Human Toxicol. (1989), **8**, 229–235

Acute Vapour Inhalation Toxicity of Acrolein and its Influence as a Trace Contaminant in 2-Methoxy-3,4-dihydro-2H-pyran

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1 The LC_{50} values for acrolein (AC) vapour to Sprague-Dawley rats (combined sexes) were determined to be 26 ppm (1 h) and 8.3 ppm (4 h). Signs of severe irritancy were present, and death was due to lung injury.
2 Exposure of rats to a 2-methoxy-3,4-dihydro-2H-pyran (MDP) saturated vapour atmosphere statically generated from liquid MDP containing 0.037% AC, caused severe irritancy and death from accumulation of AC vapour. Sparging the impure material with nitrogen gas before atmosphere generation significantly reduced or abolished lethal toxicity.
3 Dynamically generated MDP vapour atmosphere produced transient respiratory and occular irritancy, but no mortalities. The intrinsic acute vapour inhalation toxicity of MDP is low.
4 The presence of highly volatile toxic impurities in a material may confer a significant acute inhalation toxicity and hazard under conditions of low air movement. Assessment of potential inhalation hazards from liquid mixtures may require investigation by static and dynamic methods for vapour generation.

Introduction

Acrolein (CAS No. 107–02–8; AC) an α, β-unsaturated aliphatic aldehyde, is highly volatile with a vapour pressure of 29.3 kPa (20°C). It is used as an intermediate for the synthesis of various industrial chemicals, as a liquid fuel, slimicide and microbiocide, and is present in cigarette smoke and car exhaust.[1–3] It is highly irritant and injurious to the lung and other mucosae.

In view of its high volatility, AC when present in trace amounts as a contaminant of another chemical, could make a significant contribution to inhalation toxicity. Therefore, the influence of AC contamination on the acute vapour toxicity of a common industrial chemical, 2-methoxy-3,4-dihydro-2H-pyran (CAS No. 4454–05–1; MDP) was investigated. MDP was chosen because AC is a contaminant of its synthesis, and has itself a moderate vapour pressure (1 kPa at 20°C) with a theoretical maximum vapour concentration of 9868 ppm. It is of moderate acute peroral and percutaneous toxicity, and a moderate transient skin and eye irritant.[4] In order to define more precisely the acute lethal inhalation toxicity of AC vapour, a preliminary study was conducted to determine the 1 and 4 h LC_{50} values of the material in rats.

Methods

Materials
AC was obtained from Aldrich Chemical Co. (Milwaukee, WI) and stored in a refrigerated room. The material was inhibited with 3% water and 200 ppm hydroquinone. Commercially available MDP (Union Carbide Corp., Institute, WV) was used and found on analysis to be 99.12% pure and contained 0.64% MDP dimer and 0.037% AC.

Animals
Sprague-Dawley albino male and female rats weighing 200–300 g were used. There were five males and five females for each exposure group.

Exposures
Pure AC vapour exposures were to target concentrations of 15, 20, 25, 30 and 80 ppm for 1 h, and 5, 7, 9 and 12 ppm for 4 h. Chamber atmospheres were dynamically generated.

For the MDP vapour exposure the following conditions were used for 1-h exposure periods.

(i) Statically generated saturated vapour atmosphere from commercially impure AC-containing MDP. In two different studies MDP vapour concentration was measured in both experiments, and AC vapour in the second.

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(ii) Statically generated saturated vapour atmosphere from MDP previously sparged with N_2, at 30 l/min for 2 h before use, in an attempt to remove residual AC. In two separate studies MDP vapour concentration was measured in both, and AC vapour in the second.
(iii) Dynamically generated saturated vapour atmosphere from commercially prepared MDP with measurement of MDP and AC vapour concentration.
(iv) Dynamically generated atmosphere to a target MDP vapour concentration of 1000 ppm, with measurement of MDP and AC vapour concentrations. This concentration was chosen because of a requirement to determine the classification of MDP with respect to its U.S. Department of Transportation Poison Inhalation Hazard status. According to this regulation, a material is classified as a poison inhalation hazard if it has a saturated vapour concentration at 20°C equal to, or greater than, 10 times the vapour 1 h-LC_{50}, providing the LC_{50} is 1000 ppm or less.

Generation and chamber conditions
For the dynamic exposures to AC and one of the 1000 ppm MDP exposures, liquid was metered from a syringe pump into a heated glass evaporator.[5] In some cases, glass beads were added to the evaporator to increase the surface area for vapourization. The resultant vapour was carried to the exposure chamber by a stream of air passing through the evaporator. The differing concentrations of AC were obtained by varying the generation temperature, air flow rate through the generator, or air flow rate through the chamber. For the dynamically generated MDP saturated vapour atmosphere and one of the 1000 ppm exposures, compressed air was passed through liquid MDP in a gas washing bottle. The air emerging from the MDP was either taken directly to the exposure chamber (for saturated vapour study) or diluted with filtered air to produce the desired 1000 ppm before entering the chamber.

For static exposures to MDP vapour a sample of the test material (130–210 g) as received, or sparged with N_2 to remove AC, was placed in a sealed 120 l chamber and the atmosphere allowed to equilibriate overnight (16–19 h). Animals were then introduced into the chamber through a gasketed drawer designed to minimize vapour leak from the chamber. Temperature and relative humidity were monitored during each exposure.

Vapour concentration measurements
Chamber atmospheres were periodically sampled using 1 ml gas-tight Hamilton syringes for the measurement of vapour concentrations. For the 1-h exposures between 4 and 6 samples were taken; for the 4-h exposures 10 samples were taken. AC and MDP were analysed using a Perkin-Elmer Model 3920B gas chromatograph equipment with a flame ionization detector; the column was packed with Porapak Q (50/80 mesh) for AC measurements, and with 20% SP2100 (on 80/100 mesh Supelcoport) for MDP. The approximate minimum detection limit for AC was 1 ppm.

Monitors for toxicity
All animals were observed for signs of toxic and pharmacological effects on the day of exposure and daily for 14 days following exposure. Animals were weighed before exposure and on post-exposure days 7 and 14. Necropsy was carried out on animals that died and survivors sacrificed at the end of the observation period by exsanguination following Methoxyflurane[R] anesthesia. Lungs from AC-exposed animals were fixed in formalin, paraffin embedded, and sections stained with hematoxylin and eosin.

Statistics
LC_{50} values, with 95% confidence limits, were calculated by either the moving average method[6] or probit analysis.[7]

Results

Acrolein studies
Concentrations of AC vapour and mortalities for 1-h and 4-h exposures are given in Table 1. From these data it was possible to calculate the LC_{50} values for AC vapour to rats shown in Table 2.

Signs of peripheral sensory irritancy and toxicity were seen at all AC vapour concentrations. During exposure there was lachrymation, perinasal and periocular wetness, and mouth breathing. After exposure there was perinasal and perioral wetness and encrustation, mouth and audible breathing, decreased breathing rate, and hypoactivity. In general, signs of respiratory distress and hypoactivity were seen for post-exposure days 1 to 6, and their duration was related to the exposure concentrations. Body weights of survivors decreased during the first week and regained during the second, particularly for the lower exposure concentrations (Table 3).

Necropsy of animals that died revealed perinasal and perioral encrustation, mottled discoloration of the lungs and liver, clear fluid in the trachea and thoracic cavity, gas-filled stomach and intestine, and opaque or cloudy corneas. Histological features seen in the lungs of animals that died included congestion and intra-alveolor haemorrhage

Table 1 Acrolein vapour concentrations and corresponding mortalities for rats exposed to pure acrolein vapour

Exposure time (h)	*Acrolein (ppm) Mean ± s.d.*	*Mortality (no. dead/no. exposed) Males*	*Females*	*Time to death (Post-exposure)*
1	81 ± 1	5/5	5/5	3 hr – 3 days
	31 ± 2	5/5	5/5	3 hr – 6 days
	24 ± 1	2/5	1/5	1 – 3 days
	22 ± 5	0/5	1/5	2 days
	14 ± 7	0/5	0/5	—
4	12.1 ± 0.4	5/5	3/5	1–3 days
	9.1 ± 1.4	3/5	4/5	1 – 13 days
	7.0 ± 0.2	3/5	0/5	1 – 5 days
	4.8 ± 0.2	0/5	0/5	—

Table 2 LC_{50} values for pure acrolein vapour to rats calculated from the dose mortality data shown in Table 1

Exposure time (h)	*Sex*	*LC_{50} (With 95% confidence limits) as ppm*	*as mg/m^3*
1	Male	26 (23–28)	65 (58–70)
	Female	24 (20–30)	60 (50–75)
	Combined	26 (24–27)	65 (60–68)
4	Male	7.4 (5.9–9.3)	18.5 (14.8–23.2)
	Female	8.8 (7.0–11.0)	22.0 (17.5–27.5)
	Combined	8.3 (7.0–9.9)	20.8 (17.5–24.8)

Table 3 Body weight of rats exposed to acrolein vapour

Exposure time	*Acrolein[a]*		*Males*	*Body weight (g) as mean ± s.d.*	*Females*		
(h)	*(ppm)*	*0[b]*	*7 days*	*14 days*	*0[b]*	*7 days*	*14 days*
1	24	308 ± 10	295 ± 14	298 ± 28	216 ± 7	211± 8	220 ± 8
	22	300 ± 16	274 ± 23	309 ± 16	240 ± 4	230 ± 12	242 ± 7
	14	281 ± 8	271 ± 12	302 ± 12	236 ± 4	238 ± 7	245 ± 13
4	12.1	280 ± 9	—	—	179 ± 6	136 ± 16	154 ± 16
	9.1	256 ± 12	229 ± 18	170 ± 5	174 ± 4	156 ± 29	—
	7.0	239 ± 5	241 ± 1	284 ± 1	236 ± 8	233 ± 8	236 ± 12
	4.8	275 ± 11	264 ± 10	302 ± 14	191 ± 7	197 ± 8	218 ± 10

[a] All males and females died for the 1-h 81 and 31 ppm exposures.
[b] 0 = pre-exposure weight.

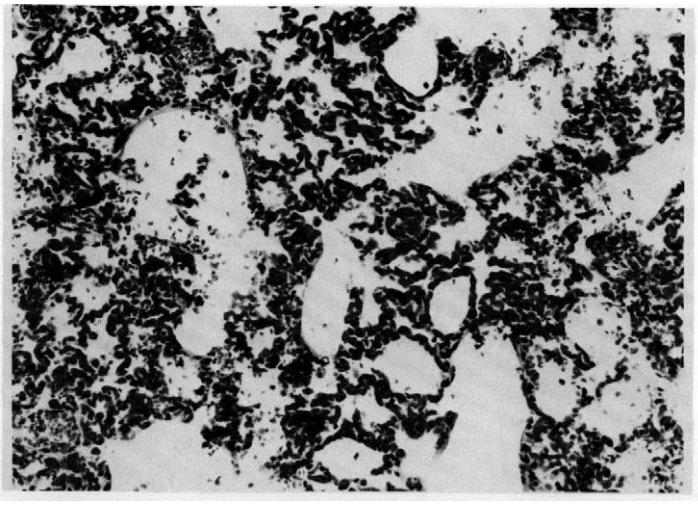

Figure 1 Pulmonary congestion and haemorrhage in a female rat dying 2 days after a 1-h exposure to 31 ppm acrolein vapour. Haematoxylin and eosin. X137

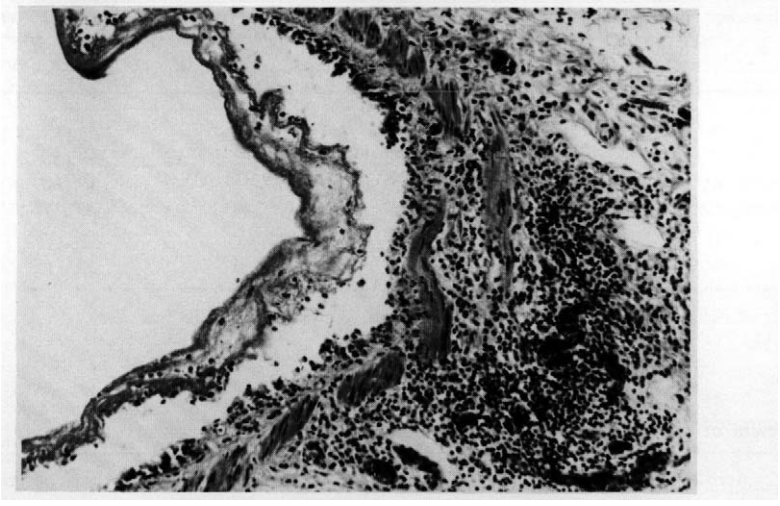

Figure 2 Bronchiolar epithelial cell necrosis and fibrin deposition within an airway of a male rat dying 2 days after a 1 hr exposure to 31 ppm acrolein vapour. Haematoxylin and eosin. X137

(Figure 1), fibrin deposition in the smaller airways, and necrosis and exfoliation of bronchiolar epithelium (Figures 2 and 3).

Methoxydihydropyran studies

All animals exposed to saturated vapour atmospheres statically generated from impure MDP died during exposure or within 24 h of exposure. Signs of irritation and toxicity seen before death included lachrymation, perioral and perinasal wetness, mouth and audible breathing, hypoactivity, prostration, absence of toe and tail pinch reflexes, and absence of surface righting reflex. Necropsy revealed red mottled discoloration of the lungs and clear fluid in the trachea and thoracic cavity. MDP vapour concentrations for the two separate exposures were 8098 ± 269 ppm and 8044 ± 878 ppm (mean ± s.d.); the AC vapour concentration measured in the second exposure was 240 ± 41 ppm.

For the first exposure to statically generated saturated vapour atmosphere from the N_2-sparged MDP, three males and two females died within 1 to 3 days of exposure. Signs of toxicity and irritancy were lachrymation, perioral and perinasal wetness, mouth and audible breathing, hypoactivity, absence of toe and tail pinch reflexes, and absence of

Figure 3 Pulmonary congestion and exfoliated bronchiolar epithelium in a male rat dying 2 days after a 1-h exposure to 31 ppm acrolein vapour. Haemotoxylin and eosin. X54

surface righting reflex. In survivors, weight losses occurred during the first post-exposure week with recovery of weight during the second week (Table 4). Necropsy of animals that died revealed mottled and dark red discolored lungs. No gross lesions were seen in survivors. The MDP vapour concentration was 9076 ± 560 ppm; AC was not measured during this exposure.

For the second statically generated saturated vapour exposure employing N_2-sparged MDP there were no deaths during or following exposure. Signs on the day of exposure were lachrymation, perioral and perinasal wetness, mouth and audible breathing, hyperactivity, and absence of toe and tail pinch reflexes; no signs were seen during the remainder of the observation period. There were slight gains in body weight during the first post-exposure week and more marked weight gains during the second week (Table 4). No gross lesions were seen at the end of the observation period. The MDP vapour concentration was 8613 ± 422 ppm and the AC concentration was below the limit of detection.

No deaths occurred when a dynamic method was used to generate a saturated vapour atmosphere from impure MDP. During exposure there was lachrymation and hyperactivity followed by hypoactivity. Periocular wetness and hypoactivity were seen immediately after exposure, but no other signs developed. Weight gains occurred post-exposure (Table 4). No gross lesions were seen at necropsy. The MDP vapour concentration was 7748 ± 524 ppm and AC could not be detected in the atmosphere.

For the two exposures to a target MDP vapour

Table 4 Body weights of rats surviving exposures to differing modes of vapour generation from methoxydihydropyran (MDP)

Generation method	*Sample*[a]	*MDP concentration (ppm)*	*Sex*	*Weight (g) as mean ± s.d.* *0*[b]	*7 days*	*14 days*
Static	Sparged	9076	M	259 ± 11	245 ± 1	282 ± 6
Static	Sparged	9076	F	235 ± 6	228 ± 14	247 ± 9
Static	Sparged	8613	M	262 ± 10	264 ± 14	286 ± 19
Static	Sparged	8613	F	234 ± 7	238 ± 11	247 ± 5
Dynamic (Bubbler)	Untreated	7748	M	272 ± 7	286 ± 12	305 ± 14
Dynamic (Bubbler)	Untreated	7748	F	233 ± 6	243 ± 3	248 ± 8
Dynamic (Bubbler)	Untreated	1064	M	247 ± 12	262 ± 14	280 ± 15
Dynamic (Bubbler)	Untreated	1064	F	242 ± 8	248 ± 15	246 ± 9
Dynamic (Evaporator)	Untreated	1095	M	277 ± 15	295 ± 16	304 ± 20
Dynamic (Evaporator)	Untreated	1095	F	241 ± 4	242 ± 6	245 ± 5

[a] Sparged = attempted removal of acrolein with N_2 stream; untreated = as received.
[b] 0 = pre-exposure weight.

concentration of 1000 ppm, the respective analytically measured vapour concentrations were 1064 ± 19 ppm (bubbler method) and 1095 ± 15 ppm (evaporator method); AC could not be detected in either exposure. Signs observed during exposure were hypoactivity and lachrymation, but no signs were seen subsequently. Weight gains occurred over the observation period (Table 4). No gross lesions were seen at necropsy.

Discussion

There is limited published information on the quantitative acute vapour inhalation toxicity of AC to rats. The 10-min LC_{50} is 355 ppm,[8] 30-min LC_{50} 131 ppm;[9] and mortalities (as no. dying/no. exposed) of 2/6, 3/6 and 4/6 in differing groups of rats have been recorded for 4-h exposures to 8 ppm.[10] The studies reported here have confirmed AC to have a high acute vapour inhalation toxicity with (combined sexes) LC_{50} values of 26 and 8.3 ppm for 1-h and 4-h exposures, respectively. Whilst the inhalation exposure doses ($LC_{50} \times$ time) are not too different for 1 h (1560 ppm min) and 4 h (1992 ppm min), those for a 30-min exposure (3930 ppm min) and 10- min exposure (3550 ppm min) suggest that Haber's law may apply only over narrow ranges for AC vapour. The significant hazard from AC vapour is emphasized by the high vapour pressure of the material and a theoretical possible saturated vapour concentration of 290 000 ppm at 20°C; i.e., 11 154 times the 1-h LC_{50}. Therefore, toxic concentrations of vapour can be readily attained with this extremely volatile material. Signs in exposed animals were typical of peripheral sensory irritancy to the eye and respiratory tract, and morbidity and mortality due to injury to the respiratory tract. The signs, gross pathology and histopathology are all compatible with death due to lung injury.

Exposure to saturated vapour atmospheres of MDP showed differing effects which clearly depended on the mode of generation. Thus, 1-h exposure to a statically generated saturated vapour atmosphere using impure material resulted in the development of signs of severe irritancy and toxicity with mortalities. However, using the same material, a dynamically generated saturated vapour atmosphere caused markedly less severe signs of irritancy and toxicity, and deaths did not occur. The effect of generation conditions suggests that the differences are due to the presence of a highly volatile and toxic trace impurity whose vapour would accumulate under static generation conditions and cause toxicity, whereas under dynamic conditions there will be no accumulation of toxic vapour. A clear candidate is acrolein, present at 0.037% in the liquid sample. This was confirmed by the 1-h static saturated vapour study with an MDP vapour concentration of 8044 ppm where all animals died and the AC vapour concentration was 240 ppm; i.e., 9.2 times the 1-h LC_{50} for AC. Further evidence of a major role for AC vapour was the reduction or abolition of mortality by prior sparging of the impure MDP with N_2 in order to remove AC. In the first study with a sparged preparation (MDP 9067 ppm), clearly some residual AC was still present since severe signs with mortality, although reduced 50%, were still present. However, in the second study with sparged material, the signs of toxicity were reduced, no deaths or gross lesions occurred and, whilst the MDP concentration was 8613 ppm, AC vapour could not be detected. Also, in the dynamically generated saturated vapour study, MDP concentration 7748 ppm, there were only short-lived signs of irritancy, no mortalities and no gross lesions. Under these conditions, the air flow prevented the accumulation of AC vapour, which could not be detected analytically in the atmosphere. Also, sparging occurs rapidly. These experimental findings have several differing implications, as follows:

(i) Exposure to MDP vapour concentrations of 1064, 1095 and 7748 ppm under dynamic conditions where AC could not be detected, caused only transient signs of ocular and respiratory irritancy and no mortality. Thus, the intrinsic acute vapour inhalation toxicity of MDP is low.
(ii) The acute inhalation toxicity, and hazard, from impure (AC-containing) MDP depends on immediate environmental conditions, particularly air movements. When there is free air movement AC vapour cannot accumulate, but in enclosed spaces or with near still air conditions AC may accumulate in the atmosphere and reach potentially hazardous concentrations. Actual vapour concentrations attained will depend on the amount of test material in the confined areas and the percentage of contaminant in the material. These considerations, of course, apply to any relatively innocuous material containing traces of volatile highly toxic impurities.
(iii) The effects of air flow on vapour MDP inhalation toxicity indicate that for mixtures or impure samples, there may be a need to investigate acute inhalation toxicity by both static and dynamic methods of generation.

In summary, these studies have confirmed the high acute vapour inhalation toxicity of AC and have demonstrated that when it is present even as a minor contaminant of other chemicals it may present a handling hazard, particularly under conditions of restricted air flow. It is, therefore, important that AC-containing chemicals should be

stored under conditions of continuous air movement in order to prevent the accumulation of potentially harmful concentrations of AC vapour. Workplaces where AC-containing chemicals are stored or handled should have the atmosphere quantitatively monitored for AC, which should not exceed the exposure guideline of an 8-h time weighted average of 0.1 ppm and a short-term exposure limit of 0.3 ppm recommended by the American Conference of Governmental Industrial Hygienists.[11]

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(Received 12 August 1988; accepted 21 November 1988)