations)

Acrolein

Dietary exposures (acute and chronic) to acrolein are not expected based on the use pattern (no direct applications of acrolein to crops except through irrigation) and available data on plant metabolism. A lettuce metabolism study indicates that acrolein is readily decomposed/incorporated into natural products showing that the only residue of concern is acrolein on the day of application by irrigation. Since it is unlikely that plants would be harvested immediately after irrigation, there is little likelihood that there would be dietary exposure from irrigation water applied to the crop.

Additionally, risks from drinking water exposures were not assessed. There is currently no Maximum Contaminant Level (MCL) set for the protection of drinking water for acrolein under the Safe Drinking Water Act. Also, the Agency did not calculate quantitative estimated environmental concentrations (EECs) for use in the risk assessment since acrolein is applied to irrigation water and there is a holding period before irrigation water is discharged to natural waters, which could serve as drinking water sources. While uncertainties remain regarding the potential for drinking water exposure, such exposures are considered unlikely due to the fact that most, if not all, of any acrolein that could reach a drinking water source from an irrigation ditch would volatilize before and during the aeration stages of drinking water treatment.

Glycidol

An assessment of potential dietary exposure of subsistence fishermen to glycidol, a metabolite of acrolein in fish, was also conducted. Based on Agency data on acrolein concentrations in fishable waters and on the location and fishing habits of tribes living in areas proximate to treated canals, the Agency believes that a subsistence fisherman scenario is possible.

No glycidol was noted in plant metabolism studies and would not be expected from animal studies since acrolein exposure is not expected for terrestrial animals.

In the residue study in fish and shellfish, glycidol accounted for as much as 10 ppb of the total radioactive residue in catfish in a study conducted at 20 ppb water concentration of acrolein. Normalizing the water concentration to account for a mean fish LC_{50} of 34 ppb would result in a estimated glycidol residue concentration of 17 ppb or 0.17 ug/g. Based on data provided in EPA's Exposure Factors Handbook Volume II dated August 1997, the mean Native American subsistence fish harvest is 70 g/day. Multiplying 0.17 ug/g by the recommended mean intake of 70 grams/day would give 11.9 ug/day or 0.0119 mg/day of glycidol. This value must be divided by the weight of an adult in kilograms (70 kg) which gives 0.00017 mg/kg/day. The maximum number of applications reported in the states of Washington, Oregon, and Idaho was 17 applications during a year for a ratio of 17/365 or 0.0466. Multiplying 0.00017 mg/kg/day by 0.0466 would give 7.9×10^{-6} mg/kg/day. This value is then multiplied by the glycidol Q_1* of 0.16 to give a conservative estimated cancer risk of 1.2×10^{-6}. Therefore, based on this conservative assessment, dietary exposure of subsistence fishermen to glycidol does not present cancer risks of concern.

The Agency recognizes that 70 grams of fish/day is a mean value and is not the maximum reported. However, the projected concentration of glycidol in fish is expected to be very conservative since it is based on the assumption that all fish consumed are exposed to 34 ppb acrolein for 17 applications per year.

4. Residential (Non-Occupational) Exposure and Risk

Acrolein products are restricted-use pesticides. The sale and use of these products is limited to certified applicators or persons under their direct supervision. The products may only be applied for uses covered by the certified applicators certification. However, inhalation

exposure to acrolein may occur from the volatilization of MAGNACIDE H from irrigation canals during treatment.

In 2005, California Air Resources Board (CARB) collected acrolein air monitoring data during the application of acrolein into an irrigation canal as part of a pilot study conducted in 2005 to determine the applicability of the proposed field test methods before proceeding to the full scale study. Six samples were collected with acrolein levels ranging from 15.9 to 59.8 ppb. In 2006, CARB collected acrolein air monitoring information during the application of acrolein (MAGNACIDE H) into an irrigation canal as part of a full scale study conducted in 2006. These data summarized in Table 7 can be found at *http://www.cdpr.ca.gov/docs/empm/pubs/tac/studies/acrolein.htm*. Air monitoring was conducted during a 4 hour application period and for 4 hours post-application. The treatment rate was 4.0 ppm with a canal flow rate of 357 cubic feet per second. Acrolein levels ranged from 8.4 to 24 ppb during application and from 1.2 to 5.3 ppb in the post-application period. These data are considered to be very high quality, but only represent one set of conditions at one location. For additional information regarding the results of the CARB monitoring, please refer to the *Acrolein HED Risk Assessment for Reregistration Eligibility Decision (RED) Document,* dated March 25, 2008, which is available in the public docket.

Table 7. Results of 2006 CARB Monitoring of Acrolein During Application

Test Location Application Rate Canal Flow	**Sampling Site (AP = Application Point) 4 Hour Application Period**	**Air Concentration (ppb)**
Kern County California 4.0 ppm 357 cfs	1. West bank AP	11
	2. West bank AP collocated sample	10
	3. East bank AP	11
	4. East bank AP collocated sample	15
	5. West bank 25 m south, 9.6 m west of AP	10
	6. East bank 19.5 m south, 10 m east of AP	9.5
	7. West bank 50 m south of AP at Canal's Edge	8.4
	8. East bank 42 m south of AP at Canal's Edge	14
	9. West bank 100 m south of AP at Canal's Edge	17
	10.East bank 88 m south of AP at Canal's Edge	20
	11. West bank 150 m south of AP at Canal's Edge	16
	12. East bank 137 m south of AP at Canal's Edge	13
	13. West bank 200 m south of AP at Canal's Edge	13
	14. East bank 187 m south of AP at Canal's Edge	18
	15. West bank 250 m south of AP at Canal's Edge	24
	16. East bank 237 m south of AP at Canal's Edge	11
Results of 2006 CARB Monitoring of Acrolein Four Hours after Application		
Kern County California 4.0 ppm 357 cfs	1. West bank AP	5.3
	2. East bank AP	3.2
	3. West bank 25 m south, 9.6 m west of AP	1.4
	4. East bank 19.5 m south, 10 m east of AP	2.2
	5. West bank 50 m south of AP at Canal's Edge	2.7
	6. East bank 42 m south of AP at Canal's Edge	2.7
	7. West bank 100 m south of AP at Canal's Edge	1.9
	8.East bank 88 m south of AP at Canal's Edge	2.2

Table 7. Results of 2006 CARB Monitoring of Acrolein During Application		
Test Location Application Rate Canal Flow	**Sampling Site (AP = Application Point) 4 Hour Application Period**	**Air Concentration (ppb)**
	9. West bank 150 m south of AP at Canal's Edge	2.6
	10. East bank 137 m south of AP at Canal's Edge	3.2
	11. West bank 200 m south of AP at Canal's Edge	1.2
	12. East bank 187 m south of AP at Canal's Edge	1.4
	13. West bank 250 m south of AP at Canal's Edge	2.4
	14. East bank 237 m south of AP at Canal's Edge	1.7

In reference to the air monitoring studies listed above, Table 8 provides a summary of the results from the MAGNACIDE H Field Air Monitoring samples that were collected in 2002. The highest result of 63 ppb occurred at the California #1 Test Location where a leak reportedly occurred. The results at the other two test locations ranged from not detectable to 30 ppb. The limit of detection was not specified but was estimated to be approximately 1 ppb based on the lowest reported result, which was 1.5 ppb.

Table 8. Results of MAGNACIDE H Field Air Monitoring (MRID 469769-12)		
Test Location, Application Rate, Canal Flow	**Sampling Site**	**Air Concentration (ppb)**
Washington, 1.98 ppm, 840 cubic feet per second (cfs)	Application point	25
	Downstream, right-of-way	4
	Downstream, right-of-way	None Detected
	Downstream, 150 feet into field	2
	Downstream, 150 feet into field	None Detected
Central California #1 8 ppm for 2 hours 200 cfs	Application point	63*
	Downstream, right-of-way	38
	Downstream, right-of-way	13
	Downstream, 150 feet into field	None Detected
	Downstream, 150 feet into field	7.8
Central California #2 7.2 ppm for 2 hours 48 cfs start 38 cfs finish	Application point	13
	Downstream, right-of-way	20
	Downstream, right-of-way	30
	Downstream, 150 feet into field	1.5
	Downstream, 150 feet into field	7.9

* Equipment leak experienced and operating vehicle entered the test site

c.) Residential Exposure

There are no residential handler (applicator) uses for acrolein. However, residential bystander exposure through the inhalation pathway can occur as a result of the application of MAGNACIDE H to irrigation canals, which may be located near residential areas. There are no requirements for the establishment of area restrictions in the proximity of the application site or the treated canal.

The acrolein exposure level at which inhalation risks are not of concern is 3 ppb. Measured air concentrations based on monitoring data associated with sites near irrigation ditches ranged from 1.5 to 63 ppb (see Table 8). The target LOC or MOE for short-term inhalation exposure to acrolein is 30. Short-term MOEs for residential exposure calculated using concentrations from the air monitoring data ranged from 1.5 to 60 (see Table 9). Therefore, depending on the scope to which residential areas are located within the vicinity of treated canals and/or non-workers are conducting activities near treated canals (during or near the time of treatment), inhalation MOEs for residential bystander exposure exceed the Agency's level of concern.

Monitoring data indicates that air concentrations of acrolein generally decrease with distance from the source (*e.g.*, the treated canal). Therefore, the highest potential risks are to persons standing adjacent to the canal and the exposures decrease away from the treated water body. It should be noted that available monitoring data provide insufficient information to determine the appropriate dimensions of a restricted area relative to the application point or area source (*e.g.*, the canal).

Although, the current acrolein label does not prohibit swimming during applications, irrigation district personnel discourage swimming in canals because of public safety concerns, particularly the risks of drowning in the canal. While a separate swimmer assessment was not conducted due to lack of appropriate endpoints for dermal and oral exposure, the Agency notes that acrolein is irritating at low concentrations and would presumably present some risks to swimmers.

5. Aggregate Exposure and Risk

The Agency has not conducted a quantitative or qualitative aggregate assessment for acrolein. An aggregate exposure assessment considers the different pathways (food, water, occupational, and residential) through which exposure to acrolein may occur when there are potential residential exposures to the pesticide. Since there are no anticipated dietary/drinking water exposures to residues of acrolein, an assessment of aggregate exposure from food and non-food sources is not required. Further, the metabolite of acrolein, glycidol, only forms in fish and depurates quickly. Although a dietary cancer assessment was conducted for glycidol, exposures to glycidol via drinking water, inhalation, and dermal pathways are not expected. Therefore, an aggregate assessment is not required.

6. Occupational Exposure and Risk

a) Occupational Handler/Application Assessment

Based on current use patterns, acrolein exposure to occupational handlers can occur. MAGNACIDE H and MAGNACIDE B are applied through a closed system transfer from steel cylinders designed to prevent applicator exposure. Both products are supplied in pressurized containers where nitrogen is used to force the liquid chemical out of the container through a metering device. It is then injected directly below the surface of moving water in the canal where it is carried along by the flow (MAGNACIDE H) or is injected into closed injection well

piping (MAGNACIDE B) through sealed hoses. It is important to note that applicators must use only specified application equipment built specifically for use of these particular products as directed by the technical registrant Baker Petrolite.

Magnacide H

During the set up and/or break down of equipment, exposure to acrolein from the application of MAGNACIDE H is not expected because applicators must comply with stringent label requirements for personal protective equipment (PPE) (i.e., full face air purifying respirator, butyl rubber gloves, etc.) throughout these activities. Use of a closed application system combined with stringent training, certification and PPE requirements is expected to effectively prevent dermal exposures of concern to workers during handling and application activities.

However, since the application of MAGNACIDE H can vary in time (30 minutes to 8 hours) and respiratory protection is not required after initial set up and prior to break down of equipment, inhalation exposures to workers during application is possible. The exposure level at which inhalation risks are not of concern is 3 ppb (90 ppb LOAEL ÷ UF 30). The target MOE for short-term inhalation exposure to acrolein is 30 and all MOE's >30 are potentially of concern.

Table 9: Estimated Inhalation Exposure and Risk to Workers and Bystanders

Range of Measured Concentrations (ppb) (see Tables 7 and 8)	LOAEL (ppb)	Target MOE	Calculated MOE
1.5 - 63 (0.0015-0.063 ppm)	90 (0.09 ppm)	30	1.5 - 60

Calculated MOE = Acute Inhalation NOAEL (90 ppb) ÷ estimated inhalation concentration (1.5 – 63 ppb).

Some of the calculated MOEs exceed the Agency's level of concern for worker exposure during the application period between set up and breakdown of equipment as well as after disassembling of the equipment has been completed.

The Baker Petrolite Corporation also submitted summary results from a MAGNACIDE H HERBICIDE Industrial Hygiene Monitoring Study. The Industrial Hygiene Monitoring Results reported by Baker Petrolite from an air sampler near the worker's breathing zone indicated that acrolein applicator exposures were all below the limit of detection (LOD), which ranged from 2.2 to 70 ppb.

Magnacide B (biocide)

Occupational exposures to acrolein from the use of MAGNACIDE B are not expected because it is applied via a closed system. MAGNACIDE B Microbiocide is applied in injection systems associated with petroleum production. The MAGNACIDE B product is applied by pumping acrolein from pressurized containers into closed injection well piping systems. The closed application system combined with stringent training and PPE requirements is intended to effectively prevent exposures of concern from any MAGNACIDE B biocide product.

b) Occupational Post-application Exposures

Post-application exposures of MAGNACIDE H to workers may also occur depending on the length of time the worker remains in the area after application has been completed and the equipment disassembled. Therefore, depending on the extent to which workers remain in the vicinity of the treated canal after acrolein has been applied and the requirement for use of a respirator is no longer applicable, inhalation MOEs for worker post-application exposure may exceed the Agency's level of concern.

7. Endocrine Disruption

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) *"may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate."* Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there were scientific bases for including, as part of the program, androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. When the appropriate screening and/or testing protocols being considered under the Agency's Endocrine Disrupter Screening Program (EDSP) have been developed and vetted, acrolein may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption.

8. Incident Reports

The following data bases were consulted for poisoning incident data on the active ingredient acrolein; OPP Incident Data System (IDS), Poison Control Centers, California Department of Pesticide Regulation, National Pesticide Telecommunications Network (NPTN), and National Institute of Occupational Safety and Health's (NIOSH) Sentinel Event Notification System for Occupational Risks (SENSOR).

Three incident reports in the OPP Incident Data System (IDS) were related to acrolein. One incident occurred in 1999, when a valve on a cylinder that contained Magnacide H, was struck by an overhead obstacle while entering a service station. A man at the site reported eye irritation, difficulty breathing, and chemical burns. Two of the incidents resulted in death, which was directly attributable to an applicator not wearing the required personal protective equipment. The incident in 1999 occurred when the applicator accidentally ran over and damaged parts of the delivery system, spilling a few gallons of the product. The applicator proceeded, without personal protective equipment, to close off the cylinder valve of the delivery system. He then washed himself off in the canal and traveled to the hospital where he was treated and then released. He was later found unconscious in his home and died the next day. The latest incident in 2007 occurred when the applicator was sprayed directly in his face with acrolein that was under pressure, after he attempted to tighten a connection in the delivery system. An initial evaluation showed signs of respiratory distress so he was transported to a medical center where he received immediate treatment. Despite the treatment, the individual died within several days.

Based on exposures reported to Poison Control Centers from 1993 through 2003, 47 cases were reported. A wide range of symptoms were reported including eye irritation/lacrimation (4 cases reported), headache (3 cases), nausea (2 cases), cough/choke (2 cases), superficial burns (2 cases), and one single event of throat irritation, vomiting, erythema skin irritation, and pruritus.

Fifteen cases submitted to the California Pesticide Illness Surveillance Program (1982-2003) were reviewed. In 14 of these cases, acrolein was used alone or was judged to be responsible for the health effects. Applicator and coincidental activities were associated with 8 of the 14 reported exposure related illnesses. These illnesses included symptoms of coughing, headache, nausea, and burns on the arm.

The National Pesticide Information Center (NPIC) received calls from 1984-1991 and acrolein was not reported to be involved in human incidents. There have been no reported incidents involving bystanders or persons exposed in residential settings. From 1998 to 2003 there was one case reported in the NIOSH SENSOR database involving acrolein. The exposed individual reported blurred vision and a feeling of warmth. Poison Control Center Data generally support the finding that acrolein's main effect is due to its irritant properties. Incidents involving more severe effects resulted from accidental exposures or misuse of the acrolein product Magnacide H.

In conclusion, if acrolein products are applied according to their labels and user manuals, the Agency does not anticipate human health incidents from registered acrolein uses.

B. Environmental Fate and Ecological Risk Assessment

The Agency conducted an environmental fate and ecological risk assessment for acrolein for the purpose of making a reregistration decision. The environmental fate and effects risk assessment is largely based on field studies and monitoring data. Since these monitoring studies only report the parent active ingredient, data are only available to consider the risks due to the parent acrolein at this time. In addition, based on available information regarding volatilization, persistence, and direct and indirect toxicity, acrolein has the potential to compromise survival and cause sub-lethal effects in non-target aquatic animals and plants, terrestrial mammals, birds and plants. As such, the assessment endpoints for acrolein include survival, reproduction and growth of birds, mammals, freshwater fish and invertebrates, estuarine/marine fish and invertebrates, terrestrial plants, insects, and aquatic plants and algae. A summary of the environmental risk assessment findings and conclusions is provided below. For more detail on the acrolein environmental exposure and risk assessment, refer to the *Environmental Fate and Ecological Risk Assessment Chapter in Support of Phase V of the Reregistration Eligibility Decision on Acrolein,* dated July 23, 2008, which is available in the public docket.

1. Environmental Fate and Transport

Acrolein is considered a contact herbicide that is phytotoxic to most submersed aquatic vegetation. Submersed aquatic plants treated with Magnicide H are intended to gradually

disintegrate into small fragments and then float downstream. Contact herbicides act quickly by destroying plant cells; however, they do not kill plant roots and re-application may be required. Due to the reactivity with organic matter, acrolein is not likely to persist in the environment. However, despite the lack of persistence, it can move considerable distances in fast moving water such as within irrigation canals.

Degradation and volatilization are believed to be the major pathways for dissipation of acrolein in water. Acrolein may also bind to plant material and this may serve as an additional route of dissipation from the water column. The Agency has no acceptable data to assess microbial degradation or photolysis. Therefore, it is unknown whether these pathways are significant routes of degradation in the environment.

No acceptable data are available for estimating desorption coefficient (K_d) values for acrolein. In the aerobic (MRID 43227101) and anaerobic (MRID 42949201) aquatic metabolism studies, acrolein was not identified in the sediment of the test vessels which suggests that acrolein does not partition into sediment to any major degree. In addition, the very high solubility (237 g/L at 25^{o}C) would indicate a very low tendency to absorb to sediment.

Acrolein does not undergo hydrolytic degradation in aqueous solution. Rather, it goes into equilibrium with a hydration product, 3-hydroxypropanal, where water has added to the double bond. The equilibrium constant is assumed to be independent of pH.

Data of the dissipation rate of acrolein from foliage is not of sufficient quality to allow for the estimation of a degradation rate. However, even though the data was limited in its quality; the Agency was able to utilize a 1-day foliar dissipation half-life. Usually, in the absence of this information, the Agency relies on a default foliar dissipation half-life of 35 days to estimate potential residues on terrestrial animal forage items. However, given the volatility and reactivity of acrolein, the default value of 35 days is not justifiable. Thus, given the uncertainties of the submitted data, a 1-day foliar dissipation half-life was used. It is noteworthy that monitoring studies, included in the ecological risk assessment, show the toxicity of acrolein is such that even with a dissipation half-life of less than 1 day, acrolein is persistent enough to move long distances with concentrations that remain a concern for wildlife.

2. Ecological Exposure and Risk

In ecological risk assessments, the ecological effects characterization describes the types of effects a pesticide can potentially produce in an animal or plant. This characterization is generally based on registrant-submitted studies that describe acute and chronic effects information for various aquatic and terrestrial animals and plants; however, these data may also be supplemented by data reported in ECOTOX (http://www.epa.gov/med/Prods_Pubs/ecotox.htm) or open/public literature sources that have met Agency criteria for acceptability.

To estimate potential ecological risk, the EPA integrates the results of exposure and ecotoxicity studies using the risk quotient method. The risk quotient (RQ) approach is used in

this assessment to reach conclusions regarding the potential for adverse effects associated with the proposed use of acrolein. The basis of the RQ approach is a comparison of the ratio of exposure concentrations to effects endpoints with predetermined levels of concern (LOCs). Risk quotients are calculated by dividing estimated environmental concentrations (EECs), based on environmental fate characteristics, by ecotoxicity values (acute and chronic) for various wildlife and plant species. RQs are then compared to LOCs, and when the RQs exceed the level of concern for a particular category, the Agency presumes a potential risk of concern to that category.

Although risk is often defined as the likelihood and magnitude of adverse ecological effects, the risk quotient-based approach does not provide a quantitative estimate of likelihood and/or magnitude of an adverse effect. These LOCs are indicators of whether a pesticide, used as directed on the label, has the potential to cause adverse effects on non-target organisms. See Table 10 for the Agency's LOCs. Risk characterization provides further information on potential adverse effects and the possible impact of those effects by considering the fate of the chemical and its degradates in the environment, organisms potentially at risk, and the nature of the effects observed. To the extent feasible, the Agency seeks to reduce environmental concentrations in an effort to reduce the potential for adverse effects to non-target organisms.

Table 10. EPA's Levels of Concern and Associated Risk Presumptions

Risk Presumption	RQ	LOC
Terrestrial animals (birds and wild mammals)		
Acute High (Non-listed) Risk	EEC[1]/LC_{50} or LD_{50}/ft^2 or LD_{50}/day[3]	0.5
Acute Endangered (Listed) Species	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day	0.1
Chronic Risk	EEC/NOAEC	1
Aquatic animals		
Acute High (Non-listed) Risk	EEC[4]/LC_{50} or EC_{50}	0.5
Acute Endangered (Listed) Species	EEC/LC_{50} or EC_{50}	0.05
Chronic Risk	EEC/NOAEC	1
Terrestrial and Semi-Aquatic Plants		
Acute High (Non-listed) Risk	EEC[5]/EC_{25}	1
Acute Endangered (Listed) Species	EEC/EC_{05} or NOAEC	1
Aquatic Plants		
Acute (Non-listed) Risk	EEC[6]/EC_{50}	1
Acute Endangered (Listed) Species	EEC/EC_{05} or NOAEC	1

[1] abbreviation for Estimated Environmental Concentration (ppm) on avian/mammalian food items

[2] $\frac{\text{mg/ft}^2}{LD_{50} * \text{wt. of bird}}$ [3] $\frac{\text{mg of toxicant consumed/day}}{LD_{50} * \text{wt. of bird}}$ [4] EEC = (ppm or ppb) in water [5] EEC = lbs ai/A [6] EEC = (µg/L or mg/L) in water

a. Terrestrial Organisms Exposure and Risk

1) Bird and Mammal Toxicity

Avian

Acrolein is very highly toxic (LD_{50} <10 mg/kg) to birds on an acute oral exposure basis. The acute oral toxicity of acrolein to the mallard duck (*Anas platyrhynchos*) and northern bobwhite quail (*Colinus virginiana*) was assessed in separate single-dose studies. Male mallard ducks were dosed with 92% acrolein, which resulted in a LD_{50} of 9.1 mg/kg a.i. with sub-lethal effects including weakness, withdrawal, muscular debility, and imbalance. Sub-lethal effects were also observed at 3.3 mg/kg treatment intervals (MRID 00117668). Another acceptable mallard duck study via oral dosing with 95.09% acrolein resulted in a LD_{50} of 28 (18-38) mg a.i./kg-bw. Sub-lethal effects were noted such as lethargy, labored breathing, tremors, anorexia among others. Body weight and food consumption reductions were also noted (MRID 42183301). In addition, data in a supplemental study for the oral toxicity of 92% acrolein to bobwhite quail resulted in a LD_{50} of 19 mg/kg (MRID 92001003). Therefore, the most sensitive endpoint used to assess the acute oral toxicity of acrolein is 9.1 mg a.i./kg-bw. Refer to Table 11 below for a complete listing of the acute toxicity values for birds used in the risk assessment.

Table 11. Summary of acute toxicity data for terrestrial organisms (birds) exposed to acrolein.

Species (common name)	Measure of effect	End-point	Mean Concentration (C.I.)	Test Substance (% a.i.)	Study Classification	Reference (MRID)
Mallard duck *Anas platyrhynchos*	Mortality	LD_{50}	9.11 mg a.i./kg (6.32-13.1)	92	Acceptable	00117668

No data are available to evaluate the subacute dietary toxicity (LD_{50}) of acrolein to birds either through registrant-submitted data or through a search of the open literature contained in ECOTOX.

In addition, no data are available to evaluate the chronic toxicity of acrolein to birds either through registrant-submitted data or through a search of the open literature contained in ECOTOX. Therefore, due to a lack of chronic toxicity data for birds, only acute, dose-based exposures to birds were considered in the assessment.

In order to assess the risk to birds from inhalation, it is necessary to estimate the inhalation LD_{50} for acrolein from rat inhalation toxicity data since there are no direct measurements of acrolein inhalation toxicity available for birds. The oral LD_{50} for gulls and songbirds were estimated using the weight of the bird relative to the weight and LD_{50} of the mallard. The method for calculating the expressed values listed below in Table 12 are outlined in the *Environmental Fate and Ecological Risk Assessment Chapter in Support of Phase V of*

the Reregistration Eligibility Decision on Acrolein, dated July 23, 2008, which is referenced in Appendix D of this document.

Table 12. LD_{50} for representative birds estimated from the acute oral LD_{50} for mallard duck and the adjusted rat inhalation LD_{50}.

Species	Body weight (g)	Oral LD_{50} ($mg \cdot kg^{-1}$)	Inhalation LD_{50} ($mg \cdot kg^{-1}$)
Mallard	1580	9.1	0.574[3]
Ring-bill gull	350	7.25[1]	0.458[2]
Songbird	20	4.72[1]	0.298[3]

[1] oral $LD_{50(oral, A)} = LD_{50(oral, mallard)}(BW_A/BW_{mallard})^{(1.15-1)}$

[2] inhalation $LD_{50(inh, gull)} = LD_{50 (or, gull)} /(LD_{50(or, rat)} * Fre / LD_{50(inh,rat)})$, Fre calculation is in text)

[3] adjusted $LD_{50(inh)}$ for mallard and songbird: $LD_{50(inh,A)} = LD_{50(inh, gull)}(BW_A/BW_{gull})^{(1.15-1)}$

Mammal

Acrolein is highly toxic (LD_{50} 10-50 mg/kg) to mammals on an acute oral exposure basis. In an acute study on rats (*Rattus norvegicus*; MRID 41257001), acrolein was administered by gavage to male and female rats. The acute oral LD_{50} for male and female rats was 10.3 and 11.8 mg/kg, respectively. The rats were observed for 4 hours, with sublethal signs of toxicity that included lethargy, hypothermia, changes in respiration and weight loss. Acrolein is also considered to be a skin/mucous membrane and eye (lacrimator) irritant. In addition, due to the volatility of acrolein, wildlife may also be exposed through the inhalation route. The inhalation LC_{50} for acrolein is 17 mg/m^3/4 hours in rats. However, for assessment purposes, this value was converted to a dose-based toxicity value to determine risks from inhalation of acrolein. For further detail on the process of this conversion, please refer to the Environmental Fate and Ecological Risk Assessment referenced above in this section. Table 13 provides a summary of the results for acute oral toxicity.

Table 13. Summary of acute toxicity data for terrestrial organisms (mammals) exposed to acrolein.

Species (common name)	Measure of effect	End-point	Mean Concentration (C.I.)	Test Substance (% a.i.)	Study Classification	Reference (MRID)
Laboratory rat *Rattus norvegicus*	Mortality	LD_{50}	Males: 10.3 (6.4-16.7) mg/kg Females: 11.8 (7.9-17.6) mg/kg	96.58	Acceptable	412570-01

In a 2-generation (rat) reproduction study (MRID 41869101), a LOAEL of 6 mg/kg/day was determined for parental toxicity. This value was based on decreases in body weight and in food consumption as well as other adverse effects. The NOAEL was determined to be 3 mg/kg/day for parental toxicity. Likewise, the LOAEL and NOAEL for offspring toxicity are 6 mg/kg/day based on body weight decrease in the F_1 generation, and 3 mg/kg/day, respectively.

2) Bird and Mammal Exposure

The EEC values for residues on food and feed items used for terrestrial exposure are derived from the Kenaga nomograph, as modified by Fletcher *et al.* (1994). Risk quotients are based on the most sensitive LD_{50} for birds (mallard) and LD_{50} and NOAEL values from rat studies.

In order to estimate risks to terrestrial mammals and birds inhabiting and eating in fields irrigated with acrolein-treated water, it was necessary to calculate the application rate of acrolein to a field in units of lbs a.i./A. This calculation requires conversion from the concentration of acrolein in irrigation water (mg/L) to the amount of acrolein that could potentially remain on the foliage after an irrigation event. Note: this method is relevant when sprinkler irrigation is used in order that the irrigation water is applied to the foliage. Thus, dietary exposure (other than drinking water) should not be a concern for flood or furrow irrigation as there is little contact of the irrigation water with the above ground foliage. To achieve this estimate, a measure of the amount of irrigation water that sticks to the crop was required. Therefore, in order to provide conservative estimates of risk, the CINTCP value (a parameter used in the Pesticide Root Zone Model (PRZM) that defines the maximum interception storage of a crop) for orchards was utilized to estimate exposures to terrestrial mammals and birds consuming food in fields receiving irrigation water containing various concentrations of acrolein.

Although acrolein is applied directly to irrigation water and terrestrial plants are not initially treated with acrolein, the label requires that treated water is to be applied to fields and thus, terrestrial organisms may potentially be exposed to acrolein-treated water. Typically, screening-level ecological risk assessments do not take drinking water exposure into account; however, terrestrial animals could potentially drink water from treated irrigation canals. Therefore, in order to assess potential risks, dose-based exposures were estimated for several representative mammalian and avian species, including mink, river otter, spotted sandpiper, belted kingfisher, herring gull, osprey, mallard duck, great blue heron and bald eagle.

The EEC values used to assess exposure to birds and mammals can be found in the *Environmental Fate and Ecological Risk Assessment Chapter in Support of Phase V of the Reregistration Eligibility Decision on Acrolein*, dated July 23, 2008, which is available in the public docket.

3) Bird and Mammal Risk

Birds

For the drinking water only exposure, no acute risk LOC is exceeded for non-listed birds, the acute risk LOC for Federally listed endangered and threatened (listed) species (RQ>0.1) is exceeded for the spotted sandpiper and belted kingfisher. Therefore, the Agency's screening-level assessment indicates a potential for acute risk to listed birds (especially smaller birds) consuming drinking water treated with acrolein at the maximum label rate. Thus, an analysis will be conducted to determine if any listed or candidate species may co-occur in the area of acrolein application or areas downstream that could be contaminated from drift or

runoff. If it is determined that listed or candidate species may be present in the proposed application areas (irrigation canals and reservoirs), further biological assessment will be undertaken. The extent to which listed species may be at risk then determines the need for the development of a more comprehensive consultation package as required by the Endangered Species Act. Refer to Table 14 for additional information.

Table 14. Acute dose-based RQ values for birds exposed to Acrolein through drinking water.

Avian Species	BW (kg-bw)	DW (L/kg-bw/d)	EEC (mg/kg-bw/d)	Adjusted Toxicity Values (mg/kg-bw)	Acute RQs
Spotted Sandpiper	0.043	0.167	2.500	5.31	**0.471**[1]
Belted kingfisher	0.148	0.111	1.662	6.39	**0.260**[1]
Herring gull	1.1	0.057	0.858	8.63	0.099
Osprey	1.5	0.052	0.774	9.04	0.086
Mallard duck	1.58	0.051	0.761	9.11	0.084
Great blue heron	2.39	0.044	0.664	9.69	0.068
Bald eagle	4.65	0.036	0.533	10.71	0.050

[1] Exceeds the acute LOC (0.1) for listed species.

The acute risk LOC (RQ>0.5) is exceeded for all-sized birds feeding on all forage categories except large birds (1000 g) feeding on fruits/pods/large insects at application rates of 0.54 lbs a.i./A representing water treatment rate of 15 mg a.i./L. At an application rate of 0.05 lbs a.i./A (representing water treatment rate of 1.5 mg a.i./L), the acute risk LOC is exceeded for small (20 g) and medium (100 g) birds feeding on short grasses, tall grasses and broadleaf plants/small insects (RQ range 0.78 – 3.09). The acute listed species LOC is exceeded across all-sized birds feeding in all forage categories except fruits/pods/large insects at application rates equivalent to 0.05 lbs a.i./A or greater. At the lowest application rate evaluated (0.005 lbs a.i./A) (representing water treatment rate of 0.15 mg a.i./L), the acute risk to listed species LOC is exceeded for small birds feeding on all forage categories except fruits/pods/large insects and for medium sized birds feeding on short grasses (Table 15).

Table 15. Acute dose-based RQs for birds of different size and feeding classes exposed to acrolein on foodstuffs treated with irrigation water.

Food Type	Small (20 g)	Medium (100 g)	Large (1000 g)
Application Rate: 0.535 (15 mg/L in water)			
Short Grass	**30.92**[1, 2]	**13.85**[1, 2]	**4.39**[1, 2]
Tall Grass	**14.17**[1, 2]	**6.35**[1, 2]	**2.01**[1, 2]
Broadleaf plants/sm insects	**17.39**[1, 2]	**7.79**[1, 2]	**2.47**[1, 2]
Fruits/pods/lg insects	**1.93**[1, 2]	**0.87**[1, 2]	**0.27**[2]
Application Rate: 0.0535 (1.5 mg/L in water)			
Short Grass	**3.09**[1, 2]	**1.38**[1, 2]	**0.44**[1, 2]
Tall Grass	**1.42**[1, 2]	**0.63**[1, 2]	**0.20**[1, 2]
Broadleaf plants/sm insects	**1.74**[1, 2]	**0.78**[1, 2]	**0.25**[1, 2]
Fruits/pods/lg insects	**0.19**[2]	**0.09**	**0.03**

Table 15. Acute dose-based RQs for birds of different size and feeding classes exposed to acrolein on foodstuffs treated with irrigation water.			
Food Type	**Small (20 g)**	**Medium (100 g)**	**Large (1000 g)**
Application Rate: 0.00535 (0.15 mg/L in water)			
Short Grass	**0.31**[1, 2]	**0.14**[2]	**0.04**
Tall Grass	**0.14**[2]	**0.06**	**0.02**
Broadleaf plants/sm insects	**0.17**[2]	**0.08**	**0.02**
Fruits/pods/lg insects	**0.02**	**0.01**	**0.00**

[1] Exceeds LOC (RQ≥0.5) for acute exposures to non-listed terrestrial birds.
[2] Exceeds LOC (RQ≥0.1) for acute exposures to listed terrestrial birds.
Foliar dissipation half life: 1 day
Number of applications: 1
Avian LD50: 9.11 (mallard duck)

Lower bound RQs are calculated from CARB monitoring data and do not exceed the Agency's LOC except for listed, small birds. Upper-bound risk quotients for acute mortality to birds based on inhalation exceed the acute risk LOC for all birds. Upper-bound concentrations used to calculate these RQs assume that the air along the sides of the canal is in equilibrium with the canal water. However, because volatilization from the water's surface is a time-dependent process, the water is moving, and the air in and around the canal is unbounded; it is unlikely that equilibrium would ever be approached (Table 16).

Table 16. Acute risk RQs for birds via the inhalation route.					
Species	**Inhalation LD_{50}** mg·kg^{-1}	**Lower Bound VID** mg·kg^{-1}	**Upper Bound VID** mg·kg^{-1}	**Lower Bound RQ** mg·kg^{-1}	**Upper Bound RQ** mg·kg^{-1}
Mallard	0.574	2.21 x 10^{-2}	2.17 x 10^{1}	0.04	**38**[2]
Gull	0.458	3.12 x 10^{-2}	3.0.7 x 10^{1}	0.07	**67**[2]
Songbird	0.298	6.03 x 10^{-2}	5.93 x 10^{1}	**0.20**[1]	**199**[2]

[1]Exceeds LOC (RQ > 0.1) for listed birds and for restricted use for birds
[2] Exceed LOC (RQ > 0.5) for high risk to birds

Mammals

Although no acute risk LOC is exceeded for non-listed mammals, for the drinking water only exposure scenario the acute risk LOC for endangered species (RQ>0.1) is exceeded for mammals. Therefore, there is a potential for acute risk to listed mammals consuming drinking water treated with acrolein at the maximum label rate. No chronic risk LOC is exceeded for mammals (Table 17).

Table 17. Acute and chronic dose-based RQ values for mammals exposed to Acrolein through drinking water.							
Mammalian Species	**BW (kg-bw)**	**DW (L/kg-bw/d)**	**EEC (mg/kg-bw/d)**	**Adjusted Toxicity Values (mg/kg-bw)**		**Risk Quotients**	
				Acute	**Chronic**	**Acute**	**Chronic**
Mink	1.0	0.099	1.485	7.92	2.31	**0.187**[1]	0.644
River otter	8.0	0.080	1.206	4.71	1.37	**0.256**[1]	0.879

[1] Exceeds the acute LOC (0.1) for listed species.

The acute risk LOC (RQ>0.5) is exceeded for all sized mammals feeding on all forage categories except large mammals (1000 g) feeding on grass, broadleaf plants and small insects at application rates of 0.54 lbs. a.i./A (representing water treatment rate of 15 mg a.i./L). At the highest application rate of acrolein, RQs exceed the LOC for listed species of all sizes and feeding categories of mammals, with the exception of granivores. At an application rate of 0.0535 lbs a.i./A (representing water treatment rate of 1.5 mg a.i./L), the acute risk LOC is exceeded only for small-sized (15 g) mammals feeding on short grasses (RQ=0.54). The acute listed species LOC for mammals is exceeded for all sized mammals feeding on short grass, broadleaf plants and small insects (Table 18).

Table 18. Acute dose-based RQs for mammals of different size and feeding classes exposed to acrolein.

Food Type	Small (15 g)	Medium (35 g)	Large (1000 g)
Application Rate: 0.534 lbs a.i.A (15 mg/L in water)			
Short Grass	**5.41**[1, 2]	**4.62**[1, 2]	**2.48**[1, 2]
Tall Grass	**2.48**[1, 2]	**2.12**[1, 2]	**1.13**[1, 2]
Broadleaf plants/sm insects	**3.04**[1, 2]	**2.60**[1, 2]	**1.39**[1, 2]
Fruits/pods/lg insects	**0.34**[2]	**0.29**[2]	**0.15**[2]
Seeds (granivore)	**0.08**	**0.06**	**0.03**
Application Rate: 0.0534 lbs a.i./A (1.5 mg/L in water)			
Short Grass	**0.54**[1, 2]	**0.46**[2]	**0.25**[2]
Tall Grass	**0.25**[2]	**0.21**[2]	**0.11**[2]
Broadleaf plants/sm insects	**0.30**[2]	**0.26**[2]	**0.14**[2]
Fruits/pods/lg insects	**0.03**	**0.03**	**0.02**
Seeds (granivore)	**0.01**	**0.01**	**<0.01**

[1] Exceeds LOC (RQ≥0.5) for acute exposures to non-listed terrestrial mammals.
[2] Exceeds LOC (RQ≥0.1) for acute exposures to listed terrestrial mammals.
Foliar dissipation half life: 1 day
Number of applications: 1
Mammalian LD_{50}: 10.30

The inhalation LC_{50} for rats exposed to acrolein is 18 mg/m^3/4 hours with an LD_{50} of 2.0 mg·kg^{-1}. For mammals, the lower bound inhalation RQ based on monitoring is >0.1 and the calculated upper bound inhalation RQ is 20 which exceeds the LOC (RQ >0.5) for acute risks to mammals.

4) Non-target Terrestrial Plants

There are no terrestrial plant toxicity data with which to evaluate potential risks to terrestrial plants; however, there is an incident report of adverse effects to agricultural crops to which acrolein-treated water is routinely applied to dissipate the chemical. It has been hypothesized that the waxy cuticle of terrestrial plants that protects them from dehydration may also serve to protect them from the toxic effects of acrolein. However, no data have been submitted with which to evaluate this hypothesis. Residue data collected from terrestrial plants indicates dicysteine residues in terrestrial plants treated with acrolein; these data suggest that in terrestrial plants acrolein can cross-link sulfhydryl residues in proteins.

5) Non-target Insects

There are no data available in order to evaluate the acute toxicity of acrolein to beneficial insects. However, risk is presumed for insects in the absence of data and based on the chemical's mode of action.

b. Aquatic Organism Exposure and Risk

On an acute exposure basis acrolein is very highly toxic to freshwater fish and invertebrates, estuarine/marine invertebrates and it is highly toxic to estuarine/marine fish. Chronic exposure to acrolein resulted in reduced growth and survival in fish and reduced survival in aquatic invertebrates. Available toxicity data indicate that aquatic animals are just as sensitive, if not more so, to acrolein than aquatic plants.

1) Fish, Invertebrate, and Aquatic Plant Toxicity

Freshwater Fish/Amphibians

There are several 96-h LC_{50} values available to describe the acute toxicity of acrolein to freshwater fish and amphibians. The most conservative value identified to describe the toxicity of acrolein to freshwater vertebrates is a 96-h LC_{50} of 7 µg a.i./L for larval African clawed frog (*Xenopus laevis)* (Holcombe *et al.* 1987). Supplemental data submitted to the Agency using guideline test species indicate that the 96-hr LC_{50} of acrolein (96.4% a.i.) to bluegill sunfish (*Lepomis macrochirus)*, and rainbow trout (*Oncorhynchus mykiss)*, under flow-through exposures is 22.4 and <31 µg a.i./L, respectively (MRIDs 415132-01 and 415132-03). Thus, acrolein is classified as very highly toxic to freshwater fish on an acute exposure basis. The most sensitive endpoint used to assess the acute toxicity of acrolein to freshwater fish is the 96-hr LC_{50} for fathead minnow (*Pimephales promelas)* of 14 µg a.i./L (Geiger *et al*. 1990; Holcombe *et al*. 1987); for aquatic-phase amphibians, the most sensitive endpoint is the African clawed frog 96-h LC_{50} of 7 µg a.i./L.

The chronic toxicity of acrolein to fathead minnow (*Pimephales promelas)* and flag fish *(Jordanella floridae)* were assessed. The NOEC from an EPA fish lifecycle study on fathead minnow study was 11.4 µg a.i./L (MRID 05008271). Other toxicity data for fathead minnow indicate that the NOEC for growth and survival are 14 and 35 µg a.i./L, respectively. Additional data from chronic exposures of flag fish to acrolein indicate a NOEC for growth of 32 µg a.i./L. Thus, the most sensitive endpoints used to assess the chronic toxicity of acrolein to freshwater vertebrates (fish) was a NOEC value of 11.4. Refer to Table 19.

Table 19. Summary of acute and chronic toxicity data for freshwater fish exposed to acrolein.					
Species (common name)	**Measure of Effect**	**End-point**	**Duration (days)**	**Mean concentration (µg a.i./L)**	**Ref. (MRID)**
African Clawed Frog *Xenopus laevis*	Mortality	LC_{50}	4	7.0	Holcombe 1987*
Bluegill Sunfish *Lepomis macrochirus*	Mortality	LC_{50}	4	22.4	41513201
Fathead minnow *Pimephales promelas*	Mortality	LC_{50}	4	14	Geiger, 1990*
Fathead minnow	Growth and reproduction	NOEC	32	9.1	Sabourin 1986*
Fathead Minnow	Survival of newly hatched fry	NOEC	60	11.4	05008271
Fathead Minnow	Survival	NOEC	32	14	Spehar 1989*
Flagfish *Jordanella floridae*	Survival and Growth	NOEC	32	16	Spehar 1989*

*Data value identified in ECOTOX literature search.

Freshwater Invertebrates

An acute 48-hour toxicity study was conducted to determine the effects of acrolein on freshwater invertebrates. Data available for the waterflea (*Daphnia magna)* shows that the 48 hour EC_{50} values for immobilization are <31 and 57 µg a.i./L, based on two submitted studies. Additional values describing the acute toxicity of acrolein to freshwater invertebrates (e.g. midge) were identified in the ECOTOX literature search; however, these values were greater (*i.e.*, less sensitive) than those submitted to the Agency. Thus, the most sensitive endpoint used to assess the acute toxicity of acrolein to freshwater invertebrates was <31 µg a.i./L. Acrolein is classified as very highly toxic to freshwater invertebrates on an acute exposure basis.

In an EPA study (MRID 05008271), three generations of water flea were exposed to flow-through concentrations of acrolein for three weeks. A NOEC for survival of 7.1 µg a.i./L was determined after two generations with a NOEC of 16.9 µg a.i./L after the third generation. The draft aquatic life criteria from the Office of Water cited this study and used the higher NOEC determined after the third generation. For calculating RQs, the lower value was chosen consistent with more conservative assumptions used in a screening level risk assessment. Refer to Table 20 for a summary of this study.

Table 20. Summary of chronic toxicity data for freshwater invertebrates exposed to acrolein.					
Species (common name)	**Measure of Effect**	**End-point**	**Duration (days)**	**Mean concentration (µg a.i./L)**	**Ref. (MRID)**
Water Flea *Daphnia magna*	Survival	NOEC	3 Gener-ations	7.1	05008271

Estuarine/Marine Fish

Results of an EPA study on acrolein for longnose killifish (*Fundulus similis)*, and sheepshead minnow (*Cyprinodon variegates)* indicated that the 48-h LC_{50} value for longnose killifish was 240 µg a.i./L and the 96-h LC_{50} for sheepshead minnow was 428 µg a.i./L. The most sensitive endpoint used to assess the acute toxicity of acrolein to estuarine/marine fish is the 48-hr LC_{50} value for longnose killifish. Acrolein is classified as highly toxic to estuarine/marine fish on an acute exposure basis. Refer to Table 21 for referenced values.

Table 21. Summary of acute toxicity data for estuarine/marine fish exposed to acrolein.					
Species (common name)	**Measure of Effect**	**End-point**	**Duration (days)**	**Mean concentration (µg a.i./L)**	**Ref. (MRID)**
Longnose killifish *Fundulus similis*	Mortality	LC_{50}	48	240	40228401
Sheepshead minnow *Cyprinodon variegatus*	Mortality	LC_{50}	96	428	43225202

No data are available to estimate the chronic toxicity of acrolein to estuarine/marine fish. Thus, in the absence of data, risk is presumed for estuarine/marine fish.

Estuarine/Marine Invertebrates

A 96-hour acute toxicity study was conducted to determine the effect of acrolein on Eastern oyster (*Crassostrea virginica)*, brown shrimp (*Penaeus aztecus)*, and mysid shrimp (*Americamysis bahia)*. The reported 96-h EC_{50} values for Eastern oyster are 55 and 106 µg a.i./L. Data available for brown and mysid shrimp are 48-h EC_{50} of 100 µg a.i./L, and a 96-h LC_{50} of 500 µg a.i./L, respectively. The most sensitive endpoint used to assess the acute toxicity of acrolein to estuarine/marine invertebrates is 55 µg a.i./L. Acrolein is classified as very highly toxic to the estuarine/marine invertebrates on an acute exposure basis. Refer to Table 22 for referenced values.

Table 22. Summary of acute toxicity data for estuarine/marine invertebrates exposed to acrolein.					
Species (common name)	**Measure of Effect**	**End-point**	**Duration (days)**	**Mean concentration (µg a.i./L)**	**Ref. (MRID)**
Eastern oyster *Crassostrea virginica*	Shell Growth	EC_{50}	96	55	40228401
Brown Shrimp *Penaeus aztecus*	Immobility	EC_{50}	48	100	40228401
Eastern oyster *Crassostrea virginica*	Shell Deposition	EC_{50}	96	106 (73-183)	43164302
Mysid shrimp *Americamysis bahia*	Mortality	LC_{50}	96	500 (390-650)	43164301

No data are available to estimate the chronic toxicity of acrolein to estuarine/marine invertebrates. Thus, in the absence of data, risk is presumed for estuarine/marine invertebrates.

Aquatic Plants

In separate Tier 2 (non-vascular) acute toxicity tests, green algae (*Pseudokirchneriella subcapitatum*), blue-green algae (*Anabaena flos-aquae*), freshwater diatom (*Navicula pelliculosa*) and marine diatom (*Skeletonema costatum*) were exposed to acrolein for 5 days (MRIDs 426209-01, 426209-02, 426209-03 and 426209-05). The most sensitive species tested is the marine diatom, which has an EC_{50} for reduction of cell density of 28 µg a.i./L

Also, in a freshwater vascular plant toxicity test for duckweed the NOAEC is 25 µg a.i./L and the EC_{50} is 72 µg a.i./L (MRID 42620904). Median effect concentrations for vascular and non-vascular aquatic plants are 36 and 72 ug/L, respectively.

2) Fish, Invertebrate, and Aquatic Plant Exposure

Estimated Environmental Concentrations (EECs) for characterizing aquatic exposure were represented by the maximum application rate of acrolein (15 mg/L) as well as by available data from monitoring conducted in Washington State for the purpose of the National Pollution Discharge Elimination System (NPDES).

3) Fish, Invertebrate, and Aquatic Plant Risk

At currently registered maximum treatment rates (15 mg/L for up to 8 hours), non-target aquatic animals and plants in treated water ways will be exposed to acrolein and thus exposure will likely result in acute mortality of aquatic animals and plants following a single treatment. Monitoring data collected for NPDES permitting indicate that while many

application events result in non-detections, several detections in the receiving water bodies at the compliance points result in exceedances an order of magnitude above the Agency's LOC. One data point showed acrolein concentrations up to 67 ppb have been measured up to 61 miles from the point of application and up to 54 hours after application. These data were measured in Washington State which has a SLN allowing discharge to receiving waters 48 hours after treatment.

RQs in Table 23 were calculated based on the maximum application rate in the canal and the highest measured concentrations from the discharge point of an irrigation canal in Washington State following release after a two day holding period. For additional information and an extensive review of the monitoring data, please refer to the *Environmental Fate and Ecological Risk Assessment Chapter in Support of Phase V of the Reregistration Eligibility Decision on Acrolein*, dated July 23, 2008, which is available in the public docket.

Table 23. Acute RQs for aquatic animals and plants.

Species	Toxicity Endpoint (µg/L)	EEC (µg/L) Max app rate	RQ* From max app rate	EEC (µg/L) Monitored Concentration	RQ*[a] (from monitoring)
Fathead Minnow *Pimephales promelas*	14	15,000	1,071	67	5
African clawed frog *Xenopus laevis*	7	15,000	2,143	67	10
Water Flea *Daphnia magna*	<31	15,000	>484	67	>2
Sheepshead Minnow *Cyprinodon variegatus*	428	15,000	35	67	0.15
Eastern Oyster *Crassostrea virginica*	55	15,000	273	67	1.2
Blue-green Algae *Anabaena flos-aquae*	36	15,000	417	67	1.8
Duckweed *Lemna gibba*	72	15,000	208	67	0.9

* Risk Quotient = EEC/Toxicity
a Monitoring value from a Washington State NPDES permit Measurement at compliance point with release of canal water after 2-day holding consistent with the Washington State Special Local Needs label.

Freshwater Fish/Amphibians

At the maximum treatment rate of 15 mg/L, acrolein concentrations in the canals exceed acute risk LOCs (RQ≥0.5) with RQs of up to 1071 for freshwater fish and up to 2143 for aquatic-phase amphibians. Monitoring data collected for NPDES permitting indicate that while many application events result in non-detections, several detections in the receiving water bodies at the compliance points result in exceedances an order of magnitude above the Agency's LOC. Calculated RQs for fish and amphibians are up to 10 based on the highest concentration observed in the monitoring studies after the required holding times. Most of the NPDES monitoring values result in no risks of concern at the compliance points.

Freshwater Invertebrates

Although aquatic invertebrates are less sensitive than fish and aquatic-phase amphibians to acrolein, the acute risk level of concern (RQ≥0.5) for freshwater invertebrates in the canal is >484. As discussed above for the freshwater fish, the highest observed concentration in the monitoring data would also result in an RQ of 2 which is above the Agency's LOC. Most of the NPDES monitoring values result in no risks of concern at the compliance points.

Estuarine/Marine Fish

Using the sheepshead minnow as a surrogate, the acute risk RQ for estuarine fish in the canal is 35 which is above the LOC (RQ≥0.5) for estuarine/marine fish; however, the acute risk at the compliance point for the highest observed concentration in the monitoring data gives an RQ of 0.15 which does not exceed the LOC.

Estuarine/Marine Invertebrates

Based on the toxicity of acrolein to the Eastern oyster, in the canal, the acute RQ is 273 which exceeds the LOC (RQ≥0.5) for estuarine/marine invertebrates. The highest observed concentration in the monitoring data resulted in an RQ of 1.2 at the compliance point. Most of the NPDES monitoring values result in no risks of concern at the compliance points.

Aquatic Plants

Aquatic plants are particularly sensitive to acrolein. In the canal, the acute risk LOC for vascular and non-vascular aquatic plants (RQ≥1.0) is exceeded with RQs of 208 and 417, respectively. In addition, RQ values exceed the acute risk to endangered species LOC (RQ≥1.0) for vascular and nonvascular plants with RQs of 600 and 1,250, respectively. For the highest observed monitoring value, the RQs are 0.9 for vascular plants and 1.8 for non vascular plants and the corresponding endangered plant RQs are 2.7 and 5.4. Most of the NPDES monitoring values result in no risks of concern for listed or non-listed plants at the compliance points.

c.) Listed Species Risk

Table 24 provides a summary of potential direct and indirect effects to listed species in the irrigation canals. It is unlikely that listed species would be found in or around treated irrigation canals since the canals are designed to deliver water to agricultural fields. While as noted above, fish and invertebrate listed species are potentially at risk from the highest observed concentration in the monitoring data, calculations using most of the compliance monitoring data would not indicate risks of concern for listed species. Any inadvertent release of treated canal water may have an effect on listed species in the immediate area, but these risks are not assessed here.

Table 24. Potential listed species risks associated with direct or indirect effects due to treatment of irrigation canals with acrolein.

Listed Taxon	RQ	Direct Effects from Acute Exposures	Indirect Effects
Aquatic			
Aquatic vascular plants	1,250	Yes	Yes[6]
Freshwater invertebrates	>484	Yes	Yes[4,5]
Marine/estuarine invertebrates	273	Yes	Yes[4,5]
Freshwater fish	1,071	Yes	Yes[4,5]
Marine/estuarine fish	35	Yes	Yes[4,5]
Aquatic phase amphibians	2,143	Yes	Yes[4,5]
Terrestrial			
Semi-aquatic plants	presumed[1]	presumed[1]	presumed[2]
Terrestrial plants	presumed[1]	presumed[1]	presumed[2]
Insects	presumed[1]	presumed[1]	presumed[2]
Birds	0.47	Yes	Yes[3,4]
Terrestrial phase amphibians	0.47	Yes	Yes[3]
Reptiles	0.47	Yes	Yes[3,4]
Mammals	0.26	Yes	Yes[3,4]

[1] No toxicity data are available to define RQ values for this exposure.
[2] Since the risks of direct effects to semi-aquatic and terrestrial plants are unknown, risks of indirect effects to organisms relying upon these plants are unknown.
[3]Direct effects to small mammals, amphibians, reptiles and birds could result in indirect effects to animals that rely upon them as food.
[4]Direct effects to aquatic animals could result in indirect effects to animals that rely upon them as food.
[5]Direct effects to aquatic plants (including unicellular and vascular) could result in indirect effects to animals that rely upon them as food.
[6]Direct effects to aquatic plants (including unicellular and vascular) could result in alterations in the plant community structure through changes in species interactions.

3. Risk Characterization

The Agency has considered the ecological risks associated with the use of acrolein. Based on the EPA's assessment and taking into account its use pattern, the use of acrolein according to label directions may potentially result in direct acute or chronic effects to fish, aquatic invertebrates and/or aquatic plants. Risk is expected for all aquatic organisms in the canals at all recommended application rates. Risks in the natural fish bearing waters which

receive canal outflow range from 0 up to an RQ of 10 for aquatic phase amphibians when label required holding times are observed. The NPDES permit monitoring data are not extensive, but most detected concentrations are low and do not violate the permit level of 21 ug/L And as indicated by reported incidents, inadvertent releases can result in mortality for large numbers of fish.

Risk is presumed for terrestrial plants and insects in the absence of data and the chemical's mode of action. Based on the most sensitive endpoint for each of the taxa evaluated, the RQ values for acute effects to listed and non-listed species exceed the LOC for acrolein. The potential for chronic risk of acrolein is uncertain and highly dependent on location and treatment regimen. Additionally, the acute toxicity of acrolein suggests that few biological receptors would survive the initial contact with the chemical; reducing the likelihood of chronic exposure to acrolein.

Although there is no acute risk LOC exceeded for non-listed birds, there is a potential for acute risk to listed birds consuming drinking water treated with acrolein at the maximum label rate. There is also a potential for acute risk to listed mammals consuming drinking water treated with acrolein at the maximum label rate. Based on upper-bound estimated environmental concentrations for acrolein in the air surrounding treated canals, there is a risk of acute mortality for both birds and mammals through inhaling acrolein fumes. Terrestrial mammals and birds foraging on vegetation, seeds and insects in agricultural fields where acrolein is applied as irrigation may also experience acute mortality depending on the size of the animal and the nature of the forage material.

Although the potential for chronic risk cannot be precluded for acrolein, there are no avian chronic toxicity data available with which to evaluate potential risk; this data gap contributes to uncertainty. While there are chronic toxicity data for mammals, the potential for chronic risk to mammals and/or birds is considered low since acrolein residues in treated water are expected to deter most animals from consuming the water. Additionally, field monitoring studies indicate that acrolein residues in treated fields dissipate with half-lives of less than 1 day; therefore, potential chronic exposure does not appear to be likely. While multiple applications may represent a potential source of repeated exposure, frequent repeat applications are conducted at much lower treatment concentrations than the maximum rate modeled in this assessment and as discussed previously would be more appropriately characterized as pulsed acute exposures. Therefore, the potential for chronic exposure is considered low and as such, potential chronic risk from the use of acrolein is considered low.

a) Endangered Species

The Agency has developed the Endangered Species Protection Program to identify pesticides whose use may cause adverse impacts on endangered and threatened species and to implement mitigation measures that address these impacts. The Endangered Species Act (ESA) requires federal agencies to ensure that their actions are not likely to jeopardize listed species or adversely modify designated critical habitat. To analyze the potential of registered pesticide uses that may affect any particular species, EPA uses basic toxicity and exposure data and considers ecological parameters, pesticide use information, geographic relationship between specific pesticide uses and species locations, and biological requirements and

behavioral aspects of the particular species. When conducted, these analyses take into consideration any regulatory changes recommended in this RED being implemented at that time.

The ecological assessment that EPA conducted for this RED does not, in itself, constitute a determination as to whether specific species or critical habitat may be harmed by the pesticide. Rather, this assessment serves as a screen to determine the need for any species-specific assessment that will evaluate whether exposure may be at levels that could cause harm to specific listed species and their critical habitat. The species-specific assessment refines the screening-level assessment to take into account information such as the geographic area of pesticide use in relation to the listed species and the habits and habitat requirements of the listed species. If the Agency's specific assessments for acrolein result in the need to modify use of the pesticide, any geographically specific changes to the pesticide's registration will be implemented through the process described in the Agency's *Federal Register* Notice (54 FR 27984) regarding implementation of the Endangered Species Protection Program.

4. Ecological Incidents

A review of the Ecological Incident Information System (EIIS) database identified a total of 14 incidents that have been reported to the Agency, which may have involved exposures of acrolein between 1971 and 2007. Of the 14 reported incidents, 1 involved terrestrial plants; 12 involved effects to fish, amphibians and/or aquatic invertebrates and 1 involved effects to aquatic birds. About half of all reported incidents occurred in California. It should be noted that many more incidents may have occurred due to acrolein exposures, but may not have been reported due to various factors, such as a lack of reporting, or a lack of witnessing effects. Therefore, the lack of an incident report may not accurately indicate an overall absence of incidents.

In nearly all reported incidents involving acrolein, hundreds to tens of thousands of fish were reportedly killed. Some of the incidents were classified as accidental misuse, while the majority was considered "probable" to "highly probable".

One incident occurred in 2004 to a private fish pond resulting in a fish kill involving Koi (*Cyprinus carpio*), listed by the South San Joaquin Irrigation District in their monitoring report. It was reported that the owner was not properly informed that the irrigation water for his pond was being treated with acrolein.

The most recent incident (2007) associated with the use of acrolein involved the loss of approximately 2400 game fish and 2800 non-game fish on a 1.5 mile stretch of the Cub River in Idaho. Various species of fish were killed as a result of the application of Magnacide® H to the Cub River Canal adjacent to the Cub River. A leaky gate was observed 2 miles above the fish kill; however, no dead fish were noted in the river above the beaver ponds which impounded the water. No residues were collected, nor were any other pollutants reported; investigators determined the incident was "unlikely" to be directly attributed to acrolein.

In 2008, one of the irrigation districts in Idaho noted that aquatic herbicides applied in a particular irrigation district had traveled from the irrigation canals through a shallow 'karst-

like' aquifer and resulted in fish kills at aquaculture facilities. For clarification purposes, it is noted that at least some of these Idaho incidents are believed to be due to xylene. This district no longer uses acrolein.

These reported incidents, as were previously mentioned, may not accurately reflect the actual number that may be associated with the use of acrolein as an herbicide. Current data indicate that roughly one third of the reported incidents resulted from the registered use of acrolein; however, the incidents involving the highest level of mortality resulted from misuses. To date, the largest loss of aquatic animals, *i.e.*, 338,600 animals killed in 1977, resulted from an inadequate holding time. However, given the toxicity of acrolein at maximum application rates, direct contact of any aquatic animal would likely prove lethal within a relatively short period of time. For a more detailed account of each reported incident, please refer to the *Environmental Fate and Ecological Risk Assessment Chapter in Support of Phase V of the Reregistration Eligibility Decision on Acrolein*, dated July 23, 2008, which is available in the public docket.

IV. Risk Management and Reregistration Decision

A. Determination of Reregistration Eligibility

Section 4(g)(2)(A) of FIFRA calls for the Agency to determine, after submission of relevant data concerning an active ingredient, whether or not products containing the active ingredient are eligible for reregistration. The Agency has previously identified and required the submission of the generic (i.e., active ingredient-specific) data required to support reregistration of products containing acrolein as an active ingredient. The Agency has completed its review of these generic data, and has determined that the data are sufficient to support reregistration of all products containing acrolein. The Agency has determined that acrolein is eligible for reregistration provided that the risk mitigation measures and label amendments specified in this RED are implemented.

B. Public Comments and Responses

Through the Agency's public participation process, EPA worked extensively with stakeholders and the public to reach the regulatory decisions for acrolein. EPA released its revised risk assessments on acrolein for public comment on April 2, 2008, for a 60-day public comment period (Phase 5 of the public participation process). During the public comment period on the risk assessments, which closed on June 2, 2008, the Agency received comments from the Washington Department of Ecology, Boise Project Board of Control, U.S. Department of the Interior (Bureau of Reclamation), commercial applicators, a registrant (SePRO Corporation), several irrigation districts and canal companies in the western U.S., as well as the technical registrant Baker Petrolite. These comments in their entirety, responses to the comments, as well as the preliminary and revised risk assessments, are available in the public docket for acrolein (EPA-HQ-OPP-2007-0588) in the EPA's electronic docket at http://www.regulations.gov.

Benefits Analysis

The following is a summary of the Agency's review of submissions containing use (application timing, pest spectrum, etc.) and economic information in response to comments received during the latest comment period referenced above. The use of acrolein as a herbicide in irrigation conveyance systems is considered by irrigation districts to be "vital" to their mission to efficiently provide irrigation water at a low cost and with a minimal loss of water (e.g. overflow). Based on the data provided in the comments, the cost of using acrolein is substantially less than other possible alternatives; such is the case with mechanical methods and biological controls. Although mechanical control does seem to be feasible in some situations, the expenses of using these methods are substantially more than acrolein, and are often debated whether or not they tend to expose workers to additional risks.

The alternatives to acrolein have other drawbacks, in addition to higher cost, that make their use less than ideal. Labor intensive, cumbersome equipment, lack of accessibility, damage to concrete lined and earthen canals, as well as problems with suspended plant debris and sediment in the water are some of the many drawbacks. While there are many aquatic herbicides on the market, a common restriction of these herbicides is that most are not labeled for use in irrigation water. Those that are labeled for irrigation water have required holding periods following application and prior to irrigation. Those herbicides that do not have a holding or containment time in the canal will need to provide a rapid kill through either a high use rate to maximize contact time with weeds, or will need to be able to work with a minimum of contact time.

It is the flow of the irrigation water that presents challenges for weed control. The primary need by water managers is an herbicide to control submerged aquatic vegetation as well as an algaecide. Although the Agency believes that irrigation districts and canal companies in the West would still be able to deliver irrigation water if alternative methods were imposed, it is clear that costs to users would be substantially higher, and it is possible that increased weed growth would adversely impact the delivery of needed irrigation water.

C. Risk Mitigation and Regulatory Position

The following is a summary of the rationale for managing risks associated with the use of acrolein. For the use in the petroleum industry, no changes to the label or use pattern are required for MAGNICIDE B based on the assumptions of completely closed delivery and use systems. For acrolein use as an herbicide, labeling revisions are required and specific language is set forth in the summary tables of Chapter V of this document.

1. Human Health Risk Management

There were several potential human health risks of concern identified for acrolein. Based on the current use pattern, acrolein exposure to occupational handlers can occur. This is due to the fact that the application of MAGNICIDE H can vary in time (depending on site) and respiratory protection is not required after initial set up and prior to break down of equipment. However, this period of time after initial setup and prior to break down of equipment can also potentially be a concern for post-application risk to workers remaining in the vicinity of the

treated canal that are not using respirators. However, if proper safety precautions are followed as outlined in the *Magnacide H Herbicide Application and Safety Manual*, for acrolein applicators, potential exposure can be limited.

In order to address risks to occupational handlers/workers, the following mitigation is required:

- Instead of requiring the registrant-provided applicator training a minimum of every three years, the training requirement will be annual.
- Upon request, the registrant must provide State Lead Agencies the names of all applicators who have received registrant-specific training.
- During application, two trained applicators must be on site at all times.
- All applications must be made during daylight hours.

In order to reduce exposures to bystanders, the following mitigation is required:

- Applicators must post "Do Not Enter" "DANGER" "Pesticide Application in Progress" signs at the site of application and around the application equipment.
- Certified applicators may only apply at sites where the irrigation district managers or owners have "No Swimming" signs posted.

Additionally, the following changes to the March 2005 version of the *Magnacide H Herbicide Application and Safety Manual* are required because these statements could be interpreted to mean that acrolein is less toxic than it is:

- On Page 5, paragraph 2 the following language must be **removed** from the Note to Physician: "Because of the extreme lacrymatory effect, the concentration tolerable by man is far below the minimum lethal concentration."
- On Page 24, in Appendix A, **remove** the table indicating "probable human response" to acrolein at various concentrations and times of exposure.
- On Page 24, in Appendix A, the paragraphs describing drinking water studies must be **removed**.
- The following language should be **added** to Appendix A:
    - Adverse health effects have been shown to occur in humans at concentrations as low as 0.09 ppm;
    - serious irreversible health effects may occur at concentrations as low as 0.4 ppm for 10 minutes;
    - OSHA does not allow workers to be exposed to concentrations over 0.3 ppm for longer than 15 minutes;
    - the 8-hour workplace standard is 0.1 ppm; and
    - the IDLH is 2.0 ppm

2. Ecological Risk Management

There were several ecological risks of concern identified for acrolein. Based on the current use pattern, acrolein exposure to wildlife can occur. It is required that application of acrolein directly to water be made only through close adherence to established standard operation procedures (SOPs) provided in the acrolein manual. This will limit the extent to which acrolein can move beyond targeted treated areas. In order to limit non-target effects, rigorous SOPs should be adhered to for the application of acrolein to agricultural fields.

- The registrant is required to include a module on reducing wildlife exposures in the annual training program and in the *Magnacide H Herbicide Application and Safety Manual*. This module should focus on risks to fish and aquatic organisms and should include information on the importance of limiting the contamination of natural fish bearing waters by release of acrolein treated canal water. The current label statement *"Water treated with Magnacide H herbicide must be used for the irrigation of fields, either crop-bearing, fallow or pasture, where the treated water remains on the field OR must be held for 6 days before being released into fish bearing waters or where it will drain into them."* should remain on the label and be included in the training and manual along with instructions and examples of how to contain the irrigation water while the acrolein is degrading.

Additionally, the following application restrictions are required to be added to all acrolein product labels:

- Maximum of eight (8) applications- annually.
- Minimum two (2) week re-treatment interval per application.

V. What Registrants Need to Do

The Agency has determined that products containing acrolein (PC Code: 000701) are eligible for reregistration provided that the risk mitigation measures identified in this document are adopted and label amendments are made to reflect these measures. Additional data are required to fill data gaps identified and to confirm this decision. The Agency intends to issue Data Call-In Notices (DCIs) requiring product-specific data and generic (technical grade) data. Generally, registrants will have 90 days from receipt of a DCI to complete and submit response forms or request time extension and/or waiver requests with a full written justification. For product specific data, the registrant will have 8 months to submit data and amend labels. For generic data, due dates can vary depending on the specific studies being required.

For acrolein technical grade active ingredient products, the registrant needs to submit the following items:

Within 90 days from receipt of the generic data call in (DCI):

1. Completed response forms to the generic DCI (i.e. DCI response form and requirements status and registrant's response form); and

2. Any time extension and/or waiver requests with a full written justification.

Within the time limit specified in the generic DCI:

1. Citations of any existing generic data that address data requirements or submit new generic data responding to the DCI.

Please contact Laura Parsons at (703) 305-5776 with questions regarding generic reregistration.

By U.S. Mail:
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By express or courier service:
Document Processing Desk (DCI/SRRD)
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For end-use products containing the active ingredient acrolein, registrants need to submit the following items for each product.

Within 90 days from receipt of the product-specific data call-in (PDCI):

(1) completed response forms to the generic DCI (i.e. DCI response form and requirements status and registrant's response form); and
(2) any time extension and/or waiver requests with a full written justification.

Within eight months from receipt of the PDCI:

(1) submit two copies of the confidential statement of formula, EPA form 8570-4;
(2) a completed original application for reregistration (EPA form 8570-1). Indicate on the form that it is an "application for reregistration";
(3) five copies of the draft label incorporating all label amendments outlined in Table 7 of this document;
(4) a completed form certifying compliance with data compensation requirements (EPA Form 8570-34);
(5) if applicable, a completed form certifying compliance with cost share offer requirements (EPA Form 8570-32); and
(6) the product-specific data responding to the PDCI.

Within the time limit specified in the PDCI:

(1)Citations of any existing generic data that address data requirements or submit new generic data responding to the DCI.

Please contact Karen Jones at 703-308-8047 with questions regarding product reregistration and/or the PDCI. All materials submitted in response to the PDCI should be addressed:

By U.S. Mail:
Document Processing Desk (DCI/SRRD)
Karen Jones
Office of Pesticide Programs (7508P)
1200 Pennsylvania Ave., NW
Washington, DC 200460

By Express or Courier Service:
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Arlington, VA 22202

A. Manufacturing Use Products

1. Additional Generic Data Requirements

The generic database supporting the reregistration of acrolein has been reviewed. The risk assessments identified the potential need for certain ecological, environmental fate, and residue chemistry data. The studies are as follows:

Ecological and Environmental Fate

- Photodegradation (Water); {GDLN 835.2240)
- Photodegradation (Soil); {GDLN 835.2410}
- Photodegradation (Air); {GDLN 835.2370}
- Aerobic Aquatic Metabolism; {GDLN 835.4300}
- Aerobic Soil Metabolism; {GDLN 835.4100}
- Anaerobic Aquatic Metabolism; {GDLN 835.4400}
- Anaerobic Soil Metabolism; {GDLN 835.4200}
- Leaching (Adsorption/Desorption); {GDLN 835.1240/ 835.1230}
- Seedling Emergence/Vegetative Vigor; {GDLN 850.4100/ 850.4150}

Residue Chemistry

- Registrants need to submit the data required for the acrolein TGAIs/MPs, and must either certify that the suppliers of beginning materials and the manufacturing processes for these TGAIs/MPs have not changed since the last comprehensive product chemistry review or submit complete updated product chemistry data packages.
- An enforcement analytical method must be developed and validated, including validation by an independent laboratory, for the determination of glycidol in fish and shellfish, if the registrant continues to support the SLN use in reservoirs and labels for these uses are not revised to provide effective fishing prohibitions (e.g., posting, restricted entry, etc.).
- If the registrant continues to support the SLN use in reservoirs and labels for these uses are not revised to provide effective fishing prohibitions (e.g., posting, restricted entry, etc.), magnitude of the residue of acrolein and glycidol in fish and shellfish are required. The submission of a protocol is preferable prior to beginning any study.
- A confirmatory nature of the residue study in root and tuber (preferably radish) is required.

2. Labeling for Technical and Manufacturing Use Products

To ensure compliance with FIFRA, technical and manufacturing use product (MP) labeling should be revised to comply with all current EPA regulations, PR Notices and applicable policies. In order to be eligible for reregistration, the technical registrants also must amend all product labels to incorporate the risk mitigation measures outlined in Section IV.

The technical and MP labeling should also bear the labeling statements contained in Table 26, the Label Changes Summary Table.

B. End-Use Products

1. Additional Product-Specific Data Requirements

Section 4(g) (2) (B) of FIFRA calls for the Agency to obtain any needed product-specific data regarding a pesticide after a determination of eligibility has been made. The registrant must review previous data submissions to ensure they meet current EPA acceptance criteria and if not, commit to conduct new studies. If a registrant believes that previously submitted data meet current testing standards, then the study MRID numbers should be cited according to the instructions in the Requirement Status and Registrations Response Form provided for each product.

2. Labeling for End-Use Products

Labeling changes are necessary to implement measures outlined in Section IV above. Specific language to incorporate these changes is specified in Table 25, the Label Changes Summary Table.

C. Labeling Changes Summary Table

In order to be eligible for reregistration, amend all product labels to incorporate the risk mitigation measures outlined in Section IV. The following table describes how language on the labels should be amended.

Table 25: Summary of Labeling Changes for Acrolein

Description	Amended Labeling Language	Placement on Label
End Use Products Intended for Occupational Use		
Restricted Use Requirement	"Restricted Use Pesticide due to a high acute toxicity. For retail sale to and use by certified applicators and only for those uses covered by the certified applicator's certification."	Top of the front panel
Manual	"THIS PRODUCT MUST BE ACCOMPANIED BY AN EPA-APPROVED PRODUCT LABEL AND THE EPA-APPROVED '*Magnacide H Herbicide Application and Safety Manual*.' THE *Magnacide H Herbicide Application and Safety Manual* IS LABELING. READ AND UNDERSTAND THE ENTIRE LABELING AND MANUAL PRIOR TO USE. ALL PARTS OF THE LABELING AND MANUAL ARE EQUALLY IMPORTANT FOR SAFE AND EFFECTIVE USE OF THIS PRODUCT."	Immediately below the RUP statement on the label and on the cover page of the Acrolein Manual.
PPE Requirements Established by the RED	"All certified applicators participating in the application during the setting up and breaking down of application equipment and during visual inspection must wear: • Long-sleeved shirt and long pants, • Shoes and socks, • Chemical-resistant gloves made of butyl rubber, and • a NIOSH-approved full-face respirator with either o organic-vapor-removing cartridges with a prefilter approved for pesticides (MSHA/NIOSH approval number prefix TC-23C), or o a canister approved for pesticides (MSHA/NIOSH approval number prefix TC-14G)."	Immediately following/below Precautionary Statements: Hazards to Humans and Domestic Animals

Table 25: Summary of Labeling Changes for Acrolein		
Description	**Amended Labeling Language**	**Placement on Label**
PPE Requirements Established by the RED For all Formulations	Respirator fit testing, medical qualification, and training: Employers must ensure that all acrolein handlers are: • Fit-tested and fit-checked using a program that conforms to OSHA's requirements (see 29CFR Part 1910.134) • Trained using a program that confirms to OSHA's requirements (see 29CFR Part 1910.134) Examined by a qualified medical practitioner to ensure physical ability to safely wear the style of respirator to be worn. A qualified medical practitioner is a physician or other licensed health care professional who will evaluate the ability of a worker to wear a respirator. The initial evaluation consists of a questionnaire that asks about medical conditions (such as a heart condition) that would be problematic for respirator use. If concerns are identified, then additional evaluations, such as a physical exam, might be necessary. The initial evaluation must be done before respirator use begins. Handlers must be reexamined by a qualified medical practitioner if their health status or respirator style or use-conditions change.	PPE Requirements Established by the RED For all Formulations
User Safety Recommendations	"User Safety Recommendations Users should wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet. Users should remove PPE immediately after handling this product. As soon as possible, wash thoroughly and change into clean clothing."	Precautionary Statements under: Hazards to Humans and Domestic Animals immediately following Engineering Controls
User Safety Requirements	"User Safety Requirements If acrolein is spilled or leaked on clothing, gloves, or shoes, immediately remove them and wash thoroughly with soap and water. Follow manufacturer's instructions for cleaning/maintaining PPE. If no such	Precautionary Statements: Hazards to Humans and Domestic Animals immediately following the PPE requirements

Table 25: Summary of Labeling Changes for Acrolein		
Description	**Amended Labeling Language**	**Placement on Label**
	instructions for washables exist, use detergent and hot water. Keep and wash PPE separately from other laundry." "Discard clothing, gloves, shoes, and other absorbent materials that have come into contact with acrolein. Do not reuse them."	
Engineering Controls	"Engineering Controls "Handlers must use a closed system that is designed by the manufacturer to prevent dermal and inhalation exposures by removing the product from the container and applying the product below the water's surface. At any disconnect point, the system must be equipped with a dry disconnect or dry couple shut-off device that will limit drippage to no more than 2 ml per disconnect. The closed system must function properly and be used and maintained in accordance with the manufacturer's written operating instructions. Handlers must wear the personal protective equipment required on this labeling."	Precautionary Statements: Hazards to Humans and Domestic Animals (Immediately following PPE and User Safety Requirements.)
Environmental Hazards	"The pesticide is extremely toxic to fish and wildlife.	Precautionary Statements immediately following the User Safety Recommendations
Application Restrictions: Certified Applicator Requirements	"At least two certified applicators must be at the application site and able to maintain visual contact with all certified applicators participating in the application." "No handlers are allowed to participate in the application unless they are state certified applicators and have completed the registrant's training program with in the last 12 months."	Directions for Use
Application Restrictions	Maximum number of applications: 8 application per year	Directions for Use

Table 25: Summary of Labeling Changes for Acrolein		
Description	**Amended Labeling Language**	**Placement on Label**
	Minimum retreatment interval: 2 weeks	
Application Restrictions: Posting of Application Equipment Area	"Posting of Application Equipment Area" "The Certified Applicator in charge of the application must post signs around the perimeter of the application equipment area (truck, hoses, and skids). Signs must be no more than 15 feet apart and contain the following information:" * Skull and crossbones symbol * "DANGER/PELIGRO" * "DO NOT ENTER/NO ENTRE: Pesticide Application/Aplicacíon de Pesticidas" * The name of the product applied * The start date and time of application * The end date and time of application. * The name, address, and telephone number of the Certified Applicator in charge of the application "Signs must remain legible during the entire posting period and must be removed once the application is completed and no later than 3 days after treatment."	Directions for Use under the heading "Posting of Application Equipment Area"
Other Application Restrictions	"Applications with [Magnacide H] may only be made in canals with posted no swimming signs. Contact the local irrigation district if the signs are not posted."	Directions for Use

ACROLEIN APPENDICES

Appendix A. Non-Food and Non-Feed Use Patterns Subject to the Reregistration of Acrolein

Product Type	Product Use Site	Max % A.I.	Max AR
Occupational Uses			
PRL	Non-food Crops-Irrigation Canals	95	15 ppm (15mg/L)
PRL	Non-food Crops-Deep Well Injection	95	0.25 lb a.i./1000 sq. ft

FORMULATION CODES
PRL: Pressurized Liquid

Appendix B. Data Supporting Guideline Requirements for Acrolein

Data Supporting Guideline Requirements for the Reregistration of Acrolein		
Guideline Number	**Study Description**	**Citation(s)**
PRODUCT CHEMISTRY		
830.1550	Product Identity and Composition	CSF (1-23-04)
830.1600	Description of Materials Used	CSF (1-23-04)
830.1700	Preliminary Analysis	41896901, 46181201
830.1750	Certified Limits	41896901, CSF (1-23-04)
830.1800	Enforcement Analytical Method	41896901, 46181201
830.6302	Color	40840601
830.6303	Physical State	40840601
830.6304	Odor	40840601
830.6313	Stability	40840601
830.7000	pH	40840601
830.7200	Melting Point	N/A
830.7220	Boiling Point	40840601
830.7300	Density	40840601
830.7370	Dissociation Constant	N/A
830.7550 830.7570	Octanol / Water Partition Coefficient	40840604
830.7840 830.7860	Solubility	40840601
830.7950	Vapor Pressure	40840603
ECOLOGICAL EFFECTS		
850.1010	Aquatic Invertebrate Acute	40228401, 41513202, 05008271
850.1025	Oyster Acute Toxicity Test	40228401
850.1035	Mysid Acute Toxicity Test	43164301
850.1045	Penaeid Acute Toxicity Test	40228401
850.1075	Fish Acute Toxicity – freshwater	41513201, 41513203, 45205107
	Fish Acute tox estuarine/marine	40228401, 43225202
850.2100	Avian Acute Oral Toxicity (Duck)	42183301,
850.2400	Mammal Toxicity (Rat)	41257001, 41869101
850.4100	Seedling Emergence and Growth	Data gap
850.4150	Vegetative Vigor	Data gap
850.4400	Aquatic Plant Growth	42620904
850.4500	Algal Plant Toxicity	42620901, 42620905, 4260902
TOXICOLOGY		
870.1100	Acute Oral Toxicity	41257001
870.1200	Acute Dermal Toxicity	00141028
870.1300	Acute Inhalation Toxicity	40945404
870.2400	Acute Eye Irritation	00141025
870.2500	Acute Dermal Irritation	00141026
870.3200	21/28 -Day Dermal Toxicity-Rabbit	00141030
870.3700	Prenatal Developmental Toxicity—Rabbit	40392401

	Prenatal Developmental Toxicity--Rat	00156438
870.3800	Reproduction and Fertility Effects, 2-Generation Reproduction	41869101
870.4100	Chronic Oral Toxicity--Dogs	41071701
	Chronic/Carcinogenicity Feeding--Rats	41306401, 46568001, 46568002
870.4200	Carcinogenicity	41334901
870.5300	In vitro Mammalian Cell Gene Mutation Assay	41579501
870.5375	In vitro mammalian chromosomal aberration assay- CHO	00141033
870.5900	In vitro sister chromatid exchange	00141032
870.7485	Metabolism and Pharmacokinetics	42031001, 43177101, 43275901
RESIDUE CHEMISTRY		
860.1300	Nature of residue in plants (lettuce)	43607101, 42295101
860.1300	Nature of residue in plants (root	Data gap
860.1300	Nature of residue in animals	43942101, 43938701
860.1300	Nature of residue in fish	43225201
860.1400	Potable water monitoring	41855401
ENVIRONMENTAL FATE		
835.1230	Leaching and Adsorption / Desorption	Data gap
835.2120	Hydrolysis	40945401
835.2240	Photodegradation in Water	Data gap
835.2410	Photodegradation in Soil	Data gap
835.2370	Photodegradation in Air	Data gap
835.4100	Aerobic Soil Metabolism	Data gap
835.4200	Anaerobic Soil Metabolism	Data gap
835.4300	Aerobic Aquatic Metabolism	Data gap
835.4400	Anaerobic Aquatic Metabolism	Data gap

Appendix C. Technical Support Documents

Additional documentation in support of the acrolein RED is maintained in the OPP Regulatory Public Docket, located in Room S-4400 One Potomac Yard (South Building), 2777 S. Crystal Drive, Arlington, VA. It is open Monday through Friday, excluding legal holidays, from 8:30 a.m. to 4:00 p.m. All documents may be viewed in the OPP Docket room or viewed and/or downloaded via the Internet at http://www.regulations.gov. The Agency's documents in support of this RED include the following:

1.) Daiss, B. Acrolein HED Risk Assessment for Reregistration Eligibility Decision (RED) Document. March 25, 2008.

2.) Garber, K., Jones, R.D., and Steeger, T. Environmental Fate and Ecological Risk Assessment for the Reregistration of Acrolein, 2nd Revision. July 18, 2008

3) Morton, T., Revised Dietary Risk and Exposure Estimate For Acrolein Through Subsistence Diets for Indigenous People of United States. March 25, 2008

4) Phillips, W., Berwald, D., Acrolein Alternatives Assessment Summary and Uncertainties. March 26, 3008

5) Daiss, B., Acrolein: Occupational and Residential Exposure Assessment and Recommendations for the Reregistration Eligibility Decision. March 25, 2008

6) Morton, T., Acrolein: Revised Product and Residue Chemistry Considerations. September 27, 2007

7) Jones., R.D., Assessment of Drinking Water Exposure and Acrolein Concentrations to which Fish May be Exposed, 2nd Revision. May 23, 2007

Appendix D. Bibliography

In addition to the studies listed in Appendix B, this bibliography contains additional citations considered to be part of the database supporting the reregistration decision for acrolein.

In addition to the MRID study references listed in Appendix B, this bibliography contains the expanded study citations as well as additional literature considered to be part of the database supporting the reregistration decision for acrolein.

MRID	Citation
Human Health References	
00141025	Dunn, G.R. and J. Goodband (1981) Summary report: Primary eye irritation study for Acrolein in rabbits. Bioassay Systems Corporation, Woburn, MA. BSC Project No.: 10258, July 20, 1981. . Unpublished. 7 pages
00141026	Dunn, G.R. and J. Goodband (1981) Summary report: Primary dermal irritation study for Acrolein in rabbits. Bioassay Systems Corporation, Woburn, MA. BSC Project No.: 10258, March 21, 1981. Unpublished. 5 pages.
00141028	Muni, I.A.(1981) Acute dermal toxicity (LD_{50}) of Acrolein (Lot No. SFSL-5993) in rabbits. Bioassay Systems Corporation, Woburn, MA. BSC Project No.: 10258, September 17, 1981. Unpublished.
00141030	Muni, I.A. (1982) 21-Day dermal test of Acrolein in rabbits. Bioassay Systems Corporation, Woburn, MA. Project No.: 10258, July 28, 1982. Unpublished. 109 pages.
00141032	Loveday, K.S. (1982) Effects of Acrolein on the *in vitro* induction of sister chromatid exchanges in Chinese hamster ovary cells. Bioassay Systems Corporation, Woburn, MA. BSC Project No.: 10258, May 11, 1982. Unpublished
00141033	Gorodecki, J. and G.M. Seixas (1982) Effects of Acrolein on the *in vitro* induction of chromosomal aberrations in Chinese hamster ovary cells. Bioassay Systems Corporation, Woburn, MA. BSC Project No.: 10258, July 23, 1982. Unpublished.
00156438	King, M. (1982) Teratology study of acrolein in rats. Bioassay Systems Corporation, Woburn, MA. Laboratory Project No.: 10258, November 12, 1982. Unpublished. 82 pages.
40392401 .	Hoberman, A.M. (1987) Developmental toxicity (embryo/fetal toxicity and teratogenic potential) study of acrolein administered orally (stomach tube) to New Zealand White rabbits. Argus Research Laboratories, Inc., Horsham, PA. Laboratory Project Id.: 603-001, May 20, 1987. Unpublished
40840601	Caravello, H. (1988) Physical Properties of Acrolein: A Summary: Study No. RD 0070.188. Unpublished study prepared by Baker Performance Chemicals, Inc. 41 p.
40840603	Robillard, K. (1988) Vapor Pressure of Acrolein: Laboratory Project ID: HAEL No.: 88-0300: Study No. EN-030-UKA001-1. Unpublished compilation prepared by Health and Environment Laboratories. 31 p.
40840604	Matherly, R.; Hackerott, J.; Nguyen, N. (1987) Octanol/Water Parti- tion Coefficient of Acrolein: Study No. RD0008.287. Unpublished study prepared by Baker

	Performance Chemicals. 24 p.
40945404	Nachreiner, D.J. and D.E. Dodd. (1987) Acute inhalation toxicity of Acrolein vapor by one and four hour exposures. Union Carbide Corp., Bushy Run Research Center, Export, PA. Laboratory Report No.: 49-170, February 2, 1987. MRID 40945404. Unpublished. 45 pages.
41071701	Long, J.E. (1987) Acrolein - chronic (12 month) oral toxicity study in the dog. Tegeris Laboratories, Inc., Laurel, MD. Laboratory Project ID.: TL 85016, October 23, 1987. Unpublished. 1047 pages.
41068801	Long, J.E. (1986) Acrolein - range finding oral toxicity study in the dog. Tegeris Laboratories, Inc., Laurel, MD. Laboratory Project ID.: TL 86003, August 7, 1986. Unpublished. 54 pages.
41257001	David, R.M. (1989) Acute oral toxicity study of Acrolein, inhibited in rats. Microbiological Associates Inc., Bethesda, MD. Laboratory Study No.: G-7230.220, September 19, 1989. Unpublished. 87 pages.
41306401	Long, J.E. and J.A. Johnson (1989) 24-month chronic toxicity and oncogenicity study in the rat with acrolein. Tegeris Laboratories, Inc., Temple Hills, MD. Laboratory Project Id.: TL 85047, September 6, 1989. Unpublished. 3701 pages.
41334901	Long, J.; Johnson, J. (1989) 18-Month Oncogenicity Study in the Mouse with Acrolein: Lab Project Number: TL/86057. Unpublished study prepared by Tegeris Laboratories, Inc. 2267 p.
41579501	Harbell, J. (1989) CHO/HGPRT Mutation Assay with Confirmation: Acrolein: Lab Project Number: T8403.332001. Unpublished study prepared by Microbiological Associates, Inc. 36 p.
41855401	Jacobson, B.; Gresham, M. (1991) Magnitude of the Residue for Acrolein in Potable Water--Arizona Site: Lab Project Number: 38983. Unpublished study prepared by ABC Laboratories, Inc. 178 p.
41869101	Hoberman, A. (1991) Reproductive Effects of Acrolein Administered Orally via Gavage to Crl:CD (SD)BR Rats for Two Generations, with one Litter per Generation: Lab Project Number: 603/003: RD/ 0155/191. Unpublished study prepared by Argus Research Laboratories, Inc. 1298 p.

41896901 Matherly, R.; Doane, B.; Caravello, H. (1991) Acrolein: Analysis and Certification of Product Ingredients: Lab Project Number: RD 0139.190. Unpublished study prepared by Baker Performance Chemicals, Inc. 110 p.

42031001 Sharp, D. (1991) Metabolism of Acrolein in Rats: Lab Project Number: HLA 6318-101. Unpublished study prepared by Hazleton Labs America, Inc. 363 p.

42295101 Ewing, A.; Kimmel, E.; Ruzo, L. (1992) Interim Report ?carbon 41| Labeled Acrolein Accumulation and Metabolism in Leaf Lettuce: Lab Project Number: 165W-1: 165W. Unpublished study prepared by PTRL West, Inc. 122 p.

43177101 Sharp, D. (1994) Supplement No. 1 to Metabolism of Acrolein in Rats: (Preliminary and Definitive Phases): Final Report: Lab Project Number: HWI/6318/101. Unpublished study prepared by Hazleton Wisconsin, Inc. 247 p.

43225201 Biever, R. (1994) (Carbon 14)-Acrolein (Magnacide H): Nature and Magnitude of Residues Study Using Freshwater Fish and Shellfish: Final Report: Lab Project Number:

93-3-4701: 12167-0691-6102-145. Unpublished study prepared by Springborn Labs., Inc. 389 p.

43275901 Sharp, D. (1994) Metabolism of Acrolein in Rats (Preliminary and Definitive Phases): Supplement No. 2: Final Report: Lab Project Number: HWI 6318-101. Unpublished study prepared by Hazleton Wisconsin, Inc. 29 p.

43607101	Mao, J. (1994) (Carbon 14)Acrolein Accumulation and Metabolism in Leaf Lettuce: Lab Project Numbers: 94-6-5322: 12167-0693-6108-791. Unpublished study prepared by Springborn Labs, Inc. 304 p.
43938701	Berge, M.; Paust, D. (1996) Metabolic Fate of (carbon 14)-Acrolein Orally Administered to Lactating Goats: Final Report: Lab Project Number: HWI 6318-104: 1167: 1167-3. Unpublished study prepared by Hazleton Wisconsin, Inc. 512 p.
43942101	Berge, M.; Hennes, M. (1996) Metabolic Fate of (carbon 14)-Acrolein Orally Administered to Laying Hens: Final Report: Lab Project Number: 6318-103: 1168: 1168-3. Unpublished study prepared by Hazleton Wisconsin, Inc. 489 p.
46181201	Matlach, W.; Baham, G. (2003) Determination of Acrolein and Its Impurities in Five Samples of Acrolein from Baker Petrolite's Acrolein Plant. Project Number: GLP/0074. Unpublished study prepared by Baker Petrolite Corporation. 137 p.
46568001	Gardner, F.M. and G.W. Gorder (2002) 24-month chronic toxicity and oncogenicity study in the rat with acrolein: supplement to MRID 41306401. Tegeris Laboratories, Inc., Temple Hills, MD. Laboratory Project Id.: TL 85047, September 6, 1989; Supplement June 20, 2002. Unpublished. 113 pages.
46568002	Gorder, G.W. (2005) 24-month chronic toxicity and oncogenicity study in the rat with acrolein: supplement # 2 to MRID 41306401. Tegeris Laboratories, Inc., Temple Hills, MD. Laboratory Project Id.: TL 85047, September 6, 1989; Supplement April 28, 2005. Unpublished. 50 pages.
41334901	Long, J.E. and J.A. Johnson (1989) 18-month oncogenicity study in the mouse with acrolein. Tegeris Laboratories, Inc., Temple Hills, MD. Laboratory Project Id.: TL 86057, October 16, 1989. Unpublished. 2267 pages.
41579501	Harbell, J.W. (1989) CHO/HGPRT mutation assay with confirmation. Microbiological Associates Inc., Rockville, MD. Laboratory Study No.: T8403.332001, May 25, 1989. Unpublished. 36 pages.
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43275901	Sharp, D. (1994) Metabolism of Acrolein in Rats (Preliminary and Definitive Phases): Supplement No. 2: Final Report: Lab Project Number: HWI 6318-101. Unpublished study prepared by Hazleton Wisconsin, Inc. 29 p.

Ecological and Environmental Fate References	
00117668	Tucker, R.; Hudson, R. 1970. *Acrolein: Acute Oral LD50 for Mallard Drakes*. (Unpublished study received Mar 20, 1975 under 10707-9; prepared by U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Unit of Physiological and Pharmacological Studies, submitted by Magna Corp., Santa Fe Springs, CA; CDL:165015-A
05008271	Macek, K. J., M. A. Lindberg, S. Sauter, K. S. Buxton, and P. A. Costa. 1976. *Toxicity of Four Pesticides to Water Fleas and Fathead Minnows.* Duluth, Minn.: U.S. Environmental Protection Agency, Environmental Research Laboratory. (EPA report no. EPA-600/3-76-099; available from: NTIS, Springfield, VA; PB-262 912).
40228401	Mayer, F. 1986. *Acute toxicity handbook of chemicals to estuarine organisms: EPA/600/X-86/231*. Prepared by USEPA Environmental Research Laboratory, Gulf Breeze, FL. 275 p.
40840602	Robillard, Kenneth A. *Water Solubility of Acrolein.* Performed by Health and Environmental Laboratories, Eastman Kodak Co. Rochester, NY. Study No. EN 040-UKA001-1. Submitted by Baker Performance Chemicals.. Houston, TX.
40945401	Haag, Werner. R., David Yao, Thomas Pettit, and Theodore Mill. 1988. *Estimation of Hydrolysis Rate Constants for Acrolein (Magnacide H Herbicide and Magnacide B Microbiocide) in the Environment.* Unpublished study performed by SRI, International, Menlo Park, CA. SRI Project 3562-3. Study submitted by Baker Performance Chemicals, Houston TX.
40945404	Nachreiner, D.; Dodd, D. (1987) Acute Inhalation Toxicity of Acrolein Vapor by One and Four Hour Exposures: Project Report 49-170. Unpublished study prepared by Union Carbide Bushy Run Research Center. 43 p.
41257001	David, R. (1989) *Acute Oral Toxicity Study of Acrolein, Inhibited in Rats: Final Report: Study G-7230.220.* Unpublished study pre- pared by Microbiological Associates, Inc. 87 p.
41513201	Bowman, J. 1990. *Acute Flow-through Toxicity of Acrolein to Bluegill (Lepomis macrochirus): Lab Project Number: 37669.* Unpublished study prepared by Analytical Bio-chemistry Laboratories, Inc. 35 p.
41513202	Blakemore, G. 1990. *Acute Flow-through Toxicity of Acrolein to Daphnia magna: Lab Project Number: 37671.* Unpublished study prepared by Analytical Bio-chemistry Laboratories, Inc. 269 p.
41513203	Bowman, J. 1990. *Acute Flow-through Toxicity of Acrolein to Rainbow Trout (Oncorhynchus mykiss): Lab Project Number: 37670.* Un- published study prepared by Analytical Bio-chemistry Laboratories, Inc. 285 p.
42183301	Pedersen, C., and B. Helsten. 1991. *Acrolein: 21-Day Acute Oral LD50 Study in Mallard Ducks: Lab Project Number: 118-001-04.* Unpublished study prepared by Bio-Life Associates, Ltd. 54 p.
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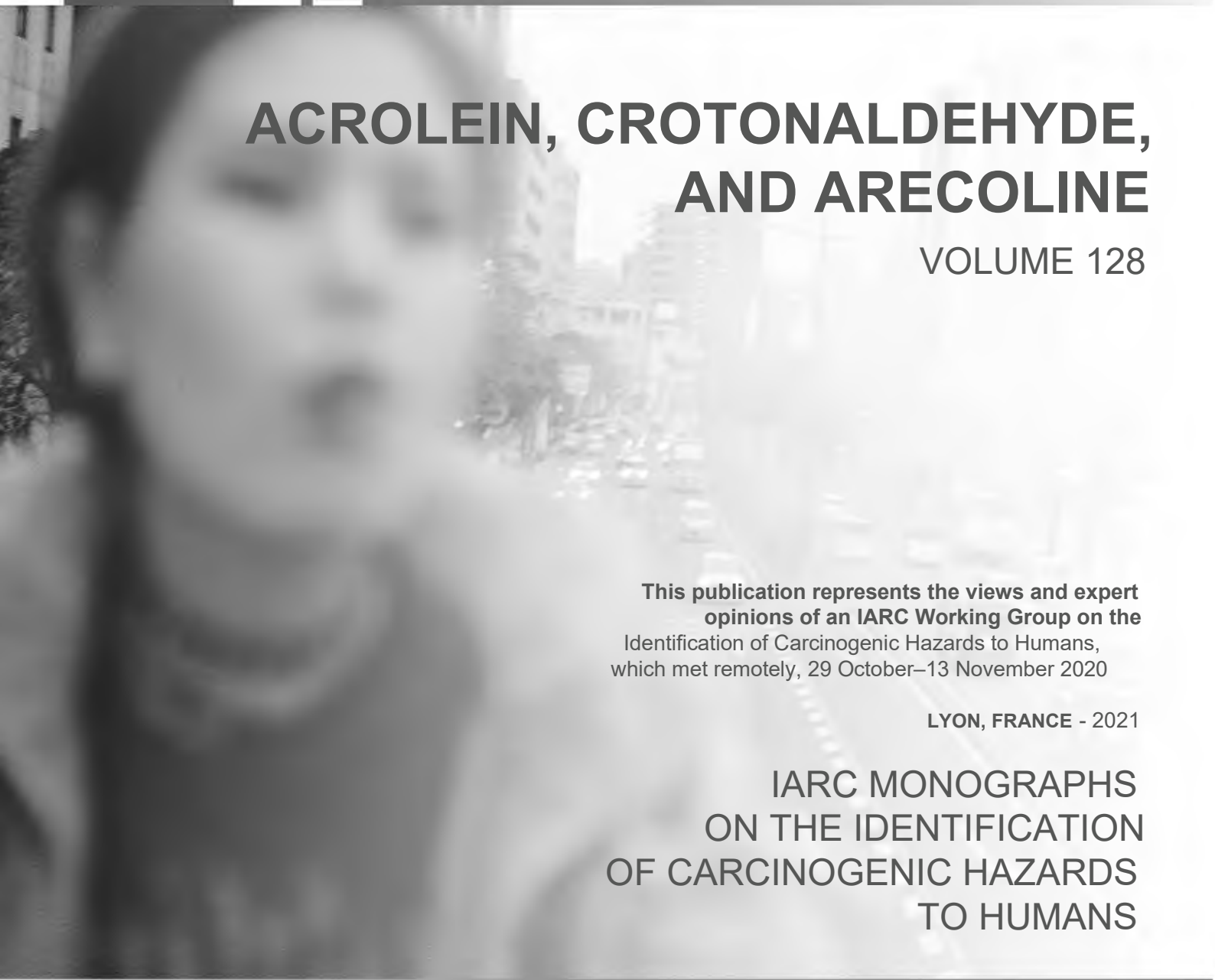


ACROLEIN, CROTONALDEHYDE, AND ARECOLINE

VOLUME 128

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 29 October–13 November 2020

LYON, FRANCE - 2021

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 107-02-8

Deleted Chem. Abstr. Serv. Reg. No.: 25314-61-8

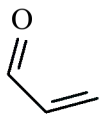
EC/List No.: 203-453-4

Chem. Abstr. Serv. name: 2-propenal

IUPAC systematic name: prop-2-enal

Synonyms: acraldehyde; acrylaldehyde; acryl - ic aldehyde; allyl aldehyde; ethylene aldehyde; propenal; 2-propenal; prop-2-en-l-al (IARC, 1995; O'Neil, 2013; ECHA, 2020).

1.1.2 Structural and molecular formulae, and relative molecular mass *Structural formula:*



Molecular formula: C_3H_4O

Relative molecular mass: 56.06 (O'Neil, 2013).

1.1.3 Chemical and physical properties

Description: colourless to yellowish liquid with extremely acrid, pungent, and irritating odour, causing lachrymation (Verschueren, 1983; IARC, 1985; O'Neil, 2013)

Boiling point: 52.5–53.5 °C (Lide, 1993)

Melting-point: –86.9 °C (Lide, 1993)

Relative density: 0.8410 at 20 °C/4 °C (Lide, 1993)

Solubility: soluble in water (206 g/L at 20 °C), ethanol, diethyl ether, and acetone (IPCS, 1992; Lide, 1993)

Volatility: vapour pressure 29.3 kPa (220 mm Hg) at 20 °C (IPCS, 1992)

Flash-point: −26 °C (IPCS, 1992)

Stability: unstable in the absence of an inhibitor (IPCS, 1992); polymerizes, especially under light or in the presence of alkali or strong acid, to form disacryl, a plastic solid (O'Neil, 2013). Inhibited acrolein undergoes dimerization above 150 °C and highly exothermic polymerization also occurs in the presence of traces of acids or strong bases even when an inhibitor is present (IPCS, 1992) *Reactivity*: reactions shown by acrolein include Diels–Alder condensation, dimerization and polymerization, additions to the carbon–carbon double bond, carbonyl additions, oxidation, and reduction (IPCS, 1992; see also Section 4.2.1)

*Octanol/water partition coefficient (*P*)*: log K_{ow}, –0.01 (O'Neil, 2013)

Odour perception threshold: 0.07 mg/m^3 (IPCS, 1992)

Conversion factor: 1 ppm = 2.29 mg/m^3 (IARC, 1995).

1.1.4 Technical products and impurities

Hydroquinone (IARC Group 3; IARC, 1999) at a concentration of 0.1–0.25% is typically used to stabilize commercially available preparations of acrolein (Etzkorn, 2009). Hydroquinone protects acrolein from polymerization, and also from hydrolysis in aqueous solutions (Kächele et al., 2014). Acrolein is available commercially with purities in the range of 90–98% and as solutions to be used as reference materials in water, methanol, and acetone (Chemical Abstracts Service, 2020). Impurities include water (up to 3.0% by weight; IPCS, 1992), acetaldehyde, and, depending on the production process, small amounts of propionaldehyde, acetone, propene oxide, and methanol, and traces of allyl alcohol and ethanol (Arntz et al., 2007).

1.2 Production and use

1.2.1 Production process

Acrolein was first prepared in 1843 by the dry distillation of fat (Redtenbacher, 1843). Commercial production of acrolein began in Germany in 1942, by a process based on the vapour-phase condensation of acetaldehyde and formaldehyde. This method was used until 1959, when a process was introduced for producing acrolein by vapour-phase oxidation of propene (Arntz et al., 2007). Several catalysts have been used in this process, including bismuth molybdate (Etzkorn, 2009). Propene oxidation is still the commercially dominant production process (Etzkorn, 2009), while research on more environmentally friendly methods of acrolein production from renewable feedstock such as glycerol, methanol, or ethanol is ongoing (Arntz et al., 2007; Etzkorn, 2009; Lilić et al., 2017). The oxidation of propene produces acrolein, acrylic acid, acetaldehyde, and carbon oxides.

1.2.2 Production volume

In 1975, global production of acrolein was approximately 59 000 tonnes (Hess et al., 1978). Worldwide production of acrolein in 1977 was estimated to have been 100–120 000 tonnes (IARC, 1979). The worldwide capacity for production of refined acrolein was estimated in the 1990s to be about 113 000 tonnes per year (Etzkorn et al., 1991). In 2007, the production capacity for acrolein in western Europe, USA, and Japan was estimated to total 425 000 tonnes per year (Arntz et al., 2007). In 2009, worldwide estimated acrolein production capacity was about 350 000 tonnes per year, which included acrolein made for captive use in methionine production (Etzkorn, 2009). Estimated global demand in 2018 was 620 000 tonnes (Zion Market Research, 2019).

Acrolein was listed by the Organisation for Economic Co-operation and Development (OECD) and the United States Environmental Protection Agency (US EPA) as a High Production Volume chemical for 2007 (IARC, 2019). About 100–1000 tonnes per year are manufactured and/or imported in the European Economic Area (ECHA, 2020).

The Chem Sources database lists 27 manufacturing companies worldwide, of which 12 are located in the USA and 5 in China (including Hong Kong Special Administrative Region) (Chem Sources, 2020).

1.2.3 Uses

Acrolein is an α,β-unsaturated aldehyde and a highly reactive, volatile organic chemical (see also Section 4.2.1). These properties contribute to the many reactions of acrolein and its commercial usefulness, either directly or (for the most part) as a chemical intermediate for the production of numerous chemical products. These include acrylic acid, which is used to make acrylates, and DL-methionine, an essential amino acid used as a feed supplement for livestock (Arntz et al., 2007; Faroon et al., 2008). Other important derivatives of acrolein are glutaraldehyde, pyridines, tetrahydrobenzaldehyde, allyl alcohol and glycerol, 1,4-butanedial and 1,4-butenediol, 1,3-propanediol, DL-glyceraldehyde, flavours and fragrances, and polyurethane and polyester resins (Sax & Lewis, 1987; Arntz et al., 2007).

The most important direct use of acrolein is as a biocide. It is used as an herbicide and to control algae, aquatic weeds, and molluscs in recirculating process water systems (at a concentration of 6–10 mg/L). It is also used to control the growth of microorganisms in liquid fuel, the growth of algae in oil fields, and the formation of slime in paper manufacture. Acrolein has been used in leather tanning and as a tissue fixative in histology (IPCS, 1992; IARC, 1995; Arntz et al., 2007; Etzkorn, 2009). Acrolein has also been used as a warning agent in methyl chloride refrigerants and other gases, in poison gas mixtures for military use, in the manufacture of colloidal forms of metals, and as a test gas for gas masks (IARC, 1979; Neumüller, 1979; O'Neil, 2013).

The market share for global acrolein production in 2017 was methionine use (61.2%), pesticide use (17.4%), glutaraldehyde use (7.3%), water treatment use (9.0%), and other applications (5.1%), with this distribution being stable (within 1%) for several consecutive years (Regal Intelligence, 2020).

1.3 Methods of measurement and analysis

Methods for the analysis of acrolein in air, water, biological media including tissue, and food have been reviewed (IPCS, 1992; IARC, 1995; Shibamoto, 2008). Representative analytical methods for a variety of sampling matrices (air, water, cigarettes, foods and beverages, and biological specimens) are presented in Table S1.1 (Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602).

1.3.1 Air

Several reference procedures are available for the analysis of acrolein in air or gaseous emissions. These include ISO 19 701 (ISO, 2013) and ISO 19 702 (ISO, 2015) for the analysis of fire effluents, JIS K0089 (JIS, 1998) and VDI 3862 Part 5 (VDI, 2008) for the analysis of gaseous emissions, and MAK Air Monitoring Methods (Hahn, 1993). Official analytical methods for air analysis by the United States (US) National Institute for Occupational Safety and Health (NIOSH) (NIOSH 2501, NIOSH 2539) and Occupational Safety and Health Administration (OSHA) (OSHA 52) are available (NCBI, 2020). Methods for the analysis of mainstream cigarette smoke (see Section 1.4.2(b)), ISO 21 160 (ISO, 2018) and Health Canada Official machine smoking regime methods are also available. Protocols are required to standardize measurements of the emissions of toxic chemicals in mainstream cigarette smoke for regulatory purposes. Although ISO methods (from the International Organization for Standardization) have been widely used for decades, Health Canada and WHO have developed more intensive smoking conditions.

The key differences between these protocols are that the ISO regime sets the machine to take 35 mL puffs every 60 seconds with ventilation holes left open, whereas the intensive regimes prescribe 50 mL puffs every 30 seconds, and, importantly, all filter ventilation holes are blocked (WHO, 2012).

[The Working Group noted that the higher values provided by the Health Canada Official method correspond better to human exposure during smoking.]

High-performance liquid chromatography (HPLC) is the routine method to quantify acrolein derivatives obtained from sorbent matrix samplers, which may be used in conjunction with ultraviolet (UV), ion trap mass spectrometry (MS), and fluorescence detectors (Alberta Environment, 2011). Gas chromatography (GC) is the routine method to quantify acrolein pre-concentrated in pressurized sampling canisters and can be used with MS (GC-MS), flame ionization, and electron capture detectors (Alberta Environment, 2011).

1.3.2 Water

Similar chromatographic methods to those used for air analysis are applied to water. Several official analytical methods for water analysis are available from the US EPA (EPA-EAD 603: US EPA, 1984a; EPA-EAD 624: US EPA, 1984b; EPA-EAD 1624: US EPA, 1984c; EPA-RCA 5030C: US EPA, 2003; EPA-RCA 8015C: US EPA, 2007; EPA-RCA 8316: US EPA, 1994) and the United States Geological Survey (USGS) National Water Quality Laboratory (USGS-NWQL O-4127-96, Connor et al., 1996) (NCBI, 2020).

1.3.3 Soil

Standardized methods for analysing acrolein in soil were not identified. However, given the extent to which acrolein is expected to volatilize from soil based on its high vapour pressure and the irreversible binding of acrolein in soil, the lifetime of acrolein in soil may be too short for concern in the context of human exposure (ATSDR, 2007).

1.3.4 Food, beverages, and consumer products

Due to its high reactivity, direct analytical determination of acrolein is difficult, specifically in complex matrices such as foods and beverages (Kächele et al., 2014). Standardized methods for analysing acrolein in foods and beverages were not identified, but several methods with a focus on analysing alcoholic beverages and fat-based products are available (Table S1.1, Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc. fr/602). Several different analytical approaches that mostly include derivatization have been suggested, typically based on HPLC or GC with various detectors including MS (Shibamoto, 2008). Several methods for acrolein analysis have applied solid-phase microextraction (SPME) for sample extraction and enrichment (Wardencki et al., 2003; Curylo & Wardencki, 2005; Saison et al., 2009; Osório & de Lourdes Cardeal, 2011; Lim & Shin, 2012; Kächele et al., 2014).

According to Kächele et al. (2014), acrolein standard solutions for calibrations should be stabilized by a suitable agent such as hydroquinone. The original hydroquinone content found in some commercial acrolein preparations as a stabilizer is not sufficient to prevent degradation if aqueous dilutions for trace analysis are prepared (Kächele et al., 2014).

1.3.5 Biological specimens

Several methods are available for the direct analysis of acrolein in saliva, urine and serum (Table S1.1, Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602) as well as the analysis of its metabolites or DNA and protein adducts (Table S1.2, Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602). Of these, the urinary biomarkers *N*-acetyl-*S*-(3hydroxypropyl)-L-cysteine (3-hydroxypropyl- mercapturic acid, HPMA) and *N*-acetyl-*S*(carboxyethyl)-L-cysteine (2-carboxyethylmer- capturic acid, CEMA) appear to be most commonly determined, and can be detected using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods (see Table S1.2, Annex 1, Supplementary material for Section 1, web only; available from: https://www. publications.iarc.fr/602).

Information regarding an internationally accepted validated biomarker for acrolein exposure was not available to the Working Group.

1.4 Occurrence of and exposure to acrolein

1.4.1 Environmental and natural occurrence

The incomplete combustion and heating of cooking oils produce acrolein, as does the photochemical degradation of 1,3-butadiene in the environment. Acrolein may also be formed endogenously (Faroon et al., 2008; see also Nath & Chung, 1994). Zhang et al. (2018) cited several sources of endogenous acrolein formation, the most important of which include the reactions of myeloperoxidase on hydroxyl-amino acids such as threonine, and the oxidation of spermine and spermidine by amine oxidase (Stevens & Maier, 2008), while other endogenous sources include peroxidation of polyunsaturated fatty acids (Uchida et al., 1998a) and oxidative ring opening of the anticancer drug cyclophosphamide and other oxazaphosphorine drugs such as ifosfamide (Brock et al., 1979). No quantitative data on endogenous production of acrolein were available to the Working Group.

Landfill leachate contained acrolein at a concentration of 0.07–2.1 ppm [0.07–2.1 mg/L] (Faroon et al., 2008). The US EPA lists acrolein as a pollutant in National Priority Superfund sites in at least 16 USA states; acrolein was detected at a concentration of 0.006–1.3 ppm [0.006–1.3 mg/L] in groundwater at half of these sites (Faroon et al., 2008). Because acrolein is highly reactive, it is not expected to bioaccumulate, but it can be formed in the environment as a breakdown product of other chemicals, in addition to occurring as a result of the direct emission of acrolein as a combustion product (Faroon et al., 2008).

1.4.2 Exposure in the general population

The most important sources of acrolein exposure in the general population include tobacco use and cooking with oil at high temperatures. Forest and residential fires, vehicle exhaust, and incinerators are other significant sources of acrolein exposure.

(a) Food, beverages, and cooking emissions

Acrolein concentrations measured in food, beverages, and cooking emissions are presented in Table 1.1.

Most food items are not considered to be major sources of acrolein in the general population. However, higher concentrations have been reported in certain food items, including frying

fats and oils (mean acrolein concentration, 276 µg/L; maximum, 1389 µg/L; *n* = 15; see Table 1.1), and cooking food in hot oil has been shown to produce emissions containing acrolein, which can be a significant source of exposure.

An analysis by Umano & Shibamoto (1987) revealed that the two most important factors in the production of acrolein during cooking were cooking duration and cooking temperature, both of which were positively associated with acrolein production; the type of oil (i.e. sunflower, beef fat, soybean, corn, sesame, and olive, in increasing order of acrolein production) was less important. While little acrolein was formed under 240 °C, emissions increased 10-fold when the temperature was increased from 280 to 300 °C, and 3-fold from 300 to 320 °C. Temperatures in home cooking were reported to rarely exceed 200 °C.

Table 1.1 Concentrations of acrolein in food, beverages, and cooking emissions

Item	Acrolein concentration (mean or range)	Country of study or purchase	Reference
Food			
French fries	1.97–4.85 mg/kg	Brazil	Osório & de Lourdes Cardeal (2011)
Domiati cheese	0.29–1.3 mg/kg	Egypt	Collin et al. (1993)
Doughnuts	0.1–0.9 mg/kg	USA	Lane & Smathers (1991)
Fried fish coating	0.1 mg/kg	USA	Lane & Smathers (1991)
Fruits	< 0.01–0.05 mg/kg	NR	Feron et al. (1991)
Vegetables	≤ 0.59 mg/kg	NR	Feron et al. (1991)
Frying fats and oils (15 tested)	Mean, 276; max., 1389 mg/kg	Germany	Kächele et al. (2014)
Beverages			
Lager beer, fresh (3 bottlings tested)	Mean, 1.6 µg/L	UK	Greenhoff & Wheeler (1981)
Lager beer, force aged (3 bottlings tested)	Mean, 5.05 µg/L	UK	Greenhoff & Wheeler (1981)
Lager beer (22 tested)	< 2.5–5.4 µg/L	Brazil	Hernandes et al. (2019)
Beer (9 tested)	All < 14 µg/L (LOD)	Germany	Kächele et al. (2014)
Wine (23 tested)	Mean, 0.7; max., 8.8 µg/L	Germany	Kächele et al. (2014)
Merlot wine	Mean, 15.9; max., 29.8 µg/L	Brazil	Ferreira et al. (2018)
Brandy/cognac (11 tested)	1.42–1.5 mg/L [1420–1500 µg/L]	Armenia	Panosyan et al. (2001)
Whiskey/bourbon (3 tested)	0.67–11.1 ppm [670–1110 µg/L]	USA	Miller & Danielson (1988)
Whiskey (15 tested)	Mean, 252; max., 915 µg/L	Germany	Kächele et al. (2014)
Vodka (4 tested)	All < 14 µg/L (LOD)	Germany	Kächele et al. (2014)
Absinthe (5 tested)	All < 14 µg/L (LOD)	Germany	Kächele et al. (2014)
Fruit spirits (28 tested)	Mean, 591; max., 2394 µg/L	Germany	Kächele et al. (2014)
Tequila (7 tested)	Mean, 404; max., 1205 µg/L	Germany	Kächele et al. (2014)
Asian spirits (16 tested)	Mean, 54; max., 477 µg/L	Germany	Kächele et al. (2014)
Grape marc (10 tested)	Mean, 487; max., 1808 µg/L	Germany	Kächele et al. (2014)
Mineral & table water (10 bottles)	All < 14 µg/L (LOD)	Germany	Kächele et al. (2014)
Water stored in cisterns	< 3–115 µg/L	Brazil	de Oliveira Moura et al. (2019)

Item (cooking oil)	Acrolein concentration in air (µg/m^3)	Emission rate of acrolein (mg/kg food per hour)	Reference
Emissions during cooking (for 5 minutes)			
Oil only (soybean)	57.9	26.67	Seaman et al. (2009)
French fries (soybean)	41.8	17.81	Seaman et al. (2009)
Chicken strips (soybean)	40	16.06	Seaman et al. (2009)
Battered fish (soybean)	64.5	27.04	Seaman et al. (2009)
Doughnuts (soybean)	32.4	12.9	Seaman et al. (2009)
Doughnuts (canola)	31.6	13.15	Seaman et al. (2009)
Doughnuts (corn)	26.4	10.68	Seaman et al. (2009)
Doughnuts (olive)	29.2	11.79	Seaman et al. (2009)
Doughnuts (no oil)	1.83	0.19	Seaman et al. (2009)

LOD, limit of detection; max., maximum; NR, not reported; ppm, parts per million.

However, Hecht et al. reported that, among non-smoking Chinese women in Singapore who cook at much higher temperatures or cook more frequently than controls (women randomly selected from the Chinese Health Study), concentrations of urinary acrolein metabolites were about 50% higher than among women who cooked less frequently (see Table 1.2; Hecht et al., 2010, 2015).

Beer typically contains acrolein at a concentration of 1–5 µg/L, although higher concentrations (up to 25 µg/L) are found in the early stages of beer making, before processing to make the final product; the acrolein in other alcoholic drinks ranges from 0.02 to 11 µg/L, (Greenhoff & Wheeler, 1981; Ferreira et al., 2018; Hernandes et al., 2019). A study of 117 alcoholic beverages found that over half had detectable levels of acrolein (limit of detection, 14 µg/L), some at much higher concentrations (Kächele et al., 2014). None of 9 beers, 4 vodkas, and 5 absinthes tested had detectable concentrations, nor did only 21 out of 23 wines tested. However, over 85% of the 15 whiskey samples, 7 tequilas, 28 fruit spirits, and 10 grape marc samples tested were positive; the average acrolein concentration in all the samples was 276 µg/L, but some tequilas, fruit spirits, and grape marc were over 1000 µg/L (Kächele et al., 2014). Rainwater to be used as drinking-water and stored in polyethylene cisterns in Brazil was found to contain acrolein in 75% of the 36 cisterns tested, with concentrations up to 115 µg/L (de Oliveira Moura et al., 2019). No acrolein was detected in 10 bottles of mineral and table water in Germany (Kächele et al., 2014).

(b) Tobacco products and tobacco-related products

Acrolein is present in smoke from cigarettes, cigars, bidis, and hookahs, as well as in emissions from electronic cigarettes and "heatsticks" (Table 1.3). Average concentrations in mainstream smoke from bidis and small cigars are slightly higher than in cigarette smoke. The apparent variability in acrolein yield in mainstream smoke from cigarettes smoked according to the outdated ISO 3308 method is greatly reduced when using the Health Canada Intensive method recommended by WHO, with most products producing 100–200 µg of acrolein/rod. In general, sugars (which are natural components of tobacco and which may also be added during the manufacturing process) increase the emissions of acrolein in tobacco smoke by 20–70% (Talhout et al., 2006). Hookahs (waterpipes, narghile) produce approximately 900 µg of acrolein in mainstream smoke and 1100 µg of acrolein in sidestream smoke per session, which lasts for approximately

1 hour, meaning that secondhand acrolein exposure from waterpipes may exceed that from cigarettes, at 140 µg/rod (Al Rashidi et al., 2008; Daher et al., 2010). Although the fluid in electronic cigarettes ("e-liquid") does not contain acrolein, it is apparently formed during the heating of the fluid, at an amount that is dependent on the composition of the fluid and the temperature of the coil (Conklin et al., 2018); a single puff contains 3–15 ng of acrolein (Herrington & Myers, 2015). Increasing voltage from 3.8 V to 4.8 V increased the acrolein yield more than 4-fold (Kosmider et al., 2014), and the addition of humectants, sweeteners, and flavourings increased the production of acrolein from nondetectable to several hundred micrograms per gram of e-liquid (Khlystov & Samburova, 2016). [The Working Group noted that newer devices contain voltage/temperature controls that can increase the delivery of nicotine and also enhance acrolein production, indicating that acrolein exposures among current users may be much greater than reflected in the recent literature.] Heatsticks, which have been available in over 40 countries for the past 5 years, each discharge about 5 µg of acrolein in mainstream and 0.7 µg of acrolein in sidestream emissions (Cancelada et al., 2019). The acrolein exposure from the heatsticks is reduced by a factor of

Study, Group (if No. of samples country applicable) HPMA/CEMA

Table 1.2 Levels of acrolein metabolite biomarkers measured in human urine

				HPMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		CEMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		Reference
		Unexposed[a]	Exposed	Unexposed	Exposed	Unexposed	Exposed	
Cigarette smoking								
NHANES 2005–2006, USA		2467/NR	601/NR	219 (140, 353)[b]	1089 (469, 2012)	78.8 (51.8, 121)	203 (111, 338)	Alwis et al. (2015)
PATH Study, USA	Cigarettes only	1571/1517	2284/2176	272.4	1143.5	98.14	271.5	Goniewicz et al. (2018)
European multicentre observational study, Germany, Switzerland, and UK	< 10 cigarettes/day		467/NR		1.12 mg/24 h			Lindner et al. (2011)
	10–19 cigarettes/day		557/NR		2.10 mg/24 h			
	≥ 20 cigarettes/day		135/NR		2.98 mg/24 h			
German university study		54/NR	40/NR	146[b]	884[b]			Eckert et al. (2011)
					4123 (2341, 6808) [911 (517, 1505)][c,d]			Park et al. (2015)
					6007 (3947, 9606) [1328 (872, 2123)][c,d]			
					6738 (3885, 1057) [1489 (859, 2422)][c,d]			
					3480 (186, 5908) [769 (412, 1306)][c,d]			
					5344 (3163, 8596) [11 851 (699, 1900)][c,d]			

Multiethnic cohort study, USA	African American	362/NR			
	Native Hawaiian	329/NR			
	White	438/NR	Latino	449/NR	
	Japanese American	704/NR			

Table 1.2 (continued)

Study, Group (if country		No. of samples HPMA/CEMA		HPMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		CEMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		Reference
		Unexposed[a]	Exposed	Unexposed	Exposed	Unexposed	Exposed	
Betel-quid chewing								
Healthy subjects in a study of smoking, betel quid chewing and oral cancer, Taiwan, China	Cigarettes only		111/NR		5.8 [1282][c]			Tsou et al. (2019)
	Betel quid only		12/NR		3.6 [796][c]			
	Cigarettes + betel quid		107/NR		8.9 [1967][c]			
E-cigarettes								
PATH Study, USA	E-cigarettes only	1571/1517	212/198	272	315	98	108	Goniewicz et al. (2018)
	Cigarettes only				1144		272	
	E-cigarettes + cigarettes		767/739		1318		302	
Cooking								
Study of Chinese female regular home cookers, Singapore	Frequent home cooking vs random	50/NR	54/NR	1370 [303][d]	1959 [433][d]			Hecht et al. (2010)
	Cook > 7×/wk vs < 1×/wk	90/NR	95/NR	1901 [420][d]	2600 [575][d]			
Non-source–related								
Shanghai cohort Study, China	Control participants		392/NR		6712 (5845, 7707) [1483 (1292, 1703)][d]			Yuan et al. (2012)
National Children's Study, USA	Pregnant women	488/NR		240 µg/L[b]		71.8 µg/L[b]		Boyle et al. (2016)

applicable)

Table 1.2 (continued)

Study, country	Group (if applicable)	No. of samples HPMA/CEMA		HPMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		CEMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		Reference
		Unexposed[a]	Exposed	Unexposed	Exposed	Unexposed	Exposed	
Pregnant women, Guatemala	Fasting	23/NR		268 (178, 399)[c]				Weinstein et al. (2017)
	After sauna	23/NR		572 (429, 1041)[c]				

CEMA, *N*-acetyl-S-(2-carboxyethyl)-L-cysteine (2-carboxyethylmercapturic acid); e-cigarette, electronic cigarette; HPMA, *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (3-hydroxypropyl-mercapturic acid); NR, not reported; vs, versus.

[a] Unexposed/exposed applies to exposures in subheadings (e.g. *Cigarette smoking*).

[b] Median.

[c] Median (interquartile range).

[d] pmol/mg creatinine [converted to µg/g creatinine].

[e] µmol/g creatinine [converted to µg/g creatinine].

Table 1.3 Concentrations of acrolein in smoke from tobacco products

Product and method details	Reported measurements				Reference
	Method: ISO 3308 (µg/product)		Method: Health Canada Intensive (µg/product)		
	Range	Median	Range	Median	
Cigarettes					
12 brands, mainstream smoke			51–223[a]	163[a]	Borgerding et al. (2000)
12 brands, sidestream smoke			342–523[b]	412[b]	Borgerding et al. (2000)
6 Thai & 2 US brands (90% market share)	79.9–181				Mitacek et al. (2002)
35 brands	30.8–82.6		139–213		Cecil et al. (2017)
3 brands + 1 reference cigarette	24.9–52.2	48.5	100–125	117	Eldridge et al. (2015)
Cigars					
Sheet-wrapped cigars (15 brands)	34.3–105		105–185		Cecil et al. (2017)
Bidis					
76 mm unfiltered bidi – one selected sample	67 µg				Hoffmann et al. (1974)
	Mean total yield	**Mean mainstream yield**	**Mean sidestream yield**	**Sidestream/mainstream yield ratio**	
Narghile/hookah (waterpipe)					
Narghile	145.5 µg/g tobacco				Al Rashidi et al. (2008)
Narghile, per session	892 µg				Al Rashidi et al. (2008)
Narghile, per session		1135 µg	0.7		Daher et al. (2010)
	Various metrics				
Electronic cigarettes					
Aerosol	0.003–0.015 µg/mL (≈20–230 g of acrolein per cigarette assuming 400–500 × 40 mL puffs)				Herrington & Myers (2015)

Aerosol from neat PG	< LOD (0.03×10^{-3} µg/puff)	Conklin et al. (2018)
Aerosol from neat VG	0.08 ± 0.002 µg/puff	Conklin et al. (2018)
Aerosol from 25–75% PG in VG	0.04 µg/puff	Conklin et al. (2018)
Aerosol – 'brand I' (unflavoured)	ND	Khlystov & Samburova (2016)
Aerosol – 'brand III' (unflavoured)	ND	Khlystov & Samburova (2016)
Aerosol – 'brand I' (flavoured)	172 ± 27 to 347 ± 37 µg/g of e-liquid	Khlystov & Samburova (2016)
Aerosol – 'brand II' (flavoured)	ND	Khlystov & Samburova (2016)
Aerosol – 'brand II' (flavoured)	ND to 237 ± 61 µg/g of e-liquid	Khlystov & Samburova (2016)

Table 1.3 (continued)

Product and method details	Reported measurements					Reference
	Mean mainstream emissions	Mean sidestream	Mean emissions concentration	environmental	Range of % of conventional cigarette	
"Heatsticks"						
Heated tobacco device: "iQOS blue"[c]	5.4 ± 0.7 µg per heatstick	0.6 ± 0.3 µg per heatstick				Cancelada et al. (2019)
Heated tobacco device: "iQOS amber"	4.9 ± 0.6 µg per heatstick	0.8 ± 0.3 µg per heatstick				Cancelada et al. (2019)
Heated tobacco device: "iQOS yellow"	5.3 ± 0.7 µg per heatstick	0.7 ± 0.3 µg per heatstick				Cancelada et al. (2019)
Heated tobacco device: "iQOS"			4.6 ± 3.2 µg/m^3		1.8–2.3%	Ruprecht et al. (2017)

LOD, limit of detection; ND, not detected; PG, propylene glycol; VG, vegetable glycerin.

[a] Massachusetts machine smoking protocol. [b] Sidestream smoke, Massachusetts machine smoking protocol. The median value was calculated by multiplying the median value for mainstream smoke by the median value for the sidestream/mainstream smoke ratios for the 12 commercial cigarette brands, which was 2.53. [c] iQOS is a brand name.

about 10 compared with conventional cigarettes (Lachenmeier et al., 2018).

While acrolein metabolites (the mercapturic acids HPMA and CEMA) have been detected in the urine of 99% of Americans (Alwis et al., 2015), concentrations of these metabolites were three to five times higher in smokers than in non-smokers (Eckert et al., 2011; Lindner et al., 2011; Alwis et al., 2015; Goniewicz et al., 2018; Table 1.2), with concentrations increasing with the number of cigarettes smoked per day (Lindner et al., 2011) and with increasing urinary concentration of cotinine (a metabolite of nicotine) (Alwis et al., 2015). Acrolein metabolite concentrations were slightly higher in electronic-cigarette smokers than in non-smokers, but four times higher in dual users of cigarettes and electronic cigarettes (Goniewicz et al., 2018). Passive exposure to secondhand smoke led to comparable increases in urinary acrolein metabolites among hookah smokers and non-smokers alike after visiting a hookah lounge or attending a hookah social event at home (Kassem et al., 2018), probably due to the abovementioned high sidestream emission of acrolein from hookahs. Levels of urinary acrolein metabolites were significantly higher in children living with daily hookah smokers than in children from non-smoking homes (Kassem et al., 2014).

Park et al. (2015) reported significantly different concentrations of acrolein metabolites for smokers from different racial and ethnic groups. Similarly, the National Health and Nutrition Examination Survey (NHANES) found that the 25th percentile of the HPMA concentrations for tobacco smokers was greater than the 75th percentile for non-tobacco users, for all age groups, and that HPMA concentrations among non-tobacco users were similar for both sexes, and were lower for non-Hispanic White people and non-Hispanic Black people than for Mexican Americans or for people of other Hispanic origins or for other or multiple ethnicities. However, among Mexican Americans, metabolite concentrations for smokers were much lower (36%) than those of non-Hispanic White people (Alwis et al., 2015).

In Taiwan, China, healthy subjects who chewed betel quid had HPMA concentrations that were significantly elevated, but significantly lower than in cigarette smokers, and those who both smoked cigarettes and chewed betel quid had the highest urinary HPMA levels (3600, 5800, and 8900 pmol/mg creatinine [796, 1282, and 1967 µg/g creatinine], respectively, see Table 1.2 (see also Table 2.1); Tsou et al., 2019). In contrast, in patients with oral squamous cell carcinoma who both smoked cigarettes and chewed betel quid, urinary levels of HPMA were only 7% those of healthy people with matched smoking and betel-quid use history, despite the fact that their NNAL (4-(methylnitrosamino)-1- (3-pyridyl)-1-butanol)/creatinine levels were com - parable.

(c) Indoor air (i) In the home

Activities in the home, especially tobacco smoking and cooking with oils and fats heated to high temperatures, are the primary indoor source of acrolein (see Section 1.4.2(a)). Cooking can increase air concentrations of acrolein by 26 to 64 µg/m^3 (Seaman et al., 2009). Other indoor sources of acrolein include gas stoves, woodburning fireplaces and stoves, burning candles, and incense. When indoor air in the home and outdoor air are measured simultaneously, the indoor concentration of acrolein is usually 2–10 times greater than the outdoor concentration. Azuma et al. (2016) reviewed surveys of Japanese homes and reported an average indoor concentration of acrolein of 0.267 µg/m^3, which was three times higher than the outdoor concentration, but much lower than that found in homes in other countries.

A survey of 130 homes in Beijing, China, reported much higher concentrations, with an average of 2.1 µg/m^3, although neither smoking nor cooking occurred during the sampling period (Liu et al., 2014). A study of acrolein concentrations outdoors and inside occupied homes and unoccupied, newly constructed, model homes (expected to have high emissions from construction materials) reported morning indoor concentrations in occupied homes (2–8 µg/m^3) that were generally more than 10 times higher than the outdoor concentrations (0.1–0.3 µg/m^3) in Davis and surrounding towns in California, USA. Similarly, in new model homes, the indoor concentrations were also 10 times higher than the outdoor concentrations. The outdoor concentrations in occupied homes in Los Angeles averaged 5–10 times higher than those around Davis (0.8–1.7 µg/m^3), but indoor concentrations were comparable (Seaman et al., 2007). The greatest increases in indoor acrolein concentrations in occupied homes in all three counties studied (Los Angeles, Placer, Yolo) were associated with cooking with fats and oils. Homes with frequent, regular cooking activity had the highest morning acrolein levels. In four unoccupied new houses, indoor acrolein concentrations were increased by 10-fold compared with those outdoors, although no cooking or smoking had taken place. However, the particle board and lumber used to construct these houses was found to emit acrolein (1–8 ng acrolein/g). The Relationship of Indoor, Outdoor and Personal Air (RIOPA) study of 398 homes in the USA found quite different average acrolein concentrations in the three cities studied. The average concentration in these cities ranged from 1.0 µg/ m^3 in Elizabeth, New Jersey, and 1.2 µg/m^3 in Los Angeles, California, to 3.1 µg/m^3 in Houston, Texas (Weisel et al., 2005).

In Prince Edward Island, Canada, acrolein concentrations were consistently two and a half times higher in homes with smokers than in homes without. Similarly, concentrations were higher in homes with new carpets than in those without new carpets. No significance was found for the presence of wood stoves, the type of heating, or painting (Gilbert et al., 2005). Subsequent studies of over 250 homes in Edmonton, Halifax, Regina, and Windsor, Canada, also found that homes with smokers had distinctly higher concentrations of acrolein than homes without, and also that indoor concentrations of acrolein were higher than outdoor concentrations; median indoor concentrations ranged from 1.3 to 8.1 µg/m^3, while paired outdoor concentrations were more than 60% lower (ranging from 0.2 to 2.2 µg/m^3) (Health Canada, 2020).

Other sources of acrolein in homes include burning incense and using kilns. Burning incense increases acrolein concentrations by 2.67–8.14 ppm/g [6000–19 000 µg/m^3 per g] burned (Lin & Wang, 1994). Hirtle et al. (1998) measured acrolein concentrations greater than 20 ppb [46 µg/m^3] in three homes with kilns.

Overall, the acrolein concentrations in homes ranged from less than 0.01 to 39 µg/m^3, with median concentrations of 1 to 8 µg/m^3.

(ii) Primary schools

In a study of 408 primary schools (attended by 6590 students) in France, 14% of the children were found to be exposed to acrolein at concentrations greater than 1.55 µg/m^3 in their classrooms (Annesi-Maesano et al., 2012). [The Working Group noted that the aldehyde (acrolein, formaldehyde, and acetaldehyde) concentrations inside the classrooms in this study were greater than the outdoor concentrations in the same cities, which indicates that there might be indoor sources, but these were not identified. Possibilities include smoking by staff or emissions from building materials.] Similarly, a study of seven schoolrooms in Mira Loma, California, USA, reported that acrolein concentrations in the

classroom were greater than outdoor concentrations. The authors attributed the higher indoor acrolein concentrations to building elements such as carpet, drywall, and adhesives (Sawant et al., 2004).

(iii) Hospitality sites

Hospitality sites where smoking was permitted had higher indoor concentrations of acrolein. Measurements made in the 1970s and early 1980s in France found acrolein concentrations in cafés to be between 12 and 43 µg/ m^3. Acrolein concentrations in restaurants and taverns in the Netherlands were between 1 and 8 µg/m^3, and concentrations in a car with three smokers increased from 13 µg/m^3 with the windows open to ten times that level when the windows were closed (Triebig & Zober, 1984). Löfroth et al. (1989) reported acrolein concentrations on two evenings to be 21 and 24 µg/m^3 in a tavern in the USA. [The Working Group noted that the advent of smoke-free regulations has presumably lowered these concentrations substantially.]

(d) Outdoor air pollution

The major sources of acrolein in the outdoor environment are forest fires and exhaust from motor vehicles and aircraft. Acrolein is released directly into the ambient air from vehicle exhaust and is also formed by photo-oxidation of 1,3-butadiene and other hydrocarbons (Faroon et al., 2008,). These reactions comprised an estimated 39% of total acrolein emissions in California, USA, in 2012 (OEHHA, 2018). Other sources of acrolein, which may be important in nearby local areas, include emissions from manufacturing processes such as pulp and paper, coal/gas/oil-fired power plants, waste-disposal emission, and the volatilization of biocides.

The seasonal effect for acrolein is opposite to that for many other pollutants in that concentrations decrease in winter. For example, the median summer concentration measured in several European cites was 2 µg/m^3, while the median winter concentration was 0.6 µg/m^3 (Campagno- lo et al., 2017), which may be partially attributable to the decline in frequency of photochemical reactions with seasonal reduction in solar intensity.

Outdoor concentrations of acrolein in the USA are typically 0.5–3.2 ppb [1–7 µg/m^3] (Faroon et al., 2008), although acrolein concentrations measured outside 124 homes in Houston, Texas, averaged 17.9 µg/m^3 (Weisel et al., 2005). Median concentrations in California were 0.041 µg/m^3 in coastal areas, 0.068 µg/m^3 in intermediate areas, 0.101 µg/m^3 in the San Francisco Bay area, and 0.32 µg/m^3 in the Los Angeles air basin (Cahill, 2014); concentrations outside 15 homes averaged 0.60 µg/m^3 (Seaman et al., 2007). Based on measurements throughout the state, acrolein exposures in California increased between 2004 (0.51 ppb) [1.2 µg/m^3] and 2014 (0.66 ppb) [1.5 µg/ m^3], although concentrations of volatile organic compounds other than aldehydes have declined, and acrolein emissions from gasoline-related sources decreased by two thirds between 1996 and 2012. The increase in acrolein emissions from non-gasoline related sources in 2012 was attributed primarily to a higher estimate of emissions from waste disposal (OEHHA, 2018).

Exhaust from gasoline- and diesel-powered vehicles is one of the most important, ubiquitous sources of acrolein in outdoor air. With the introduction of engine and fuel improvements due to stricter regulations to reduce exhaust emissions, this contribution has declined in North America and Europe. Schauer et al. (2002) reported that tailpipe emissions of acrolein from several gasoline-powered vehicles equipped with early catalytic converters (1981–1994) were greatly reduced compared with those from vehicles without these converters (1969–1970), from 3800 to 60 µg/km. The estimated acrolein emissions

from on-road vehicles in the 48 contiguous states of the USA in 2007 were less than half the estimated emissions in 1996 (10 185 versus 21 266 metric tonnes/year). This decrease was almost entirely due to reductions from gasoline-powered vehicles and was attributed to changes in gasoline formulation and implementation of stricter Tier 2 emission standards for light-duty vehicles (IARC, 2013).

(i) Local sources

Local sources may increase acrolein concentrations. The importance of nearby industry and traffic is illustrated by the results of 2 years of sampling in the Pittsburgh area, Pennsylvania, USA. Four locations were sampled every sixth day: one near downtown (near the city centre) with heavy traffic; one remote from both traffic and industry; and two in residential areas within 0.8 km of heavy industry. In the two residential areas near industry, acrolein concentrations were approximately double those in the rural area, while the downtown area had the highest average and 95th percentile concentrations (Logue et al., 2010). Other evidence of the importance of local sources included measurements made in the vicinity of a petrochemical plant: acrolein concentrations were 640 µg/m^3 at a distance of 1 km, and 2000 µg/m^3 at 100 m. Concentrations measured 50 m from a perfume factory ranged from 40 to 480 µg/m^3 (Izmerov, 1984).

Acrolein is used as a biocide in irrigation canals and volatilizes quickly after application. In the San Joaquin Valley of California, USA, a major agricultural area through which pass the 640 km California Aqueduct and numerous irrigation canals, an estimated 90 tonnes of acrolein were volatilized into the air in 2001 (CEPA, 2002). The estimate for 2012 was 33 tonnes (OEHHA, 2018). [The Working Group noted that no measurements of ambient acrolein concentrations were made while acrolein was in use, but these could affect local concentrations.]

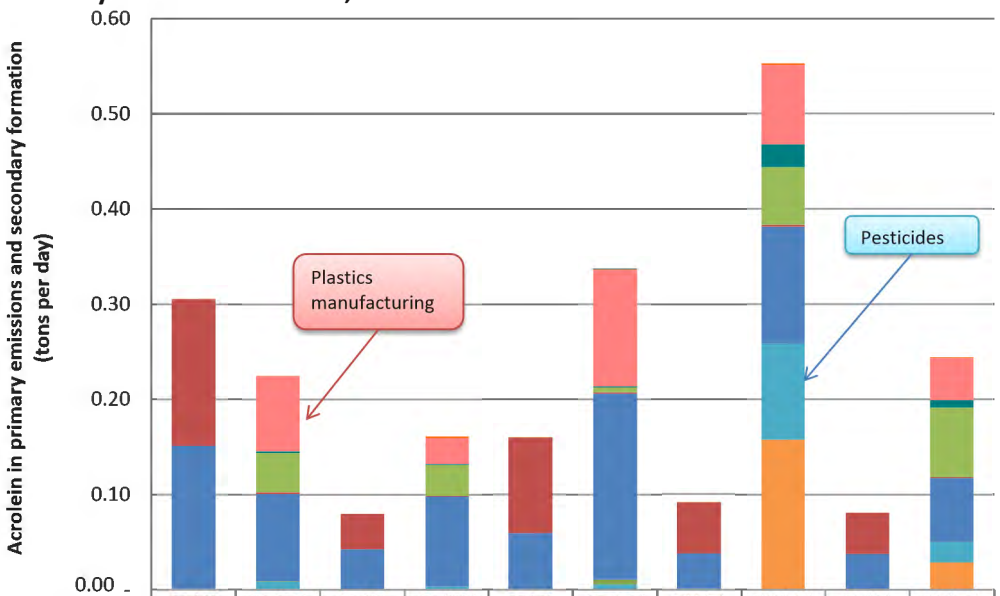
(ii) Diesel and biodiesel

A study of emissions from the engines of two heavy-duty trucks found that both pollution-control technology and fuel were major determinants of acrolein emissions (Cahill & Okamoto, 2012). The truck engine built in 2008 was equipped with a diesel oxidation catalyst/diesel particulate filter, while the truck engine built in 2000 was not, although it complied with the environmental regulations of the time. Emissions from the truck engine without pollution controls (without the catalyst/filter) were 2–10 times greater than those from the engine with these controls, depending on the fuel type used; the difference was least for ultra-low sulfur diesel fuel (ULSD; a petroleum product) and greatest for soy biodiesel blend (50% soy biodiesel and 50% ULSD). More fuels were tested with the 2008 engine than with the 2000 engine. These included: ULSD, a soy biofuel, an animal biofuel, a "renewable" fuel (hydrotreated biofuel), and 50:50 blends of each of the biofuels and ULSD. Acrolein emissions from the renewable fuels (hydrotreated biofuel, and 50:50 hydrotreated biofuel and ULSD) were comparable to those from the petroleum-based fuel (ULSD); the animal biofuel and blend emitted 40% more acrolein than the ULSD fuel, and the soy biofuel emitted the most acrolein (two and a half to three times that of the ULSD).

(iii) Gasoline and other sources of acrolein

Gasoline- and diesel-powered road motor vehicles are the major quantified source of acrolein in outdoor air in Canada. Annual releases from these were estimated to be 209 000 to 2 730 000 kg. However, unquantified but possi - bly greater sources of acrolein are other vehicles that are not fitted with pollution-control devices, such as aircraft, railway and marine vehicles, as well as off-road motor vehicles, lawnmowers, and snowblowers. Other major anthropogenic sources include the

Fig. 1.1 Primary and secondary sources of acrolein, 2012



	SC (G)	SC (NG)	SD (G)	SD (NG)	SF (G)	SF (NG)	SJV (G)	SJV (NG)	SV (G)	SV (NG)
CLEANING AND SURFACE COATINGS	-	-	-	-	-	< 0.01	-	-	-	-
FUEL COMBUSTION	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
INDUSTRIAL PROCESSES	-	0.08	-	0.03	-	0.12	-	0.08	-	0.04
MISCELLANEOUS PROCESSES	-	< 0.01	-	< 0.01	-	< 0.01	-	0.02	-	< 0.01
NATURAL SOURCES	-	0.04	-	0.03	-	< 0.01	-	0.06	-	0.07
ON-ROAD MOTOR VEHICLES	0.15	< 0.01	0.04	< 0.01	0.10	< 0.01	0.05	< 0.01	0.04	< 0.01
OTHER MOBILE SOURCES	0.15	0.09	0.04	0.09	0.06	0.20	0.04	0.12	0.04	0.07
PETROLEUM PRODUCTION AND DISTRIBUTION	-	< 0.01	-	-	-	< 0.01	-	< 0.01	-	< 0.01
SOLVENT EVAPORATION	-	< 0.01	-	< 0.01	-	< 0.01	-	0.10	-	0.02
WASTE DISPOSAL	-	-	-	-	-	-	-	0.16	-	0.03

G, gasoline sources; NG, non-gasoline sources; SC, South Coast (Los Angeles and surrounding counties); SD, San Diego County; SF, San Francisco Bay Area; SJV, San Joaquin Valley, southern part of Central Valley, a hot, dry agricultural region with major irrigation canals; SV, Sacramento Valley, northern part of Central Valley, also agricultural.
"Other mobile sources" of gasoline-attributed emissions include recreation boats, off-road equipment including garden and lawn, (each responsible for about 40%), off-road recreational vehicles such as motorcycles and all-terrain vehicles, and agricultural equipment and fuel storage.
Each region has two bars, the first for the gasoline sources and the second for all other sources. The first bar in each region represents the portion attributable to gasoline; especially notable here is that on-road motor vehicles, which have been tightly regulated, now contribute approximately half the gasoline-attributable acrolein, while the other half comes from "other mobile sources," such as recreational boats, off-road equipment for gardens and lawns (each contributes about 40% of this category), and off-road motorcycles and all-terrain vehicles. The second bar in each set illustrates the contribution of industrial processes, natural sources, such as wildfires, waste disposal, and "solvent evaporation," which refers to the volatilization of acrolein used as a biocide. This latter contribution appears significant only in the two agricultural regions of the Central Valley, the San Joaquin Valley, and the Sacramento Valley.
From OEHHA (2018), with permission.

oriented strand board industry (25 664 kg/year), pulp and paper mills (18 735 kg/year), waste incineration (2435 kg/year), and coal-based power plants (467–17 504 kg/year) (Environment Canada and Health Canada, 2000).

A recent evaluation of the sources of acrolein emissions in outdoor air in California, USA, reported that the contribution of gasoline, as both a primary and secondary pollutant, declined significantly from 52% in 1996 to 28% in 2012. Despite this decline, the average exposure to ambient acrolein in California increased

from 0.51 to 0.66 ppb [1.2 to 1.5 µg/m^3] (OEHHA, 2018). Fig. 1.1 presents the dominant sources of acrolein in three urban and two agricultural areas of California in 2012.

1.4.3 Occupational exposure

Workers may be exposed occupationally to acrolein during its manufacture and use as a chemical intermediate (see Section 1.2). However, as for the population at large, workplace exposures to acrolein occur primarily from the formation of acrolein during the incomplete combustion of organic material such as tobacco, cooking oils, gasoline and diesel fuel, and forest and residential fires.

The National Occupational Exposure Survey estimated that approximately 1300 workers were potentially exposed to acrolein in the USA when the study was conducted in 1981–1983. Approximately one third of these workers were mechanics and repairers. Other occupations identified with potential exposure to acrolein included painters and spray painters, machinists, sheet metal workers, chemical technicians, janitors, and water and sewage treatment plant operators (NIOSH, 1990). [The Working Group noted that the survey did not include agricultural production, mining activity, or railroad transportation. During the subsequent 40 years, occupational exposures in manufacturing in the USA have evolved significantly and these numbers have probably changed substantially due to changes in product usage, export of chemical manufacturing, and automation, to name a few examples.] Between 1993 and 2009, 8 cases of acrolein-related illness from pesticide usage were identified in Washington State and California, USA (Rodriguez et al., 2013).

Occupational exposure to acrolein in firefighting, manufacturing, welding, food processing, and traffic-related occupations is presented in Table 1.4 and detailed below.

(a) Firefighting

Firefighters are exposed to high concentrations of acrolein produced during the incomplete combustion of burning materials. Structural fires and wildland fires are fought by distinctly different crews who have different exposure profiles. The exposures of wildland firefighters and urban firefighters are presented in Table 1.4.

The two distinct phases of fighting structural fires are: (i) knockdown, when the visible flames are extinguished; and (ii) overhaul, during which smouldering material is searched for embers and hidden flames. Jankovic et al. (1991) collected short-term personal samples from 22 fires, mostly residential, in the USA and reported that half the samples from during knockdown exceeded the short-term exposure limit (STEL) for acrolein at the time – 300 ppb [690 µg/m^3] – and that the maximum value was 3200 ppb [7330 µg/m^3]. Their data were similar to those reported by Burgess et al. in 1979 and plotted in the Jankovic publication. Together, these data provided a median of 500 ppb [1100 µg/m^3], with a 95th percentile of 5000 ppb [11 000 µg/m^3] and a maximum of 15 000 ppb [34 000 µg/m^3]. During knockdown, firefighters wear a self-contained breathing apparatus; some samples collected inside the breathing mask measured as high as 900 ppb [2000 µg/m^3]. During overhaul, when a self-contained breathing apparatus is not generally worn, measured acrolein concentrations were as high as 200 ppb [500 µg/m^3] in the Jankovic publication and 300 ppb [700 µg/m^3] in a study of 25 fires in the USA by Bolstad-Johnson et al. (2000). Of the 96 30-minute samples collected by BolstadJohnson et al. (2000), only 7 exceeded the limit of detection (11 ppb [25 µg/m^3]). The mean for these 7 samples was 123 ppb [282 µg/m^3].

Wildland firefighters do not wear respiratory protection. The three types of wildland firefighting are: (i) initial attack – the first day of a fire, during which all but 5% of

fires are extinguished; (ii) project fires – the second and successive days of fighting those few fires that continue past the first day; and (iii) prescribed burns – intentionally set and controlled fires in an established area. In the USA, Reinhardt & Ottmar (2004) reported geometric mean (GM) acrolein concentrations of 1 ppb [2 µg/m^3] during 13–14 hour shifts for the initial attack day (45 samples) and also for the subsequent days (84 samples), while the GM during prescribed burns was 9 ppb [21 µg/m^3] (11.5-hour average shift, 200 samples), and the maximum concentrations were 11, 15, and 60 ppb [25, 34, and 140 µg/m^3], respectively. Similar results for prescribed burns in the USA

Table 1.4 Occupational exposure to acrolein

Job, task or industry	Country	No. of sites	No. of samples	Acrolein air concentration	Reference
Firefighting					
Overhaul (structure fires)	USA	25	96	Mean, 0.123 ppm [282 µg/m³] Max., 0.3 ppm [687 µg/m³]	Bolstad-Johnson et al. (2000)
Initial attack – fireline (wildfires)	USA	NR	45	Geometric mean, 5 ppb [11.5 µg/m³] Max., 11 ppb [25 µg/m³]	Reinhardt & Ottmar (2004)
Project fires – fireline (wildfires)		NR	84	Geometric mean, 2 ppb [4.6 µg/m³] Max., 16 ppb [34 µg/m³]	
Prescribed burns – fireline (wildfires)		NR	200	Geometric mean, 15 ppb [34.4 µg/m³] Max., 98 ppb [225 µg/m³]	
Prescribed burns – pre- to post-shift timeweighted averages	USA	NR	65	Mean, 0.01 ppm [22.9 µg/m³] Max., 0.041 ppm [94 µg/m³]	Slaughter et al. (2004)
Manufacturing					
Phenol-formaldehyde resins (abrasive materials)	Poland	13	NR	Range, 0–0.003 mg/m³ [0–3 µg/m³]	Pośniak et al. (2001)
Phenol-formaldehyde resins (friction linings)		11	NR	Range, 0–0.01 mg/m³ [0–10 µg/m³]	
Plastics	USA	130[a]	23[b]	Mean[c], 39 ppb [89 µg/m³] Max., 240 ppb [550 µg/m³]	OSHA (2020)
Tyres and inner tubes		1	1 [b]	Max: 11 ppb [25 µg/m³]	
Copper foundries		17 [a]	6 [b]	Mean[c], 12 ppb [27 µg/m³] Max., 45 ppb [103 µg/m³]	
Photographic equipment		4 [a]	3 [b]	Mean[c], 1.2 ppb [2.7 µg/m³] Max., 1.8 ppb [4.1 µg/m³]	
Packing and crating		3[a]	3[b]	Mean[c], 6.7 ppb [15 µg/m³] Max., 8.5 ppb [19 µg/m³]	
Potters	Canada	10	50	Range, < 28–110 ppb [< 64–252 µg/m³]	Hirtle et al. (1998)
Welding and flame cutting					
Welding (unspecified)	USA	3[a]	1[b]	Max., 21 ppb [48 µg/m³]	OSHA (2020)
Food production					
Tortilla manufacturing	USA	8[a]	6[b]	Mean[c], 14 ppb [32 µg/m³] Max., 26 ppb [60 µg/m³]	OSHA (2020)

Food production including tortilla	USA	22[a]	13[b]	Mean[c], 29 ppb [66 µg/m³] Max., 74 ppb [169 µg/m³]	OSHA (2020)
Restaurants					
University catering kitchen	Iran	16	NR	Mean, 670 ppb [1534 µg/m³] Range, 210–910 ppb [481–2084 µg/m³]	Neghab et al. (2017)

Table 1.4 (continued)

Job, task or industry	Country	No. of sites	No. of samples	Acrolein air concentration	Reference
Restaurants, hotels and burger chains	Norway	44	NR	Mean, 10 ppb [23 µg/m³] Max., 32 ppb [73 µg/m³]	Svendsen et al. (2002)
Gasoline and diesel exhaust-related exposures					
Bus drivers	Poland	10 drivers serving 5 bus lines	NR	Range, 0.01–0.035 mg/m³ [10–35 µg/m³]	Brzeźnicki & Gromiec (2002)
Toll station operators	USA	NR	6	Range, 0.031–0.14 µg/m³	Destaillats et al. (2002)
Toll station operators	Spain	15 attendants at 2 toll stations	17	Range, < 0.5–2.75 µg/m³]	Belloc-Santaliestra et al. (2015)
Highway construction	USA	12[a]	3[b]	Mean[c], 91 ppb [208 µg/m³] Max., 155 ppb [355 µg/m³]	OSHA (2020)
Transportation	USA	12[a]	2[b]	Mean[c], 9 ppb [21 µg/m³] Max, 20 ppb [46 µg/m³]	OSHA (2020)
Waste management and incineration					
Waste management	USA	3[a]	1[b]	Max, 13 ppb [29.3 µg/m³]	OSHA (2020)
Working near burn pit and incinerator operations at an airfield	Afghanistan	3 sites within close proximity	78	Site means, 9–19 ppb [21–44 µg/m³] Site maxima, 39–140 ppb [89–321 µg/m³]	Blasch et al. (2016)

NR, not reported; ppb, parts per billion. [a] Number of measurements.

[b] Number of measurements above the limit of detection. [c] Mean value of measurements above the limit of detection was calculated by the Working Group.

were reported by Slaughter et al. (2004): a timeweighted average (TWA) mean of 10 ppb [23 µg/ m³] and a maximum of 41 ppb [94 µg/m³] for 65 samples. Task-specific (~2 hours) concentrations ranged from < 1 ppb [< 2.3 µg/m³] at the engine and 5 ppb [11 µg/m³] while igniting the fire to 30 ppb [69 µg/m³] for the holding boss and 18 ppb [41 µg/m³] for others holding the fire within prescribed boundaries. A 30-minute exposure during direct attack to extinguish flames that had escaped these boundaries was 62 ppb [140 µg/m³] (Reinhardt & Ottmar, 2004).

(b) Manufacturing operations

The manufacture of acrolein can lead to very high exposures of 43–3526 ppb [98–4075 µg/m³] (Izmerov, 1984). Various plastic-manufacturing processes use or produce acrolein. Polyethylene extrusion operations and phenol–formaldehyde resins led to exposures under 13 ppb [< 30 µg/m³] (Tikuisis et al., 1995; Pośniak et al., 2001).

(c) Welding

In a study in Ukraine, Protsenko et al. (1973) found that, while metal untreated with primer emitted no measurable acrolein, some primers coated onto metals resulted in significant acrolein emissions during both gas cutting and automatic submerged arc welding, with acrolein concentrations reaching 447 ppb [1024 µg/m³]. While exposures during welding in new ship outfitting averaged 9 ppb [21 µg/m³], with maximum values reaching 28 ppb [64 µg/m³], exceeding the occupational exposure limit (OEL) for the European Union (EU), exposures during ship repair were even higher, reaching 64 ppb [150 µg/m³], and over half the shipbreaking samples exceeded the EU OEL, with one sample at 600 ppb [1400 µg/m³]. Although in most short-term (15-minute) samples collected in engine and garage repair shops acrolein was not detectable (i.e. < 65 ppb [< 150 µg/m³]), one sample contained acrolein at 260 ppb [595 µg/m³].

(d) Food processing, traffic-related, and other occupations

Exposures (summarized in Table 1.4) mea - sured in restaurant kitchens are highly variable, probably reflecting emissions from cooking fuels. Similarly, those who work near gasoline exhaust, such as bus drivers, garage workers, and highway construction workers, and those who work at or near incineration facilities, have highly variable and significant exposures, from 10 ppb to > 100 ppb [~23 to > 230 µg/m³]. Klochkovskii et al. (1981) reported that 37% of 800 samples collected in quarry operations in an area of the former Soviet Union exceeded permissible limits, and that acrolein concentrations in exhaust gases and workplace air averaged 900–3100 ppb [2100–7100 µg/m³].

(e) Occupational exposure to acrolein from secondhand smoke

Workers, especially hospitality workers, may also be subject to significant exposures to acrolein in places where smoking is permitted. Acrolein concentrations in a tavern in North Carolina, USA, with moderately high levels of secondhand smoke (on average, particles, 430 µg/m³; and nicotine, 66 µg/m³) were measured at 21 µg/m³ and 24 µg/m³ on two sampling trips of 3–4 hours each (Löfroth et al., 1989). In open offices where smoking was allowed in Massachusetts, USA, the 90th percentile of weekly average concentrations of nicotine was 34 µg/m³ (Hammond et al., 1995), so office exposures may exceed 5 ppb [11 µg/m³] acrolein (Mitova et al., 2016). Ayer & Yeager (1982) reported that acrolein concentrations reached > 50 ppm [114 000 µg/m³] in the smoke plume of cigarettes. Thus, secondhand smoke can

be an important source of both peak and TWA exposure to acrolein.

(f) Occupational Safety and Health Administration compliance data

OSHA maintains a publicly available database of industrial hygiene samples collected in the USA as part of its compliance monitoring programme, the Chemical Exposure Health Data (OSHA, 2020). The results for 1220 samples and blanks collected by OSHA inspectors and analysed for acrolein between 1984 and 2019 provide some information from inspections for those 35 years (OSHA, 2020). These values should be compared with the 8-hour TWA OSHA permissible exposure limit of 100 ppb [250 µg/m^3], the EU OEL of 20 ppb [50 µg/m^3] for 8-hour TWA and 50 ppb [114 µg/m^3] STEL (for 15 minutes) as well as the American Conference of Governmental Industrial Hygienists (ACGIH) ceiling value of 100 ppb [250 µg/m^3]. Only about 10% of the samples were above the limit of detection, and only 3 of the nearly 200 samples collected for less than 1 hour had detectable concentrations of acrolein, but of these 2 were of concern: the 15-minute sample was 115 ppb [263 µg/m^3] acrolein and the 24-minute sample was 69 ppb [158 µg/m^3], both in excess of the EU short-term limit of 50 ppb [120 µg/m^3]; the limit of detection in air for these shorter-timed samples would have been higher than that for the 8 hour samples, but these values were not in the database. Because of the intense irritation caused by acrolein, the ACGIH recommends neither an 8 hour nor a 15-minute STEL, but, rather, a ceiling of 100 ppb [250 µg/m^3] that should never be exceeded. [The Working Group noted that, while none of the OSHA samples contained detectable levels of acrolein after such short exposures, the higher concentrations clearly indicated that this recommendation was exceeded for many samples.] Of the samples with detectable levels of acrolein, 40% exceeded the EU OEL of 20 ppb [50 µg/m^3], and half of these samples contained acrolein at more than twice that OEL (Table 1.4).

The highest acrolein concentration reported was from samples collected in late 2018 at a company that manufactured plastic pipes and pipe fittings. Four workers wore the sampling equipment for 90–180 minutes and their exposure concentrations were less than detectable, 17, 25, and 240 ppb [39, 57, and 550 µg/m^3] (sampling times were 90, 140, 180, and 170 minutes, respectively). Only 17% of the 134 personal samples collected at approximately three dozen plastic-manufacturing establishments were above the limit of detection. Those samples that were detectable ranged from 3 to 240 ppb [7 to 550 µg/m^3] acrolein, with an average of 39 ppb [89 µg/m^3] and a median of 21 ppb [48 µg/m^3]; one 24-minute sample averaged 69 ppb [158 µg/m^3], above the EU STEL of 50 ppb [120 µg/m^3] (Table 1.4; OSHA, 2020).

Over half of the personal samples collected from food production workers had detectable concentrations of acrolein, and both the mean and median values of those samples (29 and 25 ppb [66 and 57 µg/m^3], respectively) exceeded the EU OEL of 20 ppb [50 µg/m^3] (OSHA, 2020).

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

Acrolein is a severe irritant to the eyes, mucous membranes, and the respiratory tract at concentrations lower than 1 ppm, and this is the basis for OELs. At higher concentrations, acrolein can cause pulmonary oedema and death (10 ppm; 23.3 mg/m^3) (ATSDR, 2014; ACGIH, 2019).

In 1946, the ACGIH recommended that 8-hour TWA exposure to acrolein should not

exceed 0.5 ppm [1100 µg/m³]. This value was lowered to 0.1 ppm [230 µg/m³] in 1963. In 1976, a STEL of 0.3 ppm [690 µg/m³] was added to this recommendation, and in 1998 both the TWA and the STEL were replaced by a ceiling value of 0.1 ppm [230 µg/m³] that should not be exceeded for any duration. These ACGIH Threshold Limit Values® were intended as recommendations to industrial hygienists but have been adopted by many countries as OELs directly, by reference, or as the basis upon which national OELs were developed. Currently the EU has an 8-hour TWA OEL of 0.02 ppm or 0.05 mg/m³ and a STEL of 0.05 ppm or 0.12 mg/m³ (European Commission, 2017). Within the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) registration of acrolein, the derived no-effect level (DNEL) of long-term exposed workers was set at 0.2 mg/m³ for both local and systemic effects, and the DNEL for long-term skin exposure at 0.08 mg/kg body weight (bw) per day (ECHA, 2020).

Table 1.5 presents the OELs for various countries. Many countries use the EU OEL of 0.02 ppm [0.05 mg/m³], or the older ACGIH OEL (TWA, 0.1; STEL, 0.3) or the current ACGIH ceiling value of 0.1 ppm [0.23 mg/m³].

(b) Environmental exposure limits

The US EPA reference concentration for inhalation exposures is 2×10^{-5} mg/m³, and the reference dose for oral exposures is 0.5 µg/kg per day (US EPA, 2003). The US Agency for Toxic Substances and Disease Registry (ATSDR) set the minimal risk level for ingestion of acrolein at 4 µg/kg per day for 15–364 days on the basis of forestomach squamous epithelial hyperplasia in mice (ATSDR, 2007). The International Programme on Chemical Safety tolerable intake levels are 0.17 ppb [0.4 µg/m³] for inhalation exposures and 1.5 µg/mL (corresponding to 7.5 µg/kg bw per day) for drinking-water exposures (IPCS, 1992).

For subchronic exposures, e.g. 8 hours, environmental guidelines were 0.03–4.8 ppb [0.07–11 µg/m³], whereas OELs were 20–100 ppb [0.05–0.23 mg/m³], although some guidelines suggested ceiling values of 100 ppb [230 µg/m³] that should never be exceeded.

The occupational guidelines for acute exposures (50–100 ppb [120–250 µg/m³]) are approximately 10–100 times the environmental guidelines for acute exposures. Acute Exposure Guideline Levels (AEGLs) have been established for acrolein (National Research Council, 2010). The lethal level of exposure (AEGL-3) is reached after 10 minutes of exposure to acrolein at 6.2 ppm [14 000 µg/m³], whereas exposure to acrolein for any duration from 10 minutes to 8 hours at 30 ppb [69 µg/m³] leads to slight eye irritation and discomfort.

Table S1.3 (Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602) presents some guidelines for acrolein concentrations in the air.

1.5.2 Reference values for biological monitoring of exposure

A metabolite of acrolein (the mercapturic acid HPMA) has been measured as an indicator of exposure. The German Committee for the determination of occupational exposure limits (the "MAK-Commission") suggests a biological reference value for workplace substances (BAR) for HPMA of 600 µg/g creatinine in the urine in non-smokers (Jäger, 2019).

1.6 Quality of exposure assessment in key epidemiological studies

Table S1.4 and Table S1.5 (Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602) provide a detailed overview and critique of the methods used for exposure assessment in cancer epidemiology studies and mechanistic studies in humans that have been included in the evaluation of acrolein. Methods for the exposure assessment varied according to type of study. In the cancer studies in humans, two occupational cohort studies assigned exposure to acrolein, on the basis of expert evaluation of company

Table 1.5 Occupational exposure limits for acrolein in various countries

Country or agency	8-hour TWA		Short-term (15 minutes)		Ceiling		Reference
	ppm	mg/m³	ppm	mg/m³	ppm	mg/m³	
Argentina					0.1	0.23	ACGIH (2019), IOHA (2018)
Australia	0.1	0.23	0.3	0.69			IFA (2020)
Austria	0.02	0.05	0.05	0.12			IFA (2020)
Belgium	0.02	0.05	0.05	0.12			IFA (2020)
Brazil					0.1	0.23	ACGIH (2019)
Canada – Ontario					0.1		Government of Ontario (2020)
Canada – Québec	0.1	0.23	0.3	0.69			IFA (2020)
Chile					0.1	0.23	ACGIH (2019), IOHA (2018)
China						0.3	IFA (2020)
Columbia					0.1	0.23	ACGIH (2019), IOHA (2018)
Denmark	0.02	0.05	0.04	0.1			IFA (2020)
European Union	0.02	0.05	0.05	0.12			IFA (2020)
Finland	0.02	0.05	0.05	0.12			IFA (2020)
France	0.02	0.05	0.05	0.12			IFA (2020)
Germany – AGS	0.09	0.2	0.18	0.4			IFA (2020)
Hungary		0.23		0.23			IFA (2020)
India	0.1	0.25	0.3	0.8			Government of India (2015)
Ireland	0.02	0.05	0.05	0.12			IFA (2020)
Japan – JSOH	0.1	0.23					IFA (2020)
Latvia	0.02	0.05	0.05	0.12			IFA (2020)
Mexico					0.1	0.23	ACGIH (2019), IOHA (2018)
New Zealand	0.1	0.23					IFA (2020)
Poland		0.05		0.1			IFA (2020)
Romania	0.02	0.05	0.05	0.12			IFA (2020)
Singapore	0.1	0.23	0.3	0.69			IFA (2020)

South Africa	0.1	0.25	0.3	0.8			South Africa Department of Labour (1995)
Republic of Korea	0.1	0.25	0.3	0.8			IFA (2020)
Spain	0.02	0.05	0.05	0.12			IFA (2020)
Sweden	0.02	0.05	0.05	0.12			IFA (2020)
Switzerland	0.02	0.05	0.05	0.12			IFA (2020)
USA – ACGIH					0.1	0.23	ACGIH (2019)
USA – NIOSH	0.1	0.25	0.3	0.8			IFA (2020)
USA – OSHA	0.1	0.25					IFA (2020)

Table 1.5 (continued)

Country or agency	8-hour TWA		Short-term (15 minutes)		Ceiling		Reference
	ppm	mg/m³	ppm	mg/m³	ppm	mg/m³	
USA – Cal/OSHA					0.1	0.25	State of California (2020)
United Kingdom	0.02	0.05	0.05	0.12			IFA (2020)
Venezuela					0.1	0.23	ACGIH (2019), IOHA (2018)

ACGIH, American Conference of Governmental Industrial Hygienists; AGS, Ausschuss für Gefahrstoffe (German Committee on Hazardous Substances); Cal/OSHA, California Division of Occupational Safety and Health; JSOH, Japan Society for Occupational Health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; ppm, parts per million; TWA, time-weighted average.

records on the use of chemicals and also on job history information. No quantitative exposure assessment methods were applied. Other studies used internal markers of exposure to acrolein, based on urinary acrolein metabolites (HPMA), acrolein–DNA adducts from buccal cells, or analyses of acrolein–protein conjugates in serum samples (see Section 4.2.1 for further discussion of acrolein-derived DNA and protein adducts). In these studies, some information on possible external sources of exposure (e.g. smoking, betel-quid chewing, air pollution) was collected through questionnaires. The mechanistic studies in humans showed a partial overlap with the cancer studies in humans, applying internal markers of exposure showing similar limitations regarding assessment of external exposure.

1.6.1 Quality of exposure assessment in key cancer epidemiology studies

Two studies of occupational exposure identified workers exposed to acrolein using information from available records (Bittersohl, 1975; Ott et al., 1989a). No quantitative assessment of exposure was carried out in these studies.

Bittersohl (1975) investigated cancer fre - quency in an aldehyde factory in Germany and reported that the derivatives produced contained traces of acrolein; however, no evidence was provided that this resulted in any exposure of the workforce to acrolein. Employees were exposed to other chemicals at higher levels than acrolein.

Ott et al. (1989a) investigated risk of lymphohaematopoietic cancer in a complex chemical-manufacturing facility in the USA and assessed the potential for exposure of workers to 21 specific chemicals, including acrolein. Workers were assigned as having been exposed to acrolein if they worked in an area where acrolein was used for 1 day or more. This assessment was based on linking information on job histories with records that contained information on the historical use of chemicals in each department. Intensity of exposure was not assessed, but duration of exposure was estimated. There was no evidence provided of the airborne levels of acrolein in these production facilities. Among 200 production workers, 25 (12.5%) were judged to have been exposed to acrolein for at least 1 day and 3% were exposed to acrolein for 5 years or more (Ott et al., 1989b). Workers were likely to be exposed simultaneously to other chemical agents.

Four other studies assessed exposure to acrolein using internal markers. Yuan et al. (2012, 2014) estimated exposure to acrolein in two lung cancer case–control studies of smokers and non-smokers, respectively, nested within a cohort study of men in Shanghai, China. A single void urine sample was collected from each participant at baseline and analysed to determine the concentration of HPMA, and a range of other urinary biomarkers (including cotin - ine). Information on smoking was available. No assessment of external exposure to acrolein was carried out.

Tsou et al. (2019) investigated the role of acrolein in oral cancer and estimated exposure to acrolein through analyses of urinary HPMA and of acrolein–DNA adducts in buccal cells collected from cases and controls in Taiwan, China. Information was also collected on smoking history and betel-quid chewing. Buccal cells and urine samples were collected after diagnosis. There was no statistically significant difference in buccal acrolein–DNA adduct levels between healthy controls with different smoking and betel-quid chewing histories. The urinary HPMA concentration was statistically significantly correlated with smoking years and betel-quid chewing years. [The Working Group noted that it was not clear from the data to what extent the levels of buccal

acrolein–DNA adducts and urinary HPMA levels are representative of historical exposure attributable to smoking and betel-quid chewing. The Working Group was not certain whether acrolein–DNA adducts can be considered as a marker of exposure or effect, particularly since samples were collected and analyses carried out after diagnosis.]

Finally, Hong et al. (2020) investigated the role of endogenous exposure to acrolein in a case–control study of urothelial carcinoma patients with chronic kidney disease and healthy controls in Taiwan, China. Endogenous exposure to acrolein was estimated using acrolein–DNA adducts in DNA from tumour or normal urothelial cells, HPMA in urine, and acrolein–protein conjugates in serum samples. [The Working Group noted that information on smoking and air pollution was collected, but these exposures were considered only as confounders in the analyses. The Working Group was not certain whether acrolein–DNA adducts can be considered as a marker of exposure or effect. Markers of acrolein exposure were estimated in samples collected from cases and controls after diagnosis; hence it is not clear whether endogenous exposure to acrolein preceded tumour development or was a consequence of the urothelial carcinoma.]

1.6.2 Quality of exposure assessment in mechanistic studies in humans

Common elements of the human mechanistic studies were their cross-sectional nature, the small sample size (typically 10–20 participants) and the method-development design (e.g. to facilitate and optimize the measurement of certain acrolein adducts in various human tissues) (e.g. Nath & Chung, 1994; Chen & Lin, 2011; Alamil et al., 2020).

The majority of the studies investigated smokers (mainly relying on self-reports), assuming that smoking is the predominant source of exposure to acrolein in humans (Nath et al., 1998; Zhang et al., 2007; Bessette et al., 2009; Zhang et al., 2011; Weng et al., 2018; Yang et al., 2019b). Tsou et al. (2019) included other factors besides smoking, such as alcohol consumption or betel-quid chewing (also see Section 1.6.1 above for a detailed critique of Tsou et al. (2019) in the Acrolein context of studies of cancer in humans). Wang et al. (2019) included fried food consumption in non-smokers, but insufficiently defined other external exposures.

Another large subset of studies investigated acrolein adducts in tumour tissues without considering any potential external exposure of the patients (Liu et al., 2005; Chen & Lin, 2011; Chung et al., 2012; Yin et al., 2013; Lee et al., 2014; Fu et al., 2018). Hence it is not clear whether external or internal exposure caused adduct formation, or adduct formation was a consequence of tumour development.

Several studies researched treatment with cyclophosphamide or other medicinal products of which acrolein is the principal metabolite (McDiarmid et al., 1991; Al-Rawithi et al., 1998; Takamoto et al., 2004). While external exposure attributable to the medicines is well characterized, all these studies failed to consider other external exposures except smoking.

Interestingly, endogenous exposure was not defined or assessed in most of the studies. Yang et al. (1999b) suggested that both endogenous and exogenous sources may contribute to the formation of acrolein–DNA adducts. [The Working Group noted that it was unclear whether the background exposure comes from endogenous formation or from a low external exposure such as air pollution, secondhand smoke, or consumption of fried food.] Noteworthy regarding endogenous exposure is the study of Ruenz et al. (2019), which placed non-smoking participants in defined living conditions, adhering to a defined diet, and which

provided convincing evidence for substantial background exposure to acrolein that was independent of smoking, ingestion of heat-processed food, or other nearby environmental exposures such as exhaust gases or open fires.

2. Cancer in Humans

2.1 Descriptions of individual studies

See Table 2.1.

Six studies – one cohort study, two case–control studies, and three nested case–control studies in cohorts – have been published on the relationship between cancer and exposure to acrolein. Five other studies (mainly case reports) described bladder cancers or leukaemia occurring after use of the pharmaceutical cyclophosphamide (classified in IARC Group 1, *carcinogenic to humans*) or ifosfamide to treat cancer or autoimmune disease. These studies on pharmaceutical agents were determined by the Working Group to be uninformative because the role of acrolein in causing these cancers could not be distinguished from that of other metabolites. The quality of the exposure assessment in the six studies described below is detailed in Section 1.6.

Bittersohl (1975) reported on a small cohort of 220 workers exposed to multiple aldehydes or aldehyde derivatives including acrolein (in trace amounts) in a factory in the former German Democratic Republic, who were followed up from 1967 to 1972. There were 9 cases of cancer in men (5 squamous cell lung carcinomas, 2 squamous cell carcinomas of the oral cavity, 1 adenocarcinoma of the stomach, and 1 adenocarcinoma of the colon) and 2 cases in women (1 leukaemia and 1 cancer of ovary). There was no formal comparison group except a narrative comparison with incidence rates in the general population, source unspecified. [The Working Group noted that although cancer rates were reported to be higher in the cohort than in the population of the German Democratic Republic, the study did not quantify any excess, nor specify the population rate in the German Democratic Republic. Exposure was poorly defined, and no attempt was made to assess exposure (semi-) quantitatively by measurements of duration. No

inference can be made regarding the association between acrolein exposure and cancer risk.]

In an occupational nested case–control study among male chemical workers in the USA, Ott et al. (1989a) reported on 129 workers who died from lymphohaematopoietic cancer and their controls (matched on hire decades), with the time scale being time since hire. Information on multiple chemical exposures was available (Ott et al., 1989b), with expert assessment of individual exposures based on jobs, including acrolein. Positive associations between acrolein exposure and non-Hodgkin lymphoma (NHL), multiple myeloma, and leukaemia were reported, based on small numbers of exposed cases (n = 6). Given the small sample size and multiple exposures, no inference was possible. [The Working Group noted that matching was based on hire decades. Implications for potential bias were not discussed in the paper. In addition, the exposure assessment was insufficient, limited to dichotomous (ever/never) classification, based on production records and not measured exposure, and exposure encompassed multiple chemicals in addition to acrolein.]

Yuan et al. (2012, 2014) published the results of two nested case–control studies within a cohort of 18 244 Chinese men enrolled in 1986–1989 in Shanghai, China. Besides in-person interviews, a spot urine sample was taken from each

participant at baseline and stored until laboratory analysis. Incident cases of and deaths from lung cancer were identified through annual in-person interviews of all surviving participants, the local cancer registry, and the vital statistics office. The first study (Yuan et al., 2012) was a nested case– control study on lung cancer, limited to current smokers at enrolment, and based on follow-up through 2006. Urinary biomarkers related to smoking habits were measured at enrolment, including HPMA (an acrolein-derived, mercapturic acid metabolite), NNAL, cotinine and others. Overall, 343 cases and 392 controls were included in the analysis, after exclusion of

Table 2.1 Epidemiological studies of cancer in humans exposed to acrolein

Reference, location, enrolment/ Exposed level	Population size, description, exposure assessment method deaths follow-up period, study design	Cancer type	Exposure category or	cases or	Risk estimate	Covariates	Comments (95% CI) controlled
Bittersohl (1975) Former German Democratic Republic 1967–1972 Cohort	220 workers in the chemical industry for dimerization of aldehydes. Workers were exposed to acetaldehyde, crotonaldehyde, butyraldehyde, and/or acrolein (traces). Exposure assessment method: records; exposure was assumed based on employment within the aldehyde factory	Lung (squamous cell carcinoma), incidence	Men: NR	5	NR	None	*Exposure assessment critique*: Poorly defined exposure. No evidence of acrolein exposure provided. No separate exposure assessment for different chemical agents present in the factory. *Limitations*: no inference possible for lack of comparator.
		Oral cavity, incidence	Men: NR	2	NR	None	
		Stomach, incidence	Men: NR	1	NR	None	
		Colon, incidence	Men: NR	1	NR	None	
		Leukaemia, incidence	Women: NR	1	NR	None	
		Ovary, incidence	Women: NR	1	NR	None	

Table 2.1 (continued)

Reference, location, period, study design	Population size, description, enrolment/ cases or deaths	Cancer type or category	Exposure level	Exposed	Risk estimate (95% CI)	Covariates controlled	Comments
Ott et al. (1989a) USA 1940–1978 Nested case–control	Cases: 52 cases of NHL, 20 cases of multiple myeloma, 39 cases of nonlymphocytic leukaemia, 18 cases of lymphocytic leukaemia; 129 deaths from lymphohaematopoietic cancers; in two chemical manufacturing plants; 29 139 men in the cohort Controls: 5 controls randomly selected per case (N not reported); incidence sampling design from the cohort. Exposure assessment method: expert judgement; 1020 substances, including acrolein, associated with different working areas; exposure was assumed based on whether a chemical substance was used at all in a production unit; no assessment of the intensity of exposure or estimation of cumulative exposure	NHL, mortality	Acrolein exposure (OR): Never Ever	 NR 2	 NR 2.6	Decade of hire (by matching in design)	*Exposure assessment critique*: No (semi-) quantitative exposure assessment carried out. Exposure was assumed based on assignment to production unit within factory. Exposure was not based on measurement of personal exposure. 21 chemicals were included, and workers are likely exposed to multiple agents (see Ott et al., 1989b). *Limitations* : cases had died, controls alive; small number of subjects exposed to acrolein.
		Multiple myeloma, mortality	Acrolein exposure (OR): Never Ever	 NR 1	 NR 1.7		
		Nonlymphocytic leukaemia, mortality	Acrolein exposure (OR): Never Ever	 NR 3	 NR 2.6		

Table 2.1 (continued)

Reference, location, enrolment/ period, study design	Population size, description, category or level	Cancer type or cases or deaths	Exposure assessment method	Exposed cases or controls	Risk estimate (95% CI)	Covariates controlled for follow-up	Comments
Yuan et al. (2012) Shanghai, China enrolment, 1986–1989/ follow-up, 2006 Nested case– control	Cases: 343 cases of incident lung cancers and deaths, current smokers at enrolment, identified through annual in-person interviews and reviewed through Shanghai Cancer Registry and Shanghai Municipal Vital Statistics Office; lung cancer cases and matched controls from within a cohort of 18 244 Chinese men in Shanghai	Lung, incidence	Quartile of urinary HPMA (l creatinine), current smokers (OR):		1.39 (0.86–2.23)	Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline	*Exposure assessment critique*: Internal exposure assessment only. All study subjects were smokers. Smoking history collected and included in the models. Urine samples were collected at baseline, so clearly preceded the health outcome; however, only one urine sample was collected. *Strengths*: study design; relatively large sample and long follow-up (20 yr); few losses to follow-up (4.6%); urinary biomarker was collected before disease occurrence; self-reported smoking status was verified by urinary cotinine. *Limitations*: intraindividual variation in exposure not captured; 35% of cases were not histologically confirmed.
			First quartile	49	1.60 (1.00–2.58)		
			Second quartile	74	2.00 (1.25–3.20)		
			Third quartile	92			
			Fourth quartile	128	(pmol/mg rs ıt enrolment		
			Trend-test *P* value, 0.004				
	Controls: 392 participants in the Shanghai Cohort Study; one control was selected from the same cohort, current smoker at enrolment, alive and free of cancer and matched to the index case by age (± 2 yr), date of specimen collection (± 1 month) and neighbourhood of residence at enrolment. Exposure assessment method: exposure to acrolein was determined based on measurement of urinary metabolites of acrolein (HPMA); urine samples were collected at baseline survey of the cohort, in which the case– control study was nested; smoking information was also collected	Lung, incidence	Quartile of urinary HPMA creatinine), current smoke (OR):	1	1.26 (0.77–2.05)	Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline, urinary total NNAL and PheT	
			First quartile	49	1.38 (0.84–2.26)		
			Second quartile	74	1.64 (1.01–2.66)		
			Third quartile	92			
			Fourth quartile	128			
			Trend-test *P* value, 0.046				

Table 2.1 (continued)

Reference, location, exposure assessment method	Population size, description, category or cases or deaths	Cancer type	Exposure level	Exposed (95% CI)	Risk estimate	Covariates controlled	Comments
Yuan et al. (2012) (cont.)		Lung, incidence	Quartile of urinary HPMA (pmol/mg creatinine), current smokers at enrolment (OR):			Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline, urinary total NNAL and PheT, total cotinine	
			First quartile	49	1		
			Second quartile	74	0.98 (0.59–1.65)		
			Third quartile	92	1.02 (0.61–1.72)		
			Fourth quartile	128	1.06 (0.62–1.8)		
			Trend-test *P* value, 0.772				
		Lung (squamous cell carcinoma), incidence	Quartile of urinary HPMA (pmol/mg creatinine), current smokers at enrolment (OR):			Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline	
			First tertile	NR	1		
			Second	NR	NR tertile		
			Third tertile	NR	2.56 (1.30–5.05)		
			Trend-test *P* value, < 0.05				
		Lung (squamous cell carcinoma), incidence	Quartile of urinary HPMA (pmol/mg creatinine), current smokers at enrolment (OR):			Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline, total cotinine	
			First tertile	NR	1		
			Second	NR	NR tertile		
			Third tertile	NR	NR		
			Trend-test *P* value: > 0.10				

Table 2.1 (continued)

Reference, location	Population size, description, period, study design	Cancer type	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments enrolment/ follow-up, exposure assessment method
Yuan et al. (2014) Shanghai, China enrolment, 1986–1989/ follow-up, 2008 Nested case–control	Cases: 82 cases of incident lung cancer in men, lifelong non-smokers aged 45–64 yr at enrolment; Shanghai Cohort Study consisted of 18 244 men (80% of eligible) who were aged between 45 and 64 yr at enrolment in 1986–1989 and resided in one of four small geographically defined communities in Shanghai, China. Controls: 83 members of the Shanghai Cohort Study without cancer, non-smokers and alive at the time of cancer diagnosis of the case; matched by age at enrolment (± 2 yr), year and month of urine sample collection (± 1 month) and neighbourhood of residence at recruitment. Exposure assessment method: exposure was determined based on measurement of urinary metabolites of acrolein (HPMA); urine samples were collected at baseline survey of the cohort, in which the case–control study was nested; there was no assessment of external exposure	Lung, incidence	Quartile of urinary HPMA, never smokers (OR):			Age at baseline, neighbourhood of residence at enrolment, years of sample storage and urinary cotinine level	*Exposure assessment critique*: Internal exposure assessment only. No evidence of external exposure. Smokers were excluded. Urine samples were collected at baseline, so clearly preceded the health outcome; however, only one urine sample at baseline was collected. *Strengths*: active follow-up with annual in-person interviews; after 22 yr loss to follow-up low, only 5%; urinary cotinine was also quantified to confirm non-smoking status. *Limitations*: relatively small sample size; 26% of cases not histologically confirmed; small number of cases of squamous cell cancer ($n = 16$); intraindividual variation in exposure not captured.
			First quartile	21	1		
			Second quartile	19	0.97 (0.40–2.34)		
			Third quartile	19	0.98 (0.40–2.36)		
			Fourth quartile	21	1.13 (0.47–2.75)		
			Trend-test *P* value, 0.79				

Table 2.1 (continued)

Reference, location, enrolment/ period, study design	Population size, description, category or cases or deaths	Cancer type level	Exposure (95% CI)	Exposed controlled	Risk estimate follow-up	Covariates	Comments
Tsou et al. (2019) Taiwan, China 2016–2018 Case–control	Cases: 97 cases of cancer of the oral cavity; hospital-based Controls: 230 healthy controls, not further described Exposure assessment method: questionnaire; information on smoking and betel-quid chewing history was collected during interviews with participants or relatives; urine samples were analysed for HPMA; acrolein–DNA adducts were measured in buccal cells or tumour tissues	Oral cavity, incidence	Acrolein–DNA adduct: Controls Ratio of vs controls	222 80	1 1.4 ($P < 0.001$) cases	None	*Exposure assessment critique*: Acrolein–DNA adducts and urinary HPMA were measured in samples from cases and controls; however, it is not clear to what extent acrolein–DNA adducts are a marker of effect, or of exposure. Both were measured at the time of cancer treatment (and similar period for controls). The authors indicated that urinary HPMA was significantly correlated with smoking history but no correlation coefficient was given and the correlation appeared weak in the graph. Acrolein–DNA adduct levels were higher in the tumour tissues than in the buccal swabs, but HPMA levels were lower. There was no indication whether and for how long the cases had stopped smoking or chewing before their samples were collected. *Strengths*: DNA adducts in buccal swabs for exposure assessment. *Limitations*: small sample size; controls not described.
		Oral cavity, incidence	Acrolein–DNA, cigarette smokers + betelquid chewers: Controls Ratio of vs controls	101 51	1 1.3 (P < 0.05) cases	None	
		Oral cavity, incidence	Acrolein–DNA, cases: Buccal tissue Ratio of tissue to buccal tissue	NR NR	1.8 ($P < 0.01$) tumour	None	

Table 2.1 (continued)

Reference, location	Population size, description, period, study design	Cancer type	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates	Comments	Exposure assessment method
Tsou et al. (2019) (cont.)		Oral cavity, incidence	Mean urinary HPMA (μmol/g creatinine): Controls, all	230	7.1	None		
			Controls, cigarette smokers only	111	5.8			
			Controls, betel-quid chewers only	12	3.6			
			Controls, cigarette smokers and betel-quid chewers	107	8.9			
			Cases	97	0.7 ($P < 0.001$, compared with all controls)			
Hong et al. (2020) Taiwan, China 2016–2019 Case–control	Cases: 62 cases of urothelial cancer; hospital-based, Taiwan, China; patients with CKD; no treatment with cyclofosfamide or ifosfamide Controls: 43 healthy controls; Biomarkers CKD or other diseases. Controls had not related to	Urinary bladder (urothelial cancer), incidence	Acrolein–DNA, cases: Normal urothelial cells	62	1	None	*Exposure assessment critique*: Only considered endogenous exposure due to kidney failure. External exposure to smoking was considered but only as a not described but did not have compared confounder.	
			Ratio in tumour cells with normal cells	62	1.2 ($P < 0.001$)			
			Acrolein–DNA, non-smoking cases: Normal urothelial cells	48	1	None		
			Ratio in tumour cells with normal cells	48	1.2 ($P < 0.001$)			

Reference, location	Population size, description, period, study design	Cancer type	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates	Comments	Exposure assessment method
	questionnaire; exposure of interest was endogenous exposure considered.	Urinary bladder (urothelial cancer), urothelial	Acrolein–DNA, non-smoking cases: Normal urothelial cells	48	1	None	higher levels of HPMA. Air pollution was to chronic kidney failure, HPMA. No other external exposure to acrolein due	
			Ratio in tumour cells with normal cells	48	1.2 ($P < 0.001$)			
			Acrolein–DNA, smoking cases: Normal urothelial cells	14	1	None		
			Ratio in tumour cells with normal cells	14	1.2 ($P < 0.001$)			
			Acrolein–DNA, cases: Normal urothelial cells	62	1	None		
			Ratio in tumour cells with normal cells	62	1.2 ($P < 0.001$)			
			Acrolein–DNA, non-smoking cases: Normal urothelial cells	48	1	None		
			Ratio in tumour cells with normal cells	48	1.2 ($P < 0.001$)			
			Acrolein–DNA, smoking cases: Normal urothelial cells	14	1	None		
			Ratio in tumour cells with normal cells	14	1.2 ($P < 0.001$)			

Table 2.1 (continued)

Reference, location, enrolment/ exposure assessment method	Population size, description, category or cases or controls	Cancer type level	Exposure deaths	Exposed follow-up	Risk estimate (95% CI)	Covariates	Comments
		measured through Acr–PC, acrolein–DNA adducts, acrolein, but it is not if endogenous acrolein contribute to higher levels measured, but not	Ratio in information on smoking and Smoking did not appear to Urinary bladder (urothelial Controls incidence	48 43	1.2 ($P < 0.001$) 0.26	were appropriate for and by HPMA in urine; clear from the results air pollution was collected. compared with normal cells levels are a result or a consequence of urothelial cancer), Cases of acrolein–DNA adducts or GST activity.	tumour cells
		Urinary bladder (urothelial Controls incidence	Mean plasma Acr–PC (mM) 43		0.26	None carcinomas. GSH levels Acr–PC	*Strengths*: measurement of specific DNA adducts cancer), Cases with early-stage CKD
				37	0.51 ($P < 0.001$)	were	
				25	0.48 ($P < 0.001$)	by acrolein and *TP53* mutations.	
were				12	0.56 ($P < 0.001$)	*Limitations*not described; small sample : controls	size; there is a serious flaw in the disproportion of non-smokers: all controls and 79% of cases.

Table 2.1 (continued)

Reference, location, exposure assessment method	Population size, description, enrolment/ follow-up	Cancer type	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments

cases and controls for whom urine samples were depleted or values for one or more mercapturic acid metabolites were missing. One control per case was selected from among cohort members who were current smokers at enrolment, free of cancer, and alive at the time of the cancer diagnosis of the index case, and further matched on age at enrolment, date of biological specimen collection, and neighbourhood of residence at recruitment. Comparing the highest with the lowest quartiles, risk of lung cancer associated with HPMA levels doubled in models adjusting for matching factors and number of cigarettes smoked per day and years of cigarette smoking at baseline. In models with further adjustment for metabolites of polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines (NNAL) and/or cotinine, no association was found between HPMA and lung cancer. [The Working Group noted that there were multiple correlated exposures (biomarkers). Strengths of the study included: a relatively large sample and long follow-up (20 years); few losses to follow-up (4.6%); urinary biomarkers collected before disease occurrence; and self-reported smoking status verified by urinary cotinine. The 2-fold increase in risk of lung cancer was associated with the highest quartile of HPMA concentration, adjusted for only intensity and duration of smoking. However, this effect disappeared with further adjustment for other smoking biomarkers, indicating that acrolein represented a biomarker of smoking. The Working Group judged that this study was uninformative for an evaluation of the carcinogenicity of acrolein.]

The second study (Yuan et al., 2014) had a similar study design but extended follow-up through 2008 and included only never-smokers at baseline (82 cases of lung cancer and 83 controls; same design as in the Yuan et al., 2012). The same urinary biomarkers as in the previous paper were measured. There was no association between quartile of urinary HPMA concentration and lung cancer in never-smokers (fourth quartile versus first quartile: OR, 1.13; 95% CI, 0.47–2.75) in analysis adjusting for matching factors and urinary cotinine level. [The Working Group noted that only internal exposure was assessed, and since the participants were all non-smokers, the source of external exposure to acrolein was unclear. The Working Group also noted that urinary cotinine represents a shortterm biomarker of passive smoking and therefore may not fully adjust for long-term secondhand smoke exposure.]

Tsou et al. (2019) measured acrolein–DNA adducts in buccal swabs from patients (n = 97) with cancer of the oral cavity. Acrolein–DNA adducts were also measured in buccal swabs from 230 healthy controls. Additionally, HPMA and NNAL were measured in the urine of the same 97 patients with cancer of the oral cavity and 230 healthy controls. For the patients with cancer, Tsou et al. (2019) also compared DNA– adduct levels in cancer biopsies with those in adjacent normal tissue collected from buccal swabs. Levels of acrolein–DNA adducts in buccal cells were 1.4 times higher in cases than in controls ($P < 0.001$). The ratio was 1.3 among smokers and betel-quid chewers only ($P < 0.05$). Levels of acrolein–DNA adducts were 1.8 times higher in cancer biopsy specimens than in buccal swabs from adjacent normal tissue ($P < 0.01$). However, there was no significant difference in levels of acrolein–DNA adducts among healthy controls with different cigarette smoking or betel-quid chewing histories. Smoking and betelquid chewing were associated with significantly higher levels of HPMA. Levels of urinary HPMA were lower among cases (0.7 µmol/g creatinine) than among controls (7.1 µmol/g creatinine) ($P < 0.001$), with a similar difference observed when only smokers and chewers were considered. There was no

adjustment for covariates. [The Working Group noted that, overall, the paper suggests that HPMA (but not acrolein–DNA adducts) is associated with smoking and betelquid chewing, and acrolein–DNA adducts are associated with oral cancer (cross-sectionally). There were lower levels of HPMA in the urine of cases than in controls (irrespective of smoking/ chewing status). The cross-sectional nature of the study and the fact that specimens were collected after cancer diagnosis in cases make causal inference difficult.]

Hong et al. (2020) in a case–control study in Taiwan, China, included 62 patients with urothelial carcinoma and 43 healthy controls. All cases and none of the controls had chronic kidney disease (CKD), the rationale being that CKD patients have a high risk of bladder cancer and altered metabolism that increases susceptibility to chemical exposures. Urinary HPMA, plasma acrolein–protein conjugates, DNA adducts formed by acrolein, and *TP53* mutations in frozen tissue samples were measured. Tumour biopsies showed levels of acrolein–DNA adducts that were 1.2 times higher than those in adjacent normal tissue in urothelial carcinoma patients overall ($P < 0.005$). The same ratio and *P* value were also found in cases and controls who were non-smokers. Levels of acrolein–DNA adducts were correlated with CKD severity. Also, levels of plasma acrolein–protein conjugates were twice as high in cases as in controls ($P < 0.001$). Similar results were observed for acrolein–protein conjugates in plasma in study participants with different degrees of severity of CKD and in non-smokers. Urinary HPMA levels were lower in cases (0.83 µmol/g creatinine) than in controls (1.16 µmol/g creatinine) ($P = 0.023$), this observation being attributed to binding of HPMA to glutathione (GSH) as a cellular defence mechanism. [The Working Group noted that controls were not described, and cases were all affected by CKD. The only endogenous exposure considered was due to kidney failure, while external exposure to smoking was considered only as a confounder. The study also had a small sample size, considerable age difference between cases and controls, and short follow-up period. There appeared to be a disproportionate number

of non-smokers included: all controls and 79% of cases.]

2.2 Evidence synthesis for cancer in humans

The epidemiological evidence available on acrolein in relation to cancer in humans included one occupational cohort study (Bittersohl, 1975), three nested case–control studies in occupational or population-based cohorts (Ott et al., 1989a; Yuan et al., 2012, 2014), and two hospital-based case–control studies (Tsou et al., 2019; Hong et al., 2020). There was little consistency in the cancer sites evaluated across these studies, with studies variously examining cancers of the lung (Bittersohl, 1975; Yuan et al., 2012, 2014), oral cavity (Bittersohl, 1975; Tsou et al., 2019), bladder (Hong et al., 2020), or lymphohaematopoietic cancers (Ott et al., 1989a).

2.2.1 Exposure assessment

The quality of the exposure assessment carried out within the available studies was of concern, as detailed in Section 1.6. For the studies that considered occupational exposure to acrolein (Bittersohl, 1975; Ott et al., 1989a, b), no quantitative exposure assessment was carried out, and therefore no exposure–response analyses could be performed. In addition, study participants were simultaneously exposed to multiple,

undifferentiated chemical agents, reducing the informativeness of a comparison of cancer risk between exposed and unexposed groups.

The remaining studies investigated acrolein mainly from a mechanistic point of view and looked at urinary metabolites (mercapturic acids) (Yuan et al., 2012, 2014; Tsou et al., 2019; Hong et al., 2020), acrolein–DNA adducts (Tsou et al., 2019; Hong et al., 2020), and/or acrolein–protein conjugates measured in serum (Hong et al., 2020). These studies did not consider external exposure to acrolein explicitly. Although information on smoking was available in some studies and may have been an important source of acrolein exposure, these studies adjusted for smoking through restriction or statistical adjustment (Yuan et al., 2012, 2014; Hong et al., 2020).

2.2.2 Cancers of the lung, oral cavity, and other sites

Two case–control studies (Yuan et al., 2012, 2014) nested in a population-based cohort studied several biomarkers in relation to lung cancer (one among current smokers, one among non-smokers). There was matching by smoking habits and adjustment for markers of smoking (NNAL, cotinine, and urinary HPMA) but the study did not investigate the etiological involvement of acrolein per se.

One case–control investigation (Tsou et al., 2019) studied acrolein–DNA adducts in buccal swabs of patients with cancer of the oral cavity compared with healthy controls and found higher levels in cancer cases. However, adducts were not associated with tobacco smoking or betel-quid chewing, and thus were unlikely to be markers of those exposures. Urinary HPMA (a metabolite of acrolein) was associated with smoking and betel chewing. There were lower levels of HPMA in the urine of cases than in controls (irrespective of smoking/chewing status), attributed by the authors to HPMA binding to GSH as a cellular defence mechanism.

The three other studies were considered uninformative – one occupational cohort (Bittersohl, 1975), one nested case–control study on lymphohaematopoietic cancer in an occupational cohort (Ott et al., 1989a), and a case–control study on urothelial carcinoma in patients with CKD (Hong et al., 2020) – due to small numbers, poor external exposure assessment, and flaws in design.

Taken together, these studies provide little evidence of a positive association between acrolein exposure and cancer in humans. Some of the available studies were of a mechanistic nature, i.e. they investigated the role of a urinary mercapturic acid metabolite of acrolein in smokers with null results after controlling for other smoking-related biomarkers. In other studies, the design, including external exposure assessment, was poor.

3. Cancer in Experimental Animals

In previous evaluations, the *IARC Monographs* programme concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of acrolein (e.g. IARC, 1995).

Studies of carcinogenicity with acrolein in experimental animals are summarized in Table 3.1.

3.1 Mouse

3.1.1 Inhalation

In a study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6D2F$_1$/Crlj mice (age, 6 weeks) were treated with acrolein (purity, > 98.3%; 1.42% acetaldehyde identified by GC-MS) by inhalation with whole-body exposure for 6 hours per day, 5 days per week, for up to 99 weeks. (JBRC, 2016a, b, c). The concentration in the exposure chambers was set to 0 (clean air, control), 0.1, 0.4, or 1.6 ppm (v/v) for males and females. The mean air concentrations, based on monitoring every 15 minutes, were the target values and the coefficients of variation were within 0.6%. The survival rates for all groups (including both male and female control groups) were decreased due to the development of renal lesions and/or amyloid deposition but were not affected by exposure to acrolein. When the survival rates for the male and female control groups were lower than 25%, the study was terminated by

Table 3.1 Studies of carcinogenicity with acrolein in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Full carcinogenicity Mouse, B6D2F$_1$/Crlj (M) 6 wk 93 wk JBRC (2016a, b)	Inhalation (whole-body) > 98.3% Clean air 0, 0.1, 0.4, 1.6 ppm 6 /day, 5 days/wk 50, 50, 50, 50 11, 15, 14, 15	*Nasal cavity*: adenoma Incidence: 0/50, 0/50, 0/50, 1/50 (2%) *Lymph node*: malignant lymphoma Incidence: 1/50, 3/50, 2/50, 4/50	NS NS	Principal strengths: multiple dose study; use of males and females; study complied with GLP. Historical control data in B6D2F$_1$/Crlj male mice for nasal cavity adenoma: 1/499 (0.2%; range, 0–2%); the incidence of hyperplasia of the respiratory tract (nasal cavity) was significantly increased in treated animals compared with controls; the Working Group considered hyperplasia of the respiratory tract to be a pre-neoplastic lesion.
Full carcinogenicity Mouse, B6D2F$_1$/Crlj (F) 6 wk 99 wk JBRC (2016a, b)	Inhalation (whole-body) Clean air 0, 0.1, 0.4, 1.6 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 11, 18, 14, 19	*Nasal cavity*: adenoma Incidence: 0/50, 0/50, 16/50 (32%)* *Lymph node*: malignant lymphoma Incidence: 12/50 (24%), 8/50 (16%), 6/50 (12%), 17/50 (34%) *Uterus* Principal strengths: multiple dose study; use of males and females; study complied with GLP. Histiocytic sarcoma	carcinogenicity > 98.3% *P < 0.0001, Fischer exact test; *P* < 0.0001, Peto trend test (prevalence method) and Cochran–Armitage trend test *P* = 0.0347, Cochran–Armitage trend test	Historical control data in B6D2F$_1$/Crlj female mice for malignant lymphoma: 169/500 (33.8%; range, 28–46%); uterus histiocytic sarcoma: 114/500 (22.8%; range, 18–34%); nasal cavity adenoma: 0/500; the incidence of hyperplasia of the respiratory tract (nasal cavity) was significantly increased in treated animals compared with controls; the Working Group considered hyperplasia of the respiratory tract to be a pre-neoplastic lesion.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of	Significance	Comments
Study design **(sex)** **Age at start**	**Vehicle** **Dose(s)**	**tumours**		
		Incidence: 6/50 (12%), 13/50 (26%), 14/50 (28%)*, 6/50 (12%) Endometrial stromal polyp Incidence: 1/50, 1/50, 2/50, 3/50 *Liver*: histiocytic sarcoma Incidence: 0/50, 2/50, 0/50, 3/50	*P = 0.0392, Fischer exact test NS NS	
Duration **Reference**	**No. of animals at start** **No. of surviving animals**			
Full carcinogenicity Mouse, CD-1 (M) 8 wk 18 mo Parent et al. (1991a)	Oral administration (gavage) 94.9–98.5% (hydroquinone, 0.25–0.31%) Deionized water 0, 0.5, 2.0, 4.5 mg/kg bw per day 1×/day 70, 70, 70, 75 NR	*All sites*: no significant increase in the incidence of tumours		Principal strengths: use of males and females; use of multiple doses; large number of animals per group. Principal limitations: all major tissues and gross lesions from the control and high-dose groups were examined microscopically; only the lungs, liver, kidneys, and gross lesions from the groups at the low and intermediate dose were examined microscopically; histopathological data from mice found dead or killed because moribund were to have been collected according to the protocol, but data were not reported; dosing volume not reported; trend towards reduced survival, and decreased survival in the group at the highest dose.
Full carcinogenicity Mouse, CD-1 (F) 8 wk 18 mo Parent et al. (1991a)	Oral administration (gavage) 94.9–98.5% (hydroquinone, 0–25–0.31%) Deionized water 0, 0.5, 2.0, 4.5 mg/kg bw per day 1×/day 70, 70, 70, 75 NR	*All sites*: no significant increase in the incidence of tumours		Principal strengths: use of males and females; use of multiple doses; large number of animals per group. Principal limitations: microscopic examination was reported for all major tissues and gross lesions from the control and high-dose groups, but only for lungs, liver, kidneys, and gross lesions from the groups at the low and intermediate dose; histopathological data from mice found dead or killed because moribund were to have been collected according to the protocol, but data were not reported; dosing volume not reported.

Table 3.1 (continued)

Route Study design Species, strain (sex) Age at start	Purity Vehicle Dose(s)	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) Neonatal (8 days) 12 mo Von Tungeln et al. (2002)	Intraperitoneal injection NR DMSO 0, 150 nmol Injections with one-third and two-thirds of the total dose in 30 µL DMSO at age 8 and 15 days, respectively 24, 23 24, 23	*Liver* Adenoma Incidence: 0/24, 1/23 Carcinoma Incidence: 0/24, 0/23 Adenoma or carcinoma (combined) Incidence: 0/24, 1/23 Multiplicity: 0, 2.0	 NS NA NS NR	Principal strength: use of males and females. Principal limitations: use of single dose; lack of bodyweight data; rationale for dose not given, only data regarding liver tumours were reported.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of	Significance	Comments
Study design **(sex)** **Age at start**	**Vehicle** **Dose(s)**	**tumours**		
Duration **Reference**	**No. of animals at start** **No. of surviving animals**			
Full carcinogenicity Mouse, B6C3F$_1$ (F) Neonatal (8 days) 12 mo Von Tungeln et al. (2002)	Intraperitoneal injection NR DMSO 0, 150 nmol Injections with one-third and two-thirds of the total dose in 30 µL DMSO at age 8 and 15 days, respectively 23, 24 23, 23	*Liver* Adenoma Incidence: 0/23, 0/23 Carcinoma Incidence: 0/23, 0/23	 NA NA	Principal strength: use of males and females. Principal limitations: use of single dose; lack of body-weight data; rationale for dose not given; only data regarding liver tumours were reported.
Full carcinogenicity Mouse, B6C3F$_1$ (M) Neonatal (8 days) 15 mo Von Tungeln et al. (2002)	Intraperitoneal injection NR DMSO 0, 75 nmol Injections with one-third and two-thirds of the total dose in 30 µL DMSO at age 8 and 15 days, respectively 24, 24 24, 24	*Liver* Adenoma Incidence: 4/24, 5/24 Carcinoma Tumour incidence: 0/24, 0/24 Adenoma or carcinoma (combined) Incidence: 4/24, 5/24 Multiplicity: 1.3, 1.0	 NS NA NS NR	Principal strength: use of males and females. Principal limitations: use of single dose; lack of body-weight data; rationale for dose not given; only data regarding liver tumours were reported.
Full carcinogenicity Mouse, B6C3F$_1$ (F) Neonatal (8 days) 15 mo Von Tungeln et al. (2002)	Intraperitoneal injection NR DMSO 0, 75 nmol Injections with one-third and two-thirds of the total dose in 30 µL DMSO at age 8 and 15 days, respectively 24, 24 24, 24	*Liver* Adenoma Incidence: 0/24, 0/24 Carcinoma Incidence: 0/24, 0/24	 NA NA	Principal strength: use of males and females. Principal limitations: use of single dose; lack of body-weight data; rationale for dose not given; only data regarding liver tumours were reported.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Full carcinogenicity Mouse, NR, "partly inbred albinos" (F) ~3 mo ≤ 21–24 mo Steiner et al. (1943)	Subcutaneous injection NR Sesame oil 0.2 mg 1×/wk in 0.1 mL sesame oil for 24 wk 15 0 (11, 6, 3 and 1, at 12, 15, 18 and 21 mo, respectively)	*Subcutaneous tissue*: sarcoma Incidence: 0/15	NA	Principal limitations: use of females only; small number of mice; use of single dose; lack of body-weight data; limited information on sesame oil control group (see below); histopathological reporting limited to the induction of sarcomas; poor survival; justification of the dose was not provided. The authors stated: "at 12 months, the number of mice [sex distribution unspecified] living that had been injected with unheated sesame oil, used as vehicle for other substances in these experiments, was 61. None developed tumours at the site of injection".
Initiation–promotion (tested the dose was not provided. Mouse, S NR NR 21–22 wk Salaman & Roe (1956)	Skin application NR 0, 12.6 mg (total dose) Untreated (control) or 1×/wk application for 10 wk of 0.5% acrolein (in [presumably] 0.3 mL acetone); 25 days after 1st application, 1×/wk application of 0.17% croton oil for 18 wk (dose reduced to 0.085% for the 2nd and 3rd application) 20, 15 19, 15	*Skin*: papilloma Incidence: 4/19, 2/15 [NS]	Principal limitations: small number of mice per group; use of a single dose; limited reporting; justification of as initiator) Acetone No.: 4, 3	NR

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of	Significance	Comments
Table 3.1 (continued)				
Study design (sex) Age at start	**Vehicle Dose(s)**	**tumours**		
Full carcinogenicity Rat, F344/ DuCrlCrlj (M) 6 wk 104 wk JBRC (2016d, e)	Inhalation (whole-body) > 98.3% Clean air 0, 0.1, 0.5, 2 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 41, 40, 37, 42	*Nasal cavity*: squamous cell carcinoma Incidence: 0/50, 0/50, 0/50, 1/50 (2%)	NS	Principal strengths: multiple dose study; used males and females; study complied with GLP. Historical control data in F344/DuCrlCrlj male rats for nasal cavity squamous cell carcinoma, 0/599; the incidence of hyperplasia of the respiratory tract (nasal cavity) was significantly increased in treated rats compared with controls; the Working Group considered hyperplasia of the respiratory tract to be a pre-neoplastic lesion.

Table 3.1 (continued)

Route		Incidence (%),	Significance	Comments
Study design				
Species, strain (sex)	**Purity**	**multiplicity, or no. of tumours**		
Age at start	**Vehicle**			
Duration	**Dose(s)**			
	No. of animals at start			
				Principal strengths: multiple dose study; used males and females; study complied with GLP.
				Historical control data in F344/DuCrlCrlj female rats for nasal cavity squamous cell carcinoma, 0/600; nasal cavity rhabdomyoma, 0/600; pituitary gland adenoma, 165/599 (27.5%; range, 22–42%); the incidence of hyperplasia of the respiratory tract (nasal cavity) was significantly increased in treated rats compared with controls; the Working Group considered hyperplasia of the respiratory tract to be a pre-neoplastic lesion.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Study design **(sex)** **Age at start** **Reference**	**Vehicle** **Dose(s)** **No. of surviving animals** Full			
Inhalation (whole-body)	*Nasal cavity*			
carcinogenicity Rat, F344/ DuCrlCrlj (F) 6 wk 104 wk JBRC (2016d, e)	> 98.3% Clean air 0, 0.1, 0.5, 2 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 43, 42, 41, 34	Squamous cell carcinoma or rhabdomyoma (combined) Incidence: 0/50, 0/50, 0/50, 6/50 (12%)*	*P = 0.0133, Fischer exact test; P < 0.0001, Peto trend test (prevalence method and combined analysis) and Cochran–Armitage trend test	
		Rhabdomyoma Incidence: 0/50, 0/50, 0/50, 4/50 (8%)	$P \leq 0.0007$, Peto trend test (prevalence method) and Cochran–Armitage trend test	
		Squamous cell carcinoma Incidence: 0/50, 0/50, 0/50, 2/50 (4%)	NS	

Table 3.1 (continued)

Route		Incidence (%),	Significance	Comments
Study design **Species, strain (sex)** **Age at start** **Duration**	**Purity** **Vehicle** **Dose(s)** **No. of animals at start**	**multiplicity, or no. of tumours**		
Full carcinogenicity Rat, F344 (M) 7–8 wk ≤ 124–132 wk Lijinsky & Reuber (1987)	Oral administration (drinking-water) NR, stabilized with hydroquinone (concentration, NR) Tap water 0 (control), 100 (for 124 wk), 250 (for 124 wk), 625 (for 104 wk) mg/L 5×/wk for 104–124 wk 20, 20, 20, 20 NR	*Liver*: tumours Incidence: 2/20, 8/20*, 0/20, 3/20	*[$P < 0.0324$, one-tail Fischer exact test]	Principal strengths: long-term study (> 2 yr); use of males and females; use of multiple doses. Principal limitations: small number of rats per group; variable duration of treatments between groups; body-weight and survival data not reported. Median week of death: 115 (range, 92–124) (control), 119 (83–130), 116 (53–130), and 129 (95–132) wk, respectively; total acrolein consumption: 0, 1.2, 3.1, and 6.5 g, respectively; liver tumours were mainly neoplastic nodules, with a few hepatocellular carcinomas.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Study design **(sex)** **Age at start** **Reference**	**Vehicle** **Dose(s)** **No. of surviving animals**			
Full carcinogenicity DuCrlCrlj (F) 6 wk	Adenoma or adenocarcinoma (combined) Rat, F344/	*Pituitary gland* 21/50, 17/50 Adenoma	Incidence: 14/50, 17/50, *P* = 0.0215, Peto trend test (standard method)	
JBRC (2016d, e) (104 wk cont.)	Incidence: 14/50 (28%), 1517/50 (34%/50 (30%), 20/50 (40%),)			*P*test (standard method) = 0.0115, Peto trend
		Adenocarcinoma Incidence: 0/50, 2/50, 1/50, 0/50	NS	

Table 3.1 (continued)

Route	Study design Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Full 4/20	Oral administration NS	*Liver*: tumours males and females.			Principal strengths: long-term study (> 2 yr); use of carcinogenicity (drinking-water) Incidence: 2/20,
Rat, F344 (F)		NR, stabilized with	*Adrenal gland*: tumours		Principal limitations: small number of rats per group;
7–8 wk hydroquinone (concentration, NR) Incidence: 1/20, 5/20 NS variable duration of treatments between groups; body-weight data and survival not reported; use of a single ≤ 124–132 wk					
Lijinsky & Reuber (1987)		Tap water 0 (control), 625 (for 104 wk) mg/L 5×/wk for 104 wk 20, 20 NR			dose. Median week of death: 118 (range, 82–124) (control), and 117 (58–132) wk, respectively; total acrolein consumption: 0 and 6.5 g, respectively; liver tumours were mainly neoplastic nodules, with a few hepatocellular carcinomas.
Full carcinogenicity Rat, SpragueDawley (M) ~6 wk 102 wk Parent et al. (1992)		Oral administration (gavage) 94.9–98.5% (hydroquinone, 0.25–0.31%) Deionized water 0.0, 0.05, 0.5, 2.5 mg/kg bw 1×/day 70, 70, 70, 70 NR	*Adrenal gland* Cortical adenoma Incidence: 0/60, 4/60, 3/60, 0/60 Cortical carcinoma Incidence: 0/60, 0/60, 1/60, 1/60	NS NS	Principal strengths: use of males and females; multiple dose study; long-term study. Principal limitations: all major tissues and gross lesions from the control and high-dose groups were examined microscopically; only the lungs, liver, kidneys, and gross lesions from the groups at the low and intermediate dose were examined microscopically; reporting of adrenal gland tumours only. 10 rats per dose group were killed after 1 year.
Full carcinogenicity Rat, Sprague-Dawley (F) ~6 wk 102 wk Parent et al. (1992)		administration (gavage) (hydroquinone,	*Adrenal gland*: cortical adenoma Incidence: 2/60, 3/60,	NS	Principal strengths: use of males and females; multiple 94.9–98.5% dose study; long-term study.
Oral					

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Study design **(sex)** **Age at start**	**Vehicle** **Dose(s)**			
Deionized water 0.0170NR0×/day.25–0.31%), 70, 70, , 0.05, 0.5, 2.5 mg/kg bw 70 0/60, 0/60	Principal limitations: trend towards reduced survival, and decreased survival in the high-dose group; all major tissues and gross lesions from the control and high-dose groups were examined microscopically; only the lungs, liver, kidneys and gross lesions from the low- and mid-dose groups were examined microscopically;			reporting of adrenal gland tumours only. 10 rats per dose group were killed after 1 year.
Duration **No. of surviving animals**	**No. of animals at start**	**Reference**		
Initiation–promotion (tested as initiator) Rat, F344 (M) 5 wk 32 wk Cohen et al. (1992)	Intraperitoneal injection 97% (containing 3% water and 200 ppm hydroquinone) Distilled water Acrolein at 2 mg/kg bw followed by control diet (negative control), 0 mg/kg bw followed by uracil (sham control), 2 mg/kg bw followed by uracil, 0.2% FANFT followed by uracil (positive control) Intraperitoneal injection of 2 mg/kg bw acrolein or of distilled water, 2×/wk for 6 wk; followed by 3% uracil in the diet for 20 wk then 6 wk of control diet, or by control diet for 26 wk 30, 30, 30, 30 NR	*Urinary bladder* Papilloma Incidence: 0/30, 8/30, 18/30*, 9/30 Carcinoma Incidence: 0/30, 1/30, 1/30, 21/30	*[$P < 0.02$, Fischer exact test; acrolein + uracil group vs sham control (uracil only)] [NS]	Principal strength: sufficient duration. Principal limitations: use of single dose; data from stomach, lungs, oesophagus, liver, and kidney were not reported.

Table 3.1 (continued)

Route Study design Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Initiation–promotion (tested as promoter) Rat, F344 (M) 5 wk 53 wk Cohen et al. (1992)	Intraperitoneal injection 97% (containing 3% water and 200 ppm hydroquinone) Distilled water Untreated (negative control), FANFT followed by distilled water (sham control), 2 mg/kg bw acrolein, followed by acrolein (see comments for regimen), FANFT followed by acrolein (see comments for regimen) Intraperitoneal injection of 2 mg/kg bw acrolein or of distilled water, 2×/wk for 6 wk, or 0.2% FANFT in the diet for 6 wk; followed by intraperitoneal injection of acrolein (see comments for regimen) or of distilled water until experimental wk 53, or by control diet until experimental wk 53 30, 30, 30, 30 NR	*Urinary bladder* Papilloma Incidence: 0/30, 0/30, 0/30, 0/30 Carcinoma Incidence: 0/30, 1/30, 0/30, 0/30 Simple or papillary/nodular (combined) hyperplasia Incidence: 0/30, 14/30, 16/30*, 22/30 Simple hyperplasia Incidence: 0/30, 14/30, 14/30*, 22/30 Papillary/nodular hyperplasia Incidence: 0/30, 0/30, 2/30, 0/30	NA [NS] *$P < 0.001$, Fischer exact test; acrolein + acrolein vs negative (untreated) control *$P < 0.001$, Fischer exact test; acrolein + acrolein vs negative (untreated) control NS	Principal limitations: use of single dose; data from stomach, lungs, oesophagus, liver, and kidney were not reported. The protocol (originally for a 100 wk-study) had to be revised for the two acrolein-treated groups, because of severe toxicity, and the acrolein treatment regimen was revised as follows: intraperitoneal injections of acrolein at 2 mg/kg bw, 2×/wk, during experimental wk 1–9; 1.5 mg/kg bw, 1× at experimental wk 10; 1.5 mg/kg bw, 2×/wk, during experimental wk 11–17; and 1.0 mg/kg bw, 1× at experimental wk 18, 2× at experimental wk 19, and 1× at experimental wk 20 and 21.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of	Significance	Comments
Study design **(sex)** **Age at start** **Duration** **Reference**	**Vehicle** **Dose(s)** **No. of animals at start** **No. of surviving animals**	**tumours**		
Full carcinogenicity Hamster, Syrian golden (M) 6 wk 81 wk Feron & Kruysse (1977)	Inhalation (whole-body) NR Filtered air 0 (unexposed control), 9.2 mg/ m^3 7 h/day, 5 days/wk for 52 wk 30, 30 7 (at 80 wk), 7 (at 80 wk)	*Respiratory tract*: all tumours (nasal cavity, larynx, trachea, bronchi or lung, combined) Incidence: 0/30, 0/30	NA	Principal strengths: use of males and females. Principal limitations: small number of animals per group; short duration of exposure; use of single dose; histopathological data were reported only for respiratory tract tumours; justification of the dose was not provided; lower survival. 15 hamsters per group also received intratracheal instillations of 0.2 mL 0.9% saline 1×/wk for 52 wk; the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined microscopically; in acrolein-treated animals, inflammation and epithelial metaplasia of the nasal cavity were observed.
Full Hamster, Syrian golden (F) 6 wk	Inhalation (whole-body)	*Respiratory tract*: all tumours (nasal cavity, larynx,		Principal strengths: use of males and females.

carcinogenicity NRFiltered air0[m]730 (unexposed control), 9.2 mg/ h/day, 5 days/wk for 52 wk[3], 30
trachea, bronchi or lung, combined)Incidence: 0/30, 1/30 NS
respiratory tract tumours; justification of the dose was not provided.Principal limitations: small number of animals per group; short duration of exposure; use of single dose; histopathological data were reported only for

81 wk

Table 3.1 (continued)

Route	Study design Species, strain (sex) Age at start	Purity Vehicle Dose(s)	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
	Feron & Kruysse (1977)	16 (at 80 wk), 13 (at 80 wk)			15 hamsters per group also received intratracheal instillations of 0.2 mL 0.9% saline 1×/wk for 52 wk; the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined microscopically; in acrolein-treated animals, inflammation and epithelial metaplasia of the nasal cavity were observed.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Study design **(sex)** **Age at start** **Duration** **No. of surviving animals**	**Vehicle** **Dose(s)** **No. of animals at start Reference**			
Co-carcinogenicity Hamster, Syrian golden (M) 6 wk 81 wk Feron & Kruysse (1977)	Inhalation (whole-body) NR Filtered air 0 mg/m³ acrolein + 0.175% B[*a*]P, 9.2 mg/m³ acrolein + 0.175% B[*a*]P, 0 mg/m³ acrolein + 0.35% B[*a*]P, 9.2 mg/m³ acrolein + 0.35% B[*a*]P, 0 mg/m³ acrolein + NDEA, 9.2 mg/m³ acrolein + NDEA Exposure to acrolein was 7 h/day, 5 days/wk for 52 wk; together with either weekly intratracheal instillations of a suspension of 0.175 or 0.35% B[*a*]P (in 0.2 mL 0.9% saline), or 1×/3 wk subcutaneous injections of 0.0625% NDEA in 0.2 mL saline 30, 30, 30, 30, 30, 30 8 (at 80 wk), 7 (at 80 wk), 11 (at 80 wk), 9 (at 80 wk), 1 (at 80 wk), 7 (at 80 wk)	*Respiratory tract*: all tumours (nasal cavity, larynx, trachea, bronchi or lung, combined) Incidence: 4/29, 7/30, 19/30, 19/29, 12/29, 10/30 No.: 5, 7, 27, 29, 15, 11	NS NS	Principal strengths: use of males and females. Principal limitations: small number of animals per group; short duration of exposure; use of single dose; histopathological data were reported only for respiratory tract tumours; justification of the dose was not provided; lower survival. 15 hamsters per control group also received intratracheal instillations of 0.2 mL 0.9% saline 1×/wk for 52 wk; the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined histologically; in acrolein-treated animals, inflammation and epithelial metaplasia of the nasal cavity were observed.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Co-carcinogenicity Hamster, Syrian golden (F) 6 wk 81 wk Feron & Kruysse (1977)	Inhalation (whole-body) NR Filtered air 0 mg/m³ acrolein + 0.175% B[*a*]P, 9.2 mg/m³ acrolein + 0.175% B[*a*]P, 0 mg/m³ acrolein + 0.35% B[*a*]P, 9.2 mg/m³ acrolein + 0.35% B[*a*]P, 0 mg/m³ acrolein + NDEA, 9.2 mg/m³ acrolein + NDEA Exposure to acrolein was 7 h/day, 5 days/wk for 52 wk; together with either weekly intratracheal instillations of a suspension of 0.175 or 0.35% B[*a*]P (in 0.2 mL 0.9% saline), or 1×/3 wk subcutaneous injections of 0.0625% NDEA in 0.2 mL saline 30, 30, 30, 30, 30, 30 21 (at 80 wk), 17 (at 80 wk), 18 (at 80 wk), 17 (at 80 wk), 11 (at 80 wk), 20 (at 80 wk)	*Respiratory tract*: s (nasal cavity, larynx, trachea, bronchi or lung, con Incidence: 3/27, 8/29, 7/24, 15/30, 11/27, 11/28 No.: 3, 8, 9, 22, 13, 15	s NS NS	Principal strengths: use of males and females. Principal limitations: small number of animals per group; short duration of exposure; use of single dose; histopathological data were reported only for respiratory tract tumours; justification of the dose was not provided. 15 hamsters per control group also received intratracheal instillations of 0.2 mL 0.9% saline 1×/wk for 52 wk; the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined histologically; in acrolein-treated animals, inflammation and epithelial metaplasia of the nasal cavity were observed.

B[*a*]P; benzo[*a*]pyrene; bw, body weight; DMSO, dimethyl sulfoxide; F, female; FANFT, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; GLP, Good Laboratory Practice; h, hour; M, male; NA, not applicable; NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; mo, month; ppm, parts per million; vs, versus; wk, week.

necropsy; this was done at week 93 for males and week 99 for females. Survival in the groups at 0, 0.1, 0.4, and 1.6 ppm was: for males, 11/50, 15/50, 14/50, and 15/50, respectively, at week 93; and for females, 11/50, 18/50, 14/50, and 19/50, respectively, at week 99. Body weights of male mice at 1.6 ppm were significantly decreased from the first week of exposure and throughout the exposure period compared with the control value. The relative final body weight in males at 0.1, 0.4, and 1.6 ppm were 89%, 95%, and 83% of the control value, respectively. Body weights of female mice at 1.6 ppm were significantly decreased from the first week of exposure until week 82, compared with the control value. The relative final body weight in females at 0.4 ppm was slightly but significantly increased. The relative final body weight for females at 0.1, 0.4, and 1.6 ppm was 104%, 111%, and 101% of the control value, respectively. All mice underwent complete necropsy, and all organs and tissues were examined microscopically.

In treated male mice, there was no significant increase in the incidence of any tumour. One (1/50, 2%) adenoma of the nasal cavity was observed in a male at 1.6 ppm; this incidence was at the upper bound of the historical control range (incidence, 1/499 (0.2%); range, 0–2%).

In female mice, the incidence of malignant lymphoma was significantly increased with a positive trend ($P = 0.0347$, Cochran–Armitage test). The incidence of histiocytic sarcoma of the uterus was 6/50 (12%, control), 13/50 (26%, 0.1 ppm), 14/50 (28%, 0.4 ppm), and 6/50 (12%, 1.6 ppm). The incidence in the group at 0.4 ppm (28%) was significantly increased ($P = 0.0392$, Fischer test) compared with the control value. [The Working Group noted that this increase did not indicate a clear dose–response relationship. The Working Group considered that this increase may have been related to treatment.] The incidence of adenoma of the nasal cavity was 0/50 (control), 0/50 (0.1 ppm), 0/50 (0.4 ppm), and 16/50 (32%, 1.6 ppm) and showed a significant positive trend ($P < 0.0001$, Peto test prevalence method and Cochran–Armitage test). The incidence in the group at 1.6 ppm was significantly increased ($P < 0.0001$, Fischer test) compared with the value for the control group and was clearly in excess of the value for historical controls (0/500).

Regarding non-neoplastic lesions in the respiratory tract (see also Section 4 of this monograph), for males at 1.6 ppm there was a significant increase in the incidence and/or severity of: eosinophilic change, inflammation, squamous cell metaplasia, regeneration, and hyperplasia in the respiratory epithelium; respiratory metaplasia and atrophy in the olfactory epithelium; respiratory metaplasia in the nasal glands; hyperplasia in the transitional epithelium; atrophy and adhesion in the turbinate; and exudate in the nasal cavity was observed. For females at 1.6 ppm, there was a significant increase in the incidence and/or severity of: inflammation, squamous cell metaplasia, regeneration, and hyperplasia in the respiratory epithelium; respiratory metaplasia and atrophy in the olfactory epithelium; respiratory metaplasia in the nasal glands; and exudate in the nasal cavity was observed. The incidence of inflammation and hyperplasia in the respiratory epithelium was also increased in female mice at 0.4 ppm. [The Working Group considered the hyperplasias of the respiratory tract observed in both males and females to be pre-neoplastic lesions.]

[The Working Group noted this was a GLP study conducted with multiple doses, and with both males and females.]

3.1.2 Oral administration (gavage)

In a study performed by Parent et al. (1991a), groups of 70–75 male and 70–75 female CD-1 mice (age, 8 weeks) were given acrolein (purity, 94.9–98.5%; containing 0.25–0.31% hydroquinone as a stabilizer) at a dose of 0 (control, deionized water only), 0.5, 2.0, or 4.5 mg/kg body weight (bw) per day by daily gavage [dosing volume not reported] for 18 months. In treated males, there was a significant negative trend in survival, and a significant decrease in survival in the group of males at the highest dose. Excess mortality was reported in all groups and attributed to trauma during gavage dosing, mis-dosing, or reasons unknown. [The number of surviving animals was not provided.] In males treated with the highest dose, a significant reduction in bodyweight gain was observed. Histopathological examination was reported for all major tissues and gross lesions from mice in the control group and at the highest dose, but only for the lungs, liver, kidneys, and gross lesions from mice in the groups receiving the lowest and intermediate dose. In addition, tumour incidence was reported only for about half of the experimental animals (271/570) killed at 18 months. For males, data were reported for 30, 29, 30, and 27 animals for controls and each dose group respectively. For females, data were reported for 42, 30, 40, and 43 animals for controls and each dose group, respectively. [According to the protocol, histopathological data from mice found dead or killed in a moribund state were to be collected, but data were not shown.]

No significant increase in the incidence of tumours was observed.

[The Working Group noted the principal strengths of the study: the use of males and females, the large number of mice per group at start, and the use of multiple doses. The principal limitations of the study were that data were obtained from a limited number of mice assessed for histopathology after killing; that full histopathological examination was performed only for mice in the control group and at the highest dose; and that survival was lower in treated males.]

3.1.3 Intraperitoneal injection

In the first experiment in a carcinogenicity study by Von Tungeln et al. (2002), which focused on the induction of liver and lung tumours in newborn mice, groups of 23 male and 24 female B6C3F$_1$ mice (age, 8 days) were given two intraperitoneal injections of acrolein [purity not reported] at a total dose of 150 nmol. One third [50 nmol] and two thirds [100 nmol] of the total dose were given in dimethyl sulfoxide (DMSO) at age 8 and 15 days, respectively. Control groups of 24 males and 23 females were given intraperitoneal injections of DMSO only. There was no significant effect on survival. The mice were killed at age 12 months and underwent a complete necropsy; livers, lungs, and gross lesions were examined microscopically. Only one male mouse, in the treated group, developed liver adenomas (controls, 0/24; treated, 1/23). No liver tumours were observed in treated females and control females.

In a second experiment in the study by Von Tungeln et al. (2002), groups of 24 male and 24 female B6C3F$_1$ mice (age, 8 days) were given two intraperitoneal injections of acrolein at a total dose of 75 nmol. One third [25 nmol] and two thirds [50 nmol] of the total dose were given in DMSO at age 8 and 15 days, respectively. Control groups of 24 males and 24 females were given intraperitoneal injections of DMSO only. There was no significant effect on survival. Mice were killed at age 15 months. Control and treated males developed liver adenomas (incidence: controls, 4/24; treated, 5/24). No liver tumours were observed in control or treated females. [The Working Group

noted the principal strength of the study: the use of males and females. The principal limitations were that a single dose was used, justification for the dose used was not given, only data regarding liver tumours were reported, and body-weight data were not provided.]

3.1.4 Subcutaneous injection

A group of 15 female mice [strain not reported, referred to as "partly inbred albinos", of unspecified age ("around 3 months")] were given weekly subcutaneous injections of 0.2 mg of acrolein [purity not reported] in sesame oil for 24 weeks (total dose, 4.8 mg) to assess the induction of sarcoma. Survival was poor, with 11, 6, 3, and 1 mice alive at 12, 15, 18, and 21 months, respectively. After 21–24 months, no sarcomas were observed (Steiner et al., 1943). [The Working Group noted the principal limitations of the study: the small number of animals, poor survival, use of females only, use of a single dose, limited reporting on a sesame oil control group, lack of body-weight data, absence of justification for the dose used, and unspecified histopathological assessment for tumours other than sarcoma. The study was considered inadequate for the evaluation of the carcinogenicity of acrolein.]

3.1.5 Initiation–promotion

A group of 15 strain S mice [sex and age not reported] was given 0.5% acrolein [purity not reported] in acetone by skin application, once per week, for 10 weeks (total dose, 12.6 mg). Twentyfive days after the first application, the mice were given 0.17% croton oil [purity not reported] in acetone by skin application, once per week, for 18 weeks (on the second and third week of treatment the dose was reduced to 0.085%). A group of 20 control animals was given croton oil only by skin application following the same schedule: 0.17% croton oil in acetone was applied once per week, for 18 weeks (on the second and third week of treatment, the dose was also reduced to 0.085%). One control mouse died before the end of the study. After experimental weeks 21–22, no increased incidence of papilloma of the skin was observed in mice initiated with acrolein compared with controls (Salaman & Roe, 1956). [The Working Group noted the principal limitations of the study: the limited reporting of the study, absence of justification for the dose used, and the use of a small number of animals and a single dose. The study was considered inadequate for the evaluation of the carcinogenicity of acrolein.]

3.2 Rat

3.2.1 Inhalation

In a study that complied with GLP, groups of 50 male and 50 female F344/DuCrlCrlj rats (age, 6 weeks) were treated by inhalation with acrolein (purity, > 98.3%; 1.42% acetaldehyde identified by GC-MS) by whole-body exposure for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2016d, e, f). The concentration in the exposure chambers was set to 0 (clean air, control), 0.1, 0.5, or 2 ppm for males and females and was monitored every 15 minutes. The mean air concentrations (± standard deviation) for these groups were 0.10 ± 0.00, 0.50 ± 0.00, and 2.01 ± 0.02 ppm, respectively. At 104 weeks, survival of females at 2 ppm was significantly decreased, compared with controls. Survival in the groups at 0, 0.1, 0.5, and 2 ppm was: for males, 41/50, 40/50, 37/50, and 42/50, respectively; and for females, 43/50, 42/50, 41/50, and 34/50, respectively. Male rats at 2 ppm showed a decrease in body-weight gain from the first week of exposure and throughout the exposure period, compared with controls. The relative final body weight in males at 0.1, 0.5, and 2 ppm was 96%, 99%, and 88% of the control value, respectively. Body weights of female rats at

2 ppm were slightly but significantly decreased (maximum, 10%) from the first week of exposure and throughout the exposure period, compared with controls. The relative final body weight in females at 0.1, 0.5, and 2 ppm was 101%, 98%, and 95% of the control value, respectively. All rats underwent complete necropsy, and all organs and tissues were examined microscopically.

In treated male rats, there was no significant increase in the incidence of any tumours. One (1/50, 2%) squamous cell carcinoma of the nasal cavity was observed in the group of males at 2 ppm, which was in excess of the value for historical controls (0/599). [The Working Group considered that this rare squamous cell carcinoma of the nasal cavity may have been related to exposure.]

Of the females, two rats (2/50, 4%) developed squamous cell carcinoma of the nasal cavity in the group at 2 ppm. Although not significantly increased compared with controls, the incidence of this rare tumour exceeded the historical control rate (0/600). The incidence of rhabdomyoma of the nasal cavity was 0/50 (control), 0/50 (0.1 ppm), 0/50 (0.5 ppm), and 4/50 (8%, 2 ppm), and showed a significant positive trend ($P \leq 0.0007$, Peto test (prevalence method) and Cochran–Armitage test), and the incidence in the group at 2 ppm exceeded the historical control rate (0/600). The incidence of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity was 0/50 (control), 0/50 (0.1 ppm), 0/50 (0.5 ppm), and 6/50 (12%, 2 ppm), and showed a significant positive trend ($P < 0.0001$, Peto test (prevalence method and combined analysis) and Cochran–Armitage test). The incidence in the group at 2 ppm (12%) was significantly increased ($P = 0.0133$, Fischer test) compared with the control value. [The Working Group considered that rare squamous cell carcinomas and rhabdomyomas of the nasal cavity observed in female rats were related to exposure. The Working Group also noted that these two tumours have different histotypes.] The incidence of adenoma in the pituitary gland was 14/50 (28%, control), 15/50 (30%, 0.1 ppm), 20/50 (40%, 0.5 ppm), and 17/50 (34%, 2 ppm), and showed a significant positive trend ($P = 0.0115$; Peto test, standard method). The incidence of adenocarcinoma of the pituitary gland was 0/50 (control), 2/50 (4%, 0.1 ppm), 1/50 (2%, 0.5 ppm), and 0/50 (0%, 2 ppm). The incidence of adenoma or adenocarcinoma (combined) of the pituitary gland, was 14/50 (28%, control), 17/50 (34%, 0.1 ppm), 21/50 (42%, 0.5 ppm), and 17/50 (34%, 2 ppm), and showed a significant positive trend ($P = 0.0215$; Peto test, standard method); however, the incidence of adenocarcinoma of the pituitary gland was not significantly increased. [The Working Group considered that the occurrence of adenoma and/ or adenocarcinoma of the pituitary gland may not be related to exposure, because of the high background incidence of adenoma of the pituitary gland in ageing rats, because the increased incidence was seen in females only, and because the incidence of adenocarcinoma of the pituitary gland was not significantly increased.]

Regarding non-neoplastic lesions in the respiratory tract (see also Section 4 of the present monograph), for males at 2 ppm there was a significant increase in the incidence and/ or severity of: inflammation and squamous cell metaplasia in the respiratory epithelium; eosinophilic change, respiratory metaplasia, and atrophy in the olfactory epithelium; respiratory metaplasia in the nasal glands; hyperplasia in the transitional epithelium; adhesion in the turbinate; goblet cell hyperplasia; inflammation with foreign body; oedema in the lamina propria; and proliferation of striated muscle was observed in the nasal cavity. For females at 2 ppm, there was a significant increase in the incidence and/ or severity of: inflammation and squamous cell metaplasia in the respiratory epithelium; respiratory metaplasia and atrophy in the

olfactory epithelium; respiratory metaplasia in the nasal glands; hyperplasia in the transitional epithelium; goblet cell hyperplasia; inflammation with foreign body; and oedema in the lamina propria was observed in the nasal cavity. [The Working Group considered that hyperplasias of the respiratory tract observed in both males and females were pre-neoplastic lesions.]

[The Working Group noted this was a GLP study conducted with multiple doses and using males and females.]

3.2.2 Oral administration (drinking-water)

Groups of 20 male and 20 female Fischer 344 rats (age, 7–8 weeks), were given drinking-water containing acrolein at a concentration of 0 mg/L (control), 100 mg/L (males only), 250 mg/L (males only), or 625 mg/L [purity not reported] stabilized with hydroquinone [concentration not reported], for 5 days per week (the other 2 days per week, the rats were given tap water) for 124 weeks (except for 104 weeks for the highest dose) (Lijinsky & Reuber, 1987; Lijinsky, 1988). The rats were killed at age 124–132 weeks There was little or no difference in survival [data were not reported]. [Body-weight and water-consumption data were not reported.] Histopathological examination was performed on all lesions, major tissues, and organs. There was a significant increase in the incidence of liver tumours (mainly neoplastic nodules, and a few hepatocellular carcinomas) in the group of males at the lowest dose compared with controls. [The Working Group noted the principal strengths of the study: this was a long-term study (> 2 years) that used multiple doses in males, and both males and females. The principal limitations were the small number of animals per group, the variable duration of treatments between groups, and the use of a single dose in females.]

3.2.3 Oral administration (gavage)

In a study by Parent et al. (1992), groups of 70 male and 70 female Sprague-Dawley rats (age, about 6 weeks), received acrolein (purity, 94.9–98.5%; stabilized with 0.25–0.31% hydroquinone; in deionized water) at a dose of 0 (control), 0.05, 0.5, or 2.5 mg/kg bw by daily gavage for up to 102 weeks. The dosing volume was 10 mL/kg bw. Excess mortality was reported in all groups; this was attributed to trauma during gavage dosing, mis-dosing, or reasons unknown. Ten rats of each sex per dose group were killed after 1 year, and surviving rats were killed [the number of surviving rats was not reported] after 102 weeks. There was a negative trend in survival and a decrease in survival at the highest dose that was significant among males and females during the first year, but only significant in females throughout the entire treatment period. There was no significant effect on body weight. All major tissues and gross lesions from rats in the control group and at the highest dose were examined microscopically; only the lungs, liver, kidneys, and gross lesions from the groups at the lowest and intermediate dose were examined microscopically. In treated males, the incidence of cortical cell adenoma of the adrenal gland was increased, but the effect was not statistically significant. Cortical cell carcinoma of the adrenal gland was seen in two male rats, one in each group at the intermediate and highest dose. In females, the incidence of cortical cell adenoma of the adrenal gland in treated rats was not significantly increased, and no adrenal gland carcinomas were observed in any group. [The Working Group noted the principal strengths of the study: this was a long-term study (> 2 years) that used multiple doses, and both males and females. The principal limitations were the reduced survival among treated females; that histopathological data

were reported only for adrenal gland tumours; and that full histopathological examination was performed only for rats in the control group and at the highest dose, while only the lungs, liver, kidneys, and gross lesions from rats in the groups at the lowest and intermediate dose were examined microscopically.]

3.2.4 Initiation–promotion

To evaluate the initiating activity of acrolein, two groups of 30 male Fischer 344 rats (age, 5 weeks) were given intraperitoneal injections of acrolein (purity, 97%; containing approximately 3% water and 200 ppm hydroquinone; in distilled water), at a dose of 0 (sham control, distilled water) or 2 mg/kg bw, twice per week, for 6 weeks, followed by feed containing 3% uracil for 20 weeks, and then control feed for 6 weeks. Another group (negative control group) of 30 male rats was given intraperitoneal injections of acrolein at a dose of 2 mg/kg bw, twice per week, for 6 weeks, followed by control feed for 26 weeks. A positive control group of 30 male rats was given feed containing 0.2% *N*-[4-(5nitro-2-furyl)-2-thiazolyl]formamide (FANFT) for 6 weeks, followed by feed containing 3% uracil for 20 weeks, and then control feed for 6 weeks. The rats were killed at experimental week 32. The stomach, lungs, oesophagus, liver, kidneys, and bladder were processed for histopathological examination. A significant increase [$P < 0.02$, Fischer exact test] in the incidence of urinary bladder papilloma was observed in rats initiated with acrolein and then exposed to the promotor uracil, compared with sham controls. The incidence of urinary bladder carcinoma was not significantly increased (Cohen et al., 1992). [The Working Group noted that the principal strength of the study was the sufficient duration. The principal limitations were the use of a single dose, and that data from stomach, lungs, oesophagus, liver, and kidneys were not reported.]

To evaluate the promoting activity of acrolein, two groups of 30 male Fischer 344 rats (age, 5 weeks) were given feed containing 0.2% FANFT for 6 weeks during the first phase, followed by intraperitoneal injections of acrolein (purity, 97%; containing 3% water and 200 ppm hydroquinone; in distilled water) at 0 (sham control, distilled water) or various concentrations (described below) during the second phase. Another group (acrolein-only group) received intraperitoneal injections of acrolein for the first and second phases. The intraperitoneal injections of acrolein were given as follows: 0 or 2 mg/kg bw, twice per week, during experimental week 1–9; 0 or 1.5 mg/kg bw, once at experimental week 10; 0 or 1.5 mg/kg bw, twice per week, during experimental week 11–17; and 0 or 1.0 mg/kg bw, once at experimental week 18, twice at experimental week 19, and once at experimental weeks 20 and 21. A negative control group was given the control feed only. The rats were killed at experimental week 53. The stomach, lungs, oesophagus, liver, kidneys, and bladder were processed for histopathological examination. No papilloma or carcinoma of the urinary bladder developed in any of the four groups of rats, apart from one rat bearing a carcinoma in the FANFT-only group (sham control). Regarding pre-neoplastic lesions, there was a significant increase ($P < 0.001$) in the incidence of simple or papillary/nodular (combined) hyperplasia of the urinary bladder in the acrolein-only group (16/30) compared with the negative (untreated) control group (0/30) (Cohen et al., 1992). [The Working Group noted that the principal limitations of the study were the use of a single dose, and that data from stomach, lungs, oesophagus, liver, and kidneys were not reported.]

3.3 Hamster

3.3.1 Inhalation

In a study by Feron & Kruysse (1977), groups of 30 male and 30 female Syrian golden hamsters (age, 6 weeks), were treated with acrolein at 0 mg/ m^3 (filtered air, control), or 9.2 mg/m^3 [purity not reported] by inhalation with whole-body exposure for 7 hours per day, 5 days per week, for 52 weeks, and the hamsters were then killed at 81 weeks. Half of the hamsters also received intratracheal instillations of 0.2 mL of 0.9% sodium chloride, once per week, for 52 weeks [but mortality and tumour results were reported and combined for all 30 animals of each sex per group]. Survival in males was low, but acrolein exposure did not affect survival rate. Seven male controls, 7 treated males, 16 control females, and 13 treated females were alive at 80 weeks. All hamsters were subject to full necropsy, but only the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined microscopically. No respiratory tract tumours were observed in any group, apart from a single papilloma of the trachea that was found in a treated female. Exposure to acrolein vapour caused inflammation and a slight to moderate degree of epithelial metaplasia in the nasal cavity. [The Working Group noted that the principal strength of the study was the use of males and females. The principal limitations were the small number of animals per group; the short duration of the exposure; absence of justification for the dose used; the lower survival in males; reporting of pathological data only for respiratory tract tumours; and the use of a single dose.]

3.3.2 Administration with known carcinogens

In a study by Feron & Kruysse (1977), groups of 30 male and 30 female Syrian golden hamsters (age, 6 weeks), were treated with acrolein [purity not reported] at a concentration of 0 mg/m^3 (filtered air, control groups), or 9.2 mg/m^3 by inhalation with whole-body exposure for 7 hours per day, 5 days per week, for 52 weeks, together with either weekly intratracheal instillations of a suspension of 0.175% or 0.35% benzo[*a*] pyrene (B[*a*]P, purity > 99%) in 0.9% sodium chloride (B[*a*]P total dose, 18.2 or 36.4 mg/ animal) or subcutaneous injections of 0.0625% *N*-nitrosodiethylamine (NDEA) in 0.2 mL of 0.9% sodium chloride once per 3 weeks (NDEA total dose, 2.1 µL/animal), and the hamsters were then killed at 81 weeks. Half of the control animals received also intratracheal instillations of 0.2 mL of 0.9% sodium chloride, once per week, for 52 weeks [but mortality and tumour results were reported and combined for all 30 control animals per sex]. Survival in males was low, but acrolein exposure did not affect survival rate. All hamsters were subject to full necropsy, but only the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined microscopically.

There were no significant differences in the number or incidence of total respiratory tract tumours, or in the incidence of tumours of the nasal cavity, larynx, trachea, bronchi, or lungs. Tumours appeared slightly earlier in the groups of males and females exposed to acrolein plus NDEA compared with their respective NDEAonly controls. Exposure to acrolein vapour caused inflammation and a slight to moderate degree of epithelial metaplasia in the nasal cavity. [The Working Group noted the principal strength of the study: the use of males and females. The principal

limitations were: the small number of animals per group; the short duration of the exposure; that justification for the dose was not provided; the lower survival in males; that pathological data were reported only for respiratory tract tumours; and the use of a single dose.]

3.4 Evidence synthesis for cancer in experimental animals

The carcinogenicity of acrolein has been assessed in one study that complied with GLP in male and female mice and rats treated by inhalation with whole-body exposure. The carcinogenicity of acrolein in mice and rats was also evaluated by other routes of exposure in studies that did not comply with GLP. Specifically, in mice, there was one study in males and females treated by oral administration (gavage), and there were two studies in newborn males and females treated by intraperitoneal injection. In addition, one study in females treated by subcutaneous injection and one initiation–promotion study (sex not reported) were available, but these studies were judged to be inadequate for the evaluation of the carcinogenicity of acrolein in experimental animals. In rats, there were two studies in males and females treated by oral administration (one drinking-water study and one gavage study), and two initiation–promotion studies in males. The carcinogenicity of acrolein has been assessed in hamsters in one study in males and females treated by inhalation with whole-body exposure, both in the presence and absence of two known carcinogens.

In the inhalation study that complied with GLP in B6D2F1/Crlj mice, acrolein significantly increased the incidence of histiocytic sarcoma of the uterus in treated females, but without a clear dose–response relationship, and caused a significant positive trend in the incidence of malignant lymphoma in treated females. In treated females, there was also a significant positive trend and significant increase in the incidence of nasal cavity adenoma, which is a very rare tumour in the mouse strain used in the study (JBRC, 2016a, b, c). In the inhalation study that complied with GLP in F344/DuCrlCrlj rats, there was a significant positive trend in the incidence of rhabdomyoma of the nasal cavity and of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity in treated females. The incidence of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity was also significantly increased in treated females. Both tumour types are very rare in the rat strain used in the study (JBRC, 2016d, e, f).

In studies of oral administration, acrolein administered by gavage to male and female CD-1 mice or Sprague-Dawley rats did not cause an increased tumour incidence (Parent et al., 1991a). When administered in drinking-water in a study in male Fischer 344 rats, acrolein increased the incidence of liver tumours (mainly benign) (Lijinsky & Reuber, 1987; Lijinsky, 1988).

When tested by intraperitoneal injection, acrolein did not cause an increased tumour incidence in newborn B6C3F$_1$ mice (Von Tungeln et al., 2002). Acrolein increased the incidence of urinary bladder papilloma in male Fischer 344 rats when administered as a tumour initiator with uracil as a tumour promoter (Cohen et al., 1992). The incidence of urinary bladder tumours was not increased when acrolein was tested as a promoter in male Fischer 344 rats, with FANFT as the initiator (Cohen et al., 1992).

Tumours did not occur in mice treated with acrolein by subcutaneous injection (Steiner et al., 1943), and acrolein did not increase the incidence of skin tumours in an initiation–promotion study in strain S mice, with croton oil as the promoter (Salaman & Roe, 1956). However, these studies were judged to be inadequate for the evaluation of the

carcinogenicity of acrolein in experimental animals.

In Syrian golden hamsters treated by inhalation with whole-body exposure, acrolein did not increase the incidence of tumours, either in the presence or absence of known carcinogens (B[*a*]P or NDEA) (Feron & Kruysse, 1977).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

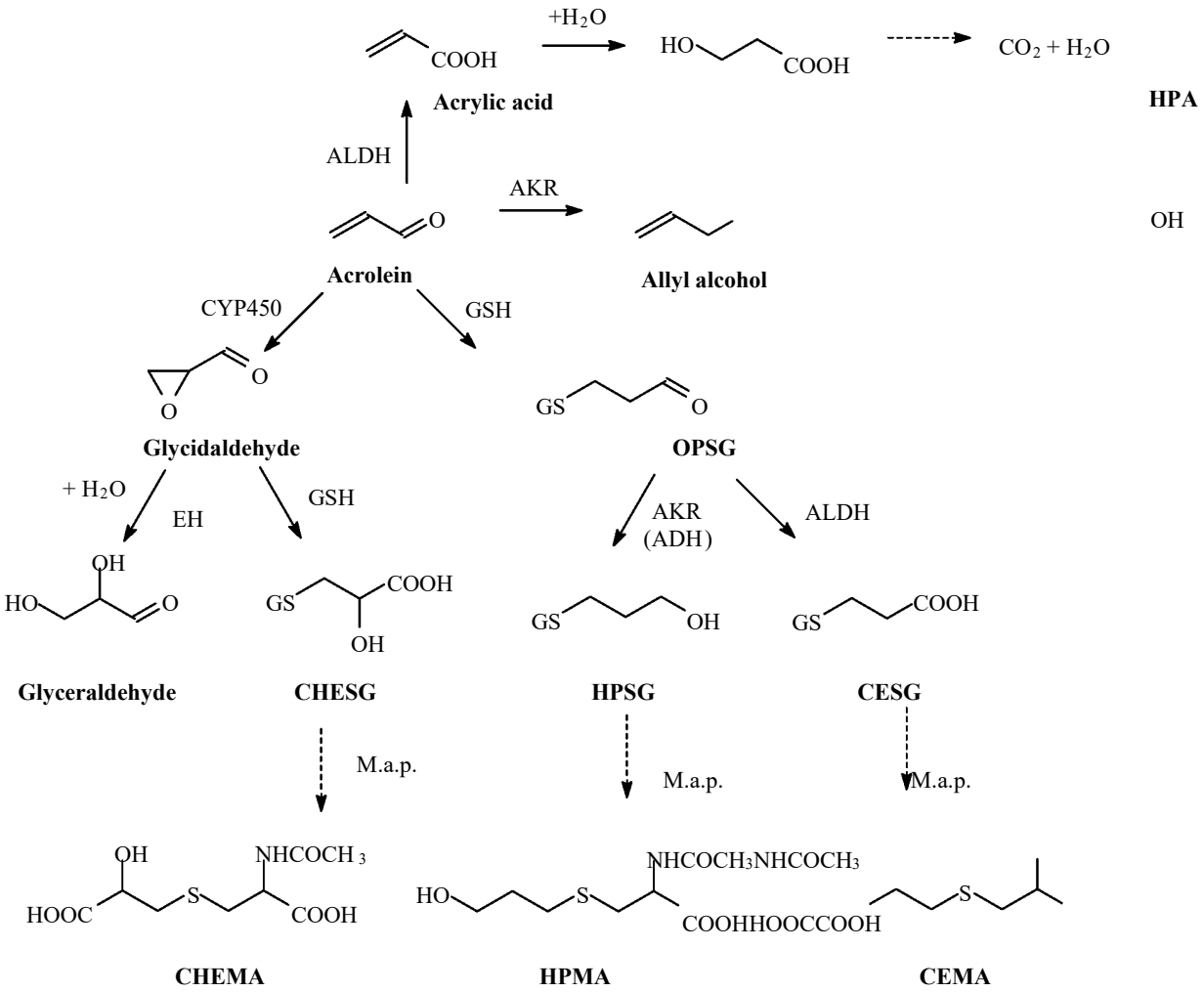
(a) Exposed humans

No data on the absorption or distribution of acrolein by inhalation were available to the Working Group.

The main metabolic pathways of acrolein are depicted in Fig. 4.1.

Two acrolein-derived mercapturic acids, HPMA and CEMA, were found in the urine of both smokers and non-smokers, HPMA being consistently the more common. Tobacco smokers showed significantly higher levels of both HPMA and CEMA (Alwis et al., 2012, 2015). A significant increase in acrolein-derived urinary mercapturic acids was also reported shortly after being served heat-processed food containing acrolein (Wang et al., 2019; Watzek et al., 2012). These mercapturic acids were also found in a limited exploratory toxicokinetic study on a single subject within

Fig. 4.1 The main pathways of acrolein metabolism



ADH, alcohol dehydrogenase; AKR, aldo-keto reductase; ALDH, aldehyde dehydrogenase; CEMA, *N*-acetyl-*S*-(carboxyethyl)-L-cysteine (2-carboxyethylmercapturic acid); CESG, *S*-(2-carboxyethyl)glutathione; CHEMA, *N*-acetyl-*S*-(2-carboxy-2-hydroxyethyl)-L-cysteine (2-carboxy-2-hydroxyethylmercapturic acid); CHESG, *S*-(2-carboxy-2-hydroxyethyl)glutathione; CYP450, cytochrome P450; EH, epoxide hydrolase; GSH, glutathione; HPA, 3-hydroxypropanoic acid; HPMA, *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (3-hydroxypropylmercapturic acid); HPSG, *S*-(3-hydroxypropyl)glutathione; M.a.p., mercapturic acid pathway; OPSG, *S*-(3-oxopropyl)glutathione. Adapted from Patel et al. (1980), Parent et al. (1998), and Kurahashi et al. (2014).

24 hours after oral uptake of acrolein at a dose of 7.5 µg/kg bw in drinking-water. For HPMA and CEMA, respectively, elimination half-times were 8.9 hours and 11.8 hours, and maximum urinary concentrations reached 2 hours after ingestion were 1.61 and 1.05 µmol/g creatinine (Watzek et al., 2012). A similar elimination half-time for acrolein (9 hours) based on urinary metabolite HPMA profile was reported in a study on subjects who were served fried food containing acrolein (Wang et al., 2019).

Acrolein can be produced endogenously, including as a result of lipid peroxidation (Nath & Chung, 1994; Stevens & Maier, 2008).

Acrolein can be excreted unchanged in exhaled air (Andreoli et al., 2003; Ligor et al., 2008; Ruenz et al., 2019).

Free acrolein was also found in the urine and saliva (Korneva et al., 1991) of patients treated with cyclophosphamide; acrolein is a metabolite of cyclophosphamide. Once formed, acrolein appeared to be rapidly excreted in urine because its urinary concentration peaked shortly (1–12 hours) after treatment with cyclophosphamide (Takamoto et al., 2004).

A role of glutathione *S*-transferases (GSTs) in detoxification of acrolein was demonstrated by a randomized clinical trial in which a significant increase in the excretion of HPMA was observed in individuals who received 2-phenethyl isocyanate, an inducer of GST mu 1 (GSTM1) and GST theta 1 (GSTT1), compared with controls (Yuan et al., 2016). The role of GSH and GSTs is discussed further below (see Section 4.1.2).

(b) In vitro

A low absorption rate of 0.480 ± 0.417 μg/cm^2 in 30 minutes was observed in experiments with human skin in vitro at 153 ppm (351 mg/m^3) of acrolein in air (Thredgold et al., 2020).

The reaction of acrolein with GSH in vitro is efficiently catalysed by human GST ε, μ, and π, the last one isolated from human placenta being the most catalytically active (Berhane & Mannervik, 1990). Significant differences were found in catalytic efficiency (k_{cat}/K_m) between four allelic variants of the π isoenzyme (hGSTP1-1) (Pal et al., 2000). However, in a search for genetic variants related to acrolein metabolism to mercapturic acids (GST polymorphism) by a genome-wide association study, no association with HPMA levels in smokers after adjusting for total nicotine equivalents was found (Park et al., 2015). [The Working Group noted that these results, together with the known high electrophilic reactivity of acrolein, suggest that its conjugation with GSH leading eventually to the excretion of HPMA is mainly a spontaneous non-catalysed process.]

Acrolein can be reduced by human aldo-keto (Al-Rawithi et al., 1998; Takamoto et al., 200 reductases with catalytic efficiencies that vary greatly among the superfamily members. Thus, aldose reductase (EC 1.1.1.21) catalysed acrolein reduction with k_{cat}/K_m = 1.09 μM^{-1} min^{-1} and was significantly induced (7–20-fold) towards a variety of aldehydes by acrolein (Kolb et al., 1994). Human aldo-keto reductase AKR1A showed a much lower catalytic activity (k_{cat}/ K_m = 0.29 × 10^{-3} μM^{-1} min^{-1} (Kurahashi et al., 2014), whereas AKR1B1 and ABR1B10 showed k_{cat}/K_m values of 0.12 and 1.07 μM^{-1} min^{-1}, respectively (Shen et al., 2011). AKR1B1, which is ubiquitously expressed in humans, also efficiently reduced the acrolein–GSH conjugate (*S*-(3-oxopropyl)glutathione, OPSG) with k_{cat}/ K_m = 0.355 μM^{-1} min^{-1}, whereas AKR1B10 expressed mainly in the gastrointestinal tract showed a much lower catalytic efficiency, k_{cat}/ K_m = 0.004 μM^{-1} min^{-1} (Shen et al., 2011). Downregulation of the *AKR1B10* gene increased the susceptibility of a colorectal cancer cell line to cytotoxicity caused by acrolein (Yan et al., 2007).

4.1.2 Experimental systems

(a) In vivo

Due to high electrophilicity and solubility in water, a significant portion of inhaled acrolein is taken up in the upper respiratory tract. Experiments on Fischer 344 rats with surgically isolated upper respiratory tract in vivo showed that the nasal uptake efficiency decreased with increasing acrolein concentration, time of exposure, and inspired air flow rate (Morris, 1996; Struve et al., 2008). At the inspired air flow rate of 100 mL/minute, the uptake efficiency averaged over an 80-minute exposure period was 98%, 68%, and 50% at 0.6, 1.8, and 3.6 ppm, respectively. At 300 mL/minute these values fell to 85%, 48%, and 38%, respectively (Struve et al., 2008).

Somewhat lower time-averaged values were obtained earlier by Morris (1996), namely, 62%, 38%, and 28% at the exposure concentrations 0.87, 4.4, and 8.7 ppm, respectively (inspiratory flow rate, 200 mL/minute).

GSH concentrations in nasal epithelium were markedly lowered in a concentration-dependent manner in rats exposed for 80 minutes. However, when the rats were pre-exposed to acrolein at 3.6 ppm during 14 days (6 hours per day, 5 days per week), the depletion was nearly compensated by an adaptive response (Struve et al., 2008). A marked depletion in rat nasal GSH was also reported earlier by Lam et al. (1985). [The Working Group noted that these results indicate a marked influence of tissue reactivity on uptake in the upper respiratory tract.]

Mercapturic acids, namely, HPMA and CEMA, were identified in the urine of rats dosed subcutaneously (Kaye, 1973; HPMA only) or orally with acrolein (Draminski et al., 1983; CEMA only), as well as in mice after inhalation and intraperitoneal injection (Linhart et al., 1996). Due to its high electrophilic reactivity, acrolein forms protein adducts in vivo (Gan & Ansari, 1989; Kautiainen et al., 1989; see also Section 4.2.1). A gradual accumulation of protein-adducted acrolein was reported in mice exposed to acrolein by inhalation at 1.5 ppm for 30 minutes twice per day for 3 weeks. At the same time, a gradual increase in urinary HPMA excretion was observed (Tully et al., 2014).

The metabolism and disposition of [2,3^{14}C]acrolein was studied in male and female Sprague-Dawley rats treated by oral and intravenous administration (Parent et al., 1996a, 1998). The rats were divided into five groups of 5 males and 5 females and were given a single dose of [2,3-^{14}C]acrolein intravenously at 2.5 mg/ kg bw, or orally by gavage at 2.5 or 15 mg/kg bw. One group of rats was pre-exposed to unlabelled acrolein for 14 days at 2.5 mg/kg-day before oral administration of [2,3-^{14}C]acrolein at 2.5 mg/kg bw. Urine, faeces, and expired air were collected for 7 days. In all exposure groups, about 26–31% of the radiolabel was exhaled as carbon dioxide while < 1.2% was tissue-bound. Rats given a single intravenous injection of [2,3^{14}C]acrolein at 2.5 mg/kg bw excreted 66–69% of the radiolabel in urine and < 2% in faeces. The main urinary metabolites were identified by HPLC/MS analysis using authentic standards as 3-hydroxypropanoic acid, HPMA, CEMA, and *N*-acetyl-*S*-(2-carboxy-2-hydroxyethyl)-Lcysteine (2-carboxy-2-hydroxy ethylmercapturic acid, CHEMA) and traces of malonic acid. After oral doses, less radiolabel was excreted in the urine (lower dose, 52%; higher dose, 36.5%) and more in the faeces (lower dose, 13%; higher dose, 31%). Two additional urinary metabolites, oxalic and malonic acid, were identified (Parent et al., 1998). No significant effect on the excretion pattern was observed after pre-treatment with acrolein. The main portion of radiolabel was excreted within 48 hours after dosing, but excretion was delayed in the group receiving the higher oral dose. The analysis of faeces did not reveal any distinct peaks in the excretion of radiolabel over time. [The Working Group noted that faeces probably contained polymers of acrolein or polysaccharide, or protein adducts resulting from the reaction of acrolein with food components.]

A computational fluid dynamics model was developed to predict nasal dosimetry of acrolein in rats and humans using parameters adjusted to fit experimental uptake efficiency data from Struve et al. (2008) and Morris (1996). In humans, calculated nasal uptake efficiencies for inhaled acrolein were 16% and 28% at exposure concentrations of 3.6 ppm and 0.6 ppm,

respectively, and were consistently lower than those in rats. These predictions capture the overall trend of increased uptake when exposure concentrations decrease (Schroeter et al., 2008). [The Working Group noted that because of oral breathing, delivery of acrolein to the lower respiratory tract could be higher in humans than in rats, which are obligate nasal breathers.]

(b) In vitro

Acrolein reacts spontaneously with GSH to form OPSG (Esterbauer et al., 1975; Mitchell & Petersen, 1989; Horiyama et al., 2016), which is subsequently oxidized by rat liver aldehyde dehydrogenase (ALDH) to *S*-(2-carboxyethyl)glu - tathione (CESG) and, in a lesser extent, reduced by rat liver alcohol dehydrogenase (ADH) to *S*-(3-hydroxypropyl)glutathione (HPSG) as the affinity of ADH (K_m = 877 µM) was low compared with the high-affinity cytosolic (K_m = 310 µM) and mitochondrial (K_m = 198 µM) ALDH forms (Mitchell & Petersen, 1989). Rat AKR7A1 catalysed reduction of both acrolein and its GSH conjugate. Chinese hamster V79 cells expressing rat AKR7A1 were efficiently protected against acrolein-induced mutations (Gardner et al., 2004) (see Section 4.2.2b).

The carbonyl group of acrolein can be oxi - dized by ALDH and reduced by aldo-keto reductases (AKR). Recombinant mouse ALDH1a1 and ALDH 3a1 efficiently oxidized acrolein to acrylic acid, ALDH1a1 showing comparable catalytic efficiency (V_{max}/K_m >> 23) but a higher affinity (K_m = 23.2 µM) than ALDH3a1 (K_m = 464 µM) (Makia et al., 2011). Significant catalytic ALDH activities were found in the microsomes, cytosol, and mitochondria of rat liver (Rikans, 1987). In mitochondria, two different ALDH activities were found: a high-affinity one with K_m = 0.017 mM, V_{max} = 42.2 nmol min^{-1} mg^{-1}, and a low-affinity one with K_m = 0.430 mM, V_{max} = 29.2 nmol min^{-1} mg^{-1}. Similarly, in the cytosolic fraction, there was a high-affinity ALDH form with K_m = 0.026 mM, V_{max} = 14.9 nmol min^{-1} mg^{-1}, and a low-affinity form with K_m = 0.725 mM, V_{max} = 7.1 nmol min^{-1} mg^{-1}. In the microsomes, a single low-affinity ALDH activity with K_m = 1.5 mM and V_{max} = 30.5 nmol min^{-1} mg^{-1} was reported. Hence, the low K_m ALDH in mitochondria was found to have the highest metabolic activity (Rikans, 1987). [The Working Group noted that both oxidation by ALDHs and reduction by AKRs are important detoxication pathways in the metabolism of acrolein.]

Metabolic activation of acrolein to glycidaldehyde and its detoxification to acrylic acid were described in rat liver and lung preparations by Patel et al. (1980). Notably, oxidation to acrylic acid was not observed in the lung preparations. Glycidaldehyde is a substrate for epoxide hydrolases as well as for cytosolic GSTs in rat lung and liver (Patel et al., 1980). However, metabolic activation was not necessary for conjugation with GSH. A weak increase in GSH conjugation as measured by GSH depletion was observed only when cytochrome P450 (CYP) in rat liver microsomes was induced by pre-treatment of rats with phenobarbital (Garle & Fry, 1989). Experiments with [^{14}C]-labelled acrolein proved its covalent association with rat microsomal CYP and further metabolism to an epoxide (Marinello et al., 1984). [The Working Group noted that conjugation of glycidaldehyde with GSH should lead to urinary CHEMA, a confirmed metabolite of acrolein.]

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether acrolein is electrophilic or can be metabolically activated to electrophiles; is genotoxic; alters DNA repair or causes genomic instability; induces oxidative

stress; is immunosuppressive; induces chronic inflammation; alters cell proliferation, cell death, or nutrient supply; induces epigenetic alterations; modulates receptor-mediated effects; and causes immortalization.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) DNA binding (i) Studies in humans

Acrolein is a chemically reactive aldehyde that directly interacts with DNA as a result of its α,β-unsaturated carbonyl function. As further described in Section 4.2.1(b), it forms four isomeric α- and γ-hydroxy-1,N^2- propano-2′-deoxyguanosine adducts (α-OH-PdG and γ-OH-PdG, two of each), and their ringopened precursors (Chung et al., 1999). Acroleininduced DNA adducts have been found in various tissues in studies in humans, including lung, buccal cells, leukocytes, peripheral blood, liver tissues, sputum, brain tissues, bladder, and urothelial mucosa (Weng et al., 2018; Zhang et al., 2007; Yang et al., 2019a; Chung et al., 2012; Nath et al., 1998; Bessette et al., 2009; Wang et al., 2019; Tsou et al., 2019; Zhang et al., 2011; Yin et al., 2013; McDiarmid et al., 1991; Alamil et al., 2020; Nath & Chung, 1994; Fu et al., 2018; Chen & Lin, 2011; Liu et al., 2005; Lee et al., 2014; Hong et al., 2020; see Table 4.1 and Table 2.1). Furthermore, significantly higher levels of acrolein–DNA adducts were found in bladder tumour tissues, hepatocellular carcinoma tissues, and brain tissues from patients with Alzheimer disease (Liu et al., 2005; Lee et al., 2014; Fu et al., 2018; see Table 4.1 and Table 2.1).

Acrolein–DNA adduct formation has been detected in the leukocytes of 40% of a group of patients treated with cyclophosphamide compared with none of the controls (McDiarmid et al., 1991). Several studies reported higher levels of acrolein–dG adducts in the buccal cells and lung tissues of tobacco smokers (Nath et al., 1998; Zhang et al., 2007; Weng et al., 2018). Similar results have been reported using immunochemical, ^{32}P-postlabelling 2D thinlayer chromatography/high-performance liquid chromatography (TLC/HPLC) and LC-MS/MS methods for mea suring acrolein–dG adducts in buccal cells (Nath et al., 1998; Weng et al., 2018; Wang et al., 2019). [The Working Group noted that different methods were used in these studies, which may account for differences in levels detected.] Using the immunochemical method and ^{32}P-postlabelling 2D TLC/HPLC, Weng et al. (2018) reported that γ-OH-PdG accumulated significantly more in smokers than in non-smokers (Weng et al., 2018). Using the LC-MS/MS method, Chung et al. (2012) also confirmed that γ-OH-PdG is the major acrolein– DNA adduct formed in the human lung tissues. Alamil et al. (2020) reported higher levels of acrolein–DNA adducts in peripheral blood in a smoker than in a non-smoker. On the other hand, it has been reported that two isomers, α- and γ-OH-PdG, formed almost equally in the lung tissues of smokers and non-smokers; and that the level of acrolein–DNA adducts in smokers is not significantly different from that in non-smokers (Ma et al., 2019; Yang et al., 2019a). The levels of acrolein–dG adducts detected were about 10–100 times lower than those reported by other laboratories, and the levels of acrolein–DNA adducts in leukocytes and lungs were similar (Chung et al., 2012; Ma et al., 2019; Zhang et al., 2011; Weng et al., 2018; Yang et al., 2019a; Alamil et al., 2020). [Since tobacco smoke contains substantial amounts of acrolein (see Section 1.4.2(b)), the Working Group noted that the lack of differences in acrolein–DNA adduct formation in both lung

tissues and leukocytes of smokers and non-smokers may be explained by other exposure sources.]

(ii) Human cells in vitro

There is ample evidence demonstrating that acrolein can adduct DNA in various primary human cells and in cultured human cell lines in vitro (Wilson et al., 1991; Feng et al., 2006; Pan et al., 2009, 2012, 2016; Greenspan et al., 2012; Wang et al., 2012; see Table 4.2). Feng et al. (2006) reported that acrolein treatment in normal human bronchial epithelial cells and normal human lung fibroblasts induces acrolein– DNA adducts that were preferentially formed at lung cancer *TP53* mutational hotspots, and that acrolein preferentially adducts guanines at cytosine methylation CpG sites. Wang et al. (2009a, 2012), using shuttle vectors containing the s*upF* gene, showed that cytosine methylation at CpG sites enhanced acrolein–DNA adduct formation and mutations at these sites; and that in human lung cells, acrolein induced γ-OH-PdG (95%)

Table 4.1 Acrolein-derived DNA adducts in exposed humans

Biosample	Location, exposure level	Adduct frequency (analytical method)	Response (significance)	Comments	Reference
Lung	Normal lung tissue of tobacco smokers (obtained from marginal tissues during tumour resection) and non-smokers (obtained from the Lung Tissue Research Consortium of the National Heart Lung and Blood Institute)	Smokers (n = 41) Non-smokers (n = 13)	Adducts/10^5 dG (^{32}P postlabelling TLC/HPLC) γ-OH-PdG: 1–24 in smokers vs 1–8 in non-smokers (statistically significant; P value, NR)	Lung, buccal cells, and sputum samples are from different individuals; smoking histories were from < 20 to > 50 packs/year.	Weng et al. (2018)
Lung	Normal tissue obtained at surgery from The Cancer Tissue Procurement Facility, University of Minnesota	Smokers (n = 5) Ex-smokers (n = 23) non-smokers	Adducts/10^9 dG (LC-MS/MS) γ-OH-PdG: 49 in smokers vs 25 in 1 mo to 26 yr; samples were from	Average calculated by the Working Group; ex-smokers quit smoking self-reported smokers; moderately well-defined exposure; other sources of acrolein exposure except smoking not considered.	Zhang et al. (2007) Center
Lung	Tissues obtained during surgery for lung cancer through the Tissue Procurement Facility, University of Minnesota	Smokers (n = 24) Non-smokers (n = 13)	Adducts/10^9 dG (LC-MS/MS) γ-OH-PdG: 20 in smokers vs 15 in non-smokers (NS)	Moderately well-defined exposure; other sources of acrolein exposure except smoking not considered.	Yang et al. (2019a)
Lung	Tissues obtained after surgery from the Histopathology & Tissue Shared Resource of the Lombardi Comprehensive Cancer Center, Georgetown University	n = 5	Adducts/10^9 dG (LC-MS/MS) γ-OH-PdG: 4–10	Poorly defined exposure; unclear whether the adducts were from endogenous exposure or from any unknown external exposure.	Chung et al. (2012)
Buccal cells	Buccal mucosa from subjects free of lung cancer at the time of the initial screening	Smokers (n = 33) Non-smokers (n = 17)	Adducts/10^7 dG (immunochemical method) γ-OH-PdG: 10–250 in smokers vs 5–15 in non-smokers (P < 0.0001)	Lung, buccal cells, and sputum samples are from different individuals; smoking histories were from < 20 to > 50 packs/year.	Weng et al. (2018)
Buccal cells (gingival tissue)	Samples from surgery at a periodontal clinic of New York University Dental Center, New York	11 smokers (4M, 7F); 12 non-smokers (8M, 4F)	Adducts/10^6 dG (^{32}P postlabelling and HPLC) Adduct levels significantly higher in smokers 1.36 ± 0.90 than in non-smokers 0.46 ± 0.26 (P = 0.003)	Small study; self-reported exposure.	Nath et al. (1998)

Table 4.1 (continued)

Biosample	Location, setting	Exposure level and no. of exposed and controls	Adduct frequency (analytical method) Response (significance)	Comments	Reference
Buccal cells	Smokers	$n = 5$	Adducts/10^7 dG (loss-triple stage with linear quadruple ion trap MS) > 5 per 10^7 unmodified DNA bases in buccal cell DNA	Tobacco smokers, smoking > 20 cigarettes per day, and on a noncontrolled diet.	Bessette et al. (2009)
Buccal cells	Healthy subjects after consumption of fried food from three commercial restaurants	$n = 19$	Acr–dG (immunochemical method) Fried food causes a 50% increase in Acr–dG levels, 2–24 hours after meal ($P < 0.005$)	Urinary HPMA, 30% increase; poorly defined exposure.	Wang et al. (2019)
Buccal cells	Healthy subjects Patients with oral squamous cell carcinoma	$n = 222$ $n = 80$	Acr–dG (immunochemical method) 25% increase ($P = 0.001$)	Moderately well-defined exposure. Sources of acrolein exposure other than smoking, alcohol drinking, and betel-quid chewing were not considered.	Tsou et al. (2019)
Leukocytes	Samples from smokers and non-smokers obtained at the University of Minnesota Tobacco Use Research Center	Smokers ($n = 25$) Non-smokers ($n = 25$)	γ-OH-PdG/10^9 nucl (LC-MS/MS) Adduct levels: smokers, 7.4 ± 3.4 adducts/10^9 nucl; non-smokers, 9.8 ± 5.5 adducts/10^9 nucl; (NS)	No difference between smokers and non-smokers; poorly defined exposure; unclear whether the adduct levels are from endogenous exposure or from any unknown external exposure.	Zhang et al. (2011)
Leukocytes	Provided by five subjects	$n = 5$	γ-OH-PdG/10^8 nucl (LC-MS/MS) 7.5–11 adducts/10^8 nucl (mean, ~9.0 ± 1.3 adducts/10^8 nucl)	Acr–dA, Acr–dC and etheno– DNA also detected. Poorly defined exposure; unclear if the adduct levels are from endogenous exposure or from any unknown external exposure.	Yin et al. (2013)
Leukocytes	Patients treated with cyclophosphamide Untreated matched patients	$n = 12$ 6 positive results in $n = 15$	Acr–dG (immunochemical methods) sources of acrolein exposure other cyclophosphamide treated patients vs 0 in matched controls ($P = 0.003$)	Moderately well-defined exposure; than smoking were not considered.	McDiarmid et al. (1991)

Table 4.1 (continued)

Biosample	Location, setting	Exposure level and no. of exposed and controls	Adduct frequency (analytical method) Response (significance)	Comments	Reference
Peripheral blood	Smoker vs non-smoker	$n = 1$ $n = 1$	Acr–dG/10^7 nucl (LC-MS/MS) 4.1 in smokers, NR in non-smokers	Reduced FA–dG and MDA–dG detected; compared single samples, one a heavy smoker, for first-level validation of methods; no measurements supporting smoking extent provided (e.g. cotinine); smoker was said to have regularly smoked 30 cigarettes/day; acrolein adducts not seen in non-smoker.	Alamil et al. (2020)
Liver	Autopsy samples from Colombia University, New York	$n = 5$	γ-OH-PdG/10^6 nucl (^{32}P postlabelling and HPLC) 0.03–0.74 adducts/10^6 dG	The health status of these individuals was unknown; demonstration study.	Nath & Chung (1994)
Liver	Tissues obtained after surgery from the Histopathology & Tissue Shared Resource of the Lombardi comprehensive Cancer Center, Georgetown University	$n = 5$	γ-OH-PdG/10^9 nucl (LC-MS/MS) 1.11 adducts/10^9 dG	Poorly defined exposure; unclear if the adduct levels are from endogenous exposure or from any unknown external exposure.	Chung et al. (2012)
Liver	Liver biopsies or HCC specimens from patients who had liver biopsies or curative resection of HCC as part of standard medical care; Georgetown University Medical Center		γ-OH-PdG (immunostaining)	Biomarker for predicting the risk of human HCC recurrence.	Fu et al. (2018)
	HCC patients	$n = 90$	High γ-OH-PdG levels in the HCC specimens were strongly correlated ($P < 0.0001$) with poorer survival in these patients.		
	HCC recurrence 500 days after surgery	$n = 45$	Patients with tumours with low γ-OH-PdG experienced a significantly longer HCC recurrence-free survival than patients with tumours with high γ-OH-PdG ($P < 0.007$)		

Table 4.1 (continued)

Biosample	Location, setting	Exposure level and no. of exposed and controls	Adduct frequency (analytical method) Response (significance)	Comments	Reference
Sputum	From subjects free of lung cancer at the time of the initial screening	Smokers ($n = 22$) Non-smokers ($n = 8$)	Adducts/10^7 dG (immunochemical method) γ-OH-PdG: 5–240 in smokers vs 5–130 in non-smokers ($P < 0.05$)	Lung, buccal cells, and sputum samples were from different individuals; smoking histories were from < 20 to > 50 packs/year.	Weng et al. (2018)
Saliva	Healthy individuals	$n = 27$	γ-OH-AdG/10^8 nucl (LC-MS/MS) 13–218 adducts/10^8 dG	Etheno–dG detected (68–752 adducts/10^8 nucl); no information on external exposure.	Chen & Lin (2011)
Brain	Brain specimens removed at autopsy from subjects with Alzheimer disease and agematched control subjects	Alzheimer disease, $n = 8$ (4M, 4F) Controls, $n = 5$ (3M, 2F)	γ-OH-PdG/10^6 nucl (LC-MS/MS) γ-OH-PdG: 5.1 in specimens from patients with Alzheimer disease vs 2.8 in healthy participants ($P < 0.025$)	Poorly defined exposure; unclear whether the adduct levels are from endogenous exposure or from any unknown external exposure.	Liu et al. (2005)
Bladder mucosa	Bladder tumours	$n = 10$	63 ± 25/10^7 dG in bladder tumours vs 25 ± 10 in normal urothelial mucosa ($P < 0.001$)	External exposure not defined.	Lee et al. (2014)
	Normal urothelial mucosa	$n = 19$			
	CKD early	$n = 40$	30% increase ($P < 0.01$)		
	CKD late	$n = 22$			
Urothelial tissue (nonsmokers)	Normal tissue Tumour tissue	$n = 48$ $n = 48$	30% increase ($P < 0.005$)		

Acr, acrolein; CKD, chronic kidney disease; dG, deoxyguanosine; F, female; FA, formaldehyde; HCC, hepatocellular carcinoma; LC-MS/MS, liquid chromatography-tandem mass spectrometry; M, male; MDA, malondialdehyde; mo, month; MS, mass spectrometry; NR, not reported; NS, not significant; nucl, nucleotide; γ-OH-PdG, γ-hydroxy-1,N^2-propano-2′deoxyguanosine; TLC/HPLC, thin-layer chromatography/high-performance liquid chromatography; vs, versus; yr, year.

Table 4.2 Acrolein-derived DNA adducts in human cells in vitro

End-point	Tissue, cell line	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA adducts (^{32}P-postlabelling)	Xeroderma pigmentosum fibroblasts, GM 5509	+	1 µM		Wilson et al. (1991)
DNA adducts (^{32}P-postlabelling)	Primary normal bronchial fibroblasts, human skin fibroblasts from a cystic fibrosis patient, GM 4539	+	100 µM	Only one concentration tested.	Wilson et al. (1991)

DNA adducts (^{32}P-postlabelling)	Lung epithelial cells and fibroblasts	+	5–20 µM	Acrolein induced DNA damage at *TP53* mutational hotspots and inhibited DNA repair.	Feng et al. (2006)
DNA adducts (^{32}P-postlabelling)	Colon HT-29 cells	+	20 µM		Pan et al. (2009)
DNA adducts (^{32}P-postlabelling)	Colon HT-29 cells	+	200 µM		Pan et al. (2012)
DNA adducts (LC-MS/MS-MRM)	BEAS-2B (bronchial epithelial cells)	+	20 µM		Greenspan et al. (2012)
DNA adducts (TLC/HPLC)	Normal bronchial epithelial cells, normal lung fibroblasts, cultured lung adenocarcinoma cells (A549)	+	25–100 µM		Wang et al. (2012)
	Human colon cancer HCT116 + ch3 cells	+	200 µM		Pan et al. (2016)

HIC, highest ineffective concentration; LC-MS, liquid chromatography-mass spectrometry; LEC, lowest effective concentration; MS-MRM, mass spectrometry multiple reaction monitoring; NT, not tested; TLC/HPLC, thin-layer chromatography/high-performance liquid chromatography. [a] All tests were conducted without metabolic activation.

and α-OH-PdG (5%). These studies also investigated subsequent mutagenesis as well as effects on DNA repair (see Sections 4.2.2 and 4.2.3).

(iii) Experimental systems: reactions with deoxyribonucleosides

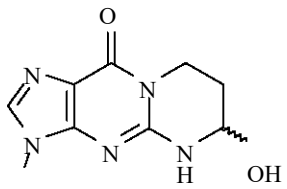
Acrolein is a strong electrophile and readily undergoes reactions with deoxyribonucleosides forming covalent adducts via Michael addition. The reactions of acrolein with deoxyguanosine, deoxyadenosine, and deoxycytidine have been well studied. The electron-rich purine bases are more reactive towards acrolein than are pyrimidine bases. These reactions involve an initial nucleophilic attack of a nitrogen in the bases to the terminal (β) olefinic carbon of acrolein, followed by the addition of a second nitrogen to the aldehydic carbon, leading to the formation of a new ring structure. The end products consist of a class of structurally unique cyclic adducts (Chung et al., 1986). Specifically, upon reaction with deoxyguanosine, acrolein yields cyclic 1,N^2-propano-2′-deoxyguanosine (PdG) adducts as a pair of regioisomers, designated as α and γ-OH-PdG (formerly as Acr–dG 1/2 and 3, respectively), depending on which deoxyguanosine nitrogen is involved in the Michael addition (Chung et al., 1984; Fig. 4.2). The α-isomers are a pair of diastereomers that exist in equilibrium due to interconversion via ring opening. The reaction of acrolein with deoxyadenosine yields cyclic 1,N^6-propano-2′-deoxyadenosine derivatives (1,N^6-PdA) with the possible formation of either 9- or 7-OH substituted regioisomers (Sodum & Shapiro, 1988, Smith et al., 1990a; Pawłowicz et al., 2006a); however, studies were mostly focused on the 9-OH isomer (Fig. 4.2). The 9-OH-1,N^6-PdA adduct can further react with another acrolein molecule, forming a 2:1 adduct (Pawłowicz et al., 2006a); [the Working Group noted that such adducts are unlikely to be formed under physiological conditions in vivo]. In addition to the above-mentioned adducts, the exocyclic amino group in deoxyadenosine can be involved in two Michael additions with two acrolein molecules, followed by intramolecular aldol condensation, which gives rise to a 6-(3-formyl-1,2,5,6-tetrahydropyridyl) substituted adduct (Pawłowicz et al., 2006b). The reaction of acrolein with deoxycytidine forms a 3,N^4-substituted cyclic adduct (7-hydroxy-3,N^4-propano-2′-deoxycytidine) as a pair of diastereomers (Chenna & Iden, 1993). However, only one of the two possible regioisomers, the one resulting from Michael addition of the endocyclic N3 to the acrolein β-carbon, has been described (Fig. 4.2). Alkylated adducts, sometimes 2:1 adducts with deoxyadenosine and thymidine, which result from Michael addition to acrolein without subsequent ring closure, have also been described; these appear to be minor products (Lutz et al., 1982; Chenna et al., 1992; Pawłowicz et al., 2006a; Pawłowicz & Kronberg, 2008). Interestingly, under strenuous conditions (DMSO at 100 °C for 5 days) γ-OH-PdG, one of the cyclic adducts of acrolein with deoxyguanosine, can further react with another molecule of deoxyguanosine forming a cyclic bis-nucleoside, γ-OH-PdG–dG (Kozekov et al., 2001). [The Working Group noted that, despite the somewhat harsh conditions, the identification of the bis-nucleoside adduct suggests the possibility that interstrand dG–acrolein–dG crosslinks can be formed in duplex DNA.] Table 4.3 summarizes the reported reaction conditions between acrolein and deoxyribonucleosides/deoxyribonucleotides and the identity of the resulting adducts.

(iv) Experimental systems: reactions with DNA in vitro

Using synthetic adducts from the reactions with deoxyribonucleosides as reference standards, several studies, mostly with calf thymus DNA, have shown that acrolein can also modify DNA, forming some of the same adducts as with deoxyribonucleosides. Adducts formed in the acrolein-modified DNA have been detected and quantified, mainly after hydrolysis, by a variety of methods, including HPLC with fluorescence detection, ^{32}P-postlabelling, immune-based assays, or LC-MS/MS (Chung et al., 1984; Liu et al., 2005; Pawłowicz et al., 2006a; Pawłowicz & Kronberg, 2008; Pan et al., 2012; Chen et al., 2019a). The levels of adduct modification in these reactions are considerably lower than those with the monomers; however, the levels of modification may be significantly increased using denatured or single-stranded DNA, or oligomers. As the most nucleophilic base in DNA, guanine reacts to the greatest extent, in what constitutes a major pathway of DNA modification by acrolein.

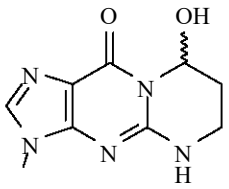
Unlike its reactions with the monomers, the formation of cyclic adducts by acrolein with deoxyguanosine and deoxyadenosi

Fig. 4.2 Structures of the major acrolein–deoxyribonucleoside adducts



dR

7-hydroxy-3,N^4-propano-2′-deoxycytidine

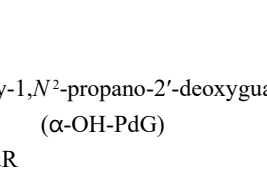


dR

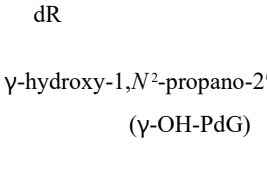
9-hydroxy-3,N^4-propano-2′-deoxycytidine

dR, 2′-deoxyribosyl.
Compiled by the Working Group.

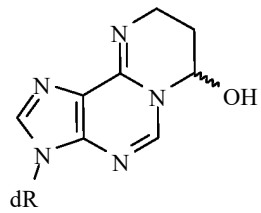
ne in DNA appears to be regioselective. For example, γ-OH-PdG predominates over the α-isomer in DNA (Chung et al., 1984). Similarly, 9-OH-1,N^6-PdA was reported to be the product in acrolein-modified DNA, not the 7-OH isomer (Smith et al., 1990a). Studies were carried out to



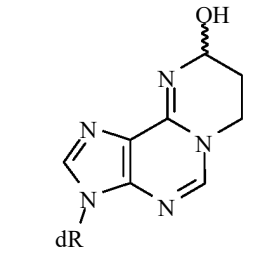
α-hydroxy-1,N^2-propano-2′-deoxyguanosine
(α-OH-PdG)



γ-hydroxy-1,N^2-propano-2′-deoxyguanosine
(γ-OH-PdG)

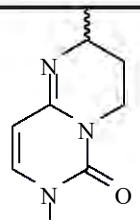


7-hydroxy-1,N^6-propano-2′-deoxyadenosine



9-hydroxy-1,N^6-propano-2′-deoxyadenosine

OH



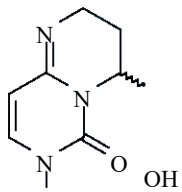


Table 4.3 Detection of acrolein-derived adducts with deoxynucleosides or deoxynucleotides in acellular systems

Nucleoside or deoxynucleotide	Conditions	Adduct	Reference
dG	PBS at 37 °C	γ-OH-PdG & α-OH-PdG	Chung et al. (1984)
dA		N^6-alkylated dA (Michael addition)	Lutz et al. (1982)
dA 5′-mp	PBS at 37 °C	9-OH-1,N^6-PdA-5′p	Smith et al. (1990a)
dA 3′,5′-bp	PBS at 37 °C	9-OH-1,N^6-PdA-3′,5′-bp	Smith et al. (1990a)
T	PBS at 37 °C	N3-alkylated	Chenna et al. (1992)
dC	PBS at 37 °C	7-OH-1,N^6-PdC	Chenna & Iden (1993)
dU	PBS at 37 °C	N3-alkylated	Chenna & Iden (1993)
dG/ γ-OH-PdG	DMSO at 100 °C	γ-OH-PdG–dG	Kozekov et al. (2001)
dG 5′-mp	ω-3 and ω-6 olyunsaturated fatty acids with ferrous sulfate/tris buffer at 37 °C	γ-OH-PdG	Pan & Chung (2002)
dA	PBS at 37 °C	1:1 and 2:1 (acrolein:dA) 1,N^6-PdA 1:1 and 2:1 (acrolein:dA) N^6-alkylated	Pawłowicz et al. (2006a, b)
T	PBS at 37 °C	1:1 N3-alkylated and four 2:1 (acrolein:T) N3-alkylated	Pawłowicz & Kronberg (2008)

1,N^6-PdA, 1,N^6-propano-2′-deoxyadenosine; 7-OH-1,N^6-PdC, 7-hydroxy-1,N^6-propano-2′-deoxycytosine; 9-OH-1,N^6-PdA, 9-hydroxy-1,N^6-PdA; α-, γ-OH-PdG, α-, γ-hydroxy-1,N^2-propano-2′-deoxyguanosine; dA, deoxyadenosine; dA 5′-mp, dA 5′-monophosphate; dA 3′,5′-bp, dA 3′,5′bisphosphate; dC, deoxycytosine; dG, deoxyguanosine; dG 5′-mp, dG 5′-monophosphate; DMSO, dimethyl sulfoxide; dU, deoxyuridine; PBS, phosphate-buffered saline; T, thymidine.

shed light onto the molecular basis for the regioselectivity. Possible explanations involve the tertiary structure of DNA and/or an intermediacy of the Schiff's base between acrolein and amines (Chung et al., 2012). The 2:1 adduct of acrolein with deoxyadenosine, but not thymidine or deoxycytidine, was also observed in the reactions with DNA in vitro (Pawłowicz et al., 2006b; Pawłowicz & Kronberg, 2008). The formation of cyclic adducts of acrolein involves covalent binding with the nitrogens that participate in hydrogen bonding in the double helical structure of DNA.

Interestingly, the cyclic bis-nucleoside adduct of γ-OH-PdG (γ-OH-PdG–dG) described above was also found in a DNA duplex containing γ-OH-PdG in a 5′-CpG sequence context with the exocyclic amino group of deoxyguanosine in the opposite strand, resulting from interstrand crosslinking in oligonucleotide

or DNA (Kozekov et al., 2001, 2010; Minko et al., 2009).

Although the crosslinking product can undergo reversible reaction, it was sufficiently stable to be isolated for structural characterization. Table 4.4 summarizes the reactions of acrolein with oligomers and DNA.

(v) Experimental systems: DNA adduct formation in tissues and cells

See Table 4.5 and Table 4.6.

Most in vivo studies of the acrolein-derived DNA adducts in cells and tissues have focused on γ-OH-PdG. The only acrolein-derived DNA adduct other than γ-OH-PdG reported to be formed in vivo is 9-OH-1,N^6-PdA (Kawai et al., 2003). It has been shown that γ-OH-PdG can be formed in DNA in vivo from acrolein derived from two major sources: environmental exposure, such as tobacco smoke; and endogenous production, such as lipid peroxidation and polyamine oxidation. Although diet may also be a possible source, its importance has been

Table 4.4 Detection of acrolein-derived DNA adducts with oligonucleotides and DNA

Oligomers or DNA	Conditions	Adduct	Detection method	Reference
ct-DNA	PBS pH 7 at 37 °C	γ-OH-PdG	HPLC-fluorescence	Chung et al. (1984)
ct-DNA	Tris pH 8.5 at 37 °C	γ-OH-PdG	CapLC-nanoESIMS/MS	Liu et al. (2005)
ct-DNA	PBS pH 7.4 at 37 °C	1:1 9-OH-1,N^6-PdA; 2:1 1,N^6-PdA; 1:1 N^6-alkylated dA; 2:1 N^6-alkylated dA	LC-ESI-MS/MS	Pawłowicz et al. (2006b)
ct-DNA	PBS pH 7.4 at 37 °C	1:1 N3-alkylated T	LC-ESI-MS/MS	Pawłowicz & Kronberg (2008)
ct-DNA	PBS pH 7.0 at 37 °C	γ-OH-PdG-dG (crosslinking)	LC-ESI-MS/MS	Kozekov et al. (2010)
ct-DNA/plasmid pSP189 ct-DNA	PBS pH 7.0 at 37 °C LPO	γ-OH-PdG α-, γ-OH-PdG, and other LPO-derived cyclic adducts	ELISA/slot blot UHPLC/ESI-IT-MS	Pan et al. (2012) Chen et al. (2019a)

ct, calf thymus; dA, deoxyadenosine; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; LPO, lipid peroxidation; α-, γ-OH-PdG, α-, gamma-hydroxy-1,N^2-propano-2′-deoxyguanosine; 1,N^6-PdA, 1, N^6-propano-2′-deoxyadenosine; 9-OH-1,N^6-PdA, 9-hydroxy-1, N^6-PdA; CapLC-nanoESI-MS/MS, capillary liquid chromatography-nano electrospray ionization-tandem mass spectrometry; PBS, phosphate-buffered saline; UHPLC/ESI-IT-MS, ultra high-HPLC ESI-ion trap multistage mass spectrometry.

Table 4.5 Detection of acrolein-derived DNA adducts in experimental animals in vivo

Adduct	Species	Tissue	Exposure	Method of detection	Reference
γ-OH-PdG	Dog	Lymphocytes	Cyclophosphamide (6.6 mg/kg)	^{32}P-Postlabelling	Wilson et al. (1991)
γ-OH-PdG	Mouse Rat	Liver Liver	None None	^{32}P-Postlabelling	Nath & Chung (1994)

γ-OH-PdG	Mouse Rat	Skin Brain Lung Kidney Colon Prostate Mammary gland Leukocytes	None	^{32}P-Postlabelling	Nath et al. (1996)
γ-OH-PdG	Rat	Liver	None	LC-MS/MS	Fu et al. (2018)
γ-OH-PdG	Rat	Liver	None	UHPLC/ESI-IT-MS	Chen et al. (2019a)
γ-OH-PdG	Cockerel	Aorta	Acrolein inhalation (0, 1, and 10 ppm)	^{32}P-Postlabelling	Penn et al. (2001)
γ-OH-PdG	Mouse	Lung/bladder	Sidestream smoke	Immunoassay/^{32}Ppostlabelling	Lee et al. (2015)
γ-OH-PdG	Mouse	Lung/bladder	Tobacco smoke	Immunoassay/^{32}Ppostlabelling	Weng et al. (2018)
Not identified	Rat	Lung	Diesel-exhaust inhalation	HPLC-MS/MS	Douki et al. (2018)
γ-OH-PdG	Mouse	Liver	High-fat diet	IHC and LC-MS/MS	Coia et al. (2018)
1,N^6-PdA	Rat	Kidney	Ferric nitrilotriacetate	IHC	Kawai et al. (2003)

1,N^6-PdA, 1,N^6-propoanodeoxyadenosine; γ-OH-PdG, γ-hydroxy-1,N^2-propano-2′-deoxyguanosine; IHC, immunohistochemistry; HPLC-MS/MS; high-performance liquid chromatography-tandem mass spectrometry. UHPLC/ESI-IT-MS, ultrahigh-HPLC electrospray ionization-ion trap multistage mass spectrometry.

Table 4.6 Detection of acrolein-derived DNA adducts in experimental systems in vitro

Adduct	Cells	Acrolein concentration	Method of detection	Reference
γ-OH-PdG	*Salmonella typhimurium* TA100 and TA104	1, 4, 7, 10, 13 mM	ELISA	Foiles et al. (1989)
γ-OH-PdG	Chinese hamster ovary	mM	ELISA	Foiles et al. (1990)
1,N^6-PdA	Rat liver epithelial cells	5, 10, 25, 50 µM	Immunoassay	Kawai et al. (2003)
γ-OH-PdG	*Sphingobium* spp. strain KK22	10 mM	LC-ESI-MS/MS	Kanaly et al. (2015)

ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting, flow cytometry; LC-ESI-MS/MS, liquid chromatography-electrospray ionization-tandem mass spectrometry; 1,N^6-PdA, 1,N^6-propano-2′-deoxyadenosine; γ-OH-PdG, gamma-hydroxy-1,N^2-propano-2′-deoxyguanosine.

questioned by a study in which integrated quantitative structure–activity relationship–physiologically based kinetic/dynamic (QSAR-PBK/D) modelling was used to predict formation of γ-OH-PdG (Kiwamoto et al., 2015). As acrolein is an oxidation product of lipid peroxidation from ω-3 and -6 polyunsaturated fatty acids, the acrolein-derived adducts can be formed upon incubation of these fatty acids in the presence of deoxyguanosine under oxidative conditions (Pan & Chung, 2002; Kawai et al., 2003). As lipid peroxidation occurs continuously in vivo as part of normal physiological processes, acrolein- derived DNA adducts are constantly formed in cellular DNA as endogenous background lesions. Several methods have been developed to detect acrolein-derived DNA adducts in vivo, including ^{32}P-postlabelling, LC-MS/MS, and immunohistochemistry. The availability of monoclonal antibodies against acrolein-derived deoxyadenosine and deoxyguanosine adducts has facilitated the development of immune-based methods, such as immunohistochemistry, immunocytochemistry, and dot blot, for

detecting these adducts in cells and tissues (Kawai et al., 2003 and Pan et al., 2012). However, it is generally agreed that LC-MS/MS is by far the most specific and most sensitive method for adduct detection and identification in vivo.

Acrolein-derived DNA adducts, including γ-OH-PdG, have been detected in various experimental animals in vivo (see Table 4.5). γ-OH-PdG was detected by a ^{32}P-postlabelling method in DNA of peripheral blood lymphocytes obtained from a dog given a therapeutic oral dose of cyclophosphamide at 6.6 mg/kg (Wilson et al., 1991). Studies later showed γ-OH-PdG is an endogenous background DNA lesion in livers of rodents and humans without known treatment and exposure (Nath & Chung, 1994; Nath et al., 1996). γ-OH-PdG was also detected in rats and mice given control feed (Fu et al., 2018; Chen et al., 2019a). Exposure of cockerels to acrolein (1 and 10 ppm) for 6 hours via inha lation gave rise to γ-OH-PdG in the aortic DNA (Penn et al., 2001). Exposure to tobacco smoke (mainstream, ~75 mg/m^3, 6 hours per day, 5 days per week, for 12 weeks; or sidestream, 500 μg/m^3, 6 hours per day, 5 days per week, for 8 or 16 weeks) and automobile exhaust was shown to induce γ-OH-PdG formation in the rodent lung (Lee et al., 2015; Weng et al., 2018; Douki et al., 2018). A small, but significant, increase in levels of acrolein-derived DNA adducts was found in the lung DNA of rats exposed to diesel exhaust; however, the data on the specific identity of the adduct were not reported (Douki et al., 2018). The notion that DNA adducts of acrolein can be derived from endogenous sources, such as lipid peroxidation, has been reinforced by recent studies showing that the levels of γ-OH-PdG are significantly increased in liver DNA of mice fed a high-fat diet (Coia et al., 2018). This study further demonstrated that the elevated hepatic formation of γ-OH-PdG in mice fed a high-fat diet parallels the increased risk of developing hepatocellular carcinoma in these mice. The only other acrolein-derived DNA adduct in vivo so far reported is 9-OH-1,*N*^{6}-PdA. This adduct was found in rat kidney, using an iron-induced kidney carcinogenesis model under oxidative stress conditions in which rats were exposed to ferric nitrilotriacetate (Kawai et al., 2003). However, the structural identity of the adduct was not unequivocally established in this study because the adduct was detected by a monoclonal antibody raised against acrolein-modified DNA, not specifically 9-OH-1,*N*^{6}-PdA.

Acrolein-derived DNA adducts, including γ-OH-PdG, have also been assessed in various experimental cell types in vitro (see Table 4.6). Using a monoclonal antibody developed against crotonaldehyde-derived cyclic deoxyguanosine adducts structurally analogous to γ-OH-PdG (Foiles et al., 1987), an early study demonstrated the detection of γ-OH-PdG in *Salmonella typhimurium* strains TA100 and TA104 exposed to acrolein at the concentration range in which mutations were induced (Foiles et al., 1989). The first study detecting γ-OH-PdG in mammalian cells was reported using enzyme-linked immunosorbent assay (ELISA) in Chinese hamster ovary cells exposed to acrolein at a high concentration (1 mM) (Foiles et al., 1990). This concentration, however, was too toxic for scoring mutations. Later, monoclonal antibodies were raised against 1,*N*^{6}-PdA, using acrolein-modified DNA (Kawai et al., 2003), and against γ-OH-PdG, using specifically γ-OH-PdG-conjugated bovine serum albumin (Pan et al., 2012; see Table 4.4). More recently, a DNA adductomics approach was applied to the study of γ-OH-PdG in the soil bacterium *Sphingobium* spp. strain KK22 (Kanaly et al., 2015). This study demonstrated the potential

of LC-MS/MS in DNA adductomics as a promising tool to study γ-OH-PdG and other related adducts in cells.

(b) Interactions with cellular proteins

(i) Reactions with amino acids and proteins in vitro See Table 4.7.

Acrolein shows a strong propensity to react with amino acids or proteins via Michael addition, considerably more so than with DNA bases. Cysteines and the thiols of amino acids and proteins are the major sites for covalent binding with acrolein. Because the thiols are known to play important roles in enzyme activities and redox homeostasis, their facile interactions with acrolein can profoundly alter cellular functions. On the other hand, compounds with the mercapto (-SH) group, like GSH and cysteine, are widely used as effective scavengers of acrolein, with aim of reducing its adverse effects in cells or animals (Rees & Tarlow, 1967; Gurtoo et al., 1981; Wildenauer & Oehlmann, 1982).

The N-alkylation of proteins by acrolein may also occur. These reactions, through the sidechain amino group of lysine or a ring nitrogen of histidine, are kinetically less favourable than conjugation with -SH groups. Unlike reactions with cysteines, N-alkylation is irreversible, and the end products are usually quite stable (Cai et al., 2009). Reactions of acrolein with lysine have been investigated extensively with 3-formyl-3,4-dehydropiperidine (FDP), a 2:1 adduct, as a notable product that may serve as a potential biomarker of acrolein exposure detectable by a monoclonal antibody (Uchida et al., 1998a, b). The formation of FDP lysine adducts in histone has been associated with the inhibition of chromatin assembly mediated by acrolein (Fang et al., 2016). Furthermore, acrolein can form a Schiff base with the amine of lysine, followed by Michael addition yielding *N*-(3-methylpyridium)lysine via 2:1 addition (Furuhata et al., 2003; Kaminskas et al., 2005) and intra- and inter-protein crosslinks (Burcham & Pyke, 2006; Ishii et al., 2007; Minko et al., 2008). In addition to lysine, acrolein can also react with histidine by nucleophilic attack

Table 4.7 Reactions of acrolein with amino acids and proteins

Source	Amino acid or protein	Adduct	Detection method	Reference
Cyclophosphamide and acrolein	Rat hepatic microsomal CYP450	Not identified	Radioactivity with gel electrophoresis	Marinello et al. (1984)
Acrolein	[^{3}H-lysine]albumin	Not identified	Radioactivity	Thakore et al. (1994)
Acrolein	Synthetic peptide	Not identified	HPLC-MS	Carbone et al. (1997)
Acrolein	Lysine and low-density lipoprotein	(3-Formyl-3,4-dehydropiperidino)lysine	HPLC/MS/amino acid analysis IHC (mAb5F6)	Uchida et al. (1998a, b)
Acrolein	Histidine	3-Formylethylhistidine	LC-MS and NMR	Pocker & Janjić (1988), Uchida et al. (1998a)
Acrolein	BSA	Michael adduct	Spectrophotometric method for detection of DNPH derivative	Burcham et al. (2000)
Acrolein	BSA	*N*-(3-Formyl-3,4-dehydropiperidino)lysine	HPLC-MS/amino acid analysis	Furuhata et al. (2002)
Acrolein	Peptide (B chain of insulin)	*N*-(3-Methylpyridinium)lysine	ESI-LC/MS mAb5F6	Furuhata et al. (2003)
Acrolein	BSA	Lysine mono-Michael adduct versus Schiff base and FDP cyclic adduct	ESI-MS	Kaminskas et al. (2005)
Acrolein	Bovine pancrease Ribonuclease A	Crosslinking dimerized proteins	Gel electrophoresis	Burcham & Pyke (2006)
Acrolein	Actin	Cys374	LC-ESI-MS/MS	Dalle-Donne et al. (2007)
Acrolein	Peptide (B chain of insulin)	Crosslinking adducts	LC-ESI-MS/MS	Ishii et al. (2007)
Acrolein–dG or –dA adduct	KWKK peptide	Crosslinking adducts	Gel electrophoresis	Minko et al. (2008)
Acrolein	Insulin peptides	Cys, lysine, histidine, intra-molecular Schiff base	ESI-MS, ESI-MS/MS	Cai et al. (2009)
Acrolein/lipid oxidation	BSA/LDL	*N*-(3-Propanal)histidine	ESI/LC/MS/MS	Maeshima et al. (2012)
Acrolein	Recombinant histone/ H2a and H4	Lysine FDP adduct	LC-MS/MS	Fang et al. (2016)
Acrolein	Human serum albumin	Michael addition adducts	Biotin affinity tag LC-MS/MS	Coffey & Gronert (2016)
Acrolein	Lysozyme and human serum albumin	Histidine/cysteine/lysine adducts	LC-MS/MS	Afonso et al. (2018)

BSA, bovine serum albumin; CYP450, cytochrome P450; DNPH, dinitrophenyl hydrazine; ESI-MS, electrospray ionization-tandem mass spectrometry; FDP, *N*(ε)-3-formyl-3,4dehydropiperidine; HPLC-MS, HPLC, high-performance liquid chromatography-mass spectrometry; LC, liquid chromatography; LDL, low-density lipoprotein; mAb, monoclonal antibody; NMR, nuclear magnetic resonance spectroscopy.

on the imidazole ring nitrogen (Pocker & Janjić, 1988; Maeshima et al., 2012). A recent study demonstrated that acrolein may be one of the aldehydes in tobacco smoke responsible for the inhibition of the enzymes involved in DNA repair by targeting these proteins via direct binding (Weng et al., 2018). Acrolein is a major metabolic product of certain anticancer drugs, such as cyclophosphamide, and early studies showed that the bladder toxicity of cyclophosphamide can be effectively attenuated by GSH or other SH-containing small compounds, whereas its therapeutic efficacy was not affected by GSH (Gurtoo et al., 1981). The reaction products were studied and compared between acrolein versus 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) and a synthetic peptide 128 (Carbone et al., 1997). Ample evidence shows that dithiothreitol and hydralazine also inhibit acrolein-induced cellular toxicity through their interactions with acrolein (Rees & Tarlow, 1967; Cox et al., 1988; Burcham et al., 2000; Burcham & Pyke, 2006; Chen et al., 2016). The chemical basis for the inhibition is the -SH conjugation with the former and formation of a hydrazone derivative with the latter; both reactions can effectively block acrolein's ability to bind cellular target proteins.

The identification of the binding sites of acrolein to protein is important because this knowledge may help understand the molecular basis underlying the toxicity caused by acrolein. To this end, LC-MS/MS-based proteomic methods have been developed in the past decade (Spiess et al., 2011; Coffey & Gronert, 2016; Afonso et al., 2018; Chen, Liu et al., 2019b). The application of proteomics in the determination of protein binding sites has been demonstrated with the use of model proteins as well as in cells treated with acrolein (Table 4.7).

(ii) Protein binding in human cells in vitro

Enhanced protein binding of acrolein has been demonstrated in exposed human bronchial epithelial cells (Caito et al., 2010), in human serum albumin (Colombo et al., 2010) and with the lactate dehydrogenase isozymes in human saliva (Avezov et al., 2014). Further investigations have revealed effects on protein function. For example, acrolein formed Michael adducts with sirtuin 1 (SIRT1) and reduced its activity (Caito et al., 2010). The evidence for protein dysfunction is ample; for example, Biswal et al. (2003) showed that acrolein modified c-JUN, preventing its dimerization and consequently preventing AP-1-promoter binding, and that acrolein modified B[*a*]P-induced TP53 and reduced its transcription transactivation activity. In human T cells, acrolein caused modification at Cys-61 and Arg-307 sites in p50 and IkB phosphorylation, consequently preventing DNA binding of NF-kB and reducing the expression of interleukins IL2 and IL10, interferon gamma (INFγ), tumour necrosis factor α (TNFα), and granulocyte-macrophage colony-stimulating factor. In human lung cells, acrolein at noncytotoxic levels can cause acrolein–Cys binding and consequently Hsp90 crosslinks (Burcham et al., 2007).

(iii) Protein binding in experimental animal cells and tissues

See Table 4.8.

To identify target proteins and binding sites in cells and tissues of rodents exposed to acrolein the methods currently used include immunohistochemistry, immunocytochemistry, Western blot, and LC-MS/MS. Because direct exposure to acrolein can cause overt toxicity, studies in vivo are often carried out with cancer chemotherapeutics, cigarette smoke, ethanol, and diet as indirect sources of acrolein (Gurtoo et al., 1981; Wildenauer & Oehlmann, 1982;

Günther et al., 2008; Conklin et al., 2009; Chen et al., 2016). The availability of a monoclonal antibody to acrolein-modified keyhole limpet haemocyanin, with the lysine binding as an epitope, has greatly facilitated studies of acrolein-bound proteins in cells and tissues (Uchida et al., 1998a, b). The antibody was specifically developed

Table 4.8 Detection of acrolein-derived adducts in proteins in experimental animal cells and tissues

Source	Cell or animal	Protein target	Detection method	Reference
Cyclophosphamide	Mouse/rat	Hepatic proteins	Radioactivity	Gurtoo et al. (1981)
Cyclophosphamide	Rabbit liver microsomes Human erythrocytes	Membrane and cytoplasm	Radioactivity with SDS polyacrylamide Gel electrophoresis	Wildenauer & Oehlmann (1982)
Cyclophosphamide	SCID mouse	Implanted CT26 tumour cells	IHC	Günther et al. (2008)
Tobacco smoke or Acrolein	Mouse lung, plasma, aorta		Western blot	Conklin et al. (2009)
Acrolein	Human lung epithelial cells	Proteome	LC-MS/MS	Spiess et al. (2011)
Acrolein	F344 rat	Cardiac mitochondria	Aldehyde-specific chemical labelling and LC-MS/MS	Chavez et al. (2011)
Endogenous	F344 rat	Cardiac mitochondria	Proteomics NanoLC MALDI-MS/MS	Han et al. (2012)
Alcohol or acetaldehyde	Rat hepatoma H4IIEC cells	FDP-lysine adduct	ICC	Chen et al. (2016)
Diet with 5% ethanol	Male C57BL/6J mouse	Hepatic proteins	IHC	Chen et al. (2016)
Acrolein	Proteomes of human lung cancer H1299 cells	> 2300 proteins > 500 cysteines	Aldehyde-directed aniline-based probe by LC-MS/MS	Chen et al. (2019b)

GST, glutathione-*S*-transferase, ICC; immunocytochemistry; IHC, immunohistochemistry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MALDI, matrix-assisted laser desorption ionization; MS, mass spectrometry; SDS, sodium dodecyl sulfate.

for lysine-adducted proteins. In recent years, LC-MS/MS-based proteomics has also been used to identify hundreds, if not thousands, of protein targets in cells and tissues (Spiess et al., 2011; Chavez et al., 2011; Han et al., 2012; Chen et al., 2019b; Table 4.8).

4.2.2 Is genotoxic

(a) Humans (i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

See Table 4.9.

Using the alkaline elution assay, acrolein-induced DNA strand breaks were observed in primary human bronchial epithelial cells (Grafström et al., 1986, 1988), human normal skin fibroblasts (CRL 1508) as well as xeroderma pigmentosum fibroblasts (Dypbukt et al., 1993), human myeloid leukaemia cells (K562) (Crook et al., 1986), and human lymphoblastoid cells (Namalwa) (Eisenbrand et al., 1995). [The Working Group noted that some of these experiments (Grafström et al., 1986; Dypbukt et al., 1993) were carried out at concentrations of acrolein that induced excessive cytotoxicity.] The frequency of phosphorylated H2AX proteins (γH2AX), an indicator of DNA double-strand breaks, was found to be significantly increased in acrolein-treated lung epithelial adenocarcinoma cells (A549) (Zhang et al., 2017) and human bronchial epithelial cells (BEAS-2B) (Zhang et al., 2020), and positive results were obtained in the comet assay for DNA damage in human normal lung fibroblasts (IMR-90) (Luo et al., 2013), A549 cells (Zhang et al., 2018), BEAS-2B cells (Zhang et al., 2020), human Burkitt lymphoma B lymphocytes (Raji) (Yang et al., 1999a), liver hepatoma cells (HepG2) (Li et al., 2008a), and retinal epithelial cells (ARPE-19) (Li et al., 2008b). [The Working Group noted that one experiment (Yang et al., 1999a) was carried out with acrolein at concentrations up to 500 μM with no measure of cytotoxicity.]

Acrolein-induced DNA–protein crosslinks were reported in bronchial epithelial cells (Grafström et al., 1986, 1988), in HepG2 cells (Li et al., 2008b), and in Burkitt lymphoma cells (EBV-BL) (Costa et al., 1997), but a negative result was reported in human promyelocytic leukaemia cells at a concentration (i.e. 100 μM) that resulted in a study-specific cell viability of 58% (Schoenfeld & Witz, 2000). [The Working Group noted that some of these experiments (Grafström et al., 1986; Schoenfeld & Witz, 2000) were carried out at concentrations of acrolein that induced excessive cytotoxicity.] Additionally, a negative result was reported in BEAS-2B cells exposed to acrolein at 7.5 μM; however, at this same concentration acrolein significantly enhanced the level of DNA–protein crosslinks observed when co-administered with formaldehyde (Zhang et al., 2020).

Acrolein induced a dose-dependent increase in *HPRT* mutant frequency in human DNA-repair-deficient xeroderma pigmentosum fibroblasts (Curren et al., 1988) and normal human bronchial epithelial cells (BEAS-2B) (Zhang et al., 2020), but failed to elicit a positive response in normal human fibroblasts when tested up to 2 μM (Curren et al., 1988). A positive result was obtained for micronucleus formation in lung A549 cells (Zhang et al., 2018) and BEAS-2B cells (Zhang et al., 2020), and for sister-chromatid exchanges in human primary lymphocytes (Wilmer et al., 1986). All studies in human cells were carried out in the absence of exogenous metabolic activation.

In eight experiments, plasmids containing the *supF* gene were reacted with acrolein and were

then transfected into various human cell types to allow for repair and replication; the *supF* mutant frequency was subsequently assessed in *Escherichia coli*. Six experiments reported positive results (Feng et al., 2006; Kawanishi et al., 1998; Wang et al., 2009a, 2013a; Lee et al., 2014)

Table 4.9 Genetic and related effects of acrolein in human cells in vitro

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (alkaline elution)	Primary human bronchial epithelial cells	(+)	0.1 mM [5.6 µg/mL]	Concentration tested induced excessive cytotoxicity; single concentration tested.	Grafström et al. (1986)
DNA strand breaks (alkaline elution)	Primary human bronchial epithelial cells	+	30 µM [1.7 µg/mL]	Single concentration tested.	Grafström et al. (1988)
DNA strand breaks (alkaline elution)	Human xeroderma pigmentosum fibroblasts, CRL1223	(+)	100 µM [5.6 µg/mL]	Vehicle not reported; concentrations tested induced excessive cytotoxicity.	Dypbukt et al. (1993)
DNA strand breaks (alkaline elution)	Human normal skin fibroblasts, CRL1508	(+)	300 µM [17 µg/mL]	Vehicle not reported; concentrations tested induced excessive cytotoxicity.	Dypbukt et al. (1993)
DNA strand breaks (alkaline elution)	Human myeloid leukaemia, K562 cells	+	5.4 µM [0.3 µg/mL]		Crook et al. (1986)
DNA strand breaks (alkaline elution)	Human lymphoblastoid, Namalwa cells	+	50 µM [2.8 µg/mL]		Eisenbrand et al. (1995)
DNA double strand breaks (γH2AX)	Human lung epithelial carcinoma, A549	+	80 µM [4.5 µg/mL]		Zhang et al. (2017)
DNA double strand breaks (γH2AX)	Human bronchial epithelial cells, BEAS-2B	+	7.5 µM [0.42 µg/mL]		Zhang et al. (2020)
DNA damage (comet assay)	Human normal lung fibroblasts, IMR-90	+	4 µM [0.22 µg/mL]	Minimal information in description of comet method; single concentration tested.	Luo et al. (2013)
DNA damage (comet assay)	Human, retinal epithelial cells, ARPE-19	+	75 µM [4.2 µg/mL]	Minimal information in description of methods (i.e. pH of lysis etc.); no quantification of the level of DNA damage (binary approach used: nuclei with tails vs those without).	Li et al. (2008b)
DNA damage (comet assay)	Human Burkitt lymphoma B lymphocytes, Raji	(+)	500 µM [28 µg/mL]	No cytotoxicity assessment; minimal information in description of methods (i.e. pH of lysis etc.); comets classified into three size classes; vehicle not reported.	Yang et al. (1999a)
DNA damage (alkaline comet assay)	Human liver hepatoma, HepG2	+	12.5 µM [0.7 µg/mL]		Li et al. (2008a)
DNA damage (alkaline comet assay)	Human lung epithelial carcinoma, A549	+	55 µM [3 µg/mL]	This was the lowest concentration tested.	Zhang et al. (2018)
DNA damage (alkaline comet assay)	Human bronchial epithelial cells, BEAS-2B	+	1 µM [0.056 µg/mL]		Zhang et al. (2020)

DNA–protein crosslinks (method not specified)	Primary human bronchial epithelial cells	(+)	0.1 mM [5.6 µg/mL]	Single concentration tested, which induced excessive toxicity; minimal information in description of methods.	Grafström et al. (1986)

Table 4.9 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA–protein crosslinks (modified alkaline elution assay)	Primary human bronchial epithelial cells	+	30 µM [1.7 µg/mL]	Single concentration tested.	Grafström et al. (1988)
DNA–protein crosslinks (alkaline comet assay, ProtK modified)	Human liver hepatoma, HepG2	+	50 µM [2.8 µg/mL]		Li et al. (2008a)
DNA–protein crosslinks (SDS/KCl precipitation assays)	Human bronchial epithelial cells, BEAS-2B	–	7.5 µM [0.42 µg/mL]	This was the only concentration tested; significantly enhanced DNA–protein crosslinks when co-exposed with formaldehyde.	Zhang et al. (2020)
DNA–protein crosslinks (SDS/KCl precipitation assay)	Human promyelocytic leukaemia cells, HL60	(–)	100 µM [5.6 µg/mL]	Cell viability at this dose was 58%; single concentration tested.	Schoenfeld & Witz (2000)
DNA–protein crosslinks (SDS/KCl precipitation assay)	Human Burkitt lymphoma cells, EBV-BL	+	150 µM [8.4 µg/mL]	Concentrations at which DNA–protein crosslinks were found were highly cytotoxic when assessed 4 days later by trypan blue exclusion.	Costa et al. (1997)
Gene mutation (*HPRT*)	Human xeroderma pigmentosum fibroblasts	+	0.2 µM [0.01 µg/mL]		Curren et al. (1988)
Gene mutation (*HPRT*)	Human normal fibroblasts	–	2 µM [0.1 µg/mL]		Curren et al. (1988)
Gene mutation (*HPRT*)	Human bronchial epithelial cells, BEAS-2B	+	7.5 µM [0.42 µg/mL]	Single concentration tested.	Zhang et al. (2020)
Micronucleus formation (CBMN)	Human bronchial epithelial cells, BEAS-2B	+	4 µM [0.22 µg/mL]		Zhang et al. (2020)
Micronucleus formation (CBMN)	Human lung epithelial carcinoma, A549	+	55 µM [3 µg/mL]	This was the lowest concentration tested.	Zhang et al. (2018)
Micronucleus formation (non-CBMN)	Human bronchial epithelial cells, BEAS-2B	+	7.5 µM [0.42 µg/mL]	Single concentration tested.	Zhang et al. (2020)
Sister-chromatid exchanges	Human primary lymphocytes	+	5 µM [0.28 µg/mL]		Wilmer et al. (1986)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into repair.	+	100 µM [5.6 µg/mL] and then transfected into human cells for	Plasmids were reacted with acrolein at 37 °C	Feng et al. (2006) normal human lung fibroblasts replication and

Table 4.9 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Forward mutation (*supF*)	Plasmid pMY189 (exposed acellularly); transfected into a normal human fibroblast cell line (W138-VA13)	+	26 mM [1456 µg/mL]	Plasmids were reacted with acrolein at 37 °C and then transfected into human cells for replication and repair.	Kawanishi et al. (1998)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into human repair-proficient fibroblasts (GM637) and human repairdeficient (XPA) fibroblasts (GM4427)	–	1 mM [56 µg/mL]	Plasmids were reacted with acrolein and transfected into human cells for replication and repair.	Kim et al. (2007)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into immortalized normal human lung fibroblasts (CCL-202)	+	0.5 mM [28 µg/mL]	Plasmids were reacted with acrolein at 37 °C and then transfected into human cells for replication and repair.	Wang et al. (2009a)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into normal human lung fibroblasts	+	0.5 mM [28 µg/mL]	Plasmids were reacted with acrolein at 37 °C and then transfected into human cells for replication and repair.	Wang et al. (2013a)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into immortalized normal human bladder cells (UROtsa) or normal human lung fibroblasts (CCL-202)	+	0.5 mM [28 µg/mL]	Plasmids were reacted with acrolein at 37 °C and then transfected into human cells for replication and repair.	Lee et al. (2014)

CBMN, cytokinesis-blocked micronucleus; γH2AX, phosphorylated gamma-histone 2AX; HIC, highest ineffective concentration; HPRT, hypoxanthine-guanine phosphoribosyltransferase; KCl, potassium chloride; LEC, lowest effective concentration; NA, not applicable; NT, not tested; PrtK, protein kinase; SDS, sodium dodecyl sulfate; vs, versus. [a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality. All studies in human cells in vitro were carried out in the absence of exogenous metabolic activation.

and two experiments reported negative results (Kim et al., 2007). In one of these studies, Feng et al. (2006) sequenced mutations in the recovered plasmid from normal human lung fibroblasts and found that > 50% of the acrolein-induced base substitutions in the *supF* gene were G→T transversions. In the *supF* gene of acrolein-reacted plasmids recovered from human lung fibroblasts (CCL-202), primarily G→T transversions (53%) were observed, followed by G→A transitions (30%), and G→C transversions (12%); moreover, they found that mutational hotspots occurred in sequences with runs of Gs, and that the mutations across the *supF* gene mapped to the same sequence locations as those where the acrolein-derived adducts formed (Wang et al., 2009a). In another *supF* shuttle vector study, of the acrolein-exposed plasmids recovered from a transformed normal human fibroblast cell line (W138VA13), 76% of mutations were base substitutions (46% single substitutions, 30% tandem or multiple substitutions), 21% were deletions, and 2% were insertions. Of the base substitutions, it was found that G→T predominated (44%), followed by G→A (24%), and G→C (12%) (Kawanishi et al., 1998).

A study in human xeroderma pigmentosum group V (XPV) cells transfected with a plasmid containing the α-OH-PdG adduct found that there was inaccurate translesion synthesis by both polymerases η and κ (Yang et al., 2003). Only marginal miscoding (< 1%) was observed for translesion synthesis across the γ-OH-PdG adduct in normal human fibroblasts, HeLa cells, xeroderma pigmentosum group A (XPA), and group V (XPV) cells (Yang et al., 2002a; Yoon et al., 2018). Another study in XPA cells transfected with plasmids containing either the α- or the γ-OH-PdG adduct found that the α-OH-PdG adduct strongly blocked DNA synthesis and induced base-pair substitutions (predominantly G→T) with an overall miscoding frequency of 10.4–12.5%, whereas the γ-OH-PdG adduct had neither effect (Yang et al., 2002b).

In one acellular study, human DNA polymerase ι was found to replicate past γ-OH-PdG in an error-free manner (Washington et al., 2004a), whereas in another acellular study, γ-OH-PdG was found to cause a significant replication block to human polymerase η (i.e. 100 times lower efficiency than dGTP), and caused misincorporation frequencies of approximately 10^{-2} to 10^{-1} (Minko et al., 2003).

(b) Experimental systems (i) Non-human mammals in vivo

See Table 4.10.

A negative result was obtained for formation of DNA–protein crosslinks in the nasal respiratory mucosa of male Fischer 344 rats exposed to acrolein by inhalation for 6 hours; however, acrolein enhanced the level of DNA–protein crosslinks when rats were co-exposed to both acrolein and formaldehyde (Lam et al., 1985). No significant increase in the frequency of dominant lethal mutations was observed in male ICR/ Ha Swiss mice exposed to acrolein as a single intraperitoneal injection (Epstein et al., 1972). In the micronucleus assay, a significant increase of 1.4-fold in the frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of male Sprague-Dawley rats treated with acrolein at 5 mg/kg bw per day by gavage, six times per week, for 30 days (Aydın et al., 2018). There was no dose-dependent increase in the frequency of micronucleated normochromatic erythrocytes in male and female B6C3F_1 mice exposed to acrolein at 10 mg/kg bw per day by gavage for 14 weeks. However, a significant increase of 2-

fold in the frequency of micronucleus formation was observed in the female mice at 5 mg/kg bw per day (Irwin, 2006).

(ii) Non-human mammalian cells in vitro

See Table 4.11.

An increase in the frequency of DNA strand breaks was observed via the alkaline elution assay in Chinese hamster ovary (K1) cells (Deaton et

Table 4.10 Genetic and related effects of acrolein in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA–protein crosslinks	Rat, F344 (M)	Nasal respiratory mucosa	–	1.0 mg/m^3 [2 ppm]	Inhalation, 6 h	Acrolein enhanced the level of DNA–protein crosslinks when rats were co-exposed to both acrolein and formaldehyde.	Lam et al. (1985)
Dominant lethal mutation	Mouse, ICR/Ha Swiss (M)	Early fetal death/implants	–	2.2 mg/kg bw	Intraperitoneal 1×		Epstein et al. (1972)
Micronucleus formation	Rat, SprageDawley (M)	Bone marrow (polychromatic erythrocytes)	+	5 mg/kg bw per day	Gavage, 6×/wk for 30 days; killed at day 30.	Significant increase but only 1.4-fold control value; single dose tested.	Aydın et al. (2018)
Micronucleus formation	Mouse, B6C3F$_1$ (M)	Blood (normochromatic erythrocytes)	–	10 mg/kg bw	Gavage daily, 5×/wk for 14 wk		Irwin (2006)
Micronucleus formation	Mouse, B6C3F$_1$ (F)	Blood (normochromatic erythrocytes)	–	10 mg/kg bw	Gavage daily 5×/wk for 14 wk	Positive at a single dose (2-fold, 5 mg/kg bw per day) but no dose trend; no analysis of target tissue exposure was reported.	Irwin (2006)

bw, body weight; d, day; F, female; h, hour; HID, highest ineffective dose; lowest effective dose; M, male; NT, not tested; ppm, parts per million; wk, week.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality.

Table 4.11 Genetic and related effects of acrolein in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks (alkaline elution)	Chinese hamster ovary K1	+	NT	0.022 mM [1.2 µg/mL]		Deaton et al. (1993)
DNA strand breaks (alkaline elution)	Mouse, leukaemia L1210	(+)	NT	NR	Concentration at which a positive response was observed caused substantial cytotoxicity.	Eder et al. (1993)
DNA damage (alkaline comet assay)	Mouse, Leydig cells TM3	+	NT	7.4 µM [0.4 µg/mL]	LEC was the lowest concentration tested; LEC reported was for tail% DNA. LEC for OTM and tail length was 13.6 µM.	Yildizbayrak et al. (2020)

DNA damage (alkaline comet assay)	Rat, primary hepatocytes	(–)	NT	44.1 mM [2500 µg/mL]	Concentrations tested induced excessive cytotoxicity.	Kuchenmeister et al. (1998)
DNA and/or protein crosslinks (alkaline comet assay)	Rat, primary hepatocytes	(+)	NT	11 mM [616 µg/mL]	94% of cells had condensed spot in middle of cell characteristic of DNA and/or protein crosslinks; concentrations tested induced excessive cytotoxicity.	Kuchenmeister et al. (1998)
DNA–protein crosslinks	African green monkey kidney cell, CV-1	(+)	NT	0.5 mM [28 µg/mL]	No cytotoxicity assessment.	Permana & Snapka (1994)
DNA–protein crosslinks	Rat, nasal mucosal cells	(+)	NT	3 mM [168 µg/mL]	No cytotoxicity assessment.	Lam et al. (1985)
Gene mutation (*Hprt*)	Chinese hamster lung fibroblasts V79	+	NT	1 µM [0.056 µg/mL]		Smith et al. (1990b)
Gene mutation (*Hprt*)	Chinese hamster lung fibroblasts V79	+	NT	20 µM [1 µg/mL]	Only concentration tested.	Gardner et al. (2004)
Gene mutation (*Hprt*)	Chinese hamster lung fibroblasts V79 expressing rat AKR7A1	–	NT	20 µM [1 µg/mL]	Only concentration tested.	Gardner et al. (2004)
Gene mutation (*Hprt*)	Chinese hamster ovary (CHO)	+	NT	30 µM [1.7 µg/mL]	Control value not explicitly stated however response appears to be robust.	Cai et al. (1999)
Gene mutation (*Hprt*)	Chinese hamster ovary (CHO)	–	(+)	89 µM [5 µg/mL]	Elevated mutant frequencies observed at some concentrations but no clear concentration–response relationship.	Parent et al. (1991b)
Gene mutation (*Hprt*)	Chinese hamster ovary (CHO)	–	NT	100 µM [5.6 µg/mL]	No methods reported; HIC was cytotoxic.	Foiles et al. (1990)

Table 4.11 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	
		Without metabolic activation	With metabolic activation			
Gene mutation ($Tk^{+/-}$)	Mouse, lymphoma L5178Y/$Tk^{+/-}$	+	NT	10 µM [0.56 µg/mL]		Demir et al. (2011)
Gene mutation (*cII*)	Mouse, embryonic fibroblasts from BigBlue TGR mouse	–	NT	100 µM [5.6 µg/mL]		Kim et al. (2007)
Chromosomal aberrations	Chinese hamster ovary (CHO)	–	NT	10 µM [0.56 µg/mL]	Only concentration tested that was nontoxic.	Au et al. (1980)

						Reference
Chromosomal aberrations	Chinese hamster ovary (CHO)	–	–	17.9 µM [1 µg/mL]		Galloway et al. (1987)
Sister-chromatid exchange	Chinese hamster ovary (CHO)	+	–	10 µM [0.56 µg/mL]		Au et al. (1980)
Sister-chromatid exchange	Chinese hamster ovary (CHO)	+	–	17.9 µM [1 µg/mL]	Reported as a weak positive.	Galloway et al. (1987)

AKR, aldo-keto reductase; Hprt, hypoxanthine-guanine phosphoribosyltransferase; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; OTM, olive tail moment; Tk, thymidine kinase.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality.

al., 1993) and mouse leukaemia (L1210) cells, although the latter study noted that the tested dose caused substantial toxicity (Eder et al., 1993). An alkaline comet assay in mouse Leydig cells gave a positive result for DNA damage (i.e. comet tail intensity) at the lowest concentration tested (i.e. 7.4 µM) (Yildizbayrak et al., 2020). At a dose that was higher by nearly 6000-fold (i.e. 44.1 mM), an alkaline comet assay in rat primary hepatocytes gave a negative response when cells were analysed for comet tail length/intensity. However, 94% of cells had a condensed nucleus characteristic of compounds that cause DNA and/or protein crosslinks (Kuchenmeister et al., 1998). Acrolein-induced DNA–protein crosslinks were also observed in African green monkey kidney cells (CV-1) (Permana & Snapka, 1994) and in rat nasal mucosal cells (Lam et al., 1985). [The Working Group noted that these experiments were carried out with acrolein at high concentrations that either induced excessive cytotoxicity (Kuchenmeister et al., 1998; Lam et al., 1985), or at which cytotoxicity was not assessed (Permana & Snapka, 1994).]

Acrolein was found to be mutagenic, with a positive result for *Hprt* mutations in two assays in Chinese hamster lung fibroblasts (V79) (Smith et al., 1990b; Gardner et al., 2004). However, a negative result was obtained in V79 cells that express the rat aldo-keto reductase enzyme AKR7A1 (Gardner et al., 2004). [The Working Group noted that AKR7A1 catalyses the reduction of acrolein to alcohols, indicating that rat AKR7A1 protects against acrolein-induced mutagenicity (see Section 4.1.2b).] The frequency of acrolein-induced *Hprt* mutants was also analysed in Chinese hamster ovary cells, with one study reporting a positive response at 30 µM (Cai et al., 1999). Another study reported elevated mutant frequencies at some doses, but with no clear dose–response relationship when acrolein was tested at up to 89 µM with and without metabolic activation (rat liver S9) (Parent et al., 1991b). An additional study reported negative results for *Hprt* mutations in Chinese hamster ovary cells (Foiles et al., 1990). A significant increase in the frequency of $Tk^{+/-}$ mutations was reported in mouse lymphoma (L5178Y) cells (Demir et al., 2011), but a negative response was reported for the induction of *cII* mutations in mouse embryonic fibroblasts from the Big Blue mouse (Kim et al., 2007). Chromosomal aberrations and sister-chromatid exchanges were both assessed in two different studies in Chinese hamster ovary cells, with both reporting a negative response for chromosomal aberrations, and a positive result for sister-chromatid exchanges (Au et al., 1980; Galloway et al., 1987).

Using shuttle vectors containing either adduct isomer, the α- and γ-OH-PdG adducts were found to be mutagenic in African green monkey kidney (COS-7) cells, with a similar percentage mutagenicity observed for both isomers (i.e. 8.3% and 7.4%, respectively) (Sanchez et al., 2003). The γ-OH-PdG adduct was found to be significantly mutagenic in plasmid-transfected COS-7 cells; primarily transversions were observed, but also transition mutations (Kanuri et al., 2002).

(iii) Non-mammalian experimental systems

See Table 4.12.

In *Drosophila melanogaster*, largely positive results were obtained in SMART eye and wing spot mutation studies after exposure to acrolein in feed (Sierra et al., 1991; Demir et al., 2013; Vogel & Nivard, 1993), or via inhalation (Vogel & Nivard, 1993). Acrolein was also tested in *Drosophila* for the ability to induce sex-linked

recessive lethal mutations, with negative results for all four feeding assays, but when acrolein was administered by injection, two out of three assays gave positive results (Zimmering et al., 1985, 1989; Sierra et al., 1991; Barros, et al., 1994a, b). Acrolein did not induce sex chromosome loss in *Drosophila* when administered either by injection or via feed (Sierra et al., 1991).

In *Saccharomyces cerevisiae,* acrolein did not induce DNA strand breaks and interstrand

Table 4.12 Genetic and related effects of acrolein in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster	SMART wing spot mutation	+	NA	10 mM [560 µg/mL] (feed)		Sierra et al. (1991)
Drosophila melanogaster	SMART wing spot mutation	+	NA	10 mM [560 µg/mL] (feed)		Demir et al. (2013)
Drosophila melanogaster	SMART eye spot mutation	+	NA	5 mM [280 µg/mL] (feed)		Sierra et al. (1991)
Drosophila melanogaster	SMART eye spot mutation	+	NA	8.9 mM, 500 ppm [500 µg/mL] (inhalation)	Vehicle was ethanol.	Vogel &Nivard (1993)
Drosophila melanogaster	SMART eye spot mutation	–	NA	80 mM [4480 µg/mL] (feed)	Vehicle was ethanol.	Vogel &Nivard (1993)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	5 mM [280 µg/mL] (feed)		Sierra et al. (1991)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	10 mM [560 µg/mL] (feed)		Barros et al. (1994a, b)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	14.3 mM [800 µg/mL] (feed)		Zimmering et al. (1989)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	53.6 mM [3000 µg/mL] (feed)		Zimmering et al. (1985)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	3.6 mM [200 µg/mL] (injection)		Zimmering et al. (1985)
Drosophila melanogaster	Sex-linked recessive lethal mutation	+	NA	3 mM [168 µg/mL] (injection)		Sierra et al. (1991)
Drosophila melanogaster	Sex-linked recessive lethal mutation	+	NA	3 mM [168 µg/mL] (injection)		Barros et al. (1994a, b)
Drosophila melanogaster	Sex chromosome loss	–	NA	5 mM [280 µg/mL] (feed)		Sierra et al. (1991)
Drosophila melanogaster	Sex chromosome loss	–	NA	5 mM [280 µg/mL] (injection)		Sierra et al. (1991)
Saccharomyces cerevisiae	DNA strand breaks and interstrand crosslinks	–	NT	0.1 mM [5.6 µg/mL]		Fleer & Brendel (1982)
Saccharomyces	Reverse mutation	–	NT	100 µg/mL		Izard (1973) *cerevisiae* S211 and

						Reference
S138						
		activation	**activation**			
Salmonella typhimurium TA1535 pSK 1002	DNA damage SOS (*umu*) induction assay	–	NT	5.6 µg/mL [0.1 mM]		Benamira & Marnett (1992)
Salmonella typhimurium TA1535	Reverse mutation	–	(+)	0.005 µg/mL		Hales (1982)
Salmonella typhimurium TA1535	Reverse mutation	–	–	13 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA1535	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
Salmonella typhimurium TA1535	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
Salmonella typhimurium TA1535,	Reverse mutation	–	–	28 µg/plate		Loquet et al. (1981)
Salmonella typhimurium TA1535	Reverse mutation	–	–	43 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA1535 (vapour protocol)	Reverse mutation	–	–	0.5 mL/chamber		Irwin (2006)
Salmonella typhimurium TA1535 (preincubation)	Reverse mutation	–	–	16 µg/plate		Irwin (2006)
Salmonella typhimurium TA100	Reverse mutation	+	+	10 µg/plate	Inconsistent dose–response relationship.	Parent et al. (1996b)
Salmonella typhimurium TA100	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
Salmonella typhimurium TA100	Reverse mutation	–	–	28 µg/plate		Loquet et al. (1981)

Table 4.12 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			

Table 4.12 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA100	Reverse mutation	–	(+)	38 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA100	Reverse mutation	–	–	43 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA100	Reverse mutation	+	NT	224 µg/mL		Foiles et al. (1989)
Salmonella typhimurium TA100	Reverse mutation	–	–	NR		Basu & Marnett (1984)
Salmonella typhimurium TA100	Reverse mutation	+	Toxic	NR		Eder et al. (1993)
Salmonella typhimurium TA100	Reverse mutation	+	NT	NR	Solvent NR.	Khudoley et al. (1987)
Salmonella typhimurium TA100	Reverse mutation	+	NT	NR	Solvent NR.	Eder et al. (1990)
Salmonella typhimurium TA100 (vapour protocol)	Reverse mutation	–	–	1 mL/chamber		Irwin (2006)
Salmonella typhimurium TA100 (pre-incubation)	Reverse mutation	–	–	6 µg/plate without activation, 16 µg/plate with activation	Slight toxicity at highest dose without activation.	Irwin (2006)
Salmonella typhimurium TA100 (liquid suspension)	Reverse mutation	+	–	2.1 µg/mL –S9; HIC, 4.2 µg/mL +S9		Lutz et al. (1982)
Salmonella typhimurium TA104	Reverse mutation	+	NT	224 µg/mL		Foiles et al. (1989)

						Reference
Salmonella typhimurium TA104	Reverse mutation	+	NT	14 µg/plate		Marnett et al. (1985)
Salmonella typhimurium TA1537	Reverse mutation	–	–	13 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA1537	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
		activation	**activation**			
Salmonella typhimurium TA1537	Reverse mutation	–	–	43 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA1538	Reverse mutation	–	–	43 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA1538 (preincubation)	Reverse mutation	NT	–	16 µg/plate		Irwin (2006)
Salmonella typhimurium TA97 (vapour protocol)	Reverse mutation	–	–	0.5 mL/chamber		Irwin (2006)
Salmonella typhimurium TA98	Reverse mutation	+	–	8.4 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA98	Reverse mutation	(+)	+	10 µg/plate	Weak positive (2-fold). Inconsistent dose–response relationship.	Parent et al. (1996b)
Salmonella typhimurium TA98	Reverse mutation	–	–	13 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA98	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
Salmonella typhimurium TA98	Reverse mutation	–	–	28 µg/plate		Loquet et al. (1981)
Salmonella typhimurium TA98	Reverse mutation	–	–	NR		Basu & Marnett (1984)
Salmonella typhimurium TA98	Reverse mutation	+	+	NR		Claxton (1985)

Table 4.12 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic	With metabolic			
Salmonella typhimurium TA98	Reverse mutation	+	NT	NR	Solvent NR.	Khudoley et al. (1987)
Salmonella typhimurium TA98 (vapour protocol)	Reverse mutation	–	–	1 mL/chamber		Irwin (2006)

Table 4.12 (continued)

Test system (species, strain)	End-point (LEC or HIC)	Results[a] metabolic activation	metabolic activation	Concentration	Comments	
		Without	With			
Salmonella typhimurium TA98 (pre-incubation)	Reverse mutation	–	–	16 µg/plate		Irwin (2006)
Salmonella typhimurium TA102	Reverse mutation	–	NT	NR		Marnett et al. (1985)
*Salmonella typhimurium hisD3052/nop*KM101	Reverse mutation	–	–	NR		Basu & Marnett (1984)
Escherichia coli HB101pUC13	DNA–histone crosslinks	+	NT	8.4 µg/mL		Kuykendall & Bogdanffy (1992)
*Escherichia coli PQ*37, SOS chromotest	DNA damage	+	NT	NR	Solvent NR.	Eder et al. (1990)
*Escherichia coli PQ*37, SOS chromotest	DNA damage	+	NT	NR		Eder et al. (1993)
*Escherichia coli PQ*37, SOS chromotest	DNA damage	–	NT	NR		Eder & Deininger (2002)

						Reference
*Escherichia coli PQ*37, SOS chromotest	DNA damage	+	NT	NR	Ethanol used as solvent.	Eder & Deininger (2002)
Escherichia coli AB1157	Reverse mutation	+	NT	56 µg/mL [1 mM]		Nunoshiba &Yamamoto (1999)
Escherichia coli JTG10	Reverse mutation	+	NT	56 µg/mL [1 mM]	Strain lacks glutathione; mutation frequency higher than in AB1157 strain.	Nunoshiba &Yamamoto (1999)
Escherichia coli WP2 (*uvr*A)	Reverse mutation	–	(+)	50 µg/plate	Weak positive (2-fold); inconsistent dose–response relationship.	Parent et al. (1996b)
Escherichia coli WP2 (*uvr*A)	Reverse mutation	(+)	NT	NR	Reported as weak mutagenicity.	Hemminki et al. (1980)
		activation	**activation**			
Plasmid pIF101 (acellular)	Reverse mutation A→C (*lacZ*)	+	NT	0.010 mM [0.56 µg/mL]	Plasmids were reacted with acrolein at 37 °C then transfected into AlkB proficient and deficient *E. coli* for mutation scoring.	Dylewska et al. (2017)
Plasmid pIF105 (acellular)	Reverse mutation A→T (*lacZ*)	+	NT	0.005 mM [0.28 µg/mL]	Plasmids were reacted with acrolein at 37 °C then transfected into AlkB proficient and deficient *E. coli* for mutation scoring.	Dylewska et al. (2017)
Plasmid pIF106 (acellular)	Forward mutation A→G (*lacZ*)	+	NT	0.005 mM [0.28 µg/mL]	Plasmid were reacted with acrolein at 37 °C then transfected into AlkB proficient and deficient *E. coli* for mutation scoring.	Dylewska et al. (2017)
Calf thymus DNA (acellular)	DNA damage (fluorescent screen for changes in DNA melting and annealing behaviour)	+	NA	100 mM [5600 µg/mL]		Kailasam & Rogers (2007)

AlkB, alpha-ketoglutarate B-dependent dioxygenase; A, adenine; C, cytosine; G, guanine; T, thymine; HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NR, not reported; NT, not tested; ppm, parts per million; S9, 9000 × *g* supernatant. [a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality.

crosslinks in one study (Fleer & Brendel, 1982), or reverse mutations in another study (Izard, 1973).

Acrolein has been evaluated in multiple assays in several *Salmonella* tester strains sensitive to base-pair substitutions (i.e. TA1535, TA100, TA104) and frameshift mutations (i.e. TA1537, TA1538, TA97, and TA98). However, only one assay was carried out in TA102, a strain that is used specifically for the detection of crosslinking agents. In the TA1535 base-pair substitution strain, a negative response was observed in the SOS induction assay (Benamira & Marnett, 1992) and the results were negative for reverse mutation (Hales, 1982; Haworth et al., 1983; Florin et al., 1980; Loquet et al., 1981; Lijinsky & Andrews, 1980; Irwin, 2006). In the TA100 strain, the results were mixed positive (Parent et al., 1996b; Haworth et al., 1983; Foiles et al., 1989; Eder et al., 1993; Khudoley et al., 1987; Eder et al., 1990; Lutz et al., 1982) or negative (Florin et al., 1980; Loquet et al., 1981; Lijinsky & Andrews, 1980; Basu & Marnett, 1984; Irwin, 2006), with the positive responses mainly occurring without metabolic activation (rat liver S9). Notably, only one pre-incubation assay was carried out with TA100 and a negative result was reported (Irwin, 2006). However, a positive result was obtained in TA100 when acrolein was tested in a liquid suspension assay (Lutz et al., 1982). Of the two assays reported in TA104, both gave positive results without metabolic activation (S9) (Foiles et al., 1989; Marnett et al., 1985). In the frameshift strains, all three TA1537 assays gave negative results (Haworth et al., 1983; Florin et al., 1980; Lijinsky & Andrews, 1980), both results in TA1538 were negative (Lijinsky & Andrews, 1980; Irwin, 2006), the one TA97 experiment gave negative results (using the vapour protocol) (Irwin, 2006), and four positive results (Lijinsky & Andrews, 1980; Parent et al.,1996b; Claxton, 1985; Khudoley et al., 1987) and six negative results were reported in TA98 (Haworth et al., 1983; Florin et al., 1980; Loquet et al., 1981; Basu Acrolein

& Marnett,1984; Irwin, 2006). A negative result was obtained in the crosslink strain TA102, but the highest tested dose was not reported (Marnett et al., 1985). The more sensitive pre-incubation version of the Ames assay was not carried out with any frameshift strains without metabolic activation.

In *E. coli*, a positive result for DNA-histone crosslinks was reported (Kuykendall & Bogdanffy, 1992). Several studies reported positive results for acrolein in the SOS chromotest (Eder et al., 1990, 1993; Eder & Deininger, 2002), whereas a negative response was observed in the SOS chromotest when DMSO was used as the solvent. Additional studies in *E. coli* reported positive results for reverse mutations (Nunoshiba & Yamamoto, 1999; Hemminki et al., 1980), as well as one experiment with a negative result without metabolic activation and a weak positive result with metabolic activation (Parent et al., 1996b). In one study, three different plasmids containing different mutational targets in the *lacZ* gene were reacted with acrolein and then transfected into *E. coli* for mutant frequency assessment; positive results were observed for all three mutation types (i.e. A→C, A→T, and A→G) (Dylewska et al., 2017). An increase in DNA damage, assessed via a fluorescence-based screen quantifying changes in DNA melting/annealing behaviour, was observed in calf thymus DNA reacted with acrolein in an acellular system (Kailasam & Rogers, 2007).

In the study of Kanuri et al. (2002), described above, the γ-OH-PdG adduct was found to be significantly less mutagenic in *E. coli* than in COS-7 cells transfected with the same plasmid (i.e. 0.96% mutations in *E. coli* versus 6.3–7.4% in COS-7). In *E. coli*, a study by Yang et al. (2001) found that DNA polymerase III catalysed

translesion synthesis across the γ-OH-PdG adduct in an error-free manner, but that DNA polymerase I did so in an error-prone manner, with incorporation frequencies opposite the γ-OH-PdG adduct of 93% for deoxyadenosine triphosphate (dATP), 88% for deoxyguanosine triphosphate

(dGTP), 7% for deoxycytidine triphosphate (dCTP), and 5% for deoxythymidine triphosphate (dTTP). Additionally, γ-OH-PdG was found to inhibit DNA synthesis in *E. coli* (Yang et al., 2001). In another study with *E. coli* transformed with bacteriophage vectors containing an 8-hydroxy-1,N^2-propano-2′-deoxyguanosine (OH-PdG) adduct (no stereochemistry specified), the correct base was inserted under all conditions (VanderVeen et al., 2001).

In an acellular study, γ-OH-PdG was found to cause a significant replication block to yeast polymerase η (i.e. 190 times lower efficiency than deoxyguanosine), although incorporation opposite the adduct was relatively accurate (Minko et al., 2003). In an acellular study, both α- and γ-OH-PdG caused a significant replication block to yeast DNA pol η, with α-OH-PdG being a significantly stronger blocking lesion as pairing with dCTP was strongly inhibited (Sanchez et al., 2003). When assayed for nucleotide incorporation frequency, dCTP was primarily incorporated across from both lesions, but extension with other deoxynucleoside triphosphates (dNTPs) was also observed at almost identical ratios for both stereoisomers (Sanchez et al., 2003). In other acellular studies, yeast Rev1 was demonstrated to replicate past γ-OH-PdG in an error-free manner (Washington et al., 2004b; Nair et al., 2008).

In an acellular study with bacteriophage DNA polymerase $T7^-$ and HIV-1 reverse transcriptase, OH-PdG adducts (stereochemistry not specified) were found to be miscoding, with dATP being preferentially incorporated instead of dCTP (Zang et al., 2005). In another acellular study, *Sulfolobus solfataricus* Dpo4, the prototypic Y-family DNA polymerase, was capable of bypassing γ-OH-PdG adducts in a primarily error-free manner (Shanmugam et al., 2013).

4.2.3 Alters DNA repair

(a) Humans

No studies on exposed humans were available to the Working Group.

Acrolein was found to inhibit the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) in human bronchial fibroblasts in two studies (Krokan et al., 1985; Grafström et al., 1986). [The Working Group noted that, as aldehydes are highly reactive towards thiols, this inhibition is probably due to acrolein reacting with and inhibiting the methyl-acceptor cysteine residue in MGMT (Grafström et al., 1986).] In a study using human normal skin fibroblasts and DNA-repair deficient XPA fibroblasts, it was concluded that acrolein inhibited nucleotide excision repair since there was an accumulation of DNA single-strand breaks in acrolein-treated normal skin fibroblasts, which only increased after a recovery period in fresh medium (Dypbukt et al., 1993). Indeed, several studies found that acrolein treatment causes concentration-dependent inhibition of nucleotide excision repair in primary normal human lung fibroblasts (NHLFs) (Feng et al., 2006; Wang et al., 2012), primary normal human bronchial epithelial cells (NHBEs), human lung adenocarcinoma cells (A549s) (Wang et al., 2012), and in immortalized human urothelial (UROtsa) cells (Lee et al., 2014). Acrolein also causes concentration-dependent inhibition of base excision repair in NHBEs, NHLFs, A549s (Wang et al., 2012), and UROtsa cells (Lee et al., 2014), and of mismatch repair in HeLa (epithelial adenocarcinoma) cells (Wang et al., 2012). A subgenotoxic concentration of acrolein (i.e. 50 μM) has also been demonstrated to inhibit the repair of gamma-

irradiation–induced DNA damage in human B-lymphoid cells, and the repair inhibition increased with acrolein dose (Yang et al., 1999b).

Acrolein treatment reduced the expression level of certain DNA repair genes in A549 cells (Sarkar, 2019). Other studies did not find an effect on gene expression but showed that acrolein reacts rapidly with and directly inhibits DNA repair proteins (Wang et al., 2012; Lee et al., 2014). More specifically, in NHBE, NHLF, A549, and UROtsa cells, acrolein treatment caused a dose-dependent reduction in the expression of repair proteins (i.e. XPA, XPC, human 8-oxo guanine DNA glycosylase (hOGG1), PMS2, and MLH1) that are crucial for nucleotide excision repair, base excision repair, and mismatch repair (Wang et al., 2012; Lee et al., 2014). Pre-treatment of cells with proteasome inhibitors reduced the level of protein degradation, and pre-treatment with an autophagy inhibitor caused partial reduction in the degradation of DNA repair proteins; however, repair capacity was not rescued (Wang et al., 2012; Lee et al., 2014). [The Working Group noted that these results indicate that acrolein protein modification alone is capable of causing DNA-repair protein dysfunction, and that this modification results in DNA-repair protein degradation both by proteasomes and by autophagy; see also Section 4.2.1(a).]

Wang et al. (2012) found that both α-OH-PdG and γ-OH-PdG adducts were not efficiently repaired in acrolein-exposed NHBEs and NHLFs. A study using HeLa whole-cell extracts found that α-OH-PdG and γ-OH-PdG adducts were not efficiently removed by base excision repair (Yang et al., 2002b). In a study of nuclear extracts from unexposed human normal skin fibroblasts and DNA-repair deficient human XPA cells transfected with acrolein-treated plasmids, it was found that acrolein–dG adducts (i.e. a mixture of α-OH-PdG and γ-OH-PdG) are substrates for nucleotide excision repair proteins, but are repaired at a much slower rate than other similar adducts, and that this is probably because of poor recognition and/or excision of the lesions in DNA (Choudhury et al., 2013).

(b) Experimental systems

No data were available to the Working Group.

4.2.4 Induces oxidative stress

(a) Humans

No in vivo data were available to the Working Group.

In vitro studies using human retinal pigmented epithelial and lung fibroblast cell lines have demonstrated that acrolein induces a variety of biochemical changes, including decreased nuclear protein levels of nuclear factor erythroid 2-related factor 2 (NRF2; [NFE2L2, nuclear factor, erythroid 2-like 2]) (retinal pigmented epithelial cells only), decreased superoxide dismutase and glutathione peroxidase activities, lowered cellular GSH levels, and increased generation of reactive oxygen species (ROS) and protein carbonyls (Jia et al., 2007, 2009; Li et al., 2008a). Haem oxygenase-1 (*HO-1*) gene expression is induced in human bronchial epithelial cells (HBE1 cells) after acrolein exposure, and acrolein-induced HO-1 protein levels are attenuated by pan-protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) inhibitors (Zhang & Forman, 2008). Exposure of human cultured liver (HepG2) or retinal pigment epithelial cells to acrolein results in endoplasmic reticulum stress, mitochondrial disruption, and oxidative stress (Li et al., 2008a, b; Mohammad et al., 2012). Human primary bronchial epithelial cells exposed to acrolein

vapour (0.1 and 0.2 ppm) for 30 minutes had increased IL17 expression (Johanson et al., 2020).

(b) Experimental systems

See Table 4.13.

Multiple in vivo studies in rodents have shown that acrolein administration via multiple routes of exposure, including oral administration, inhalation, and intraperitoneal injection, results in decreased tissue GSH concentrations

Table 4.13 Effects of acrolein on markers of oxidative stress in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
GSH SOD, GPx	Rat, Wistar (M)	Liver	↓	2.5 mg/kg bw per day	Gavage, 45 days		Arumugam et al. (1997)
PCO, TBARS	Rat, Sprague Dawley (M)	Spleen, thymus, PMN	↑	5 mg/kg bw per day	Oral, 6 days/wk for 30 days		Aydın et al. (2018)
GSH		Spleen, thymus, PMN	↓				
GPx NPSH GST	Rat, Wistar (M)	Nasal cavity	– ↑ ↓	0.25 ppm 0.67 ppm 1.4 ppm	Inhalation (noseonly), 6 h/day for 3 days		Cassee et al. (1996)
GSH	Rat, F344 (M)	Nasal cavity Tracheobronchial mucosa	↓	0.2 ppm 0.2 ppm	Inhalation (noseonly), 6 h		Cichocki et al. (2014)
GSH	Rat, F344 (M)	Liver	↓	89 µmol/kg (0.1 mL/100 g bw) [31.5 mg/kg]	Intraperitoneal, 1×		Cooper et al. (1992)
GSH	Mouse (F)	Liver	↓	4.5 mg/kg	Intraperitoneal, 1×	Qualitative statistics only.	Gurtoo et al. (1981)
GSH 8-OHdG	Mouse, C57BL/6 (M)	Lung	↓ ↑	10 ppm 10 ppm	Inhalation (wholebody), 12 h		Kim et al. (2018)
Lipid peroxides, TBARS	Mouse, *ApoE*$^{-/-}$ (M)	Serum, peritoneal macrophages	↑	3 mg/kg per day	Oral (drinkingwater), 1 mo	Genetic background not provided.	Rom et al. (2017)
ROS	Mouse, C57BL/6 (F)	Lung	↑	5 µmol/kg bw [0.06 mg/kg bw]	Intranasal, 1×	Elevated at day 7 postexposure only (not day 28).	Sun et al. (2014)
8-Isoprostane Total antioxidant capacity	Mouse, $gp^{91phox(-/-)}$ (M)	Liver	↑ ↓	0.5 µg/kg per day	Intraperitoneal, 7 days		Yousefipour et al. (2013)
8-isoprostane Total antioxidant capacity	Mouse, $gp^{91phox(+/+)}$ (M)	Liver	↑ ↓	0.5 µg/kg per day	Intraperitoneal, 7 days		Yousefipour et al. (2013)

Table 4.13 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
8-isoprostane Total antioxidant capacity	Mouse, $gp^{91phox(-/-)}$ (M)	Liver	↑ ↓	0.5 mg/kg per day	Intraperitoneal, 7 days		Yousefipour et al. (2017)
8-isoprostane Total antioxidant capacity	Mouse, $gp^{91phox(+/+)}$ (M)	Liver	↑ ↓	0.5 mg/kg per day	Intraperitoneal, 7 days		Yousefipour et al. (2017)

bw, body weight; F, female; h, hour; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione-*S*-transferase; HID, highest ineffective dose; LED, lowest effective dose; M, male; mo, month; NPSH, nonprotein sulfydryl groups; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PCO, protein carbonyls; PMN, polymorphonuclear leukocytes; ppm, parts per million; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; wk, week. [a]↑, increase; ↓, decrease;–, no effect.

(Arumugam et al., 1997; Aydın et al., 2018; Cassee et al., 1996; Cichocki et al., 2014; Cooper et al., 1992; Gurtoo et al., 1981; Kim et al., 2018). Oral and parenteral rodent studies have shown evidence of lipid peroxidation or protein carbonyl production after short-term (up to 1 month) exposure (Aydın et al., 2018; Rom et al., 2017; Sun et al., 2014; Yousefipour et al., 2013, 2017). A significant increase in levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) has been reported in the mouse lung after inhalation of acrolein (Kim et al., 2018). Intraperitoneal exposure of wildtype and *gp91*^{phox} knockout mice with acrolein at 0.5 µg/kg provided evidence that increased oxygen radical generation occurs via NAD(P)H oxidase activation (Yousefipour et al., 2013). In vitro studies with bovine pulmonary artery endothelial cells have likewise shown that acrolein causes increased generation of oxygen radicals by NAD(P)H oxidase activation (Jaimes et al., 2004).

4.2.5 Is immunosuppressive

(a) Humans

No data in exposed humans were available to the Working Group.

In vitro studies show that acrolein exposure is associated with apoptosis and necrosis in human alveolar macrophages and with inhibition of IL1β, TNF, and IL12 release from cells (Li et al., 1997). Human T cells treated with acrolein also demonstrated suppressed cytokine production and T-cell responses (Lambert et al., 2005). Human alveolar macrophages infected with *Mycobacterium tuberculosis* and exposed to acrolein have a reduced ability to clear these bacteria (Shang et al., 2011).

(b) Experimental systems

See Table 4.14.

Multiple studies in rodents have assessed whether acrolein inhalation alters bacterial-induced mortality, bactericidal activity, or innate immune function (Aranyi et al., 1986; Astry & Jakab, 1983; Jakab, 1993; Danyal et al., 2016; Hristova et al., 2012; Leach et al., 1987). Most of these studies have used short-term exposures (e.g. < 10 days).

Splenic cells isolated from naïve female C57/BL6 mice that were subsequently exposed to acrolein exhibited decreased T- and B-cell proliferation (Poirier et al., 2002). Immunosuppression by acrolein has been attributed to GSH depletion and interactions with redox-sensitive signalling pathways such as NF-κB or JNK (Lambert et al., 2005; Valacchi et al., 2005; Kasahara et al., 2008).

4.2.6 Induces chronic inflammation

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

See Table 4.15.

Chronic inhalation (6 hours per day, 5 days per week, for 104 weeks) of acrolein was associated with mild inflammation in the nasal respiratory epithelium in rats and mice (JBRC, 2016d, e, f; see also Section 3). A 1-year study in hamsters treated with acrolein by inhalation (7 hours per day, 5 days per week, for 52 weeks) was also associated with mild inflammation in the nasal respiratory epithelium (Feron & Kruysse, 1977; see Table 3.1). [The Working Group noted that changes in cell proliferation in response to acrolein exposure have not been evaluated in experimental systems.] Multiple studies in rodents with short-term or subchronic exposures to acrolein via inhalation have shown

that acrolein produces airway inflammation (Johanson et al., 2020; Kasahara et al., 2008; Wang et al., 2009b; Liu et al., 2009a, b; Sithu et al., 2010). Accumulation of monocytes, macrophages, and lymphocytes in the lung interstitium and mucous cell metaplasia are common features seen in many rodent inhalation studies with acrolein (Kutzman et al., 1985; Borchers et

Table 4.15 Inflammatory responses after acrolein exposure in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Respiratory epithelial inflammation	Mouse, Crj:BDF1 (M,F)	Nasal cavity	↑	1.6 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk, 104 wk		JBRC (2016a)

Table 4.14 Immunosuppression after acrolein exposure in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Streptococcus zooepidemicus induced mortality	Mouse, CD_1 (F)	Lung	–	0.1 ppm	Inhalation (whole-body); 3 h	Single exposure concentration.	Aranyi et al. (1986)
Streptococcus zooepidemicus induced mortality	Mouse, CD_1 (F)	Lung	↑	0.1 ppm	Inhalation (whole-body); 3 h/day for 5 days	Single exposure concentration.	Aranyi et al. (1986)
35S-*Klebsiella pneumoniae* clearance	Mouse, CD_1 (F)	Lung	–	0.1 ppm	Inhalation (whole-body); 3 h	Single exposure concentration.	Aranyi et al. (1986)
35S-*Klebsiella pneumoniae* clearance	Mouse, CD_1 (F)	Lung	↓	0.1 ppm	Inhalation (whole-body); 3 h/day for 5 days	Single exposure concentration.	Aranyi et al. (1986)
Staphylococcus aureus clearance	Mouse, Swiss (F)	Lung	↓	3 ppm	Inhalation (whole-body); 8 h		Astry & Jakab (1983)
Staphylococcus aureus or *Proteus mirabilis* clearance	Mouse, Swiss (F)	Lung	–	2.5 ppm	Inhalation (nose-only); 4 h/day for 4 days	Single exposure concentration.	Jakab (1993)
Antigen-induced inflammation	Mouse, C57BL/6J (NR)	Lung	↓	5 ppm	Inhalation; 1 or 4 h	Single exposure concentration.	Danyal et al. (2016)
Innate macrophage function	Mouse, C57BL/6J (M)	Lung	↓	5 ppm	Inhalation (whole-body); 4 h	Single exposure concentration.	Hristova et al. (2012)
Listeria monocytogenes-induced mortality	Rat, SpragueDawley (M)	Lung	–	3 ppm	Inhalation (whole-body); 6 h/day, 5 days/wk for 3 wk	Exposure associated with nasal pathology.	Leach et al. (1987)
Antibody plaque-forming cells			–				

F, female; h, hour; HID, highest ineffective dose; LED, lowest effective dose; M, male; NR, not reported; ppm, parts per million; wk, week.

[a] ↑, increase; ↓, decrease; –, no effect.

Respiratory epithelial inflammation	Rat, F344 (M, F)	Nasal cavity	↑	2.0 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk, 104 wk	JBRC (2016d)

CxCl2, IL6, IL17β, and TNF expression	Mouse, 129S1/SvlmJ (F) Mouse, A/J (F) Mouse, BALB/cByJ (F) Mouse, C3H/HeJ (F) Mouse, C57BL/6J (F) Mouse, DBA/2J (F) Mouse, FVB/NJ (F)	Lung	↑ CxCl2: (C57BL/6J and FVB/NJ only) ↑ IL6: (129S1/SvlmJ; BALB/cByJ; C57BL/6J and A/J only) ↑ IL17β: (129S1/SvlmJ; BALB/cByJ; and C57BL/6J only)	1 ppm	Inhalation (whole-body), 6 h/day, 4–5 days/wk, 11 wk	Single exposure concentration; TNF data incompletely reported.	Johanson et al. (2020)
Cell count and cytokine level	Mouse C57BL/6J (M)	BALF	–	5 ppm	Inhalation (whole-body); 6 h/day for 3 days	Single exposure concentration.	Kasahara et al. (2008)
Total cells, macrophages, neutrophils; TNFα, CINC-1	Rat, Sprague–Dawley (M)	BALF Lung	↑ ↑	2.5 ppm 2.5 ppm	Inhalation (whole-body); 6 h/day, 7 day/wk for 2 or 4 wk	Single exposure concentration.	Wang et al. (2009b)
Macrophage, neutrophil, leukocytes, TNFα, KC (IL8 homologue)	Mouse, Kunning (M)	BALF	↑	4 ppm	Inhalation (whole-body), 6 h/day, 7 days/wk, for 21 days	Single exposure concentration.	Liu et al. (2009a)
Mucin, macrophage, neutrophil, TNFα, IL8, IL1β	Rat, Sprague-Dawley (M)	BALF	↑	3 ppm	Inhalation (whole-body), 6 h/day, 7 days/wk, 2 wk	Single exposure concentration.	Liu et al. (2009b)
TNFα, IL6, IL1β	Mouse, C57BL/6 (M)	Lung	–	5 ppm	Inhalation (whole-body), 6 h	Single exposure concentration.	Sithu et al. (2010)
TNFα, IL6, IL1β	Mouse, C57BL/6 (M)	Lung	–	1 ppm	Inhalation (whole-body), 6 h/day for 4 days	Single exposure concentration.	Sithu et al. (2010)
Wet weight, oedema	Rat, F344 (M, F)	Lung	↑	1.4 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for 13 wk		Kutzman et al. (1985)

Table 4.15 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Macrophages, neutrophils	Mouse, FVB/N, (M)	BALF	↑	3 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk, 3 wk	Single exposure concentration.	Borchers et al. (1999)

Macrophages	Mouse, C57BL/6J, (NR) Mouse, MME (+/+), (NR) Mouse, MME (−/−), (NR)	BALF	↑ ↑ ↑	3 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk, 3 wk	Single exposure concentration.	Borchers et al. (1999)
CD8+ lymphocytes, macrophage	Mouse, C57BL/6J (NR)	Lung	↑	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 12 wk	Single exposure concentration.	Borchers et al. (2007)
CD8+ lymphocytes, macrophage	Mouse, $Cd8^{-/-}$ (NR)	Lung	↑	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 12 wk	Single exposure concentration.	Borchers et al. (2007)
Mucous cell metaplasia Macrophage accumulation	Mouse, C57BL/6J (F)	Lung	↑ ↑	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 4 wk	Single exposure concentration.	Borchers et al. (2008)
Mucous cell metaplasia Macrophage accumulation	Mouse, γδ T-cell deficient (F)	Lung	↑ ↑	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 4 wk	Single exposure concentration.	Borchers et al. (2008)
Mucous cell metaplasia Macrophage accumulation	Mouse, αβ T-cell deficient (F)	Lung	↑ –	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 4 wk	Single exposure concentration.	Borchers et al. (2008)
Interstitial inflammation, neutrophil infiltration, congestion, and oedema	Mouse, C57BL/6 (M)	Lung	↑	10 ppm	Inhalation (whole-body), 12 h	Single exposure concentration.	Kim et al. (2018)
Nasal epithelial inflammation and metaplasia	Rat, Wistar (M, F) Hamster, Golden Syrian (M, F) Rabbit, Dutch (M, F)	Nasal cavity	↑ ↑ ↑	0.4 ppm 1.4 ppm 4.9 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for 13 wk		Feron et al. (1978)
Olfactory epithelial inflammation	Rat, F344 (M)	Nasal cavity	↑	1.8 ppm	Inhalation (whole-body), 6 h/day 5 days/wk for 13 wk		Dorman et al. (2008)

Table 4.15 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Respiratory epithelial cell inflammation	Rat, F344 (M)	Nasal cavity	↑	1.8 ppm	Inhalation (whole-body), 6 h/day 5 days/wk for 13 wk		Dorman et al. (2008)
Mucus hypersecretion	Rat, Sprague-Dawley (M)	BALF	↑	3 ppm	Inhalation (whole-body), 6 h/day for 12 days	Single exposure concentration.	Chen et al. (2013a)
Inflammatory cells	Mice, C57BL/6 (M)	BALF	–	5 ppm	Inhalation (whole-body), 4 h/day, 4 days/wk for 2 wk	Single exposure concentration.	O'Brien et al. (2016)
Total protein	Mouse, 129S1/SvlmJ (F) Mouse, A/J (F) Mouse, BALB/cByJ (F) Mouse, C3H/HeJ (F) Mouse, C57BL/6J (F) Mouse, DBA/2J (F) Mouse, FVB/NJ (F)	BALF	All strains (↓)	1 ppm	Inhalation (whole-body), 6 h/day, 4–5 days/wk for 11 wk	Single exposure concentration.	Johanson et al. (2020)
Total protein Total cells Total protein	Rat, Wistar, (M)	BALF BALF NALF	↑ ↑ ↑	4 ppm 4 ppm 4 ppm	Inhalation (nose-only), 4 h/day for 2 days		Snow et al. (2017)
Total protein Total cells Total protein	Rat, Goto Kakizaki (M)	BALF BALF NALF	↑ ↑ ↑	2 ppm 4 ppm 4 ppm	Inhalation (nose-only), 4 h/day for 2 days		Snow et al. (2017)
Total cells; neutrophils; TNFα, IL1α; IL1β; KC	Mouse. BALB/c (M)	BALF	↑	1 mg/kg	Oropharyngeal aspiration	Relevance of route of exposure (anaesthetized).	Ong et al. (2012)
Bladder wet weight (oedema)	Mouse, Swiss (M)	Urinary bladder	↑	75 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Batista et al. (2006)
Bladder wet weight (oedema)	Mouse, Swiss (M)	Urinary bladder	↑	75 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Batista et al. (2007)
Bladder wet weight (oedema)	Mouse, C57 (F)	Urinary bladder	↑	6 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Bjorling et al. (2007)
Bladder wet weight (oedema)	Mouse, C3H/HeJ (F)	Urinary bladder	↑	6 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Bjorling et al. (2007)

Table 4.15 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Bladder wet weight (oedema)	Mouse, C3H/OuJ (F)	Urinary bladder	↑	6 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Bjorling et al. (2007)
Bladder wet weight (oedema)	Rat, Wistar (M)	Urinary bladder	↑	75 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Macedo et al. (2008)
Bladder wet weight (oedema)	Rat, Wistar (F)	Urinary bladder	↑	1 mM (400 µL)/ bladder	Intravesical, 1×	Relevance of exposure route.	Merriam et al. (2011)
Bladder wet weight (oedema)	Rat, Wistar (F)	Urinary bladder	↑	75 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Wang et al. (2013b)

BALF, bronchoalveolar lavage fluid; CINC, cytokine-induced neutrophil chemoattractant; F, female; h, hour; HID, highest ineffective dose; IL, interleukin; KC, mouse homologue for human IL8; LED, lowest effective dose; M, male; NALF, nasal lavage fluid; NR, not reported; TNF, tumour necrosis factor; ppm, parts per million; wk, week. [a] ↑, increase; ↓, decrease; –, no effect.

al., 1999, 2007, 2008). Interstitial inflammation, neutrophil infiltration, congestion, and oedema were reported in mouse lung (Kim et al., 2018). Increased inflammation has also been reported in rat respiratory epithelial cells and in the rat, hamster, and rabbit olfactory epithelium after acrolein inhalation (Feron et al., 1978; Dorman et al., 2008). Mucus hypersecretion has been observed in rats after acrolein inhalation (Chen et al., 2013a), and effects on bronchoalveolar lavage fluid have variously been observed in studies in mice and rats (O'Brien et al., 2016; Johanson et al., 2020; Snow et al., 2017). Oropharyngeal administration of acrolein in mice results in pulmonary inflammation as shown by the associated increase in elevated macrophage and neutrophil counts in the bronchoalveolar lavage fluid, and increased expression of production of cytokines, including interleukins IL1α, IL1β, IL6, IL17, and TNF, IFNγ, and monocyte chemotactic protein 1 (MCP-1) (Ong et al., 2012).

Some in vivo studies in rodents have investigated the role of acrolein in cyclophosphamide-induced inflammation and haemorrhagic cystitis. These studies rely on an injection of acrolein directly into the urinary bladder (Batista et al., 2006, 2007; Bjorling et al., 2007; Macedo et al., 2008; Merriam et al., 2008; Wang et al., 2013b). [The Working Group noted that these studies use a route of exposure that is unlikely to occur in humans and they involve acute exposures.]

4.2.7 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data in exposed humans were available to the Working Group.

In human cell lines, several studies suggested that acrolein is capable of inhibiting tumour suppressor genes and activating proto-oncogenes either by directly binding and modulating the protein or by disrupting signalling cascades that promote cell proliferation. Acrolein inhibited both DNA-binding activity and luciferase-reporter activity of the tumour suppressor TP53, in a B[*a*]P induction study using human lung adenocarcinoma cells (A549) (Biswal et al., 2003). The inhibitory effect of acrolein occurred in the absence of altered TP53 protein levels under basal or induced conditions (i.e. a 48-hour pre-treatment with B[*a*]P), which is probably the result of direct binding of the TP53 protein by acrolein, as well as the alteration of the intracellular redox status.

Acrolein was demonstrated to both covalently modify and inhibit phosphatase and tensin homologue (PTEN) in MCF-7 breast ductal carcinoma cancer cells (Covey et al., 2010). Perturbation of Wnt/β-catenin signalling in human embryonic kidney cells (HEK-293), which favour the retention of active protein kinase AKT, was demonstrated in both time- and dose-dependent manners. Acrolein inactivation of PTEN lead to an increase in activity of the proto-oncogene AKT, which can increase cellular proliferation and survival (Covey et al., 2010). AKT activity was further explored in a human colon carcinoma cell line (HCT 116), and in MCF-7 cells: acrolein was generated endogenously as a by-product of myeloperoxidase catalysis (quantified spectrophotometrically) and resulted in the accumulation of higher amounts of phospho-Ser^{473}AKT; when a PI3K inhibitor (wortmannin) or a myeloperoxidase inhibitor (resorcinol) were added, phospho-Ser^{473}AKT formation was suppressed (Al-Salihi et al., 2015).

Acrolein caused differential inhibition and modification (covalent adducts) of pyruvate kinase, the enzyme involved in the last step of anaerobic glycolysis, in MCF-7 cells and in a cell-free system. This change in activity has been reported during the transformation of cells to a

proliferative or tumorigenic phenotype (Sousa et al., 2019).

(b) Experimental systems

A significant increase in the incidence and/or severity of respiratory tract metaplasia and hyperplasia was observed in B6D2F$_1$/Crlj mice and F344/DuCrlCrlj rats exposed to acrolein by inhalation for 2 years (JBRC, 2016a, b, c, d, e, f) and is discussed in Sections 3.1 and 3.2 of the present monograph.

Feron & Kruysse (1977) reported an increase in epithelial metaplasia of the nasal cavity epithelium in Syrian golden hamsters repeatedly inhaling acrolein vapour (4 ppm) for 7 hours per day, 5 days per week, for 52 weeks. In a subacute toxicity study (6 hours per day, 5 days per week, for 13 weeks) in hamsters, rats, and rabbits, squamous metaplasia was only observed in rats treated with the intermediate dose of 1.4 ppm (Feron et al., 1978). [The Working Group noted high mortality in the group at the higher dose.]

Fischer 344 rats treated with acrolein (0.6 or 1.8 ppm) by inhalation for up to 65 days presented with respiratory epithelial hyperplasia and squamous metaplasia (Dorman et al., 2008). In the most sensitive location, the lateral wall, respiratory epithelial cell proliferation occurred in the two highest dose groups as detected by proliferating cell nuclear antigen (PCNA) immunohistochemistry (Dorman et al., 2008). In a separate inhalational study, acrolein (3 ppm) induced goblet cell hyperplasia in the bronchial epithelium in lungs of male Sprague-Dawley rats exposed for 6 hours per day, 7 days per week, for 2 weeks (Liu et al., 2009b). Acrolein (≥ 0.1 μM) elicited a similar increase in goblet cell number in a differentiated lung epithelium model, which mirrored, to some extent, the goblet cell hyperplasia observed in animal inhalation models and after human chronic exposure (Haswell et al., 2010).

In Sprague-Dawley rats, acrolein (3 ppm) inhalation for 3 weeks led to metaplastic, dysplastic, and hyperplastic changes in the mucous, respiratory, and olfactory epithelium of the nasal cavity (Leach et al., 1987). These changes were prominent on the septum and in the anterior and ventral areas.

In Sprague-Dawley rats given a single exposure or a sustained 3-day exposure to acrolein at 0.2 or 0.6 ppm via inhalation, a concentration-dependent increase in the proportion of 5-bromo-2′-deoxyuridine (BrdU)-labelled nasal epithelial, tracheal epithelial, or free lung cells was observed (Roemer et al., 1993). Although significantly increased compared with control at both time-points, the single exposure elicited a stronger proliferative response (Roemer et al., 1993). Similar treatment-related proliferative increases were measured by both BrdU and PCNA labelling in nasal epithelium of albino Wistar rats that were treated (≤ 0.67 ppm) for 6 hours per day, for 3 days (Cassee et al., 1996). These rats also presented with slight disarrangement, necrosis, thickening, and desquamation of respiratory/transitional epithelium (Cassee et al., 1996). [The Working Group noted necrosis associated with the highest dose.]

A single inhalational exposure (5 ppm for 10 minutes) of BALB/c mice to acrolein led to a sustained increase in levels of vascular endothelial growth factor protein that persisted for 8 weeks (Kim et al., 2019). A single inhalation exposure of Sprague-Dawley rats to acrolein (3 ppm for a 12-day period of 5 days of treatment, 2 days of rest, and another 5 days of treatment) significantly activated the Ras/ERK pathway in bronchial epithelial cells, which functions downstream of epidermal growth factor. This finding was accompanied by an increase in goblet cell hyperplasia and metaplasia, which were significantly inhibited

by simvastatin, a Ras inhibitor (Chen et al., 2010).

In oral gavage studies in B6C3F$_1$ mice and F334/N rats, acrolein treatment led to lesions associated with uncontrolled cell growth. Squamous epithelial hyperplasia in the forestomach and hyperplasia of bone marrow cells were observed in rats treated with acrolein at ≤ 10 mg/kg bw 5 days a week for 2 weeks; mice in the dose groups treated with ≤ 10 mg/kg bw
developed squamous epithelial hyperplasia of the forestomach (Irwin, 2006). [The Working Group noted the high mortality in the groups of rats and mice at the highest dose.]

Forestomach epithelial hyperplasia was observed in male and female Fischer 344/N rats and B6C3F$_1$ mice given acrolein (≤ 10 mg/kg bw) by gavage once per day, 5 days per week, for 14 weeks (Auerbach et al., 2008). The pro-toxicants, allyl acetate and allyl alcohol, which are metabolized to acrolein, were also investigated. Periportal hepatocyte hypertrophy was observed in rats treated with allyl acetate and allyl alcohol, but not acrolein. Both species treated with the highest dose of allyl acetate exhibited forestomach epithelial necrosis. [The Working Group noted the 100% mortality of this dose group for all species and sexes; the Working Group also noted low (93.3%) purity of allyl acetate.]

In a mouse model of intestinal cancer, *Apc*$^{min/+}$ mice were either treated with water or dextran sodium sulfate to induce a model of colitis. Colonocytes isolated from mice treated with dextran sodium sulfate were found to have covalent acrolein–protein adducts on the PTEN tumour suppressor from endogenously generated acrolein (myeloperoxidase catalysis), which corresponded with the activation of the *Akt* protooncogene in these samples (Al-Salihi et al., 2015).

In studies in hypertension-resistant and salt-induced rats treated with acrolein (≤ 1.4 ppm) via inhalation for 6 hours per day, 5 days per week, for 62 days, bronchiolar epithelial hyperplasia was reported that was sometimes accompanied by squamous metaplasia and fibrosis (Kutzman et
al., 1984). [The Working Group noted the toxicity associated with the highest dose, and the model of hypertension that was used for this study.]

4.2.8 Other key characteristics of carcinogens

(a) Induces epigenetic alterations

Several studies in experimental systems investigated the effect of acrolein on histone modification. Acrolein inhibited acetylation of the N-terminal tails of cytosolic histones H3 and H4 in vitro, compromising chromatin assembly in immortalized human bronchial epithelial and lung adenocarcinoma cell lines (Chen et al., 2013b). Interestingly, the effect of acrolein was specific to unmodified and newly synthesized histones; post-translational modifications seemed to protect the histone from being targeted. The mechanism behind these phenomena was further investigated by the same research group. Fang et al. (2016) determined that acrolein reacts and forms covalent adducts with lysine residues in an immortalized human bronchial epithelial cell line (BEAS-2B), including those residues important for chromatin assembly, therefore preventing these sites from undergoing physiological modifications (see Section 4.2.1). Promoter histone modifications of the *FasL* gene were enhanced by acrolein in the human liver hepatocarcinoma HepG2 cell line and in primary rat hepatocytes both alone and when co-treated with the HIV antiretroviral zidovudine (Ghare et al., 2016). When the acrolein scavenger hydralazine was added to the experiment, promoter-associated epigenetic changes were inhibited. Global DNA

methylation and accumulation of DNA damage because of silencing of DNA repair genes was observed in acrolein-treated C57BL/6 mouse bladder tissue and in cultured mouse bladder muscle cells (Haldar et al., 2015, 2016). Cox et al. (1988) showed that DNA methylase isolated from the liver and urothelium of rats (strain not reported), treated with acrolein, was inhibited by 30–50% but the mechanism behind the inhibition was unclear.

(b) Modulates receptor-mediated effects

Several receptors appear to be activated or modulated by acrolein, although the studies are limited in both number and specificity. Thyroid hormone co-treatment with acrolein, administered both as a single compound and as a component of cigarette smoke, acts as a partial agonist for the thyroid receptor through recruitment of the nuclear coactivators glucocorticoid receptor interacting protein 1 (GRIP1) and steroid receptor coactivator 1 (SCR1) (Hayashi et al., 2018). Again, independently or as a component of cigarette smoke extract, acrolein was able to recruit GRIP1 or SCR1, but this time to peroxisome proliferator-activated receptor-α (PPARα) to induce transcriptional changes (Matsushita et al., 2019).

In male Fischer 344 rats given acrolein intraperitoneally with phenobarbital, α-, 2β-, 6β-, 16α-, and 16β-hydroxylation of testosterone and androstenedione was decreased (Cooper et al., 1992). This was the result of acrolein impairing the induction of CYP by 45%.

(c) Causes immortalization

Acrolein significantly increased soft agar anchorage-independent-growth colony formation, a characteristic of tumorigenic cell transformation, in immortalized human bronchial epithelial cells (BEAS-2B) and bladder urothelial cells (UROtsa) (Lee et al., 2015).

(d) Multiple characteristics

Gene expression changes in response to acrolein exposure were investigated in several studies. Data suggested a coordination of several of the key characteristics, namely induces oxidative stress, induces chronic inflammation and, to some degree, alters DNA repair in epithelial tissue or cells.

In normal human bronchial epithelial cells treated with acrolein for up to 24 hours, a combination of high-content screening and genome-wide transcriptomics revealed induction of genes associated with cellular stress followed by proliferation, and to a lesser extent, senescence networks (Gonzalez-Suarez et al., 2014). Interestingly, NRF2 was consistently activated despite the lack of observed increases in ROS. Furthermore, an increase in phosphorylation of histone 3 (pH3) levels was not accompanied by changes in cell number, suggesting the presence of cell cycle arrest at G_2/M. Rats exposed for 6 hours to acrolein by inhalation (nose-only) exhibited similar patterns of protein and gene expression (Gonzalez-Suarez et al., 2014). In addition to the nuclear accumulation of Nrf2 protein, antioxidant genes (i.e. NAD(P)H quinone dehydrogenase 1, *Nqo1*; catalytic sub - unit of glutamyl cysteine ligase, *Gclc*; and haem oxygenase 1, *Hmox1*) were upregulated at much lower acrolein concentrations than those required to induce the expression of proinflammatory genes (i.e. chemokine-induced neutrophil chemoattractant-1, *Cinc1*; and interleukin 6, *Il6*) (Cichocki et al., 2014).

Three studies investigated the transcriptional response to acrolein in human adenocarcinoma lung epithelial (A549) cells at various time-points. Over the course of 4 hours, a strong initial downregulation of genes was observed, possibly

in response to DNA damage, followed by an increase in gene upregulation in which pro-inflammatory and pro-apoptotic pathways were dominant (Thompson & Burcham, 2008). Overall, these results indicate a dysregulation in several key characteristics of carcinogens including apoptosis, cell cycle control, and cell signalling. In a 2-hour exposure study in the same cells (A549), acrolein given alone or as a mixture with other short-chain aldehydes resulted in only one upregulated gene, *HMOX1*, a key gene in oxidative stress response (Cheah et al., 2013). A 24-hour treatment of A549 cells with acrolein at half maximal inhibitory concentration (IC_{50}) induced a robust expression of DNA repair genes, but this failed to rescue cells from apoptosis, even after acrolein washout and a recovery period (Sarkar, 2019)

4.3 Data relevant to comparisons across agents and end-points

Acrolein is one of approximately 1000 chemicals tested across the full assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) programmes supported by the US EPA (2020). In vitro assay descriptions to map Tox21 and ToxCast screening data in the context of the 10 key characteristics were previously summarized by Chiu et al. (2018). Results in this data set only include one active hit out of 235 assays. Acrolein was active in the antioxidant response element assay designed to target transcription factor activity, specifically mapping to the *NRF2* gene using a positive control of naphthoflavone. *NRF2* encodes the transcription factor NRF2, which regulates genes containing antioxidant response elements (ARE) in their promoters; this is probably the result of acrolein inducing oxidative stress. [The Working Group noted one flag for < 50% efficiency with this assay.]

5. Summary of Data Reported

5.1 Exposure characterization

Acrolein is a High Production Volume chemical that is used as a reactive intermediate and in the manufacture of numerous chemical products, including acrylic acid and methionine. It is directly used as a biocide, specifically, as an herbicide in recirculating water systems. Acrolein is formed during combustion of fuels, wood, and plastics, and is present in cigarette smoke and vapours from electronic cigarettes. In kitchens, high-temperature roasting and deep-fat frying produce measurable amounts of acrolein in the air. Acrolein is also formed during fermentation and is found in various alcoholic beverages. Acrolein is routinely measured in studies monitoring outdoor air pollution, and it has been identified in various combustion emissions in numerous reports. Firefighters are also exposed to acrolein. Occupational and environmental exposure guidelines exist for acrolein.

The urinary metabolite *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (3-hydroxypropylmer - capturic acid, HPMA) has been applied to estimate exposure, and a reference value for workplace substances is available. However, it is often challenging to differentiate endogenous from exogenous exposure due to the various external sources including air pollution, exposure to secondhand smoke, and consumption of fried and fermented foods.

5.2 Cancer in humans

One occupational cohort study, two hospital-based case–control studies, and three nested case–control studies in occupational or population-based cohorts were available, with little consistency in the cancer sites evaluated.

The study in an occupational cohort, one case–control study on urothelial cancer in patients with chronic kidney disease, and one nested case– control study on lymphohaematopoietic cancer in an occupational cohort were uninformative due to small numbers, poor external exposure assessment, and flaws in design. The other case– control study detected higher levels of acrolein– DNA adducts in buccal swabs of patients with oral cancer compared with healthy controls, but the study did not find an association between adduct levels and external exposures, including tobacco smoking or betel chewing. Finally, two nested case–control studies in a population-based cohort studied several biomarkers (including metabolites of acrolein) in relation to lung cancer among current smokers and non-smokers respectively, without demonstrating a direct etiological involvement of acrolein. In summary, all studies were judged to be uninformative and did not provide evidence on a causal relationship between acrolein exposure and cancer in humans. The studies were either of poor quality regarding design or exposure assessment, or they were of a mechanistic nature.

5.3 Cancer in experimental animals

Exposure to acrolein caused an increase in the incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species.

In an inhalation study in B6D2F$_1$/Crlj mice, female mice exposed to acrolein showed a significant positive trend in the incidence of malignant lymphoma.

In an inhalation study in F344/DuCrlCrlj rats, there was a significant positive trend in the incidence of rhabdomyoma of the nasal cavity and of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity in females exposed to acrolein. There was also a significant increase in the incidence of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity. Rhabdomyoma of the nasal cavity and squamous cell carcinoma of the nasal cavity are very rare tumours in the strain of rats used in the study.

5.4 Mechanistic evidence

The available data on absorption and distribution of acrolein in humans are scarce. Acrolein is absorbed after inhalation or oral exposure. A slow absorption rate from air was observed in experiments with human skin in vitro. In humans, the delivery of acrolein to the lower respiratory tract can be higher than in rats; in rats, which are obligate nasal breathers, a significant portion of acrolein (up to 98%) is absorbed in the upper respiratory tract. Acrolein is a reactive electrophile that reacts spontaneously with cellular glutathione as well as with nucleophilic sites in proteins and DNA. It is efficiently metabolized by three detoxification pathways: (i) conjugation with glutathione leading eventually to HPMA and *N*-acetyl-*S*-(carboxyethyl)-L-cysteine (2-carboxyethylmercapturic acid, CEMA), which are excreted in urine; (ii) reduction by aldo-keto reductases to allyl alcohol; and (iii) oxidation by aldehyde dehydrogenases to acrylic acid, which is further converted to 3-hydropropionic acid and thereby enters physiological catabolism. Metabolic activation by cytochrome P450s (CYPs) to glycidaldehyde is a minor metabolic pathway leading to 2-carboxy-2-hydroxyethylmercapturic acid through glutathione conjugation. Acrolein is excreted in urine, exhaled air, and faeces. Excretion half-time in humans is approximately 9 hours as measured by urinary HPMA levels. Small amounts of acrolein from both endogenous and exogenous sources have been detected in

exhaled air. In rats, 26–31% of both intravenous and oral doses were exhaled as carbon dioxide.

There is consistent and coherent evidence that acrolein exhibits key characteristics of carcinogens. Acrolein is a strongly electrophilic α,β-unsaturated aldehyde (enal) that readily reacts with DNA bases and proteins forming DNA and protein adducts in vivo and in vitro. Among these adducts, the most widely studied are the cyclic deoxyguanosine adducts, which are formed as a pair of α and γ regioisomers, α- and γ-hydroxy-1,N^2-propano-2′-deoxyguanosine (α- and γ-OH-PdG). γ-OH-PdG has been consis tently detected in humans in various samples (including from lung, liver, brain, urothelial mucosa, and saliva), as well as in experimental animals, with detected levels dependent on species, tissue types, exposure, and physiological conditions. Elevated levels of acrolein-derived adducts are found in tobacco smokers, or under chronic inflammatory conditions, such as non-alcoholic fatty liver disease. This indicates their formation by acrolein from tobacco smoke; their presence in tissues of non-smokers is indicative of acrolein formation by endogenous processes, including lipid peroxidation. In acrolein-treated human lung cells, acrolein–DNA adducts were preferentially formed at lung cancer *TP53* mutational hotspots, and acrolein preferentially adducted guanines at cytosine methylation CpG sites. Acroleinderived DNA adducts have been detected in the liver of untreated rodents as well as in various tissues of rodents exposed to cigarette smoke, automobile exhaust, or a high-fat diet. Acroleinderived DNA adducts have also been detected in dogs exposed to cyclophosphamide, and in cockerels exposed to acrolein.

Acrolein is genotoxic. No data in humans in vivo were available. In several studies in human primary cells, acrolein consistently induced DNA strand breaks and DNA–protein crosslinks. In cultured human cell lines, acrolein consistently induced DNA strand breaks, mutations, and micronucleus formation, and was suggestive of inducing DNA–protein crosslinks. A limited number of in vivo studies of genotoxic end-points were available and were largely negative; however, across many in vitro experimental systems acrolein was found to consistently induce DNA strand breaks, DNA–protein crosslinks, mutations, and sister-chromatid exchanges. In *Salmonella* strains tested without metabolic activation, acrolein induced both base-pair substitution and frameshift mutations. The mutagenicity of acrolein has also been demonstrated in experiments with plasmid DNA.

Acrolein alters DNA repair or causes genomic instability. No data in humans in vivo were available. Multiple studies in human cells have demonstrated that acrolein directly inhibits proteins in three major DNA-repair pathways. Acrolein induced concentration-dependent inhibition of nucleotide excision repair, base excision repair and mismatch repair in primary human lung fibroblasts and bronchial epithelial cells, as well as in cultured human lung and urothelial cells. Acrolein inhibited the DNA repair enzyme O^6-methylguanine–DNA methyltransferase in human bronchial fibroblasts. It also inhibited excision repair due to the accumulation of DNA single-strand breaks in normal skin fibroblasts.

Acrolein induces oxidative stress. No in vivo human data were available. In vitro studies using multiple human- and rodent-derived cells showed that acrolein induces biochemical changes consistent with depletion of glutathione and increased generation of ROS and protein carbonyls, indicative of oxidative stress. Multiple studies in rodents have likewise shown that acrolein administration via multiple routes of exposure including inhalation, oral, and intraperitoneal injection resulted in decreased

tissue glutathione concentrations, and increased lipid peroxidation and protein carbonyl production. A statistically significant increase in levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) has been reported in rodent lung DNA after acrolein inhalation.

Acrolein is immunosuppressive. No data in humans in vivo were available. In studies in vitro with human immune cells, acrolein exposure can impair cytokine release and result in cytotoxicity. Human alveolar macrophages exposed to acrolein have reduced ability to clear *Mycobacterium tuberculosis* infection. Multiple studies in rodents have demonstrated that acrolein inhalation alters bacterial-induced mortality, bactericidal activity, or innate immune function. Mouse splenic cells exposed to acrolein exhibited decreased T- and B-cell proliferation.

Acrolein induces chronic inflammation. No data were available in humans, but acrolein exposure can produce chronic inflammation in rodents. Shorter (i.e. acute to subchronic) rodent studies showed that acrolein administration via multiple routes, including oral and inhalation, produces inflammation at the site of entry.

Additionally, acrolein alters cell proliferation, cell death, or nutrient supply. No in vivo data were available in humans. In vitro studies using multiple human and rodent cell types showed that acrolein inhibited tumour suppressor genes and activated proto-oncogenes either by directly binding and modulating the protein or by disrupting signalling cascades that promote cell proliferation. Hyperplasia, metaplasia, and dysplasia were seen in the respiratory system of rodents exposed chronically or acutely by inhalation. After chronic exposure, rodents treated with acrolein by oral gavage developed forestomach epithelial hyperplasia.

There is suggestive evidence that acrolein induces epigenetic alterations via DNA methylation and histone modification. One study using mouse tissues and cells treated with acrolein reported alteration of global DNA methylation and accumulation of DNA damage because of silencing of DNA repair genes. This result was consistent with findings in mouse tissues and cells. DNA methylase was inhibited in two different rat strains. In vitro studies using human- and rodent-derived cells suggest that acrolein compromises chromatin assembly through inhibition of acetylation of the N-terminal tails of cytosolic histones.

Acrolein was essentially without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of acrolein.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of acrolein.

6.3 Mechanistic evidence

There is *strong evidence* that acrolein exhibits multiple key characteristics of carcinogens, primarily from studies with human primary cells and studies in experimental systems, supported by studies in humans for DNA adducts.

6.4 Overall evaluation

Acrolein is *probably carcinogenic to humans (Group 2A)*.

6.5 Rationale

The *Group 2A* evaluation for acrolein is based on *sufficient evidence* of cancer in experimental animals and *strong* mechanistic evidence. The *sufficient evidence* of carcinogenicity in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species. There is *strong* evidence that acrolein exhibits multiple key characteristics of carcinogens; acrolein is electrophilic; it is genotoxic; it alters DNA repair or causes genomic instability; it induces oxidative stress; it is immunosuppressive; it induces chronic inflammation; and it alters cell proliferation, cell death, or nutrient supply. The supporting data that acrolein exhibits these key characteristics comes primarily from studies with human primary cells and studies in experimental systems, and is supported by studies in humans for DNA adducts.

The evidence regarding cancer in humans is *inadequate*. The few available studies related to acrolein exposure and human cancer were inconsistent in the cancer sites evaluated, and most studies were small. All had poor assessment of external exposure to acrolein or could not distinguish the effects of acrolein exposure from other constituents of cigarette smoking.

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Acrolein

MAK value	**–**
Peak limitation	**–**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity (1997)	**Category 3B**
Prenatal toxicity	**–**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	acrylic aldehyde acrylaldehyde acraldehyde
Chemical name (CAS)	2-propenal
CAS number	107-02-8
Structural formula	$H_2C=CH–CHO$
Molecular formula	C_3H_4O
Molecular weight	56.06
Melting point	–88 to –87°C
Boiling point	52°C
Density at 20°C	0.84 g/cm^3
Vapour pressure at 20°C	290 hPa
log P_{ow}*	–1.10 to 0.9
1 ml/m^3 (ppm) ≙ 2.33 mg/m^3	**1 mg/m^3 ≙ 0.43 ml/m^3 (ppm)**

This document also discusses studies of cyclophosphamide which are relevant to the question of potential carcinogenic effects of acrolein, as metabolic activation of cyclophosphamide releases acrolein.

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* *n*-octanol/water distribution coefficient

1 Toxic Effects and Mode of Action

Because acrolein is highly reactive, its main effects are local toxic effects. The toxicity of acrolein is high after oral and inhalation exposure, and moderate after dermal exposure. Even in highly diluted solution, acrolein causes severe irritation of the skin and mucous membranes, so that undiluted acrolein can be expected to have corrosive effects. In studies with volunteers, acrolein caused eye irritation from concentrations of about 0.15 ml/m^3. The substance causes severe sensory irritation in the mouse with RD_{50} values around 2 ml/m^3. Cell proliferation and slight histological changes in the nasal epithelium of the rat are observed after exposing the animals three times to 0.25 ml/m^3.

In medium and long-term inhalation studies with various species, irritation was observed in the respiratory tract, mainly in the nose, and even hyperplastic and metaplastic changes in the nasal epithelium, depending on the concentration. In a 90-day study with rats, the most sensitive species, histologically detectable low-grade irritation of the nasal epithelium was observed after exposure of the animals to concentrations as low as 0.4 ml/m^3. A NOEL (no observed effect level) could not be determined for rats; for hamsters it was 0.4 ml/m^3. Only unspecific systemic-toxic effects are seen after oral administration, and the NOEL was found to be 0.5 mg/kg body weight for rats.

Acrolein was not found to have sensitizing effects in the maximization test with guinea pigs.

With oral doses up to the parentally toxic range, in rats acrolein neither has adverse effects on fertility nor does it cause embryotoxicity; in rabbits it causes neither teratogenicity nor embryotoxicity or foetotoxicity. Only at parentally toxic doses are reversible delays in the growth of the offspring seen in rats.

Acrolein reacts *in vitro* with DNA bases to form cyclic adducts. Cyclophosphamide, from which acrolein and other alkylating metabolites are formed, causes *in vivo* the formation of DNA adducts with both acrolein and the other alkylating metabolites. *In vitro*, acrolein was directly genotoxic in various test systems. In *Drosophila*, mutations were induced in both germ cells and somatic cells. Two *in vivo* studies of the mutagenicity and cytogenetics in rats yielded negative results. Carcinogenicity studies with dermal, inhalation and oral exposure of hamsters, rats and mice yielded no evidence of carcinogenic effects. In experiments with animals and after long-term therapeutic use, cyclophosphamide caused haemorrhagic cystitis, cystic toxicity and bladder tumours. The cystic toxicity is caused by acrolein formed during the metabolism of cyclophosphamide. Acrolein is probably also involved in the formation of the bladder tumours.

2 Mechanism of Action

As a result of the conjugation of the C–C double bond with the carbonyl group, acrolein is a highly reactive substance which can react with numerous nucleophilic cell

components. Acrolein and to a greater extent its glutathione adduct 3-oxopropylglutathione stimulate the release of oxygen radicals (Adams and Klaidman 1993). These findings may explain the severe local irritation and the genotoxic effects. The DNA single strand breaks probably occur, on the one hand, as a result of the known formation of alkali-labile DNA adducts via alkali-labile sites and, on the other hand, as a result of incomplete repair. In V79–HPRT mutants, the exocyclic acrolein adducts found in deoxyguanosine and deoxyadenosine and C–C intrastrand crosslinks may be involved in the production of mutations (Smith *et al*. 1996). In tests with *Drosophila melanogaster* it was shown that acrolein is not only directly mutagenic, but is also activated by CYP450 and glutathione conjugation, and inactivated by enzymes induced with phenobarbital (Barros *et al*. 1994b). For other 2-alkenals it has been demonstrated *in vitro*, however, that the induction of DNA strand breaks correlates inversely with the glutathione level in the cells (Eisenbrand *et al*. 1995). The cytotoxicity of acrolein can be reduced by glutathione, so that conjugation with glutathione is to be regarded primarily as detoxification. Complete detoxification is, however, not achieved by glutathione conjugation, as the conjugates or the resulting mercapturic acid gradually release the alkenal in the absence of glutathione. After systemic distribution, acrolein conjugates can reach tissues with low levels of glutathione and after cleavage produce genotoxic effects there (Eisenbrand *et al*. 1995).

Haemorrhagic cystitis after cyclophosphamide treatment is caused by the acrolein released in the bladder and not by the other alkylating metabolites, which do, however, also reach the bladder (see Section 3.2). This was clearly demonstrated by the toxic effects on the bladder of a cyclophosphamide derivative whose alkyl residue was not chlorinated and thus could not have an alkylating effect; metabolism of the cyclophosphamide derivative did, however, yield acrolein. Conversely, a derivative with chloroalkyl residues which could not release acrolein, had only minimal effects on the bladder (Brock *et al*. 1981a, Cox 1979). 2-Mercaptoethanesulfonate (MESNA) reduces the irritative effects of cyclophosphamide on the bladder (Brock *et al*. 1982, Kunze *et al*. 1984) and the proliferation of the urothelium induced by cyclophosphamide (Kunze *et al*. 1984), and is therefore used together with cyclophosphamide in therapy. MESNA reacts both with the 4-hydroxy-cyclophosphamide initially formed during metabolism and with acrolein, hardly, however, with the alkylating cyclophosphamide metabolites. As a result of the pharmacokinetic properties of MESNA, the reaction takes place only after the substance has passed through the kidneys. The reaction with 4-hydroxy-cyclophosphamide leads to a relatively stable condensation product, so that release of the alkylating metabolites and acrolein is reduced. The reaction with acrolein yields a non-toxic thioether. The MESNA adducts with 4-hydroxy-cyclophosphamide and acrolein have been detected in urine (Brock *et al*. 1979, 1981b). It can therefore be assumed that not only free acrolein but also the concentration of alkylating metabolites in the bladder is reduced by MESNA.

Another mechanism suggested as being responsible for the toxic effects of cyclophosphamide on the bladder is the β-elimination of acrolein from acrolein-mercapturic acids or their directly cytotoxic effects on the bladder epithelium (Ramu *et al*. 1995).

Enzyme polymorphism in man was held responsible for individual differences in metabolism of cyclophosphamide and its toxic effects on the bladder (Fleming *et al*.

1996). In animals of different strains, differences in sensitivity to the toxic effects of cyclophosphamide and acrolein have been observed. After administration of cyclophosphamide, differences between strains were also observed in excretion of acrolein and of substances which release acrolein after acidic hydrolysis (Fraiser and Kehrer 1992).

In experiments with animals, the formation of bladder tumours after doses of cyclophosphamide is prevented by the administration of MESNA (Habs and Schmähl 1983, Schmähl and Habs 1983). It was not described in the studies whether the animals developed cystitis. It is unclear whether the protective effects of MESNA on the bladder are mainly a result of its trapping the acrolein released or its stabilisation of 4-hydroxy-cyclophosphamide. The reaction with acrolein seems, however, to be more important (Brock *et al*. 1979). The tumours are thus either to be attributed to a direct genotoxic effect of acrolein in the bladder or to the strongly irritative effects of acrolein on the bladder epithelium in combination with genotoxic effects. These can be induced by acrolein or the other alkylating metabolites, which also reach the bladder.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

In the isolated upper respiratory tract of F344 rats, at a flow rate of 200 ml/min and concentrations of 0.86, 4.3 or 8.6 ml/m^3, during the last 20 minutes of the 40-minute exposure period 62 %, 38 % and 28 % of an administered acrolein dose was absorbed. Independent of the flow rate and concentration, absorption never reached equilibrium, but decreased slowly in the course of the experiment. During simultaneous exposure to the two substances, acrolein increased the absorption of acetone (Morris 1990).

Seven days after fasting rats had been given single oral doses of 2.5 mg radioactively labelled acrolein per kg body weight, 52 % to 63 % of the radioactivity was eliminated with the urine, 12 % to 15 % with the faeces and about 30 % as CO_2 in exhaled air. About 90 % of the dose had been eliminated after 24 hours. Also after repeated doses, the excretion pattern was similar. Of a single dose of 15 mg/kg body weight about 40 % was detected in urine, about 30 % in the faeces and about 30 % as CO_2. About 90 % of the dose had been eliminated after 48 hours. Intravenous administration of 2.5 mg/kg body weight led to renal excretion of about 70 % of the radioactivity and exhalation as CO_2 of 27 %. Small amounts of radioactivity were found in the organism (Parent *et al*. 1996b; see also the study of Sharp *et al*. 1994, Section 3.2).

3.2 Metabolism

3.2.1 Acrolein

The main pathway of acrolein metabolism involves enzymatic or non-enzymatic conjugation with glutathione, which, during inhalation by rats, takes place in the first tissues exposed, in the nasal mucosa: after exposure of F344 rats to 2.5 ml/m^3 for 3 hours, the glutathione concentration in the respiratory mucosa decreased by about 60 % and at 0.1 ml/m^3 by about 20 % (Lam *et al*. 1985, McNulty *et al*. 1984). In rats given acrolein doses of about 5 mg/kg body weight by intraperitoneal injection, the glutathione concentration in the liver was decreased (Cooper *et al*. 1992). In mammalian cells incubated with acrolein, complete glutathione depletion was observed from 10 µM (Dypbukt *et al*. 1993, Eisenbrand *et al*. 1995, Grafström 1990, Grafström *et al*. 1988).

After oral or intravenous administration of acrolein (for conditions see Parent *et al*. 1996b, Section 3.1) the main urinary metabolite was found to be *S*-(hydroxypropyl)-mercapturic acid. Other urinary metabolites were malonic acid, *S*-(2-carboxy-2-hydroxyethyl)-mercapturic acid, *S*-(carboxyethyl)-mercapturic acid and 3-hydroxypropionic acid. Oxalic acid was another important metabolite, but was only detected after oral administration. The metabolite pattern was comparable for all modes of administration. In the urine, 25 % to 77 % of the administered acrolein was recovered. In the faeces an inert homopolymer of acrolein (15 % of the total dose after acrolein doses of 2.5 mg/kg body weight and 30 % after 15 mg/kg body weight) was detected only after oral administration. The authors assumed that oxalic acid and the polymer were formed in the intestinal tract. From this study which was published only as an abstract, quantitative data on the metabolite pattern are not available (Sharp *et al*. 1994). The extent to which the substance was exhaled as CO_2 was not given, but is stated in another publication by the same research group (Parent *et al*. 1996b, see Section 3.1).

Further metabolites found *in vitro* were acrylic acid, glycidaldehyde and glyceraldehyde (BUA 1995).

After inhalation of acrolein in concentrations of 10, 18, 33 or 54 ml/m^3 for one hour, rats excreted 10.9 %, 13.3 %, 16.7 % and 21.5 % of the absorbed amount as the sum of the two mercapturic acids *S*-(carboxyethyl)-mercapturic acid and *S*-(hydroxypropyl)-mercapturic acid. About 30 % of intraperitoneal doses of 8.9 to 35.7 µmol/kg body weight (0.5–2 mg/kg body weight) was excreted as the mercapturic acids, independently of the dose (Linhart *et al*. 1996).

78.5 % of an oral acrolein dose of 13 mg/kg body weight was excreted by rats as *S*-(hydroxypropyl)-mercapturic acid. Other metabolites were not investigated (Sanduja *et al*. 1989).

The oral or intravenous administration of radioactively labelled acrolein to fasting rats led to the renal excretion of 34 % of the administered radioactivity as *S*-(carboxyethyl)-mercapturic acid and 7 % as *S*-(hydroxypropyl)-mercapturic acid. The other 28 % of the administered radioactivity in the urine was not identified (no further details; Parent *et al*. 1993b; c.f. the study of Sharp *et al*. 1994).

The known pathways of acrolein metabolism are shown in Figure 1.

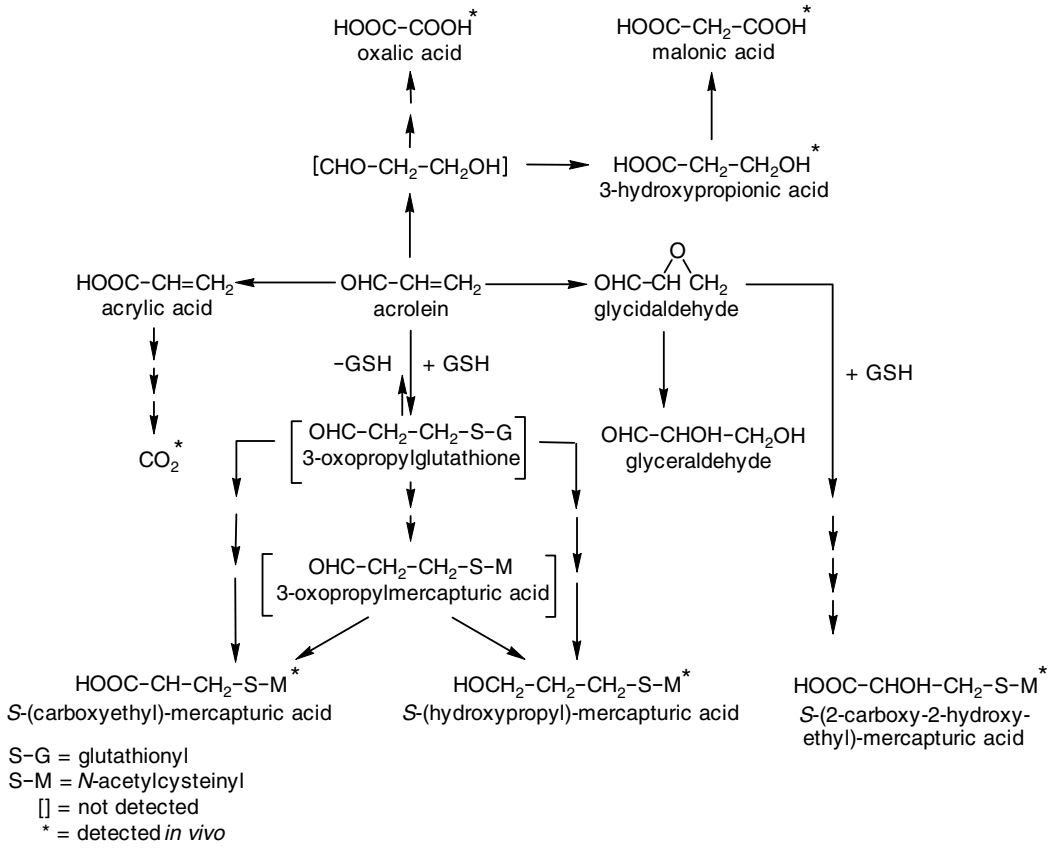


Figure 1. Pathways of acrolein metabolism

The following section describes studies of the stability and toxicity of the acrolein-mercapturic acids and the glutathione adduct.

In vitro, with increasing pH, small amounts of acrolein were released from 3-oxopropylmercapturic acid *S*-oxide, 3-oxopropylmercapturic acid and 3-oxopropylglutathione, conceivable intermediates of acrolein metabolism which have not yet been detected *in vivo*. From 3-oxopropylmercapturic acid *S*-oxide at pH 8 a maximum of 16 % acrolein was released and at pH 7.4 a maximum of 8 %, from 3-oxopropylmercapturic acid at pH 8 a maximum of 7 %, at pH 7.4 a maximum of 4 %. From 3-oxopropylglutathione and the detected metabolite *S*-(hydroxypropyl)-mercapturic acid at pH 8, a maximum of 2 % was released. The values for pH 7.4 were not given. The amount of acrolein released was evaluated by determining absorption at 210 nm over 120 minutes; after 30–60 minutes there was no further increase in absorption. Very similar values were obtained with the above substances at a pH of 8.0 also when the released acrolein was determined by means of the reaction with semicarbazide. However, when the reaction with *m*-aminophenol was used, 23 % to 75 % acrolein was shown to be released at a pH of 8.0. It was checked that the reaction product came mainly from acrolein and was not the result of the reaction with the aldehyde group of the initial substances. Also here the amount of acrolein released decreased in the order 3-oxopropylmercapturic acid *S*-oxide > 3-oxopropylmercapturic acid > 3-oxopropylglutathione > *S*-(hydroxypropyl)-mercapturic acid. The authors did not explain the quantitative differences between the methods of detection. To investigate the toxic effects on

the bladder, the above substances and acrolein were instilled in concentrations of 5 to 20 mM in buffer solutions at pH 7.4 into the bladders of rats. This led to concentration-dependent damage to the bladder, detected as increased levels of haemoglobin in the bladder. The potency of the individual substances correlated with the amount of acrolein released as described above. However, *S*-(hydroxypropyl)-mercapturic acid, the main metabolite found *in vivo*, did not have toxic effects on the bladder (Ramu *et al*. 1995). The release of acrolein from the other metabolites detected *in vivo* was not investigated.

The release of acrolein from 3-oxopropylmercapturic acid was detected indirectly, deduced from its cytotoxicity *in vitro*, which was prevented by glutathione or *N*-acetylcysteine (Perry *et al*. 1995).

3-Oxopropylmercapturic acid *S*-oxide, 3-oxopropylmercapturic acid, and to a lesser extent 3-oxopropylglutathione, were cytotoxic for lung carcinoma cells. The toxicity was decreased by glutathione and increased by glutathione depletion. The structurally similar 4-oxobutylmercapturic acid, which cannot release acrolein, was not toxic; the authors therefore concluded that the toxicity is caused by acrolein and not by the aldehyde group of the mercapturic acids. The corresponding acids, e.g. *S*-(carboxyethyl)-mercapturic acid which was found *in vivo*, were not toxic (Ramu *et al*. 1996).

Under alkaline conditions (pH 8) acrolein was released from 3-oxopropylmercapturic acid *S*-oxide, but not from 3-oxopropylmercapturic acid itself, which suggests that for acrolein to be released the oxidation of 3-oxopropylmercapturic acid to form the sulfoxide is necessary. At lower pH values less acrolein was released. 3-Oxopropylmercapturic acid *S*-oxide and 3-oxopropylmercapturic acid were found to be cytotoxic for LLC-PK1 cells and isolated proximal tubular kidney cells of the rat. With the isolated kidney cells, incubation with the monooxygenase inhibitor methimazole, which inhibits oxidation to form the sulfoxide and thus the release of acrolein, resulted in a decrease in the cytotoxicity of 3-oxopropylmercapturic acid but not of the *S*-oxide. With LLC-PK1 cells, however, the toxicity of 3-oxopropylmercapturic acid could not be reduced by incubation with methimazole. The authors tried to explain these discrepancies in terms of possible differences in the inactivation of methimazole in the two types of cell. They are of the opinion that the release of acrolein from the mercapturic acids is decisive for the toxicity (Hashmi *et al*. 1992).

To summarize, in all studies the release of acrolein was held responsible for the toxicity of the aldehydic mercapturic acids. The results of Hashmi *et al*. (1992) with LLC-PK1 cells show, however, that also the mercapturic acids themselves can be cytotoxic.

To date, the release of significant amounts of acrolein has only been demonstrated from mercapturic acids which are not found *in vivo*. It is, however, unclear whether this could have been detected under the analytical conditions in the other studies. It is unclear whether acrolein is also released under physiological conditions, as the pH of urine is lower than that used in these studies. The available results indicate that the *S*-(hydroxypropyl)-mercapturic acid found in urine is relatively stable and does not have toxic effects on the bladder. The other main metabolite, *S*-(carboxyethyl)-mercapturic acid, is not cytotoxic.

3.2.2 Cyclophosphamide

The main metabolite of cyclophosphamide is 4-hydroxy-cyclophosphamide, which is formed in the liver and is in equilibrium with aldophosphamide. Aldophosphamide decomposes spontaneously to form acrolein and phosphoramide mustard, which can be metabolized to nornitrogen mustard. The alkylating agent is phosphoramide mustard and also nornitrogen mustard. In addition dechloroethyl-cyclophosphamide, 4-keto-cyclophosphamide, alcophosphamide and carboxyphosphamide are formed; these substances do not yield alkylating species or are not as strongly alkylating or mutagenic (Chan *et al*. 1994, Ellenberger and Mohn 1977, Pool *et al*. 1988). In addition, chloroethyloxazolidin-2-one (Chan *et al*. 1994), and acrolein, and substances which release acrolein after acid hydrolysis (Al-Rawithi *et al*. 1993, Fraiser and Kehrer 1992) have been detected in urine.

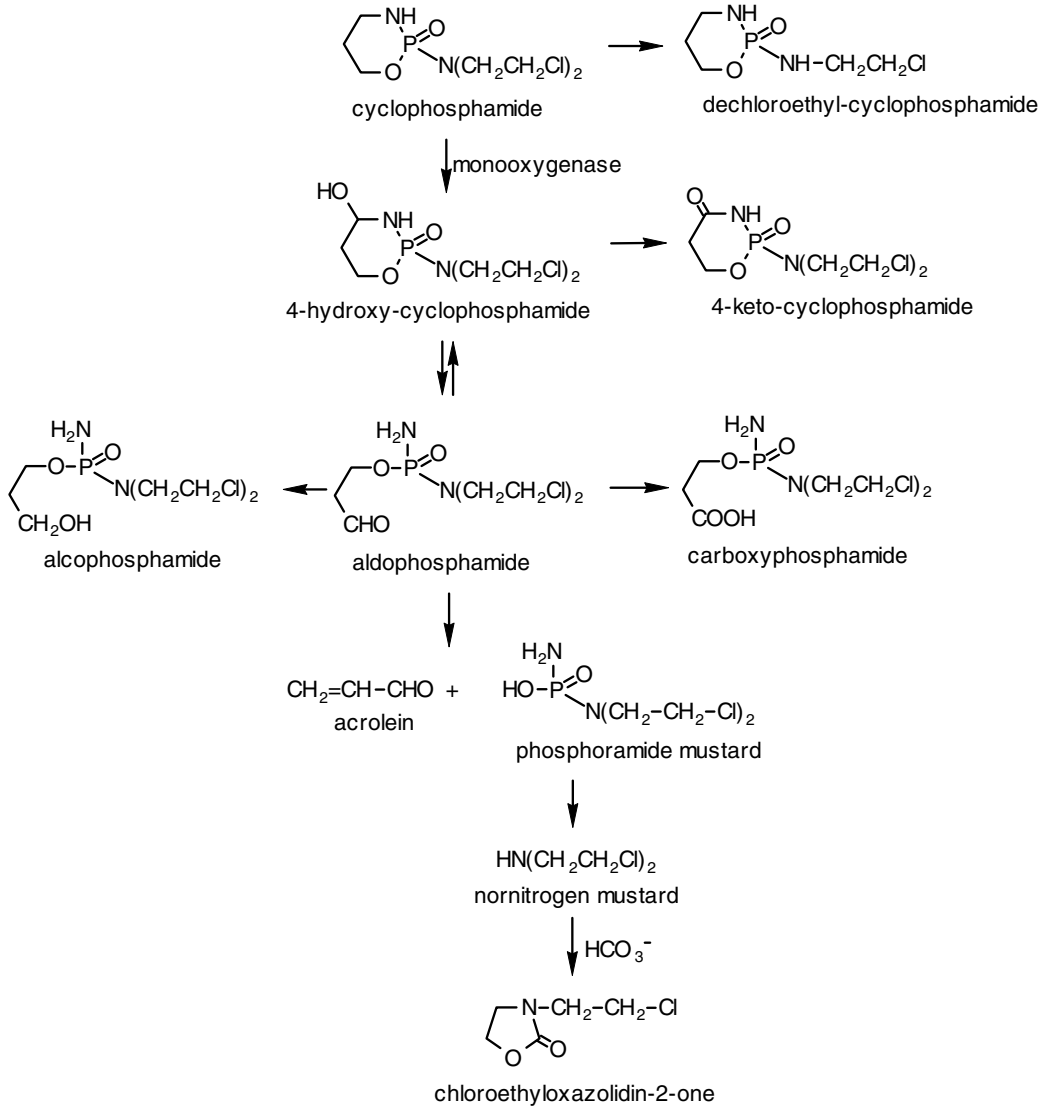


Figure 2. Metabolic pathways of cyclophosphamide

The main metabolic pathways of cyclophosphamide are shown in Figure 2 and have been described in detail elsewhere (Chan *et al*. 1994, Sladek 1988).

Children and adolescents given therapeutic doses of cyclophosphamide excreted between 0.7 % and 23.4 % as phosphoramide mustard (Tasso *et al*. 1992). In 6 patients 10.8 % was found unchanged in the urine, 0.5 % as hydroxy-cyclophosphamide and 39.0 % as phosphoramide mustard. Most of it was excreted within the first 24 hours (Chan *et al*. 1994). These patients were not given MESNA. In another publication the excretion of 18.5 % phosphoramide mustard in 24-hour urine was reported (Hadidi *et al*. 1986). These and other studies (Fleming *et al*. 1996, Newman *et al*. 1990) show that high levels of alkylating and mutagenic metabolites are excreted with the urine.

2.6 % of an oral cyclophosphamide dose of 160 mg/kg body weight was excreted by rats as *S*-(hydroxypropyl)-mercapturic acid (Sanduja *et al*. 1989). Rats given intraperitoneal cyclophosphamide doses of 50 mg/kg body weight excreted 9 % of the dose as *S*-(hydroxypropyl)-mercapturic acid. The mercapturic acid was also detected in urine from patients given cyclophosphamide (Alarcon 1976).

4 Effects in Man

There are no data available for toxic effects on human reproduction.

4.1 Single exposures

Accidental or non-accidental dermal absorption or ingestion of acrolein led to severe local irritation or corrosion at the site of contact. Intoxication after accidental inhalation of acrolein, which in some cases was lethal, has been reported (BUA 1995).

4.2 Repeated exposure

Vapour containing formaldehyde and probably acrolein produced during the processing of polyethylene bags caused irritative or allergic reactions in workers (Høvding 1969). As the level of exposure to acrolein is unclear, these results cannot be evaluated.

4.3 Local effects on skin and mucous membranes

Accidental or non-accidental exposure of the skin or mucous membranes to acrolein led to severe local irritation or corrosion (BUA 1995).

The irritative effects on the eyes of 9 to 14 test persons were estimated after exposure for 30 minutes via a mask to acrolein concentrations of 0.15, 0.27 or 0.54 ml/m^3 by determining the increase in the frequency of eye blinking. From the concentration–effect curve the authors concluded that irritation can be observed from 0.15 ml/m^3. In addition it was shown that during the 30-minute exposure to acrolein in concentrations of 0.54 ml/m^3, the blinking frequency increased with time to a maximum of 4 times the control value (van Eick 1977).

In a review of toxicological studies with acrolein, it is reported that exposure for 5 minutes to 0.25 ml/m^3 led to moderate irritation of the sensory organs. Tolerance to the irritative effects is not thought to develop (Shell 1958).

Another study cannot be evaluated as the acrolein concentration was increased during the experiment (Weber-Tschopp *et al*. 1977).

4.4 Allergenic effects

In the literature only one case of an allergic reaction to acrolein is described, in a smoker (Rappaport and Hoffman 1941).

4.5 Genotoxicity

Acrolein–DNA adducts were detected immunologically in leukocytes of patients treated with cyclophosphamide (McDiarmid *et al*. 1991). The absence of dose-dependency, the fact that adducts were still found even 4 years after the last cyclophosphamide treatment and the inadequate characterization of the monoclonal antibody call the results into question.

With ^{32}P-postlabelling, cyclic acrolein–DNA adducts, probably from endogenous sources, were found in control persons (see also Section 5.6).

4.6 Carcinogenicity

For acrolein itself there are no studies with man available. Cyclophosphamide was classified by the IARC as carcinogenic in man because its effects include an increase in the incidence of bladder tumours (IARC 1981). It is known from animal experiments (see Section 2) that acrolein is responsible for the toxic effects of cyclophosphamide on the bladder and that the formation of bladder tumours after administration of cyclophosphamide is prevented by trapping the acrolein with MESNA. To clarify the question of whether acrolein may be responsible for the bladder tumours seen after therapy with cyclophosphamide, the epidemiological studies with cyclophosphamide in which the relationship between toxic effects on the bladder and cancer of the bladder was investigated are reviewed below.

In a study with 145 patients treated with cyclophosphamide, non-glomerular haematuria was found to be an indicator of haemorrhagic cystitis and significantly ($p < 0.01$) associated with the development of cancer of the bladder (maximum total observation

period 27 years, median 8.5 years). The patients underwent urological examination at 1 to 3 month intervals. Non-glomerular haematuria occurred in 73 of the patients. In 18 patients this was diagnosed only after the end of the cyclophosphamide therapy. 70 % of the patients with haematuria also had cystitis. In all, 7 patients had cancer of the bladder and also microscopic or macroscopic haematuria. In one of the patients, cancer of the bladder was diagnosed as little as 0.6 years after the beginning of the cyclophosphamide therapy, so that a relationship with the therapy is improbable. On the basis of data from an American cancer register, the risk was said to be significantly increased by a factor of 31. Five of the six patients were smokers, while in the total group about 50 % were smokers. None of the 72 patients without haematuria developed cancer of the bladder. The study showed that the risk of developing non-glomerular haematuria increases with the duration of therapy and the total amount of cyclophosphamide administered. In addition, smokers developed non-glomerular haematuria sooner than non-smokers. The smokers developed cancer of the bladder after lower total amounts of cyclophosphamide and shorter therapy periods. The 6 patients with cancer of the bladder each received more than 100 g cyclophosphamide, while in the total group 65 % of all patients were given less than 100 g (Talar-Williams *et al*. 1996).

Of 119 patients who suffered from rheumatoid arthritis and were given cyclophosphamide, 6 of the treated patients developed bladder tumours. Two of them did not have haemorrhagic cystitis. In the similarly sized control group (patients with rheumatoid arthritis and a different treatment) no bladder tumours occurred (Baker *et al*. 1987). In the follow-up study of the same 119 treated patients, 9 bladder tumours were diagnosed (maximum total observation period 25 years). Three of these patients had haemorrhagic cystitis and 2 others microscopic haematuria. It is not described how and how often the patients underwent urological examination. Six of the patients with bladder tumours were heavy smokers. The smoking habits of the remaining treated patients were not described. The patients who developed cancer of the bladder were treated for longer periods and therefore received more cyclophosphamide than the others. In the control group no bladder tumours were found (Radis *et al*. 1995).

Of 471 patients treated with cyclophosphamide because of non-Hodgkin's lymphomas, 33 patients developed haemorrhagic cystitis and 9 a bladder tumour (maximum total observation period 17 years). The patients did not undergo urological examination until clinically visible haematuria developed. Seven of the nine patients with bladder tumours did not have haemorrhagic cystitis. The risk of developing cancer of the bladder relative to that in the general population was higher in patients with haemorrhagic cystitis (with a value of 11.3) than in patients without haemorrhagic cystitis (6.4); the difference could not, however, be shown to be statistically significant. Six of the nine patients were moderate smokers. The number of smokers in the whole group was not stated. The risk of developing haemorrhagic cystitis increased during the treatment period. Eight of the nine patients received more than 80 g cyclophosphamide, including the two patients with haemorrhagic cystitis (Pedersen-Bjergaard *et al*. 1988).

The study of Talar-Williams *et al*. (1996) revealed a clear relationship between non-glomerular microscopic haematuria and bladder tumours after cyclophosphamide therapy. The authors emphasize that the occurrence of non-glomerular microscopic haematuria allowed a subgroup of patients with a high risk of developing cancer of the

bladder to be identified. Of particular importance is the fact that none of the patients without haematuria developed cancer of the bladder. The reason why the relationship between cancer of the bladder and haemorrhagic cystitis was not so clear in the other studies may be that the patients were not subjected to such intensive urological examination and microscopic haematuria is a more sensitive indicator of toxic effects on the bladder. It must also be borne in mind that the number of tumour cases is small, which limits the statistical power of the studies. In the study of Radis *et al*. (1995), the average amount of cyclophosphamide administered was higher in the cases of cancer of the bladder than in the other patients. Also in the two other studies the patients with cancer of the bladder had received high amounts of cyclophosphamide. Smoking could also have contributed towards the toxic effects on the bladder and cancer of the bladder. Overall the results indicate that there is a relationship between damage to the bladder such as results from acrolein entering the bladder during cyclophosphamide therapy, and the formation of bladder tumours.

5 Animal Experiments and *in vitro* Studies

5.1 Acute toxicity

The 4-hour LC_{50} for rats was 21 mg/m^3 and for hamsters 58 mg/m^3. 30–minute LC_{50} values between 60 (Dow Chemical 1976) and 130 ml/m^3 (BUA 1995) have been determined. Oral LD_{50} values of 42 and 46 mg/kg body weight were given for rats (BUA 1995). The dermal LD_{50} for rabbits was found to be between 164 and 1022 mg/kg body weight, depending on the dilution and vehicle. The dermal LD_{50} of undiluted acrolein was 562 mg/kg body weight. For 10 % and 20 % aqueous acrolein solutions, dermal LD_{50} values of 335 and 1022 mg/kg body weight were obtained. The duration of exposure was not given (Shell 1958). At these concentrations skin irritation probably occurred (see Section 5.3), which accelerated the absorption of acrolein. The RD_{50} values were found to be 13.7 ml/m^3 for rats and between 1 and 2.9 ml/m^3 for mice (BUA 1995).

Focal hyperplasia of the bladder mucosa of rats was observed after intraperitoneal administration of acrolein doses of 25 mg/kg body weight, but not after oral administration of the same dose; with both modes of administration this dose was lethal for many of the animals. It is conceivable that a direct toxic effect of acrolein after diffusion from the peritoneal cavity into the bladder could be responsible for the hyperplasia. Both modes of administration resulted in multifocal eosinophilic degeneration in the liver. No adverse effects in the kidneys were found. After intraperitoneal administration of acrolein doses of 0.5, 1, 2, 4 and 6 mg/kg body weight in a concentration of 0.002 %, the incorporation of ^{3}H-methylthymidine into the DNA of the bladder mucosa, an indicator of cell proliferation, was increased 5 days after the last injection only after doses of 6 mg/kg body weight. This may also be a direct toxic effect of acrolein after diffusion from the peritoneal cavity into the bladder (Sakata *et al*. 1989).

The instillation of acrolein solutions in concentrations up to 6 mM into the mouse bladder led to haemorrhage like that seen after systemic administration of cyclophosphamide. The two strains of mouse used differed in sensitivity to both the acrolein and cyclophosphamide treatment (Fraiser and Kehrer 1992).

The acrolein–glutathione adduct 3-oxopropylglutathione was nephrotoxic in rats after intravenous administration of 0.5 or 1 mmol/kg body weight (corresponding to acrolein doses of about 28 or 56 mg/kg body weight). The toxicity was prevented by inhibition of γ-glutamyltranspeptidase (Horvath *et al*. 1992). As glutathione adducts are not transported efficiently into liver cells, intravenous administration reduces the level of oxidation or reduction in the liver whereas when the glutathione adduct is formed intracellularly in the liver it is subject to oxidative or reductive detoxification (Hashmi *et al*. 1992). Therefore the above experiment does not reflect the conditions *in vivo*.

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Groups of 5–6 rats were exposed via the nose to acrolein concentrations of 0.25, 0.67 or 1.40 ml/m^3, 6 hours/day for 3 days, and the nasal epithelium was then examined. From concentrations of 0.25 ml/m^3 slight disarrangement, basal cell hyperplasia and an increased number of cells in mitosis were observed in the respiratory and transitional epithelia. The findings were moderately severe in half of the animals exposed to 0.67 ml/m^3. The changes in this concentration group were similar in severity to those found under the same conditions in animals exposed to a formaldehyde concentration of 3.2 ml/m^3. A concentration-dependent increase in cell proliferation, measured as the expression of the proliferating cell nuclear antigen and the incorporation of BrdU into DNA, was found in the nose in standard plane of section II. In plane of section III both parameters were significantly increased, but no longer clearly concentration-dependent. No effects were found after single exposures (Cassee *et al*. 1996).

A similar study of cell proliferation in the nasal epithelium of rats (Roemer *et al*. 1993) cannot be included in the evaluation because of inadequate methods.

Male rats were exposed to analytically determined acrolein concentrations of 0.17, 1.07 or 2.98 ml/m^3 for 6 hours/day on 5 days/week for 3 weeks. No changes were found in the lungs of the animals. Only in the highest concentration group were body weight gains delayed. In the nasal cavity, metaplastic, hyperplastic and dysplastic changes in the respiratory and olfactory epithelia were found mainly in the septum and in the frontal and ventral areas. In the dorsal area, there was degeneration and, in some cases, even complete disintegration of the respiratory and olfactory epithelia and inflammation with leukocyte infiltration. The NOEL was found to be 1.07 ml/m^3 (Leach *et al*. 1987).

Groups of 10 male rats were exposed to acrolein in concentrations of 0.15, 0.51 or 1.52 mg/m^3, 24 hours/day for 61 days. From 0.51 mg/m^3 (0.22 ml/m^3) weight loss, epithelial proliferation and migration of eosinophils into the bronchi were observed.

The highest concentration resulted in bronchiolitis, bronchopneumonia, and myocardial and hepatic dystrophy. The NOEL was found to be 0.15 mg/m^3 (0.065 ml/m^3). The extent of the histopathological examination is unclear; the nasal mucosa of the animals was probably not examined (Gusev *et al*. 1966).

Groups of 12 Wistar rats, 20 Syrian hamsters and 4 rabbits were exposed to 0.4, 1.4 or 4.9 ml/m^3, 6 hours/day on 5 days/week for 13 weeks. In the rats exposed to concentrations from 0.4 ml/m^3, metaplasia of the nasal epithelium and leukocyte infiltration were found. Histopathological examination did not reveal any changes in the lung. For hamsters and rabbits the NOEL was 0.4 ml/m^3. At concentrations of 1.4 ml/m^3, metaplasia and leukocyte infiltration in the nasal epithelium were detected in hamsters, while in rabbits there was only a reduction in body weight gains. After exposure to concentrations of 4.9 ml/m^3, 6 of the 12 rats died and in all species metaplasia was observed in the nose, larynx and trachea. In addition, in rats and rabbits the bronchi, bronchioles and alveoli were inflamed. In no species was occult blood found in the urine, or lesions in the bladder, kidneys or adrenal glands (Feron *et al*. 1978).

In three other studies, rats were exposed to concentrations of 0.4, 1.4 or 4.0 ml/m^3, 6 hours/day on 5 days/week for 62 weeks. These studies are described below.

With F344 rats, concentrations of 0.4 ml/m^3 were not found to have adverse effects; the nose, however, was not investigated. The higher concentrations led to inflammation of the lower respiratory tract and increased levels of collagen in the lungs (Kutzman *et al*. 1985). The diffusing capacity for CO in the lung was determined in groups of 24 F344 rats per concentration; from 0.4 ml/m^3 it was increased. At 0.4 ml/m^3 the flow–volume dynamics were enhanced without any histological findings in the lung. At 1.4 ml/m^3 there were bronchial lesions in 10 % of the animals. At 4 ml/m^3 in the 9 surviving animals the flow–volume curve was depressed, the lung volumes increased and severe bronchial lesions were found. Only the lungs were examined histologically (Costa *et al*. 1986). Groups of 5 female Dahl rats resistant to salt-induced hypertension (DR) and 5 sensitive to salt-induced hypertension (DS) underwent histological examination 7 days after the end of exposure. In the lungs of 8/10 DS rats and 5/10 DR rats exposed to concentrations of 0.4 and 1.4 ml/m^3 slight epithelial hyperplasia, in some cases with squamous metaplasia, was found. Aggregations of intra-alveolar macrophages with foamy cytoplasm in the vicinity of damaged terminal bronchioles were observed in 7/10 DS and 5/10 DR rats. The incidences were, however, not given separately for the two concentration groups. At concentrations of 4 ml/m^3 the incidence of such findings was increased, and mortality, delayed body weight gains, oedema of the lung, and increased levels of protein, hydroxyproline and elastin in the lung were observed. There was a trend towards increase in relative lung weights in all concentration groups, but significant levels of about twice those found in the controls were reached only in the 4 ml/m^3 group. The nasal turbinates of the surviving animals were not found to be damaged; the extent of the examination is, however, not known. The nasal turbinates of the animals that died were not available for examination (Kutzman *et al*. 1984).

Groups of rats, guinea pigs, dogs and monkeys were exposed for eight hours to concentrations of 0.7 or 3.7 ml/m^3 on 5 days a week for 6 weeks; 3.7 ml/m^3 led to visible irritation and reduced body weight gains. Inflammatory changes and occasionally

emphysema were found in the lungs of the animals from concentrations of 0.7 ml/m^3; the effects were more severe in dogs and monkeys. In the animals exposed to 3.7 ml/m^3 treatment-related squamous metaplasia and basal cell hyperplasia were found in the trachea of dogs and monkeys, bronchopneumonia in dogs and obliterating bronchiolitis in monkeys. In all species unspecific inflammation was observed in the lungs, liver and kidneys. The nasal mucosa of the animals was not examined (Lyon *et al*. 1970).

Summary: in a 90-day study with intermittent exposure, no NOEL was determined in the most sensitive species, the rat, for histopathological changes in the nose. The LOEL (lowest observed effect level) was found to be 0.4 ml/m^3. For hamsters and rabbits this concentration was the NOEL. The evidence of bronchial damage in rats found in one study from concentrations of 0.4 ml/m^3 could not be confirmed in two other studies with larger numbers of animals.

5.2.2 Ingestion

The carcinogenicity studies with oral doses yielded for rats and mice NOELs in the range of 0.5–2 mg/kg body weight and day (see Section 5.7).

5.3 Local effects on skin and mucous membranes

Even 1 % acrolein solutions cause severe irritation of the rabbit skin and eye, so that corrosion is to be expected with the undiluted substance. Eye irritation was observed in various inhalation studies, but not quantified. Habituation to the irritative effects on the respiratory tract was reported (BUA 1995).

5.4 Allergenic effects

A Magnusson and Kligman maximization test yielded negative results (BUA 1995).

5.5 Reproductive and developmental toxicity

Groups of 30 male and female SD rats were given gavage doses of acrolein of 1, 3 or 6 mg/kg body weight in 5 ml water/kg body weight for 70 days before mating and during the 14-day to 21-day cohabitation period. The female animals received acrolein also during gestation and lactation. Groups of 40 F_1 animals were given the same acrolein doses as the parent animals from day 22 of life. The F_2 animals were not treated and were examined on day 21 of life. Mortality was significantly increased in the high dose group for male and female F_0 and F_1 animals; aspiration of the test substance could not be excluded as the cause of death. In many animals of the 6 mg/kg group erosion, hyperplasia and hyperkeratosis was found in the glandular mucosa of the stomach or in the forestomach. In 4 female F_0 animals of the 3 mg/kg group hyperplasia and hyperkeratosis were observed in the forestomach. The reproductive organs

of the F_0 and F_1 animals were unchanged. The following parameters did not differ in the treated animals and controls: mating behaviour, fertility index, incidence of abortion, relative number of live births, viability, growth, morphology of the young animals, number of implantations, duration of pregnancy, number of dams with abortions or stillbirths, lactation index, litter size and sex ratio. The only effect was reduced body weights after 6 mg/kg body weight in the male F_1 animals from day 1 to day 85 of life and in the female F_1 animals from day 1 to day 22 of life (Parent *et al.* 1992a). Even at the parentally toxic dose of 3 mg/kg, no impairment of fertility occurred and after 6 mg/kg body weight only delayed growth, sometimes reversible, was observed in the offspring.

Groups of 14 to 17 rabbits were given acrolein doses of 0.1, 0.75 or 2 mg/kg body weight from days 7 to 19 of gestation. Except for temporarily reduced food consumption and body weight gains during the first three days of treatment, no effects were observed. The numbers of abortions and implantations, the *corpus luteum* count, the relative number of live foetuses, the litter size, sex ratio, the number of resorptions, the number of skeletal and visceral malformations, and the ossification disturbances did not differ from those found in the control animals. In a dose-finding study, maternally toxic effects and mortality were caused by 4 and 6 mg/kg body weight (Parent *et al.* 1993a). Doses up to just below the maternally toxic range were neither teratogenic nor embryotoxic or foetotoxic.

As cyclophosphamide can have teratogenic effects under certain conditions, the following studies of cyclophosphamide are relevant in a review of the reproductive toxicity of acrolein. Teratogenic effects of cyclophosphamide have been described for the mouse, rat, rabbit and man (Beauchamp *et al.* 1985, Greenberg and Tanaka 1964). In *in vitro* studies it was shown, however, that metabolism via the microsomal monooxygenases of the liver is necessary before cyclophosphamide can have teratogenic effects.

Studies of the teratogenic effects of the two cyclophosphamide metabolites, phosphoramide mustard and acrolein, have been carried out. After intra-amniotic injection (day 13 after mating), teratogenic effects were described in rats for both substances. In these studies acrolein induced a range of malformations which were qualitatively like those induced by cyclophosphamide, but acrolein was found to have more pronounced effects than cyclophosphamide or phosphoramide mustard. After acrolein doses of 1 µg/foetus, malformations were detected in 85 % of the living foetuses, after 0.1 µg, no embryotoxic or teratogenic effects were observed. After treatment with phosphoramide mustard only part of the range of malformations described for cyclophosphamide was observed. After phosphoramide mustard doses of 1 µg/foetus no changes were detected (Hales 1982).

In an *in vitro* study with 10-day-old rat embryos, however, phosphoramide mustard was described as the "ultimate" teratogenic metabolite of cyclophosphamide. The authors observed the same effects on the growth and development of the embryos as with activated cyclophosphamide. No teratogenic effects were induced by treatment with acrolein (5 µg/ml) (Mirkes *et al.* 1981).

In a study of the toxic effects after inhalation, 3 male rats and 21 females were exposed to average acrolein concentrations of 0.56 ml/m^3 from four days before mating until the end of the gestation period. The number of pregnancies and the weights of the

foetuses were unaffected. As the exact data are not given in the study it cannot be included in the present evaluation (Bouley *et al*. 1976).

Acrolein doses of 3, 4.5 and 6 mg/kg body weight administered by intravenous injection on day 9 of gestation were lethal for rabbit embryos; the numbers of deaths increased with the dose. All the administered doses were also toxic (in some cases lethal) for the dams (Claussen *et al*. 1980).

In addition to these *in vivo* studies with acrolein, results are described from various *in vitro* studies.

Rat embryos (10.5 days old) were exposed to acrolein concentrations of 50 to 250 µM for 48 hours. The authors found the substance to be lethal to the embryos in a narrow dose range, and observed dose-dependent effects on the growth of the embryos, but no malformations (Schmid *et al*. 1981).

In a similar study, 10-day-old rat embryos were treated in a serum-free medium with acrolein concentrations of 0.005 to 5 µg/ml. From 0.5 µg/ml, dose-dependent effects on the development and survival of the embryos were observed. The malformations characteristic of cyclophosphamide were not observed (Mirkes *et al*. 1984).

The treatment of limb bud cultures of the mouse with acrolein concentrations of 3 to 10 µg/ml produced a significant, dose-dependent impairment of the differentiation of the limb buds, evidence that acrolein has embryotoxic potential. In the opinion of the authors the results could, however, also be interpreted as cytotoxic effects (Stahlmann *et al*. 1985).

5.6 Genotoxicity

In addition to studies with acrolein, also studies with cyclophosphamide are discussed below.

The genotoxicity of cyclophosphamide after metabolic activation *in vitro* and *in vivo* has been demonstrated in practically all the systems investigated. Only relevant studies are therefore discussed here, such as investigations of DNA adducts, studies on the influence of MESNA on the mutagenicity of cyclophosphamide and studies in which the genotoxicity of acrolein was compared with that of cyclophosphamide and its other metabolites.

5.6.1 *In vitro*

DNA adducts

Under physiological conditions *in vitro*, acrolein forms cyclic adducts with isolated DNA or nucleotides (BUA 1995). In *Salmonella typhimurium* TA100 and TA104 incubated with acrolein, DNA adducts were found and characterized as premutagenic lesions (Foiles *et al*. 1989). DNA adducts were found also in CHO cells (a cell line derived from Chinese hamster ovary) after incubation with acrolein (Foiles *et al*. 1990).

In vitro, cyclophosphamide caused monoalkylation of the N7 of guanine, and formation of DNA–DNA crosslinks and of phosphoester adducts resulting from reactions of DNA with metabolites such as phosphoramide mustard and nornitrogen mustard. N7 alkylation was twice as frequent as the formation of phosphoester adducts (Maccubbin *et al*. 1991).

Comparative *in vitro* studies of the frequency of monoalkyl adducts and other conceivable cyclic adducts produced by the acrolein released from cyclophosphamide are not available.

Strand breaks and crosslinks

Acrolein in concentrations from 10 µM induces DNA single strand breaks and DNA–protein crosslinks in mammalian cells *in vitro*; the concentrations effective in the various cell types are inversely correlated with the glutathione level in the cells (Dypbukt *et al*. 1993, Eisenbrand *et al*. 1995, Grafström *et al*. 1988, Grafström 1990). Glutathione is essential for DNA repair (Lai *et al*. 1989). As a result of rapid non-enzymatic glutathione depletion at acrolein concentrations from about 10 µM, even at this concentration DNA repair is almost completely inhibited (Dypbukt *et al*. 1993). In V79 cells, abasic sites (Smith *et al*. 1994) and C–C intrastrand crosslinks (Smith *et al*. 1995) are produced by acrolein.

Acrolein is more effective than formaldehyde in the induction of strand breaks, cytotoxicity, the depletion of glutathione, the increasing of intracellular calcium levels and the induction of terminal differentiation. If the effects are standardized to the same cytotoxicity, formaldehyde, with the exception of the effects on terminal differentiation, is more potent and of greater genotoxicity than acrolein (Grafström 1990).

Tests in microbial systems

Acrolein was found to be mutagenic in the *Salmonella* mutagenicity test (Table 1) with strain TA100 after preincubation (Foiles *et al*. 1989, Lutz *et al*. 1982, Parent *et al*. 1996b, Waegemaekers and Bensink 1984). The addition of S9 reduces or prevents the mutagenic effects (Lutz *et al*. 1982, Parent *et al*. 1996b). In the standard plate test (Bartsch *et al*. 1980, Basu and Marnett 1984, Lijinsky and Andrews 1980, Loquet *et al*. 1981, Rosen *et al*. 1980) and the spot test (Florin *et al*. 1980) no mutagenic effects could be found in strain TA100. Because of their unusual concentration dependency, the mutagenic effects observed in strain TA98 are questionable (Lijinsky and Andrews 1980) or marginal (Parent *et al*. 1996b).

In the *Salmonella* mutagenicity test with TA1535 and S9, the mutagenicity of cyclophosphamide was not reduced by the simultaneous presence of MESNA. In a second test, rats were given cyclophosphamide doses of 30 mg/kg body weight (0.128 mmol/kg body weight) without and with MESNA (0.09 mmol/kg body weight), and the urine of the animals was tested in strain TA1535. Neither with nor without S9 was the mutagenicity of the urine of the animals also treated with MESNA lower than that after cyclophosphamide treatment alone (about 30–50 times the spontaneous number of revertants) (Lähdetie *et al*. 1990).

In the *Salmonella* mutagenicity test with TA1535 without S9, 4-hydroperoxycyclophosphamide, nornitrogen mustard and phosphoramide mustard were found to be clearly mutagenic, while acrolein at the same concentrations (7.8–62.5 nmol/25 ml) was inactive. In TA1535 and TA100, the urine of rats given cyclophosphamide with MESNA was of lower mutagenicity than that from rats without additional MESNA treatment (Pool *et al*. 1988).

Negative results were obtained in tests for forwards mutation (Ellenberger and Mohn 1977) and reversion in *Escherichia coli* (Ellenberger and Mohn 1977, Parent *et al*. 1996b). Other results for the mutagenicity in bacteria and yeasts could not be included in the evaluation because of inadequate documentation (Hemminki *et al*. 1980, Jung *et al*. 1992, Khudoley *et al*. 1987, Marnett *et al*. 1985), test systems that have not been validated (Izard 1973) or low purity of the acrolein used (Haworth *et al*. 1983).

Table 1. Mutagenicity tests with acrolein in microbial test systems

Strain; test system	Lowest effective or highest tested concentration; toxicity	Result –S9/+S9	References
TA100, TA104; preincubation (20 mins, then the addition of GSH)	4 mM; at 13 mM reduced by GSH	+/n.d.	Foiles *et al*. 1989
TA100; preincubation (20 mins);	50 µg/plate; > 50 µg/plate	–/+	Haworth *et al*. 1983
TA98, TA1535, TA1537; preincubation (20 mins)	100 µg/plate; > 50 µg/plate	–/–	
TA100; preincubation (90 mins)	about 20 µM in the incubation mixture; ≥ about 20 µM; at 75 µM 20 % survival	+/–	Lutz *et al*. 1982
TA 104; preincubation (20 mins, then the addition of GSH)	0.35 µmol/plate; > 0.35 µmol/plate	+/n.d.	Marnett *et al*. 1985
TA100, TA98; preincubation (20 mins);	6.7 µg/plate; ≥ 20 µg/plate	+/+	Parent *et al*. 1996a
TA102, TA104, TA1535, TA1537, TA1538; preincubation (20 mins)	100 µg/plate; ≥ 33 µg/plate	–/–	
TA 100; preincubation (90 mins)	90 µM; >130 µM	+/n.d.	Waegemaekers and Bensink 1984
TA98, TA100, TA1535, TA1538; standard plate test	250 µM; not specified	n.d./–	Bartsch *et al*. 1980
hisD3052; standard plate test	not specified; not specified	n.d./–	Basu and Marnett 1984

Table 1. continued

Strain; test system	Lowest effective or highest tested concentration; toxicity	Result –S9/+S9	References
TA1535; standard plate test	not specified; 10 µg/plate	–/+(?)	Hales 1982
TA102; standard plate test	5000 µg/plate; not specified	–/–	Jung *et al*. 1992
TA98, TA100; standard plate test	not specified; not specified	–/+	Khudoley *et al*. 1987
TA98; standard plate test;	20 µg/plate; >100 µg/plate	+/–	Lijinsky and Andrews 1980
TA100, TA1535, TA1537, TA1538; standard plate test	not specified; not specified	–/–	
TA98, TA100, TA1535; standard plate test	50 µg/plate; not specified	–/–	Loquet *et al*. 1981
TA100; standard plate test	not specified; not specified	–/–	Lutz *et al*. 1982
TA100; standard plate test	not specified; ≥ 1.1 µg/plate	–/–	Rosen *et al*. 1980
TA98, TA100, TA1535, TA1537; spot test	150 µg/plate; 150 µg/plate	–/–	Florin *et al*. 1980

n.d. not determined, (?) questionably positive

Tests in mammalian cells

Acrolein can cause HPRT mutations in mammalian cells if suitable experimental parameters are selected. Mutagenicity has been detected to date only after treatment of the cells in a serum-free medium and, as a result of its extreme cytotoxicity, with acrolein concentrations of 2 µM or less (Curren *et al*. 1988, Smith *et al*. 1990). Incubation with acrolein in concentrations up to 120 µM with and without added S9, in the presence of 5 % foetal calf serum did not, however, produce HPRT mutations (Parent *et al*. 1991a). The potentially mutagenic damage to the DNA can apparently be repaired well by normal cells (Curren *et al*. 1988).

In tests for SCE (sister chromatid exchange) in CHO cells, weak concentration-dependent effects were seen with acrolein concentrations in the range from 0.1 to 1 µg/ml (1.8–18 µM) only without S9 (Au *et al*. 1980) and more clearly positive results, but with a reduced mitotic index, in the range from 10 to 100 µM (Galloway *et al*. 1987). Also in human leukocyte cultures, acrolein yielded positive results in the concentration range from 0.0014 to 40 µM. Fourteen times more SCE is induced by 3.39 µM phosphoramide mustard than by 20 µM acrolein. 40 µM acrolein was found to be cytotoxic, so that mitosis could not be evaluated. MESNA reduced the incidence of SCE and the cytotoxicity induced by acrolein (Wilmer *et al*. 1986).

Weak clastogenic effects were detected in a study with S9 and 40 µM acrolein (Au *et al*. 1980). In three other studies acrolein was not found to be clastogenic (Galloway *et al*. 1987, Wilmer *et al*. 1986, 1990). Phosphoramide mustard, however, was clearly clastogenic (Wilmer *et al*. 1986). Thus the metabolite acrolein plays only a minor role in the clastogenicity of cyclophosphamide (Wilmer *et al*. 1986, 1990).

In summary, acrolein can be shown to be mutagenic but the mutagenicity is slight compared with that of other cyclophosphamide metabolites.

Other effects

Increased terminal differentiation was observed in cultured human bronchial epithelial cells from 1 µM acrolein, an effect which is induced in these cells also by the skin tumour promoters 12-*O*-tetradecanoylphorbol-13-acetate (TPA), aplysiatoxin and teleocidin B (Grafström *et al*. 1988).

DNA cytosin-5 methyltransferase, which plays a role in the regulation of gene expression, is inhibited by acrolein (K_i: 6.7 µM) (Cox *et al*. 1988).

5.6.2 *In vivo*

DNA–protein crosslinks were induced by the incubation of a homogenate of rat nasal mucosa with acrolein. DNA-protein crosslinks were, however, not detected in a homogenate of rat nasal mucosa after the animals had inhaled acrolein concentrations of 2 ml/m^3 for 6 hours. The authors assume that acrolein is inactivated *in vivo* by reaction with SH groups (Lam *et al*. 1985).

Acrolein-DNA adducts

Studies of DNA adduct formation after administration of acrolein *in vivo* are not available.

Cyclic acrolein-DNA adducts (mainly propanodeoxyguanosine and, to a lesser extent, ethenodeoxyguanosine) were detected using ^{32}P-postlabelling in DNA from liver, mamma and leukocytes in man and in organs from untreated laboratory animals (mouse: liver, skin; rat: liver, lung, kidneys, brain, mamma, prostate, intestine and leukocytes) (Nath and Chung 1994, Nath *et al*. 1994, 1996, Swenberg *et al*. 1995). These adducts, like those with crotonaldehyde, are possibly the result of the lipid peroxidation of cell membranes. The level of propano adducts with acrolein and crotonaldehyde (only given as the sum) and of etheno adducts, which are formed with both acrolein and crotonaldehyde, was 1 adduct per 10^6–10^7 guanines (Chung *et al*. 1996). The level of the most important acrolein propano adduct in the liver DNA in man was 0.03–0.74, in rats 0.01–0.04 and in mice 0.23–0.67 µmol/mol guanine (Nath and Chung 1994).

By means of ^{32}P-postlabelling, hydroxypropanodeoxyguanosine–DNA adducts were detected in the lymphocytes of a dog treated with cyclophosphamide in doses of 6.6 mg/kg body weight, but not in untreated animals. The adducts were not quantified (Wilson *et al*. 1991).

In mice treated with cyclophosphamide, two adducts with deoxyguanosine were found in the hepatic DNA at levels of 1 adduct per 2.7–4.1 × 10^7 normal nucleotides (37.5 and 24.1 nmol/mol). Control animals were found to have adduct levels of 0.4 and 0.6 nmol/mol. The adducts had the same R_f values in two-dimensional chromatography as the adducts obtained *in vitro* with acrolein and deoxyguanosine monophosphate (Maccubbin *et al*. 1990).

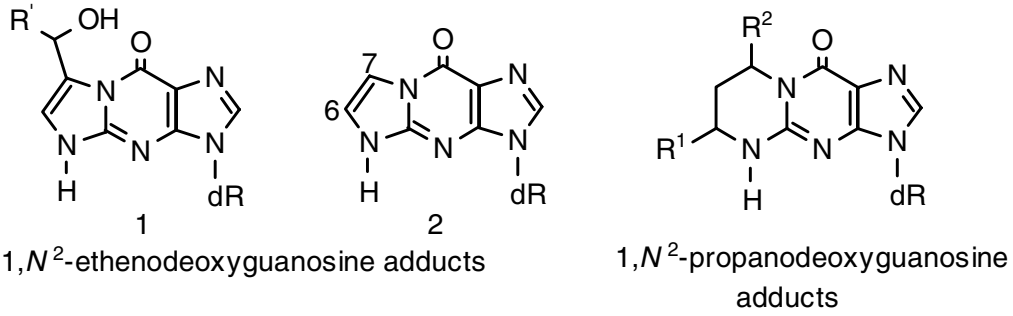
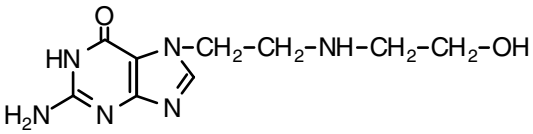


Figure 3. Cyclic adducts of acrolein or crotonaldehyde with deoxyguanosine.

Acrolein: R' = H; R^1 = OH and R^2 = H; R^1 = H and R^2 = OH
Crotonaldehyde: R' = CH_3; R^1 = OH and R^2 = CH_3; R^1 = CH_3 and R^2 = OH
dR = deoxyribose
Adduct 2 is formed by base-catalysed cleavage from adduct 1 (Chung *et al*. 1996).

DNA adducts found after administration of cyclophosphamide

After administration of cyclophosphamide, 0.2 to 0.4 guanine–N7 adducts per 10^6 nucleotides were found in the DNA of mice and rats by means of ^{32}P-postlabeling (Maccubbin *et al*. 1991). In the DNA of rats given cyclophosphamide which was radioactively labelled in the chloroethyl side chain, *N*-(2-hydroxyethyl)-*N*-[2-(7-guaninyl)-ethyl]amine was found by HPLC to be the main adduct.



The highest levels of radioactivity in the DNA were found in the bladders of the animals; 0.2 alkyl adducts were detected per 10^6 nucleotides. Adducts with acrolein were not investigated (Benson *et al*. 1988).

Drosophila tests

Acrolein (concentration not specified, yet lethal for at least 75 % of the larvae and eggs) induced in *Drosophila melanogaster* X-chromosomal recessive lethal mutations (SLRL, sex-linked recessive lethal) in 2.23 % of the male offspring compared with an incidence of 0.19 % in the controls (Rapoport 1948). The “eye spot” test with acrolein concentrations of 500 to 2000 mg/kg diet, yielded a positive result at 500 mg/kg. The higher concentrations were toxic (Vogel and Nivard 1993). Two tests (“wing spot” and

"eye spot") for somatic mutations with acrolein in concentrations of 5 to 20 mM (280 to 1120 mg/kg) in the diet yielded reproducibly positive, but not concentration-dependent results. An SLRL test with injection of 0.2 µl per animal of an acrolein solution in concentrations of 2 to 7 mM yielded concentration-dependent positive results. Another germ cell mutation test (SCL, sex chromosome loss) yielded negative results (Sierra *et al*. 1991). Adult *Drosophila* males were injected with 0.2 µl of an acrolein solution in concentrations of 2 to 5 mM and were mated with normal or repair-deficient females. Mating with normal females revealed the concentration-dependent induction of SLRL mutations. Mating with excision repair-deficient females revealed an even higher increase in mutations. After mating with females with mitochondrial DNase deficiency, the incidence of mutations was less than that seen after mating with normal females. The authors conclude from their results that acrolein lesions are repaired by the excision repair mechanism (Barros *et al*. 1994a). Adult *Drosophila* males were fed for 19 hours with phenobarbital (to stimulate xenobiotic metabolism), with phenylimidazole or iproniazid (CYP450 inhibitors) or with diethyl maleate (for glutathione depletion), before they were fed or injected with acrolein solutions. The results of the SLRL tests show that acrolein is not only a directly acting mutagen, but is also activated by CYP450 and glutathione conjugation. On the other hand, acrolein was also inactivated by the enzymes induced with phenobarbital (Barros *et al*. 1994b). Also negative results in the germ cell tests with feeding or injection have been published (Zimmering *et al*. 1985, 1989).

Chromosomal aberration and mutation tests

Groups of 5 to 7 male mice were given doses of acrolein of 1.5 or 2.2 mg/kg body weight by intraperitoneal injection and mated for 8 weeks in a mating ratio of 1:3 with untreated virgin females. Acrolein did not induce dominant lethal mutations in the germ cells. The study does not, however, meet present day standards (Epstein and Shafner 1968, Epstein *et al*. 1972).

An *in vivo* chromosomal aberration study with rats yielded negative results after intraperitoneal administration of acrolein doses of 1, 2 or 4.1 mg/kg body weight. Doses of 8.2 mg/kg body weight were lethal (BUA 1995).

5.7 Carcinogenicity

5.7.1 Initiation promotion studies

A 0.5 % solution of acrolein in acetone was applied to the skin of 15 mice for 10 weeks in doses of 0.3 ml until a total dose of 12.6 mg/animal had been applied; 25 days after the first application, croton oil was applied 18 times at weekly intervals. Three tumours were found in 2/15 animals from the acrolein group, while treatment with croton oil alone caused 4 tumours in 4/19 animals (Salaman and Roe 1956). According to the test protocol, application was not occlusive (Roe and Salaman 1955).

To investigate the initiating potential of acrolein on the bladder, groups of 30 rats were given intraperitoneal doses of acrolein of 2 mg/kg body weight twice a week for 6 weeks. *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide (FANFT) was used as a positive control, and water as a negative control. During the promotion phase 3 % uracil was administered to each group for 20 weeks (formation of bladder stones); after this period the animals were given standard diet for 6 weeks. A fourth group was given only acrolein during the initiation period, followed by standard diet for 26 weeks. In all, the animals treated with acrolein received 3.3 mg acrolein. Toxic effects developed during the administration of acrolein so that the treatment had to be interrupted. The animals developed peritonitis. In the positive control group, 9 papillomas and 21 carcinomas of the bladder were diagnosed. In the group given acrolein and uracil, 18 animals developed papillomas while 8 of the animals given only uracil developed papillomas. In both groups one carcinoma was found. From this significant increase in the incidence of papillomas the authors concluded that acrolein has initiating potential (Cohen *et al.* 1992).

In the same study the tumour-promoting potential of acrolein was investigated. In the initiation phase, acrolein or FANFT was administered as described above. For promotion the animals were given 24 intraperitoneal injections of acrolein during the next 15 weeks. According to the protocol a promotion phase of 100 weeks was planned for this test. As a result of the general toxicity, the dose and the frequency of administration had to be reduced even during the initiation phase. The animals remained untreated for a further 32 weeks. In all, the doses were between 8.3 mg acrolein per animal (initiation with FANFT) and 11.9 mg acrolein per animal (initiation with acrolein). In half of the animals hyperplasia of the bladder epithelium was observed. Also the administration of FANFT without a promoter led to a comparable incidence of hyperplasia of the bladder epithelium. Neither with FANFT nor with acrolein as initiator did bladder papillomas or carcinomas develop (Cohen *et al.* 1992). It is questionable whether acrolein has been found to have initiating effects, as the irritant effects of acrolein during the 6-week initiation phase could also have contributed to the increased incidence of papillomas. It is unclear whether acrolein or other metabolites enter the bladder during repeated intraperitoneal administration or whether acrolein diffuses directly from the peritoneal cavity into the bladder and there produces the observed practically irreversible hyperplasia of the bladder mucosa. It has been demonstrated that (mechanical) irritation of the bladder can result in papillomas.

5.7.2 Long-term studies

Groups of 20 female rats were exposed to acrolein concentrations of 8 ml/m^3, one hour a day on 5 days a week for 10 or 18 months. During the first acrolein inhalation the animals developed severe symptoms of respiratory irritation, later habituation developed. The extensive histological examination which included the bladder, adrenal gland and nose did not reveal an increased tumour incidence. The body weights of the animals were not changed relative to the control value (Le Bouffant *et al.* 1980). There

are no data available for survival, the number of animals investigated or the incidence of non-neoplastic histological changes in the nasal mucosa.

Groups of 36 male and female hamsters were exposed to acrolein concentrations of 4 ml/m^3, 7 hours a day for 52 weeks. Half of the animals received physiological saline by tracheal instillation once a week. Six animals were examined after 52 weeks, the rest 81 weeks after the end of the experiment. During the second week of the experiment the animals became adapted to the irritative effects of acrolein. After 52 weeks increased lung weights, irritative effects and metaplasia of the nasal mucosa were observed. Body weight gains were delayed, but survival was unchanged. After the last exposure all of the male animals and 28 of the females were still alive. After week 80 of the experiment, 23 males and 17 females were still alive. Thickening of the submucosa of the nasal cavity and exudate in the lumen were still detected in 20 % of the animals in the follow-up period. Lesions were not found in the bladder or adrenal glands of the animals. Treatment-related tumours were not found (Feron and Kruysse 1977). The treatment period was too short to be able to exclude carcinogenic effects. A clear local carcinogenic potential would probably, however, have been detected.

In addition, the influence of acrolein on the respiratory tract tumours caused by benzo[*a*]pyrene or diethylnitrosamine was investigated. Only the number of respiratory tract tumours caused by benzo[*a*]pyrene was increased, but not significantly, after simultaneous treatment with acrolein. The authors did not conclude that acrolein is co-carcinogenic (Feron and Kruysse 1977).

In a 2-year study, groups of 20 male F344 rats were given drinking water containing acrolein in concentrations of 100, 250 or 625 mg/l on 5 days/week. 20 female rats were given 625 mg/l. The acrolein dose taken up at this concentration was estimated to be 50 mg/kg body weight and day. The volatility and instability of acrolein in aqueous solution was not taken into account; the value is therefore too high. In the females, 5 adenomas and 2 hyperplastic nodules in the adrenal cortex were found. In the control group this tumour occurred in only one of 20 animals. In the males no adrenal tumours developed. Increased incidences of other tumours were not found (Lijinsky and Reuber 1987). The concentration dependency was not investigated and makes interpretation of the tumour findings in the female animals difficult. Tumour incidences of up to 4.8 % in the historical controls were recorded. With other substances investigated which are probably also metabolized to form acrolein—acrolein diethylacetal, acrolein oxime and allyl alcohol—no adrenal tumours developed. In a re-evaluation of the study by Parent *et al*. (1992b) no adrenal tumours were found in the treated group, but 3 phaeochromocytomas. According to the authors, however, their incidence was still within the range in the historical controls. Therefore the adrenal tumours described cannot be considered as evidence of a carcinogenic effect. Also in the other carcinogenicity studies the incidence of adrenal tumours was not increased. Other points of criticism are that the control group was introduced into the study 10 months later than the exposed group, no original sections were available for re-evaluating the female animals, the MTD (maximum tolerated dose) was not determined, and no data for body weight gains or toxicity were given. The instability of acrolein in aqueous solution was not taken into consideration.

Acrolein diethylacetal, acrolein oxime and allyl alcohol, but not acrolein, were administered by gavage to groups of 20 male hamsters for 53 to 64 weeks, depending on the substance. In each group 13 to 17 animals lived for longer than 48 weeks. After a total of 90 to 92 weeks at most, the animals were examined. In the control group the incidence of adrenal tumours was high (8/20). Tumours of the forestomach and the efferent duct of the pancreas were detected with the diethylacetal in 1/13 animals and with the oxime in 2/17 animals (Lijinsky and Reuber 1987). The data are not sufficient for evaluation but speak against the induction of adrenal tumours in hamsters by substances related to acrolein.

Groups of 60 SD rats per sex and dose were given gavage doses of acrolein of 0.05, 0.5 or 2.5 mg/kg body weight in 10 ml water per kg body weight for 102 weeks. The initial increase in mortality in the high dose group indicates that the maximum tolerated dose was reached. Necropsy yielded no evidence of a possible cause of death. In the extensive histopathological examination no substance-related increase in the incidence of tumours was found. Male animals of the high dose group which died prematurely were found to have focal reddening of the stomach. At the end of the study, however, the incidence of microscopically detectable stomach lesions was not increased (Parent *et al*. 1992b).

CD-1 mice were given acrolein doses of 0.5, 2 or 4.5 mg/kg body weight in 10 ml water/kg body weight on 5 days/week for 18 months. In the highest dose group 75 animals of each sex were used, in the other groups 70 animals of each sex per dose. In the male animals an initial increase in mortality was observed, so that the highest dose was considered to be in the range of the maximum tolerated dose. Necropsy yielded no evidence of a possible cause of death. In the extensive histopathological examination no substance-related increase in the incidence of tumours was found. At the end of the study the incidence of microscopically detectable stomach lesions was not increased. The authors discuss as a possible reason for the absence of specific systemic effects the degradation and reaction of acrolein in the stomach, e.g. with mercapto, hydroxy and amino groups, so that potential target tissues are not reached (Parent *et al*. 1991b).

In the two studies of Parent *et al*. (1991b, 1992b), no occult blood was found in the urine. The histological examination revealed no changes in the bladder or adrenal glands. Despite the initial increase in mortality, the extent of internal exposure to acrolein is unclear. The lack of systemic effects could be due to a reaction between acrolein and proteins in the feed or degradation in the stomach, as is discussed by the authors in the study of the toxic effects on reproduction with rats (Parent *et al*. 1992a) and the study with mice (Parent *et al*. 1991b). The absence of local effects is probably the result of the low concentration used (0.02 %). At a higher concentration (0.12 %), and to a lesser extent with 0.06 %, marked irritation of the stomach was found in the study of toxic effects on reproduction with rats. The relatively high renal excretion of acrolein metabolites in a metabolism study (Parent *et al*. 1996b) does not represent evidence of a generally good bioavailability of acrolein after oral administration, as the animals were fasted overnight and thus the possibility of a reaction of acrolein with the contents of the stomach is reduced. In addition, the reaction with glutathione can take place actually during absorption, as a result of the high level of glutathione in the

mucosa of stomach and intestine (Siegers *et al*. 1988, 1989), so that only the less reactive glutathione conjugate is systemically available.

6 Manifesto

Acrolein is genotoxic in *in vitro* tests and in tests in *Drosophila melanogaster*. Because of the great reactivity of acrolein and the uncertain internal exposure to the substance, the negative results of the oral carcinogenicity studies carried out with acrolein to date cannot refute the suspicion that the substance has carcinogenic effects. A valid experiment with sufficiently long inhalation exposure has not been carried out. Therefore acrolein is classified in Carcinogen category 3B of the *List of MAK and BAT Values*.

The MAK value valid until 1997 was withdrawn because acrolein is genotoxic and at present no concentration can be given at which carcinogenic effects in man can be excluded. The previous classification in Pregnancy risk group D has also been withdrawn.

As damage to the barrier layers of the skin resulting from the irritative or corrosive effects of the substance is of importance for the percutaneous absorption of acrolein and as no *in vivo* data with non-irritative concentrations are available, the substance is at present not designated with an "H".

There is no evidence from animal studies that the substance causes sensitization. The one reported case of sensitization in man does not provide justification for designating acrolein with an "Sh".

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completed 18.06.1997

2-Propenal: Human health tier II assessment

27 October 2017

CAS Number: 107-02-8

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

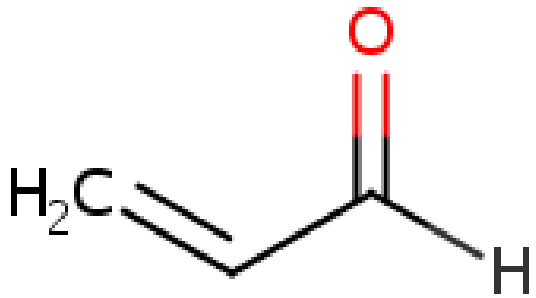
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:www.nicnas.gov.au

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	acrolein acrylaldehyde allyl aldehyde prop-2-enal
Structural Formula	
Molecular Formula	C3H4O
Molecular Weight (g/mol)	56.06
Appearance and Odour (where available)	Colourless or yellowish liquid with an acrid, pungent odour.
SMILES	C(=O)C=C

Import, Manufacture and Use

Australian

The chemical is commercially produced by vapour phase oxidation of propylene in the presence of bismuth molybdate-based catalysts. During this reaction, the major by-products produced are acrylic acid and carbon oxides, and the minor by-products are acetaldehyde, acetic acid, formaldehyde, and polyacrolein (ATSDR, 2007). Naturally occurring acrolein may be generated from the combustion of organic materials during forest fires, or from combustion of artificial materials such as tobacco products, plastics, and refined vehicle fuels. Cooking oils can also generate acrolein. A small amount of acrolein may also be generated from fermentation and ripening processes (ATSDR, 2007).

The chemical is not commercially produced in Australia. It is mainly imported from the United States of America (USA) (APVMA, 2002).

The National Pollutant Inventory (NPI) holds data for all sources of acrolein emissions in Australia. The following use information was listed on NPI:

The chemical has reported commercial and site-limited use including as:

- an intermediate for manufacturing plastics and colloidal forms of metal; and
- an additive in perfumes.

In the past, acrolein was used in military poison gas mixtures.

The chemical has reported non-industrial use as a herbicide in irrigation channels to control algae and submerged weed growth.

International

The following international uses have been identified through:

the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers; the Organisation for Economic Co-operation and Development Screening information data set International Assessment Report (OECD SIAR); Galleria Chemica; the OECD High Production Volume chemical program (OECD HPV), the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and various international assessments (Canadian Environment Protection Authority (Health Canada, 2000); US EPA, 2003).

The chemical has reported commercial uses, including:

- to promote cross-linking of protein collagen in leather tanning (US EPA, 2003);
- as a tissue fixative for histological samples (US EPA, 2003);
- controlling the formation of slime in paper manufacture (US EPA, 2003);
- as a warning agent in methyl chloride refrigerants;
- as an active ingredient to scavenge hydrogen sulfide from produced fluids in petroleum operations by oil companies (Health Canada, 2000); and
- to solubilise ferrous sulfide deposits that obstruct wells, tanks and barrels (Health Canada, 2000).

The chemical has reported site-limited uses, including:

- as an intermediate for manufacturing plastics, polymers, epoxides, colloidal forms of metal and perfumes; and
- in organic synthesis of acrylic acid, glycerol, glutaraldehyde, and pyridines.

The chemical has reported non-industrial use in pharmaceuticals, as an aquatic biocide/herbicide and in making an animal feed additive (DL-methionine).

The chemical was used as a tear gas under the name Papite in World War I (HSDB).

Although the chemical is listed as an additive in perfumes in NPI, its not listed as such in the International Fragrance Association (IFRA, 2017).

There is currently no documented use of the chemical either as a cosmetic in the Compilation of the Ingredients used in Cosmetics in United States (CIUCUS, 2011), or as a domestic use in the Household Products Database.

Restrictions

Australian

Acrolein is listed in the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) in Schedule 7 (SUSMP, 2017).

Schedule 7 chemicals are described as 'Substances with a high potential for causing harm at low exposure and which require special precautions during manufacture, handling or use. These poisons should be available only to specialised or authorised users who have the skills necessary to handle them safely. Special regulations restricting their availability, possession, storage or use may apply'. Schedule 7 chemicals are labelled with 'Dangerous Poison'. The chemical is listed with condition 1 'Not to be available **except** to authorised or licensed persons' under appendix J of the SUSMP (SUSMP, 2017).

The use of the chemical in pesticides is restricted in Schedule 4 of the Agricultural and Veterinary (Agvet) Chemicals Code Regulations 1995 (APVMA, 2017).

International

As a poisonous substance, the chemical is listed in a broad range of categories such as (Galleria Chemica):

- Canada Environmental Protection Act (CEPA) 1999 - Schedule 1 Toxic Substances List;
- Catalogue of China Strictly Controlled Toxic Chemicals for Import and Export;
- EU Annex I to Directive 67/548/EEC—Classification and Labelling of Dangerous Substances;
- International Aerospace Environmental Group (IAEG) Aerospace and Defence Declarable Substances List;
- International Global Oragnic Textile Standard - Limit values for residues in additional fibre materials and accessories;
- Japan Poisonous and Deleterious Substances Control Law;
- US OSHA List of Highly Hazardous Chemicals, Toxics and Reactives; and
- US World Doctors Association (WDA) List of Banned Medicines and Substances.

It is also restricted based on potential use in chemical warfare:

US Department of Homeland Security Chemical Facility Anti-Terrorism Standards - Chemicals of Interest.

Existing Work Health and Safety Controls

Hazard Classification

The chemical is classified as hazardous, with the following hazard categories and hazard statements for human health in the Hazardous Chemical Information System (HCIS) (Safe Work Australia):

- Acute toxicity – category 1; H330 (Fatal if inhaled), category 2; H300 (Fatal if swallowed), category 3; H311 (Toxic in contact with skin);
- Skin corrosion – category 1; H314 (Causes severe skin burns and eye damage); and
- AUH071 (Corrosive to the respiratory tract).

Exposure Standards

Australian

The chemical has an exposure standard of 0.23 mg/m^3 (0.1 ppm) time weighted average (TWA) and 0.69 mg/m^3 (0.3 ppm) short-term exposure limit (STEL) (HCIS).

International

The following exposure standards are identified (Galleria Chemica):

A TWA of 0.12–0.25 mg/m^3 (0.05–0.10 ppm) in different countries such as Canada (Quebec and Yukon), Denmark, Egypt, Germany, Greece, Iceland, India, Japan, Singapore, South Africa, Switzerland, United Kingdom and the United States of America (USA).

A STEL of 0.25–0.80 mg/m^3 (0.1–0.3 ppm) in different countries such as Canada (Quebec and Yukon), France, Hungary, Sweden, Mexico, India, Ireland, Poland, United Kingdom and the USA (Hawaii, Minnesota, Tennessee, Vermont and Washington).

Health Hazard Information

Acrolein is a reactive unsaturated aldehyde that exists as a highly volatile clear or yellow liquid. Due to its highly reactive nature, acrolein is a point of contact irritant rather than systematically toxic. As a result, effects in both humans and animals primarily involve sensory irritation and respiratory system effects.

Toxicokinetics

Following oral administration in rats, the chemical is well absorbed at low dose levels (2.5 mg/kg bw), however polymerisation occurs at higher dose levels (15 mg/kg bw) which reduces the rate of absorption. Dermal and inhalation absorption is predicted to be high. The irritant and corrosive properties of the chemical do not allow assessment of dermal absorption (EURAR, 2001; CLH, 2011).

Following inhalation exposure to atmospheres containing the vapour of the chemical (400–600 mg/m^3, 172–258 ppm) in dogs, total respiratory tract retention was high (81–84 %) and appeared to be independent of concentration (EURAR, 2001; US EPA, 2003). When administered via inhalation, the chemical is retained primarily in the upper respiratory tract (nose, throat and trachea) due to its reactive nature (US EPA, 2003).

Following oral administration of ^{14}C-acrolein in rats, the highest concentration of the radiolabel was found in the liver. Excretion was predominantly via urine, then in exhaled air (mainly as $^{14}CO_2$) and faeces. Elimination of the metabolites was observed within 48 hours of dosing in rats, at ~70–80 % following oral administration and 11–22 % following inhalation exposure (EURAR,

2001). As the chemical is rapidly metabolised, it is unlikely to bioaccumulate in the body (Health Canada, 2000). Radiolabel acrolein was also detected in the milk of lactating goats, indicating that in utero exposure of the developing foetus is possible (CLH, 2011).

The major metabolic pathways involve oxidation/hydrolysis and conjugation with glutathione (GSH). The chemical is very reactive and binds primarily at the application site. In vivo, conjugation readily occurs with glutathione (GSH) or other thiol-containing molecules to form adducts, thus, leading to GSH depletion and oxidative stress. The conjugate is excreted in rat urine as mercapturic acid metabolites (3-hydroxypropylmercapturic acid (3-HPM) and 2-carboxyethylmercapturic acid (CEMA)) (EURAR, 2001; NTP, 2006; HSDB).

In vitro, the chemical is oxidised by rat liver aldehyde dehydrogenase (ALDH) to acrylic acid. Incubation with rat liver or lung microsomes and NADPH yielded glycidaldehyde and its hydration product glyceraldehyde. These metabolites have not been demonstrated in an in vivo metabolic pathway of acrolein (EURAR, 2001).

The chemical is also capable of reacting nonenzymatically with sulfhydryl groups via Michael additions, which is proposed to contribute to cytotoxic effects if reactions with critical intracellular sulfhydryl groups occur (NTP, 2006).

The chemical may be produced in the body. It is a metabolite of allyl alcohol, allylamine, spermine, and spermidine, and is formed during lipid peroxidation. It can also be formed following exposure of the skin lipid triolein to ultraviolet (UV) radiation. The chemical has been detected in plaque deposits associated with atherosclerosis and Alzheimer’s disease (AEP, 2011).

Acute Toxicity

Oral

The chemical is classified as hazardous with hazard category ‘Acute Toxicity Category 2’ and hazard statement ‘Fatal if swallowed’ (H300) in the HCIS (Safe Work Australia). The available data support this classification (EURAR, 2001; CLH, 2011; HSDB).

The reported median lethal doses (LD50) values are:

- 10.3–46 mg/kg bw in rats; and
- 13.9–40 mg/kg bw in mice

Reported signs of toxicity in rats included lethargy, decreased motor activity, tremor, hypothermia and respiratory distress. Mice further displayed squinted eyes, rough coat, hunched posture and piloerection. At necropsy, haemorrhagic stomach and intestine were observed in both species, and reddening of the lungs was seen in mice (EURAR, 2001; CLH, 2011).

Dermal

The chemical is classified as hazardous with the hazard category ‘Acute Toxicity Category 3’ and hazard statement ‘Toxic in contact with skin’ (H311) in the HCIS (Safe Work Australia). The available data support this classification.

The dermal LD50 value in rabbits was reported between 164 to 1022 mg/kg bw depending upon the vehicle used and concentration of the chemical applied. The dermal LD50 for undiluted acrolein in rabbits was reported at 562 mg/kg bw. Clinical signs of toxicity included severe pain and hyperactive behaviour initially, followed by lethargy, respiratory distress, cyanosis, ulceration, oedema and haemorrhage of the dermis and skin discolouration. At necropsy, pulmonary effects such as red spots and collapsed lung were observed from vapourisation and inhalation of the chemical (CLH, 2011; EURAR, 2001).

Inhalation

The chemical is classified as hazardous with the hazard category ‘Acute Toxicity Category 1’ and hazard statement ‘Fatal if inhaled’ (H330) in the HCIS (Safe Work Australia). The available data support this classification.

The reported median lethal concentration (LC50) values are (EURAR, 2001; REACH):

- 16–150 mg/m^3 (vapours) for four hours in rats;
- 58 mg/m^3 for four hours in hamsters; and
- 151 mg/m^3 (vapours) for six hours in mice.

Reported signs of toxicity included eye and nose irritation, respiratory difficulties (mouth breathing, decreased respiration rate, audible respiration), body weight loss and lung and liver discolouration. Examination of the lungs revealed congestion, haemorrhages, fibrin deposition and necrosis (EURAR, 2001).

In addition to overt toxicity, several indicators of oxidative stress were reported in rats exposed to 1 ppm (2.3 mg/m^3) for 4 hours, including reduced lung levels of ascorbic acid and alpha-tocopherol, reduced glutathione and thiols, and increased superoxide dismutase activity (ATSDR, 2007).

Observation in humans

Available human case studies or exposure data mainly involve irritation (see **Irritation** section).

Human case reports of acute acrolein inhalation exposures (concentration not stated) have shown severe effects including high fever, dyspnoea, coughing, foamy expectoration, cyanosis, pulmonary oedema and death. Volunteers treated with 10 % acrolein in ethanol topically showed irritation, papillary oedema, polymorphonuclear infiltrates and epidermal necrosis after 48 hours (ATSDR, 2007).

In 1 case report, 2 young boys died from lung damage after being exposed for 2 hours to smoke from an overheated fryer. However, co-exposure to other components of the smoke may have contributed to this outcome (AEP, 2011).

Corrosion / Irritation

Corrosivity

The chemical is classified as hazardous with hazard category ‘Skin corrosion Category 1’ and hazard statement ‘Causes severe skin burns and eye damage’ (H314) in the HCIS (Safe Work Australia). The available animal and human data support this classification.

In animals, ~1 % solutions of the chemical was reported to cause serious eye and skin damage (CICAD, 2002).

In a skin irritation study, the chemical (0.5 mL) was applied on intact and abraded skin of New Zealand White (NZW) rabbits (n = 6) for 24 hours. Erythema and oedema were scored at 24 and 72 hours after exposure. During exposure, 2 animals died. Oedema up to grade 4 was observed in 1 animal. Effects were not reversible in 3 out of 4 survivors after 14 days, and were reported to become progressively severe (US EPA, 2003; CDPR, 2015).

In an eye irritation study, the chemical at 0.5 mL was severely irritating in NZW rabbits (n = 9) when applied on the lower lids with observation up to 7 days. Complete corneal opacity, deepened folds, congestion or swelling of the iris, and crimson red, swollen conjunctivae were reported. These effects were not reversible after 7 days. The reported scores were corneal opacity = 4; iris lesion = 2; redness of conjuctivae = 4; and chemosis of conjunctivae = 2 (CLH, 2011).

Exposure to the vapour of the chemical at concentrations between 1.9 and 2.6 ppm for 4 hours caused slight eye irritation in rabbits. No irritation was observed when rabbits were exposed to 0.6 ppm (1.4 mg/m^3) up to 30 days (EURAR, 2001).

Dogs and monkeys appeared to be more sensitive towards irritation than rodents to the chemical. Lacrimation, blinking or closed eyes were observed during intermediate duration exposure to 3.7 ppm, but none of these changes were reported in guinea pigs and rats exposed for the same duration (ATSDR, 2007).

Respiratory Irritation

The chemical is classified as hazardous with hazard category 'Corrosive to the respiratory tract (AU071)' in the HCIS (Safe Work Australia). Acute and repeated dose inhalation toxicity studies in rats showed irritation to the respiratory system and damage to the nasal and tracheal epithelium. Exposure to a low concentrations (0.25 ppm) caused mild nasal epithelial dysplasia, necrosis and focal basal cell metaplasia (ATSDR, 2007).

Sensory irritation of the upper respiratory tract was observed in animals after inhalation exposure. Decreased respiratory rates were observed at 1–3 ppm (2.3–6.9 mg/m^3). Mice were reported to be more sensitive than rats. The concentration required to reduce respiratory rates by 50 % (RD50) was 2.4–6.6 mg/m^3 in mice and 9.2–13.7 mg/m^3 in rats (EURAR, 2001; US EPA, 2003; ATSDR, 2007).

Exposure of Syrian golden hamsters to 6 ppm (14 mg/m^3) of the chemical for four hours caused >50 % exfoliation of ciliated cells in the bronchi, and the cells were observed to be pale and swollen at 24 and 48 hours post-exposure. Areas of irregular epithelium with early stratification and hyperplasia were observed after 96 hours. The chemical depleted sensory neuropeptides (calcitonin-gene related peptide (CGRP) and substance P) in the trachea of rats exposed to 22–249 ppm (51–571 mg/m^3) for 10 minutes (US EPA, 2003).

In vitro studies conducted in tissues from several animal species including sheep, chickens and cows showed that the chemical induced significant reduction in ciliary movement in the upper respiratory tract (CICAD, 2002).

Other

Irritation of the gastrointestinal mucosa appeared to be the primary effect following oral exposure. Acute and intermediate duration exposure to high doses (>2 mg/kg) cause increasingly severe irritation effects in the stomach, including epithelial hyperplasia, ulceration, haemorrahage, and oedema of the stomach mucosa. Species differences in gastrointestinal sensitivity were reported (ATSDR, 2007).

Observation in humans

Many human exposure studies are available. Effects following oral or inhalation exposure to the chemical have been consistently observed at the site of contact (stomach or respiratory tract) (CICAD, 2002). Exposure to the vapour or liquid forms of the chemical causes inflammation and irritation of the skin and eyes, mucous membranes and respiratory tract, progressing into delayed pulmonary oedema, chronic respiratory disease, skin and corneal burns and sensitisation dermatitis. Ingestion causes severe mouth and gastrointestinal tract irritation (HSDB).

Irritation is reported to be concentration and time dependent. In various human exposure studies, minor eye irritation (perceived as rapid-onset stinging of the eyes and increased blinking) was reported at 0.1–0.3 ppm. A lowest observed adverse effect level (LOAEL) of 0.34 mg/m^3 for eye irritation was suggested, with sensory irritation persisting 10 minutes after exposure. Rapid onset of nose and throat irritation and reduced breathing rate was reported following acute exposure to 0.3 ppm. A 5 minute exposure to 1 ppm (2.3 mg/m^3) of vapour caused lacrimation, marked eye, nose and throat irritation. At 3 ppm (7 mg/m^3), the chemical is a severe pulmonary irritant and powerful lachrymogen, affecting the conjunctivae and mucous membranes of the upper respiratory tract. At higher concentrations it causes lung injury; a 10 minute exposure to 350 mg/m^3 is lethal. Respiratory difficulties may persist for at least 18 months following exposure (ATSDR, 2007; HSDB).

Humans appear to adapt to eye irritation at low levels of vapour exposure, as a study reported increasing eye irritation up to 40 minutes in volunteers exposed to a constant level of acrolein vapours for 60 minutes, but no further increase in discomfort thereafter (ATSDR, 2007).

In a human patch test, volunteers were exposed to the chemical at concentrations of 0.01, 0.1, 1 and 10 % in ethanol (duration not stated). Positive skin reactions were observed in 6 out of 48 subjects at 1 % concentration, with 4 of the 6 exhibiting serious oedema and bullae and the other 2 with erythema. At the highest concentration (10 %), all subjects showed severe skin effects

including bullae, necrosis, inflammatory cell infiltration and papillary oedema. No adverse effects were observed at ≤0.1 % (CICAD, 2002; CLH, 2011).

Sensitisation

Respiratory Sensitisation

No data are available.

Skin Sensitisation

Based on the available data, skin sensitisation potential is indicative but not definitive. The sensitisation potential of the chemical at high concentrations cannot be ruled out.

In a poorly reported guinea pig maximisation test (similar to OECD TG 406), female guinea pigs were intradermally induced with the chemical at 0.01 % in water, followed by topical exposure at 2.5 %, and a topical challenge phase at 0.5 %. Skin reactions were scored on a scale of 0.5, 1, 2 and 3. Positive skin reactions were reported in 7/15 test animals and in one control animal at a score of 0.5. The authors defined 0.5 as patches of redness and non-confluent, which equates to a score of 1 according to the description in the OECD guidelines. The available data is insufficient to warrant classification for sensitisation (EURAR, 2001; CLP, 2001).

Repeated Dose Toxicity

Oral

Based on the available data, no systemic effects were observed. Reported effects were mainly local and were considered secondary to irritation/corrosivity; therefore, not relevant for classification.

The primary effects observed in animal studies were in the forestomach. No hepatotoxicity was reported. Stomach lesions were observed in Fischer 344 (F344) rats but not in Sprague Dawley (SD) rats, and may relate to differences in strain sensitivity (US EPA, 2003).

In a 14-week repeat dose oral gavage study conducted by the NTP, F344 rats (n = 10/sex/dose) were administered the chemical at 0, 0.75, 1.25, 2.5, 5 or 10 mg/kg bw/day, and B6C3F1 mice (n = 10/sex/dose) were administered 0, 1.25, 2.5, 5, 10 or 20 mg/kg bw/day. At the highest doses, mortalities occurred in most of the rats and in all mice. In rats, observed effects at the highest dose included significantly decreased body weight gain, clinical toxicity effects (abnormal breathing, eye or nasal discharge, ruffled fur, thinness and lethargy), significantly decreased absolute and relative thymus weights and gross lesions in the forestomach and glandular stomach. Microscopically, increased incidences of forestomach squamous epithelial hyperplasia were observed in males at ≥5 mg/kg bw/day and in females at ≥2.5 mg/kg bw/day, and increased incidences of glandular stomach haemorrhage in the 10 mg/kg bw/day groups. The no observed adverse effect level (NOAEL) was 2.5 mg/kg bw/day and 1.25 mg/kg for males and females, respectively (NTP, 2006).

In the mouse study, there was no change in body weight gain. Effects observed at the highest dose included gross lesions (red or white discolouration) in the forestomach and glandular stomach (females), significantly increased incidences of glandular stomach haemorrhage (both sexes), and glandular stomach inflammation and epithelial necrosis (females). Microscopic examinations revealed forestomach squamous epithelial hyperplasia at ≥2.5 mg/kg bw/day. The NOAEL was 1.25 mg/kg bw/day (NTP, 2006).

In a standard 90-day oral gavage study in SD rats (n = 30/sex/dose), no mortalities or treatment-related toxicity were observed at doses up to 5 mg/kg bw/day. A 2-year oral gavage study in SD rats and an 18-month study in mice did not show any treatment-related clinical signs of toxicity or any significant histopathological changes at doses up to 2.5 mg/kg bw/day in rats and 4.5 mg/kg bw/day in mice. The study otherwise resulted in early mortality in rats (0.5 mg/kg bw/day), which was considered

significant even after it was proposed to be due to dosing errors (US EPA, 2003; CLH, 2011). The disparity in effects observed between the NTP study and this study could be is due to the use of a thickening agent in the dosing vehicle for the NTP study, increasing the gastrointestinal residence time and hence, the toxicity of the chemical (ATSDR, 2007).

In a non-guideline study, beagle dogs administered the chemical in gelatin capsules up to 2 mg/kg bw/day for 12 months did not show treatment-related effects. Transient vomiting was reported at ≥0.5 mg/kg bw/day, possibibly from ingestion of the capsule. This effect decreased over time, suggesting that tolerance developed for this method of administration. (CICAD, 2002; ATSDR, 2007; CLH, 2011).

Dermal

Based on the available data, the dermal effects observed are primarily due to irritation and systemic toxicity is not expected.

In a subchronic dermal toxicity study (OECD TG 410), NZW rabbits (n = 10/sex/dose) were exposed to the chemical at 0, 7, 21 or 63 mg/kg bw/day for 21 days. Dermal application at ≥7 mg/kg bw/day resulted in local irritation which increased in severity with dose and duration. Slight to moderate erythema and oedema were observed at 7 or 21 mg/kg bw/day. Oedema was more pronounced at the highest dose. Dose-dependent increased incidences of nasal mucous discharge, interstitial pneumonia and lethargy were reported in all treated groups. Lung toxicity was stated to be a result of inhalation of the volatile chemical. No specific systemic toxicity was observed in this study (CLH, 2011).

Inhalation

Many non-guideline studies for the chemical have been conducted in rats, rabbits, guinea pigs, hamsters, dogs and monkeys, with rats being the most sensitive species. These studies had limitations in their protocol or reporting. Overall, the observed effects are similar to that observed upon acute exposure, including pulmonary inflammation, and lesions in the respiratory tract and nasal cavity. Lung effects were considered to result from repeated exposure to an irritant or corrosive atmosphere (CLH, 2011). No classification is recommended.

In several studies ranging from 62–90 days, rats were exposed (whole-body) to the chemical at concentrations ranging from 0.4 ppm (0.9 mg/m^3) to 4.9 ppm (9.2 mg/m^3). At the highest dose, significant mortality was observed. Histopathological changes in the respiratory tract (destruction and hyper- and metaplasia of the epithelial lining and inflammatory alterations) were observed with increasing severity at all doses. Histopathological changes in the lungs included bronchiolar necrosis at 1.4 ppm, and sloughing, bronchiolar oedema and focal pulmonary oedema at 4 ppm. Increased relative organ weights (lung, heart, kidneys and adrenals) was observed at 4.9 ppm. An elevated expiratory flow rate was observed in the low dose group. A dose-related decrease in body weight gain and increased severity of respiratory tract lesions (squamous cell metaplasia in the nasal cavity) were observed at all doses, while body weight change was not significant in the low dose groups. The NOAEC was determined as <0.4 ppm (<0.9 mg/m^3) (EURAR, 2001; CLH, 2011).

In a repeat dose inhalation study, rats, hamsters and rabbits were exposed (whole-body) to vapours of the chemical at 0.4, 1.4 or 4.9 ppm (0.9, 3.2 or 11.2 mg/m^3) for 62 days. Rats were the most sensitive species and effects observed are summarised above. Rabbits and hamsters did not show adverse effects at 0.4 ppm. At the highest dose, clinical toxicity effects included laboured breathing and sneezing, salivation and nasal discharge, decreased body weights, increased relative organ weights (lung, hearts and kidneys), significant increased erythrocyte count, haemoglobin content and number of lymphocytes. Histopathological changes (inflammatory changes in the nasal cavity and respiratory tract) were observed from 1.4 ppm. The NOAECs for rabbits and hamsters were determined as 0.4 ppm (0.9 mg/m^3) (EURAR, 2001; CLH, 2011).

Several short-term inhalation studies (up to 3 days, up to 1.7 ppm) in rats were conducted to study the biochemical and histopathological changes in the respiratory and olfactory epithelium of the nose and in free lung cells. Effects were examined at the microscopic level and included cell proliferation in nasal and tracheal epithelium and concentration-dependent increase in the proportion of DNA-synthesising cells, olfactory degeneration and necrosis, ulceration and basal cell hyperplasia of the respiratory epithelium (EURAR, 2001; CICAD, 2002; US EPA, 2003).

One 13-week repeat dose inhalation study in F344 rats was conducted at lower concentrations at 0, 0.05, 0.14, 0.5, 1.4 or 4.2 mg/m^3, 6 hours/day, 5 days/week and involved a more extensive examination of the nasal cavity. The NOAECs were

established as 0.5 mg/m^3 for the respiratory epithelium and 1.4 mg/m^3 for the olfactory epithelium, based on neuronal loss at 4.2 mg/m^3 (AEP, 2011). Computational fluid dynamic modelling revealed that although the reported NOAEC for the respiratory epithelium was lower, olfactory epithelium lesions arise at a lower delivered tissue dose suggesting that the ofactory epithelium is more sensitive to the effects of inhaled acrolein than the respiratory epithelium (OEHHA, 2008).

A few short-term studies in mice reported moderately severe lesions in the respiratory epithelium and olfactory epithelium up to 1.7 ppm, excessive macrophage accumulation at 3 ppm, and lung lesions at an unknown concentration (US EPA, 2003).

In 90-day studies in monkeys, inflammatory changes in sections of liver, lung, kidneys and heart, and occasional emphysema were observed at 0.22–0.70 ppm (0.5–1.6 mg/m^3). Eye irritation including ocular discharge, excessive salivation, squamous cell metaplasia and basal cell hyperplasia of the trachea were reported at 1.8 ppm (4.1 mg/m^3). Mortalities which involved pulmonary hepatic, splenic, liver lesions and lung haemorrhage were observed at the highest tested dose at 3.7 ppm (8.5 mg/m^3) (CLH, 2011).

Genotoxicity

Based on the weight of evidence, classification for mutagenicity is not warranted.

The chemical is an alkylating agent and therefore a direct-acting mutagen for bacteria. Induction of gene mutations and sister chromatid exchanges were observed, but negative results in chromosome aberrations test in mammalian cells in vitro were obtained. Positive results were generally observed in a narrow, near lethal, dose range. In vivo tests were negative.

In vitro

The following results were reported in in vitro assays:

- positive in bacterial gene mutation assays with certain strains of *Salmonella typhimurium* (TA100, TA104 and TA98), mainly in assays without metabolic activation (EURAR, 2001);
- when tested under 2 different protocols, the preincubation protocol gave weakly positive results in strain TA100, with metabolic activation, and equivocal results in TA100 and TA1535, with metabolic activation. Negative results were obtained with strains TA98 and TA1537, with or without metabolic activation. The vapour protocol gave negative results for all strains and activation conditions (NTP, 2006);
- no chromosome aberrations in four standard mammalian gene mutation tests with Chinese hamster ovary (CHO) cells, mouse embryo fibroblasts and normal human fibroblasts. Chromosome tangling was observed at cytotoxic concentrations (≥40 µM) with no chromosome breakage (EURAR, 2001; CLH, 2011);
- weakly positive in three sister chromatid exchanges (SCE) studies with CHO cells and human lymphocytes only at highest concentrations tested, without metabolic activation. Negative in another SCE study with CHO cells up to 0.75 µg/mL;
- an increase in gene mutations in a non-standard cell line, DNA repair deficient human fibroblasts (Xeroderma pigmentosum cells), but not in normal repair proficient human fibroblasts (EURAR, 2001; CLH, 2011);
- negative for petite mutations in the yeast *Saccharomyces cerevisiae* strains S211 and S138 (EURAR, 2001).

The chemical is highly toxic in bacterial and mammalian cells and; therefore, the experimental dose range is restricted. The genotoxic doses are close to or overlap cytotoxic doses (EURAR, 2001). The positive findings in in vitro bacterial systems may be due to the lack of an endogenous glutathione detoxification pathway. Glutathione reacts readily with the chemical (see **Toxicokinetics** section), thus protecting sensitive intracellular systems from damage (CLH, 2011).

In vivo

The chemical was negative in a bone marrow cytogenetics test (OECD TG 475) in male SD rats administered the chemical by gavage up to 8.2 mg/kg bw (a lethal dose). Negative results were obtained in two dominant lethal studies in mice (CLH, 2011).

The chemical appeared genotoxic in the 'somatic mutation and recombination' (SMART) test in *Drosophila melanogaster*, but did not exhibit genotoxic activity in the 'sex chromosome loss test' (SCLT), while equivocal results were obtained in the 'sex-

linked recessive lethal test' (SLRLT) (EURAR, 2001; NTP, 2006).

Carcinogenicity

Based on available animal data and the results for genotoxicity indicating lack of systemic mutagenic potential (see **Genotoxicity** section), the chemical is not considered to be carcinogenic. However, the available data are not sufficient to examine the long term potential to produce point of contact respiratory tumours as seen for formaldehyde (NICNAS, 2006).

The available oral gavage studies in rats and mice did not indicate carcinogenic potential. Available inhalation studies in rats and hamsters are inadequate for determining carcinogenicity due to study limitations.

In lifetime gavage carcinogenicity studies (OECD TG 453), SD rats (n = 70–75/sex/dose) were administered the chemical at 0, 0.05, 0.5 or 2.5 mg/kg bw/day, and CD-1 mice (n = 70–75/sex/dose) were administered the chemical at 0, 0.5, 2 or 4.5 mg/kg bw/day. No treatment-related increases in tumour incidence were observed up to the highest dose tested in either species. A statistically significant reduction in survival was observed in rats and in male mice at the highest dose. However, this was due to dosing error (EURAR, 2001; CLH, 2011).

Two long-term inhalation studies are available, 1 in rats and the other in hamsters. Both studies have limitations in reporting and of their methodology. No treatment-related tumours or metaplasia were observed in the lungs of rats exposed to the vapour of the chemical at 18.3 mg/m^3 (8 ppm) for 10 or 18 months. Syrian golden hamsters were exposed to the vapour of the chemical at 9.3 mg/m^3 (4 ppm) for 52 weeks. Nasal inflammatory changes and olfactory epithelium metaplasia were observed but were reversible after a 29-week withdrawal period. No treatment-related respiratory tract, nasal tumours or tumours at other sites were observed. Additionally, the chemical was not determined to have an enhancing (co-carcinogenic) effect with benzo[a]pyrene or *N*-nitroso-diethylamine on respiratory tract tumours. However, the experimental duration is relatively short and is not a lifetime study for hamsters (EURAR, 2001; CICAD, 2002).

The US EPA determined that the ‘data are inadequate for an assessment of human carcinogenic potential by either the inhalation or oral routes of exposure’. The highly reactive nature of the chemical and the lack of systemic toxicity suggest that the chemical is not likely to reach potential target sites at a concentration sufficient to initiate a carcinogenic process in mammalian species (US EPA, 2003).

In a case-control study of employeees of chemical manufacturing companies, slightly higher than expected incidences of non-Hodgkin's lymphoma with an odds ratio (probability of being affecting in the exposed group to that being affected in the control group) of 2.6 were reported in workers exposed to acrolein. However, these results were not statistically significant and there were co-exposures to other chemicals besides acrolein (CICAD, 2002; AEP, 2011).

Reproductive and Developmental Toxicity

Based on available data, the chemical is not likely to be a reproductive or developmental toxicant. Any reproductive and developmental effects were only observed secondary to maternal toxicity.

In a 2-generation reproductive toxicity studies (OECD TG 416), SD rats were administered the chemical (gavage) up to doses of 7.2 mg/kg bw/day (115 days) or 6 mg/kg bw/day (10 weeks). Statistically significant decreased body weight gains in the parental (F0) at 6–7.2 mg/kg bw/day were observed in both studies. Other reported parental toxicity effects (mortality, gastric lesions, histopathological stomach changes) were observed at ≥3 mg/kg bw/day. Dams developed respiratory irritation at ≥4 mg/kg bw/day and wheezing, dyspnoea and stomach lesions at 7.2 mg/kg bw/day. Reproductive parameters including male and female fertility were not affected up to the highest tested doses. In 1 study, reduced pup weights of the F1 generation pups at 6 mg/kg bw/day were observed. The NOAELs for parental toxicity were established as 1 mg/kg bw/day (stomach lesions) or 4 mg/kg bw/day. The NOAELs for developmental toxicity were established as 3 mg/kg bw/day (reduced F1 pup bodyweights during lactation) or 7.2 mg/kg bw/day (EURAR, 2001).

Developmental toxicity

In an oral teratology study, SD rats were exposed (gavage) to the chemical at doses of 0, 3.6, 6 or 10 mg/kg bw/day at gestation days (GD) 7–19. At the highest dose, increased mortality was observed in the dams. Decreases in total litter size (24 %) and mean foetal weight (18 %), increased incidences of delayed ossification (36 %) and skeletal anomalies were also observed at

the highest dose compared to the controls. Clinical toxicity (wheezing and dyspnoea) and statistically significantly reduced body weight gain for the dams were observed from 6 mg/kg bw/day. The effects occurred at doses that caused significant maternal toxicity. The NOAELs for maternal and developmental toxicity were 3.6 mg/kg bw/day and 10 mg/kg bw/day, respectively (EURAR, 2001; CLH, 2011).

In developmental toxicity study, NZW rabbits were administered (gavage) the chemical at 0, 0.1, 0.75 or 2 mg/kg bw/day at GD 7–19. At the highest dose, dams showed a transient decrease in body weight gain accompanied by decreased food consumption. An increase in mean foetal body weights was observed but not considered to be an adverse effect. No effect on pregnancy indices, implantation sites, number of live foetuses, or skeletal anomalies were observed. The NOAELs for maternal and developmental toxicity were 0.75 mg/kg bw/day and ≥2 mg/kg bw/day, respectively (EURAR, 2001; CLH, 2011).

In a poorly reported developmental toxicity study, CD-I mice were treated at doses up to 10 mg/kg bw/day, from GD 7–17. Maternal toxicity was observed at the highest dose, including lethargy, squinted eyes, dyspnoea and hunched posture. In the foetuses, an increased incidence of cleft palate was observed but occurred in mice with a high background incidence. Delayed ossification was observed only at a dose that caused maternal toxicity. An increased incidence of subcutaneous oedema was observed in foetuses and was suggestive of a dose-related response. However, the severity of this effect was not stated and it is not known if it is a localised or generalised oedema. A developmental NOEL was established at less than 4.0 mg/kg bw/day (resorptions at 10 mg/kg bw/day and generalised delayed ossification) and a maternal NOEL was established at 6.3 mg/kg bw/day (decreased body weight gain) (CLH, 2011; CDPR, 2015).

In vitro tests

A number of in vitro toxicity tests were conducted using rat embryo cultures, mouse limb bud culture and chicken eggs. Embryolethality, abnormal development and growth retardation effects were reported. These results indicate that the chemical is toxic when administered directly to the embryos or foetuses but not when administered orally in vivo. Therefore, it is proposed that the reactivity of the chemical may limit its ability to reach critical sites in the developing embryo (EURAR, 2001; EPA, 2003).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include:

- systemic acute effects (acute toxicity from oral, dermal and inhalation exposure); and
- local effects (corrosivity and respiratory irritation).

Public Risk Characterisation

Given the uses identified for the chemical, it is unlikely that the public will be exposed. The chemical is currently listed on Schedule 7 of the SUSMP and is only available to authorised or licensed persons. Hence, the public risk from this chemical is not considered to be unreasonable.

Occupational Risk Characterisation

During product formulation, dermal, ocular and inhalation exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemical at lower concentrations could also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical systemic acute and local health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise dermal, ocular and inhalation exposure are implemented. The chemical should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

Based on the available data, the hazard classification in the HCIS (Safe Work Australia) is considered appropriate.

Sensory irritation in humans following acute exposure is reported at airborne concentrations of 0.34 mg/m^3 and above, which is below the current exposure standard of 0.69 mg/m^3 STEL. Therefore, a review of the current exposure standard may be beneficial to mitigate the risk of adverse effects. Airborne concentrations of the chemical should be kept as low as reasonably practicable to minimise risk.

NICNAS Recommendation

It is recommended that Safe Work Australia consider whether current controls adequately minimise the risk to workers. A Tier III assessment might be necessary to provide further information about whether the current exposure controls offer adequate protection to workers.

All other risks are considered to have been sufficiently assessed at the Tier II level, provided that all requirements are met under workplace health and safety, and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Public Health

Products containing the chemical should be labelled in accordance with state and territory legislation (SUSMP, 2017).

Work Health and Safety

The chemical is recommended for classification and labelling aligned with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below. This does not consider classification of physical hazards and environmental hazards.

From 1 January 2017, under the model Work Health and Safety Regulations, chemicals are no longer to be classified under the Approved Criteria for Classifying Hazardous Substances system.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Not Applicable	Fatal if swallowed - Cat. 2 (H300)* Toxic in contact with skin - Cat. 3 (H311)* Fatal if inhaled - Cat. 1 (H330)*
Irritation / Corrosivity	Not Applicable	Corrosive to the respiratory tract (AUH071)* Causes severe skin burns and eye damage - Cat. 1 (H314)*

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for consumers

Products containing the chemical should be used according to the instructions on the label.

Advice for industry

Control measures

Control measures to minimise the risk from oral, dermal, ocular and inhalation exposure to the chemical should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures that could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;
- health monitoring for any worker who is at risk of exposure to the chemical, if valid techniques are available to monitor the effect on the worker's health;
- air monitoring to ensure control measures in place are working effectively and continue to do so;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemical are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemical has not been undertaken as part of this assessment.

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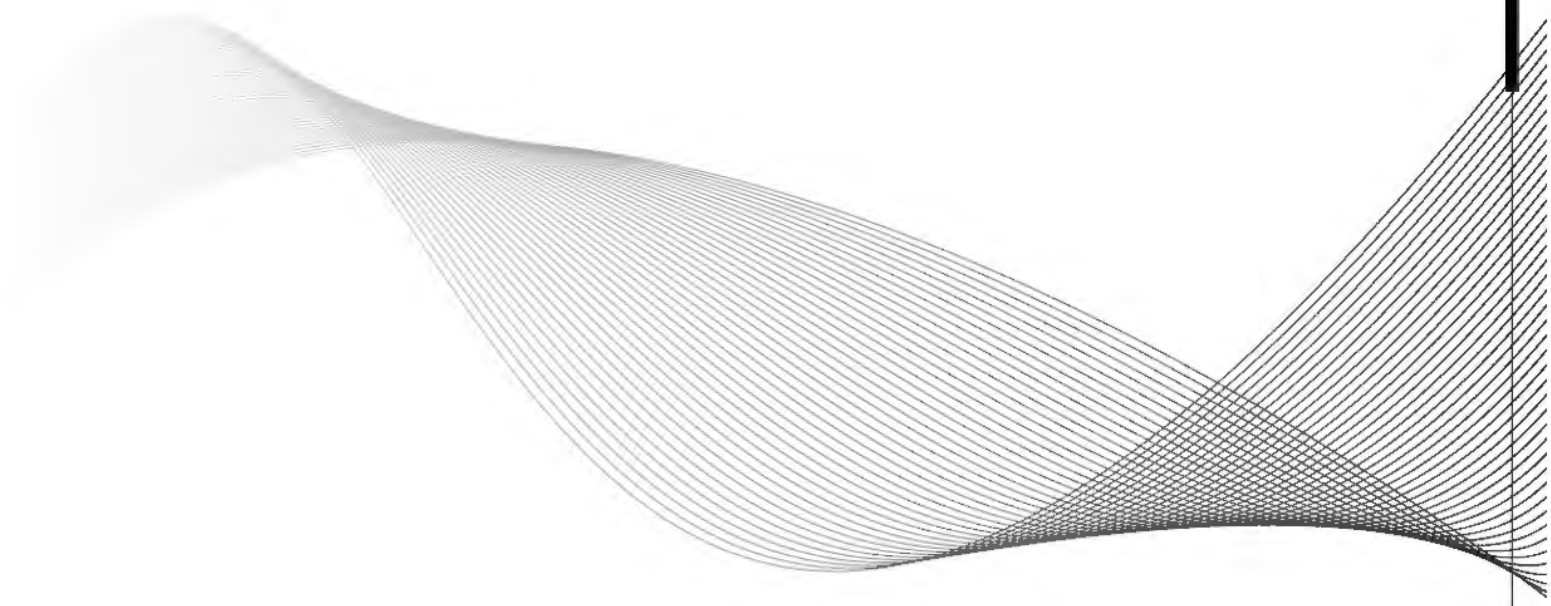

Recommendation from the Scientific Committee on Occupational Exposure Limits for acrolein

SCOEL/SUM/32

September 2007

European Commission

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Recommendation from the Scientific Committee on Occupational Exposure Limits for acrolein

8 hour TWA:	0.02 ppm (0.05 mg/m3)
STEL (15 mins):	0.05 ppm (0.12 mg/m3)
Notation:	-

Substance:

Structural formula CH_2=CH-CHO
Molecular formula C_3H_4O

Synonyms Acrylic aldehyde; allyl aldehyde; acraldehyde; 2-propenal

EINECS N° 203-453-4

EEC N° 605-008-00-3

Classification: F; R 11 T +; R26 T; R25

CAS N° 107-02-8

MWt 56.06

Conversion factor (20°C, 101 kPa) 2.33 mg/m3 = 1 ppm

1 Occurrence/use

Acrolein is a colourless liquid with an acrid odour. It has a MPt of -87.7°C , a BPt of 52.7°C and a vapour pressure of 28.7 kPa at 20°C. It has a vapour density of 1.9 times that of air and is explosive in the range 2.8 - 31 % in air. The odour threshold is about 0.2 to 0.4 ppm (0.47 to 0.93 mg/m3).

Acrolein is used in the synthesis of other chemicals. such as acrylic acid derivatives, glycerol, methionine, glutaric aldehyde and a number of chemicals used in the surface treatment of textiles and paper. It occurs after combustion of organic materials such as plastics, glycerol-containing compounds, fats and cooking oils, wood and vegetation, gasoline and diesel. Acrolein is also present in cigarettes smoke. Acrolein is formed by reaction and photodecomposition of airborne pollutants, together with other aldehydes as formaldehyde.
The production rate in the EU is in excess of 20,000 tonnes per annum.

2 Health Significance

2.1 Toxicokinetics

Acrolein is well-absorbed by inhalation (Egle, 1972). Percutaneous absorption and skin irritation was demonstrated in rabbits but has not been investigated in humans. Acrolein reacts quickly at the site of contact with protein and non-protein sulfhydryl groups , especially with glutathione (Cassee et al. 1996). The predominant pathway for the metabolism is conjugation with glutathione and conversion to N-acetylcysteine compounds (IARC 1995). Acrolein is both a product and an initiator of lipid peroxidation (Kehrer .et al. 2000) and a metabolite of the chemotherapy drug cyclophosphamide (Hales, 1982).
There are no specific human data on toxicokinetics available.

2.2 Acute toxicity: Irritation

With continuous acrolein exposure (24 h/day), changes in body weight gain, serum biochemistry and
bronchial histopathology have been reported. Similarly, Cassee et al., (1996) reported higher labelling indices and histopathological changes in the nasal respiratory epithelium in rats exposed to 0.25 or 0.67 ppm (0.58 or 1.56 mg/m3) acrolein, 6 h/d for 3 days (LOAEL 0.57 mg/m^3). The RD_{50} for acrolein, causing a 50 % reduction in respiratory rate in mice amounted to 2.4- 6.6 mg/m^3 (ICPS 1992).
There is no clear indication for a sensitizing effect of acrolein in animals or in humans.

The critical effect of acrolein in humans is irritation of the eye and the respiratory tract. In healthy volunteers, exposure to 0.09 ppm (0.20 mg/m3) for 5 min is reported to cause slight irritation in the eyes, with 0.15 ppm (0.35 mg/m3) irritating the nose (Weber-Tschopp et al. 1977). In volunteers exposed to acrolein during 5 min the eye irritation score amounted to 0.471 (on a 0 to 2 scale) at 0.06 ppm (0.14 mg/m^3), 1.2 at 1.3 – 1.6 ppm and 1.5 at 2.0-2.3 ppm (Darley et al. 1960). The odour threshold was defined (Leonardos, 1969).at 0.21 ppm (0.48 mg/ m^3).

2.3 Repeated dose toxicity

A NOAEL of 0.06 ppm (0.15 mg/m3) was identified for the rat following 61 days continuous inhalation exposure (Gusev et al., 1966). Repeated exposure in rats has shown to result in impaired weight gain from 1.4 ppm (3.3 mg/m3) 6 h/d, 5 d/w for 13 weeks (Feron et al, 1978), with a NOAEL of 0.9 ppm (1.6 mg/m3). In a 6 weeks study in rats, guinea pigs, monkeys and dogs, exposed to 0.7 and 3.7 ppm lung effects were seen and the NOAEL is concluded to be < 1.6 mg/ m^3 (Lyon et al. 1970). Minor histological changes in the bronchial mucosa were seen in Dahl rats exposed to 0.4 ppm (0.9 mg/m3) acrolein for 6 h/d, 5 d/w for 12 to 13 weeks (Kutzman et al. 1984). Roemer et al (1993) examined the proliferative response in nasal, tracheal epithelial and free lung cells of rats exposed to 0, 0.2 or 0.6 ppm (0, 0.47 or 1.40 mg/m3) acrolein for 6 h/d on one or three successive days. After a single exposure, there was an increase in proliferation in all three cell types (visualised by 5-bromodeoxyuridine labelling) following exposure to 0.6 ppm (1.40 mg/m^3) acrolein, and in the trachea and lung at 0.2 ppm (0.47 mg/m3). The response was less marked after three repeated exposures. For long-term oral exposure studies (Parent et al. 1991,1992) found a NOAEL of 0.05 mg/kg bw) for rats and dogs and 2 mg/kg bw in mice.

2.4 Mutagenicity

Acrolein is a highly reactive substance and has been shown to give positive results in a number of *in vitro* genotoxicity assays. *In vivo* tests have given mostly negative results (IARC, 1995).

In the later EU-RAR final report (2001) acrolein is considered as a mutagen for bacteria and can induce gene mutations and sister chromatid exchanges, but no chromosomal aberrations in mammalian cells in vitro. These effect are restricted to a narrow dose range due to the high toxicity of acrolein in this test systems. Most of the in vivo tests are negative.

2.5 Carcinogenicity

Acrolein has been tested for carcinogenicity in rodents by administration in drinking water (Lijinsky and Reuber, 1987), inhalation (Feron and Kruysse, 1977), skin painting (Salaman and Roe, 1956), subcutaneous injection (Steiner *el al.,* 1943) and, most recently, by gavage (Parent el al., 1992a). All studies gave negative results apart from that one using acrolein in drinking water, which gave a marginal increase in the incidence of adrenal cortical tumours in female rats at the highest dose. Shortcomings have been noted (Parent el al., I 992a) on certain experimental aspects of this study which preclude interpretation of the findings.

There are no human data on cancerogenicity.

A recent IARC evaluation (IARC, 1995) concluded that there is inadequate evidence for the carcinogenicity of acrolein in experimental animals or in humans.

2.6 Reproduction toxicity

There was no evidence of teratogenicity in rats exposed to 0.55 ppm (1.3 mg/m3) acrolein (Bouley et al. 1976). A two generation gavage study of acrolein in rats provided no evidence of specific effects on reproduction (Parent et al., 1992b).

2.7 Effects of mixed aldehyde exposure

Exposure to mixtures of aldehydes are frequent both in the occupational situation as in the general environment. Apart from acrolein are involved formaldehyde, acetaldehyde and /or crotonaldehyde.
From studies in vitro and short-term inhalation studies on the irritation and nasal cytotoxicity, there seem not to be a greater hazard from the combined exposure to aldehydes in the same target organ and exerting the same type of effect (nasal irritation) then that associated with exposure to the individual chemicals (Cassee et al. 1996a). A competitive effect between the aldeydes for the same receptor was supposed. Other experiments have shown a competitive agonism between formaldehyde, acetaldehyde and acrolein in the decrease in breathing frequency in male rats (Kane et.al. 1978)

3 Recommendation

The main health effect of exposure to acrolein is irritation of the eyes, the mucosae and the skin, both in animals and in humans. The study of Roemer et al (1993), establishing a LOAEL of 0.2 ppm (0.47 mg/m3) for damage to the bronchial mucosa of rats was considered to be the best available basis for proposing an 8-hour TWA. An uncertainty factor of 10 was considered appropriate to allow for the absence of a NOAEL and of human data on prolonged exposure. The recommended 8-hour TWA is 0.02 ppm (0.05 mg/m3).
A STEL (15 min) of 0.05 ppm (0.12 mg/m3) is proposed to limit peaks of exposure which could result in irritation. This value is in line with the EU RAR conclusion (2001) and is based upon the human volunteer study of Weber-Tschopp et al (1977), indicating a LOAEL of 0.09 ppm (0.20 mg/m3) and the short- time exposure to acrolein vapours in volunteers of over 5 minutes for eye irritation (NOAEL of 0.06 ppm, 0.14 mg/m^3)(Darley et al. 1960) .
No "skin" notation was considered to be necessary.

At the levels recommended measurement difficulties are not foreseen with established methods (e.g. NIOSH 2501, UK MDHS 70) although further validation at lower concentrations may be required.

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