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CASE REPORT

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Postmortem absorption of dichloromethane: a case study and animal experiments

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Abstract A case of accidental death after occupational exposure to an atmosphere containing dichloromethane (DCM) is reported. The concentrations of DCM in the blood and tissues of a 40-year-old man who died while observing an industrial washing machine filled with DCM vapour were blood 1660 mg/l, urine 247 mg/l, brain 87 mg/kg, heart muscle 199 mg/kg and lungs 103 mg/kg which are 3–7 times higher than previously reported fatal levels. The body was left undiscovered in the machine filled with DCM vapour for about 20 h. The present study was designed to determine whether all the DCM detected in the tissues and body fluids had been inhaled while alive using rats as the experimental model. The concentrations of DCM in the tissues and body fluids of a rat that died from DCM poisoning and was left for 20 h in a box containing DCM vapour were the same as those in the tissues and body fluids of a rat that had died from an injected overdose of barbiturates and had then been placed in the DCM box in a similar manner. Moreover, the concentrations of DCM in the tissues and body fluids of the carcasses that were exposed to the DCM vapour increased gradually throughout the period of exposure. These findings imply that DCM is able to penetrate the tissues and body fluids of rat carcasses through a route other than inhalation such as through the skin.

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Introduction

The widespread use of halogenated hydrocarbons in household products has resulted in an increase in both the frequency of intoxication as a result of accidental ingestion by children and in the degree of improper use or abuse of propellants or halogenated solvents for their narcotic effects (Oliver and Watson 1977). Dichloromethane (methylene chloride, methylene dichloride etc.) (DCM) is commonly employed commercially and industrially as an aerosol propellant, paint stripper, degreaser, fat extractant and solvent. Toxic reactions to DCM have been limited for the most part to acute exposures resulting from the direct depressant effect on the central nervous system. About 40% of an absorbed dose of DCM is not eliminated in the exhaled air and the possibility of DCM poisoning as an occupational disease has been suggested (Collier 1936; Miller et al. 1985; Trueman and Ashby 1987; Green 1997).

In the present study, a quantitative analysis of DCM concentrations in the body of a man who died from exposure to DCM is presented. In addition, the results of animal experiments to determine whether postmortem absorption of DCM is possible will be presented.

Case history and autopsy findings

The deceased, a 40-year-old man, was working a night shift in a factory where DCM was used to remove rust from aluminium sheets. The deceased was monitoring the an industrial washing machine (3.4 × 1.7 × 1.5 m (Fig. 1) containing liquid DCM. The deceased was last seen alive at 10.00 p.m. At 8.00 a.m. the following morning, the body of the deceased was found face-up between the rollers on the inner wall of the washing machine, 30–50 cm above the surface of the liquid DCM. When the body was found, the man was not wearing a mask or gloves and the top-lid of the machine was not fastened tightly. The body of the deceased was removed from the machine by emergency services at 6.00 p.m. at which time the liquid DCM was about 20 cm deep and the con-

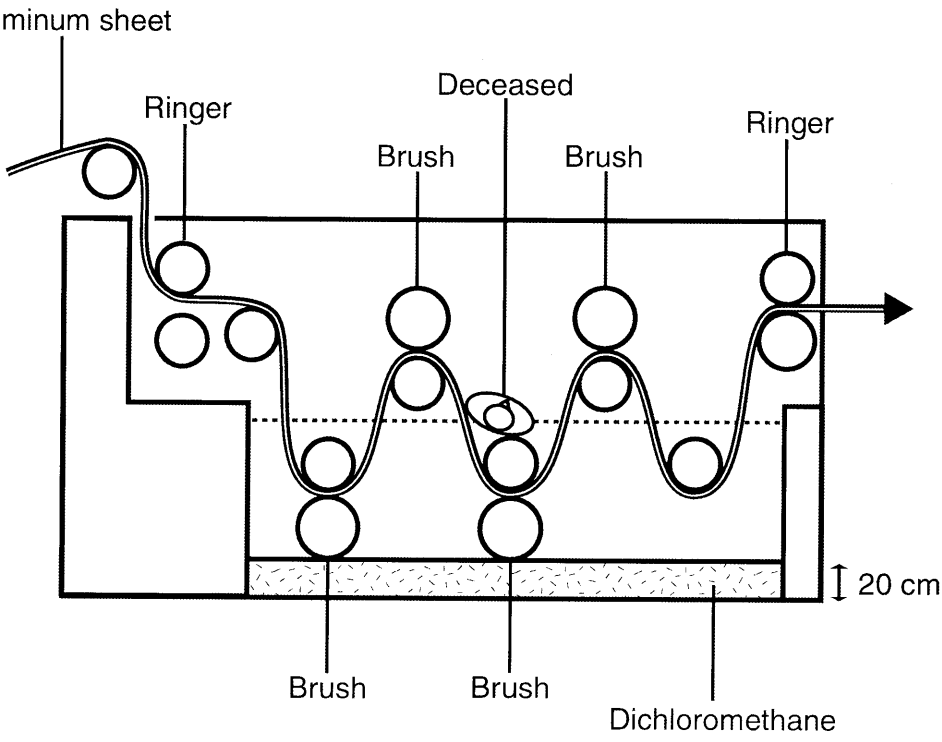

Fig. 1 Diagram of the industrial washer containing dichloromethane showing the location of the deceased. The dotted line indicates the surface level of the dichloromethane while the machine is in operation

centration of DCM vapour in the tank was 2% at a point 1 m above the surface and 20% at a point 0.5 m above the surface.

The deceased was 173 cm in height, weighed 73 kg and was of average build. The conjunctivae were congested and contained multiple petecchiae. Intense dark-red coloured parchment-like areas were observed on the face, neck, back, chest, upper limbs and lower limbs. No other signs of external trauma were present. The brain showed signs of cyanosis. The epicardial surface showed multiple petecchiae, and the heart was dilated. The lungs were congested and edematous and other viscera showed prominent signs of congestion.

Materials and methods

Analytical method

DCM was identified using gas chromatography/mass spectrometry (GC/MS) using an Automass II 120 instrument (JEOL, Tokyo, Japan) equipped with a capillary column (DB-5 ms: 30 m × 0.25 mm id × 0.5 μm df) in the electron impact ionization mode. The temperatures of the transfer line, injector and column were 280 °C, 150 °C and 35 °C, respectively. The voltage and current of ionization were 70 eV and 270 μA, respectively. The concentration of the DCM in the biological samples was determined by gas chromatography using a Shimadzu GC-18 A gas chromatograph (Kyoto, Japan) with a flame ionization detector. Samples of human and rat tissues and fluids were collected and stored at –80 °C until analysis. The samples were prepared as follows: tissue samples were weighed and homogenized in an ice bath. Mixtures of either 0.1 ml of biological fluids or 0.1 g of homogenized tissues combined with 1 ml of 0.1 M NaCl and 2 μl of internal standard (100% sec-buthanol) were then prepared. The sample vials were placed in a forced convection oven and maintained at 55 °C for 20 min. Each 100 μl of the vapour within the vial was then withdrawn using a gas-tight syringe and injected into the gas chromatograph. A wide-bore capillary column packed with DB-Wax (30 m × 1.0 mm id × 0.5 μm df) was used. The injector and detector temperatures were 220 °C and 240 °C, respectively. The column temperature was set at 40 °C for 1 min and then increased by 1°C/min until a final temperature of 60 °C was reached. The values were corrected by reference to the recovery of DCM from each tissue and body fluid. The within-run and between-run precision studies were performed at two selected concentrations of DCM in blood (i.e. at the low end and high end of the assay). The within-run precision at DCM concentrations of 10 and 4000 mg/l were 9.5% (n = 6) and 7.7% (n = 6), respectively. The between-run precision at the same two concentrations was 11.5% (n = 5) and 8.9% (n = 5), respectively. The carboxyhaemoglobin (COHb) levels in blood were determined using a modification of the method of Rodkey et al. (1979).

Animal experiment

Adult male Wistar rats weighing 300–600 g were placed in an atmosphere containing DCM and subjected to a whole body exposure. A single rat was used for each experiment. A loosely sealed glass box (25 cm long, 10 cm wide and 20 cm deep) at room temperature (17–25 °C) was used as a model of the washing machine and contained a small vessel with about 20 ml of DCM inside. The interior of the box was saturated with DCM vapour prior to the start of the experiments. All procedures involving the rats were reviewed and approved by the Institutional Animal Care and Use Committee on the base of the "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985). The following experiments were carried out:

1. Experiment 1: A single rat was placed in the box where it died from exposure to DCM 15–25 min later. The carcass was then left in the box at room temperature (17–25 °C). Blood and tissue samples were collected after 0, 5, 10 and 20 h and stored at –80 °C until analysis.
2. Experiment 2: The carcass of a rat that was terminated by an injection of barbiturates was placed in the same DCM box. Blood and tissue samples were then collected after 0.5, 1, 2, 5, 10 and 20 h and stored at –80 °C until analysis.
3. Experiment 3: The carcass of a rat that died from DCM exposure was removed from the box and placed on a laboratory benchtop under gentle ventilation. Blood and tissue samples were collected after 10 and 20 h and stored at –80 °C until analysis.

Table 1 Dichloromethane concentrations in the tissues and body fluids of the deceased (*blank spaces* represent unavailable data)

Specimen	Dichloromethane (mg/l or mg/kg)						
	Present study	Bonventre et al. 1977	Winek et al. 1981	Leikin et al. 1990	Manno et al. 1992	Tay et al. 1995	Kim et al. 1996
Blood	1660	510	298	155	507	281	252
Cerebrum	87	248[a]		109[a]			75[a]
Cerebellum	289						
Heart	199						30
Lungs	103			40			26
Stomach	95						
Greater omentum	296						
Liver	130	144		35			56
Pancreas	221						
Kidneys	71			58			59
Adrenal glands	449						
Small intestine	46						
Urine	247			22			

[a] Described as "brain" in the references

Results and discussion

The presence of DCM was confirmed and the concentration determined in the tissue and blood samples derived from the deceased human victims and from the experimental rats using GC/MS. The gas chromatogram had a typical retention time, and the mass spectrum had an atypical fragmentation pattern with a peak at m/z 49 for [CH_2Cl^+] and m/z 84 for the parent ion. These values corresponded to those of a known sample of DCM.

Case study

The concentrations of DCM in the tissues and body fluids of the victims are shown in Table 1. The concentration of DCM in blood was 3–7 times higher than previously reported values. Kim et al. (1996) and Bonventre et al. (1977) suggested that a DCM concentration in the blood of equal to or higher than 252–510 mg/l is lethal and that this value is adequate to confirm DCM poisoning as the cause of death. In our case, the body of the deceased was continuously exposed to an atmosphere containing about 10–20% DCM vapour for 20 h. We therefore strongly suspected that the DCM had penetrated the body surface and accumulated in the tissues and body fluids after death. To confirm this suspicion animal experiments were performed.

Experiment 1

The concentrations of DCM in the blood and tissues of a rat that died from DCM poisoning and had been left in an atmosphere containing DCM for 0–20 h were examined. As shown in Table 2, the concentration of DCM in the blood and tissues gradually increased with time and reached a maximum value at 20 h. Immediately after death (0 h) the concentrations of DCM were highest in the blood, lungs and greater omentum. The DCM concentrations in the blood and tissues increased by about 1.0–3.6 times (mean 1.9) after 5 h, 0.8–10 times (mean 3.5) after 10 h, and 1.5–15 times (mean 6.6) after 20 h (all values are in comparison to the initial concentrations at the time of death).

Experiment 2

The concentrations of DCM in the blood and tissues of a rat that died from a barbiturate overdose and was then placed in an atmosphere containing DCM for 0.5–20 h were examined (Table 3). Surprisingly, the presence of DCM was identified in the blood and all of the tissues tested after only 0.5 h and the concentrations increased gradually with time in a manner similar to that seen in experiment 1. The results of experiments 1 and 2 suggest that DCM vapour is able to easily penetrate the body surface and accumulate in the blood and tissues regardless of the cause of death. In the presence of an atmosphere containing DCM (Experiment 2), the concentration of DCM in the rat carcass was about 90% of the value in experiment 1after 5 h and about 97–100% of the value in experiment 1 after 10–20 h.

Experiment 3

When placed in a naturally ventilated atmosphere that did not contain DCM, the concentrations of DCM in the blood and most tissues of a rat that died of DCM poisoning decreased slowly to means of 82% and 56% of the initial concentrations after 10 and 20 h, respectively. The largest reductions in concentration were observed in the blood, liver and adrenal gland, whereas no reduction was observed in the greater omentum or small intestine after 20 h. The mean DCM concentrations in blood and tissues

Table 2 Distribution of dichloromethane in tissues and body fluids of the rats which died of dichloromethane (DCM) poisoning and were then left in the DCM atmosphere or under natural ventilation without DCM

Specimen	Atmosphere containing DCM Postmortem interval (h)				Normal atmosphere Postmortem interval (h)	
	0	5	10	20	10	20
Blood	1465 mg/l	1892	2099	2891	930	298
Cerebrum	182 mg/kg	197	138	917	138	115
Cerebellum	44 mg/kg	54	117	144	39	23
Heart	214 mg/kg	282	732	633	145	62
Lungs	288 mg/kg	1127	898	1375	388	310
Stomach	50 mg/kg	51	229	113	49	23
Greater omentum	395 mg/kg	980	771	1625	375	453
Liver	34 mg/kg	51	129	548	48	11
Spleen	25 mg/kg	57	68	278	25	16
Pancreas	119 mg/kg	225	1217	1803	111	64
Kidneys	255 mg/kg	875	1353	1017	162	104
Adrenal glands	144 mg/kg	229	278	211	32	46
Small intestine	84 mg/kg	85	111	155	97	97
Submaxillary glands	87 mg/kg	63	194	238	36	24
Testis	74 mg/kg	245	270	1421	63	29
Skeletal muscle	24 mg/kg	87	142	333	17	16

Table 3 Distribution of dichloromethane in tissues and body fluids of the rats which died of barbiturate overdosing and were then left in the dichloromethane atmosphere

Specimen	Postmortem interval (h)					
	0.5	1	2	5	10	20
Blood	58 mg/l	239	399	484	1473	2640
Cerebrum	28 mg/kg	38	38	323	282	955
Cerebellum	12 mg/kg	10	16	67	100	179
Heart	35 mg/kg	20	44	56	79	653
Lungs	59 mg/kg	78	66	368	385	1229
Stomach	16 mg/kg	20	14	68	86	119
Greater omentum	115 mg/kg	332	433	925	1148	1425
Liver	23 mg/kg	28	39	28	160	559
Spleen	54 mg/kg	36	39	79	141	167
Pancreas	19 mg/kg	20	17	384	660	1144
Kidneys	93 mg/kg	79	308	699	1335	1209
Adrenal glands	34 mg/kg	39	64	66	206	267
Small intestine	45 mg/kg	26	31	166	109	219
Submaxillary glands	11 mg/kg	13	74	239	347	217
Testis	56 mg/kg	119	247	511	1061	1375
Skeletal muscle	14 mg/kg	35	70	311	331	359

after 10 and 20 h were about 80% and 50% of the values at 0 h, respectively.

Levels of COHb in the blood in a human case study and animal experiments

The blood in the human case showed a COHb level of about 13%. In experiment 1, the COHb level was determined to be about 3–4% immediately after death (0 h) and remained unchanged thereafter. On the other hand, for all postmortem intervals in experiment 2, COHb was not detected or was below the minimum detection limit.

These results showed that DCM was detectable in the blood and tissues of a rat that had died from a barbiturate overdose and then been placed in an atmosphere containing DCM. Furthermore, DCM was detected in the blood and tissues only 0.5 h after death. After 5 h of continuous exposure, the DCM concentrations in the carcass had increased to about 90% of the values for the rat that died from DCM poisoning and was left in an atmosphere containing DCM. After 10–20 h, this had increased to about 97–100% (Tables 2 and 3). In view of these findings, we believe that the high concentration of DCM detected in the blood of the deceased (1660 mg/l) in this case was derived from two routes, inhalation through the respiratory tract during life and penetration through the body surface of the corpse from the atmosphere. Nevertheless, differences between humans and rats, such as body weight and body hair which may influence the results cannot be dismissed. Our experiments did not provide any evidence of a means to discriminate between DCM that has entered

the body through inhalation and DCM that has been absorbed into the blood. A proportion of absorbed DCM is metabolized to carbon monoxide (CO) both in vivo and in vitro (Ahmed et al. 1980). In the animal experiment, the COHb level after antemortem inhalation was about 3–4%, whereas COHb was not detected after postmortem absorption. However, although the COHb level in the human victim we examined was about 13%, a 3-h exposure to DCM vapour in a well-ventilated room was reported to result in a COHb saturation level of 8–16% (Stewart 1976). Therefore, the level of COHb in blood does not provide any useful information for discriminating postmortem absorption from inhalation in humans. This discrimination is very important for forensic investigations, since a body that has been left in an atmosphere containing DCM for several hours could have died from other causes. Further studies will be necessary to differentiate between DCM that has been inhaled and DCM that has been absorbed.

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