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Acute inhalation toxicity of smoke of fentanyl and its 1-substituted analogs in Swiss albino mice

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Abstract

Fentanyl (*N*-(1-phenethyl-4-piperidinyl)propionanilide) is a synthetic, potent narcotic analgesic agent. However, it is known to have several side effects, which led to synthesis and evaluation of its new analogs for the management of pain. We have earlier reported the comparative bioassay of fentanyl and its eight 1-substituted analogs (**1-8**) in mice. Three compounds, viz., *N*-(1-(2-phenoxyethyl)-4-piperidinyl)propionanilide (**2**), *N*-isopropyl-3-(4-(N-phenylpropionamido)piperidin-1-yl) propanamide (**5**), and *N*-t-butyl-3-(4-(N-phenylpropionamido)piperidin-1-yl)propanamide (**6**) were found to be more effective and less toxic compared to fentanyl. The present study reports the comparative acute inhalation toxicity of smoke of fentanyl and its three analogs, viz., **2**, **5**, and **6** in mice. Animals were exposed to different concentrations of smoke generated by heating the compounds. Exposure was performed in a head only all glass static exposure assembly for 15 min to determine the median lethal concentration (LC_{50}). The breathing pattern and various respiratory parameters of the animals were also monitored online using a polygraph. Out of three compounds tested, analog **5** was found to be most toxic (LC_{50} = 2820 mg/m^3) while **2** was least toxic (LC_{50} = >8000 mg/m^3). All the compounds caused long lasting respiratory depression in a dose- dependent manner, which did not completely resolve even after discontinuation of exposure. Aerodynamic median diameter and geometric standard deviation of smoke particles was determined employing eight-stage Andersen sampler. The particles were found to be within the respirable range. The study, however, concludes that due to possible decomposition of the compounds by heating or its poor absorption by the alveolar surface, the present inhalation technique cannot be employed to generate smoke of fentanyl and its analogs for any medical or surreptitious use.

Key words: Fentanyl, Opioids, Analogs, Inhalation Toxicity.

Introduction

Fentanyl (*N*-(1-phenethyl-4-piperidinyl)propionanilide) is a synthetic, potent narcotic analgesic agent that is clinically used for the management of severe and chronic pain (1, 2). It produces its effect by binding to mu opioid receptor (MOR). It has rapid onset and short duration of action, and is several times more potent than morphine (3, 4). Compared to morphine, the strong potency of fentanyl is largely due to its high lipophilicity, facilitating its entry into the central nervous system (CNS) (5). Due to high potency of fentanyl, its different analogs viz., sufentanil, alfentanil, remifentanil and lofentanil have also been synthesized with similar pharmacological characteristics, and introduced in medical practice (6, 7). Several formulations of fentanyl for intravenous (i.v.), oral (p.o.), pulmonary, intranasal, transdermal, transmucosal, and inhalation routes have been developed for clinical use in humans (8, 9).

Fentanyl has a low therapeutic index varying between 270 and 625 (10, 11), and an overdose may lead to severe breathing problems, unconsciousness, and death (12). In the recent past, there has been significant development in the synthesis and characterization of several new highly potent opioids that could possibly be used for alleviating acute pain, particularly experienced during many military operations (13). In view of this, comparative bioassay of fentanyl and its eight newly synthesized 1-substituted analogs (**1-8**) have recently been reported by us in mice (10, 14). Out of eight compounds tested, *N*-(1-(2-phenoxyethyl)-4-piperidinyl) propionanilide (**2**), *N*-isopropyl-3-(4-(N-phenylpropionamido)piperidin-1-yl)propanamide (**5**), and *N*-t-butyl-3-(4-(N-phenylpropionamido)piperidin-1-yl)propanamide (**6**) were found to be more effective and less toxic compared to fentanyl. In our both the studies, the agents were administered through oral and parenteral routes. Production of smoke by heating is a low cost and convenient method that could generate respirable particles of compounds having significant military applications and other subversive use in civil population. Recently, a non invasive method of mouse bioassay was reported by our laboratory, where thermally generated smoke of active ingredients of tear gas munitions was used (15). The present study was undertaken to evaluate the comparative acute inhalation toxicity of smoke of fentanyl and its three analogs, viz., **2**, **5**, and **6** in mice, so that we could predict if in addition to the beneficial effects, thermally generated smoke of these compounds could be put to any misuse. The study involved: (i) generation of smoke by heating the compounds, (ii) measurement of aerodynamic median diameter (AMD) of the smoke particles to predict their distribution in the respiratory tract, (iii) online monitoring of breathing pattern and va-

rious respiratory variables in mice exposed to generated smoke, and (iv) determination of median lethal concentration (LC_{50}) of the compounds following inhalation of the smoke.

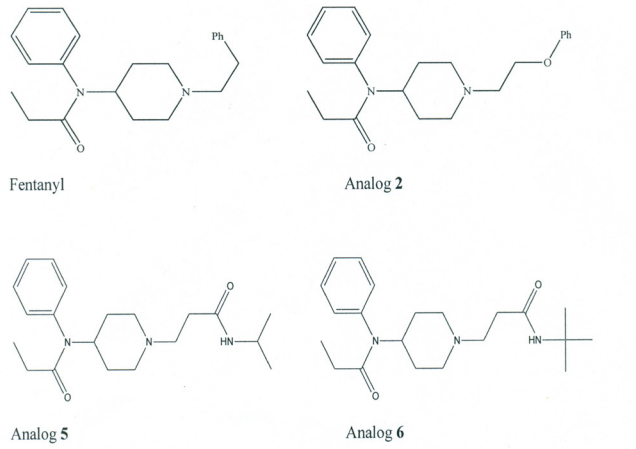
Materials and methods

Animals

Male Swiss albino mice (25-30 g) were procured from the animal facility of Defence Research and Development Establishment (DRDE), Gwalior. The animals were housed in polypropylene cages on dust free steam autoclaved rice husk as the bedding materials, with free access to food (Ashirwad Brand, Chandigarh, India*)* and water ad libitum. Prior to experiment, animals were randomized and acclimatized for seven days in controlled environmental conditions (22±2°C; relative humidity 40-60%) at a 12 h light/12 h dark cycle. The care and maintenance of the animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India, New Delhi, India. The experimental protocol was approved by the Institutional Animal Ethics Committee constituted by CPCSEA.

Chemicals

Fentanyl and its 1-substituted analogs (**2**, **5**, and **6**) were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR, GC-MS and elemental analysis in the Synthetic Chemistry Division of our establishment. The spectral data of fentanyl, **2, 5,** and **6** have been reported elsewhere (10, 14). The structures of the compounds are as follows:



Generation of test atmosphere and determination of LC_{50}

The schematic diagram of inhalation exposure assembly is given in Figure 1. Before start of the experiment, each mouse was restrained in a specially designed whole body plethysmograph (glass mouse holder), with mouse head protruding out-side the plethysmograph (28 mm ID, 92 mm length), through an air-tight diaphragm (on mouse neck; latex rubber dam, Hygienic Corp., Akron, OH, USA, and duct tape) on ground glass joint side (male B34). Thereafter, the mouse holders were fitted to the ground glass joints (female B34) of all glass exposure chamber (10 L), with mouse head protruding inside the chamber. The plethysmograph had inlet and outlet ports fitted with critical orifices for continuous ventilation (170 ml/min) of the body, and for measurement of pressure differences generated by body movements during respiration through volumetric pressure transducer (Grass Model PT5, USA). After placing the animals, two side ports and the lid of the chamber were opened and one port connected to the exhaust assembly. The assembly was operated continuously to maintain continuous fresh air flow inside the exposure chamber. Thereafter, oscilloscope and program were started for 45 min before exposure, in order to acclimatize the animals in exposure chamber and to stabilize the respiratory rate. After 45 min, the exhaust assembly was switched off, two open ports were closed, and smoke of the compounds was generated by thermal heating as discussed elsewhere (15, 16, 17). Briefly, known amount of the compound was taken in the adapter attached to the exposure chamber and heated by Bunsen flame for 45-60 sec (temp ≤ 350°C). The smoke generated was purged into the exposure chamber with the help of a rubber bellows fitted to the adapter. The animals were exposed for 15 min with 45 min pre- and 30 min post- recording of respiratory parameters with the aid of a computer program, which recorded respiratory signals at 15 sec intervals (18, 19). After completion of the exposure, two side ports of the chamber were opened and exhaust assembly was run for 30 min to maintain a continuous fresh air flow in the exposure chamber. All the animals were observed for mortality up to 14 d post exposure. The LC_{50} of the compounds was determined following the moving average method discussed elsewhere (20). In this method four animals were exposed for each concentration while determining the LC_{50}. In compliance of animal ethics, this method employs less number of animals and still generates accurate data (20). Body weight of the animals, their behaviour, and amount of food and water intake were monitored for 14 d.

Characterization of the test atmosphere

Aerodynamic particle size distribution was determined by employing an eight stage Andersen cascade impactor, Mark II (Andersen Samplers Inc., Atlanta, Georgia), with aluminium foil as a collector. At each stage, aluminium foils were weighed before and after sampling to determine the mass collected upon each stage and the cumulative percentage was calculated for the deposition of particulate matter at each stage. The cumulative percentage was plotted against the effective cut off diameter on a two-cycle logarithmic probability paper. The 50th percentile was calculated from the probability graph to determine the AMD, and the 84th percentile was calculated to determine the geometric standard deviation (σg). The rating of Andersen sampler is: 1 actual cubic feet per minute (AFCM) = 28.3 l/min direct sampling done from the glass adapter. The sampling of the generated smoke was done keeping the sampling port of the sampler opposite to the outlet of the adapter. Thereafter, percentage of particulate matter (0.43-10.0 µm size), gases and vapor (particles <0.43 µm size), total smoke generated (particulate matter + gases and vapor), and compound left in the adapter after smoke generation (residue) to the quantity of the material heated were determined. The determination

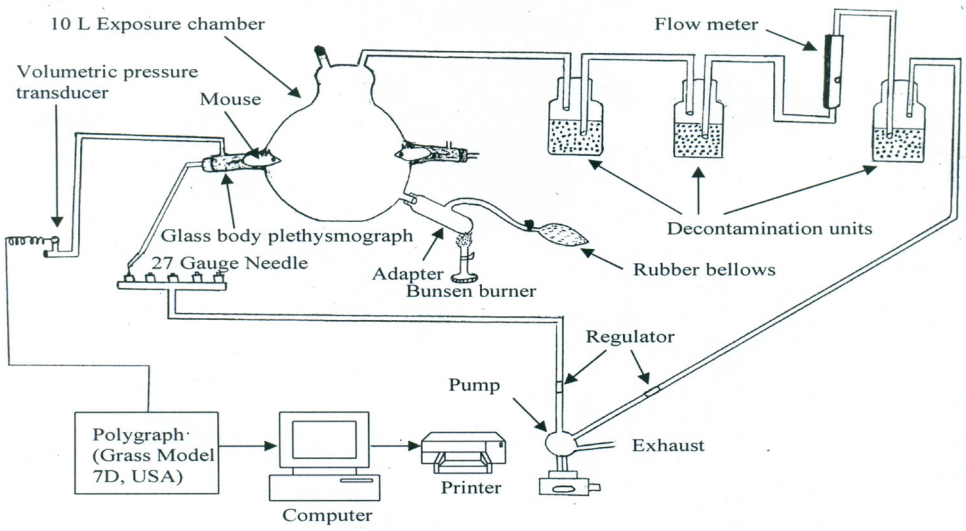


Figure 1. Schematic representation of static all glass nose only inhalation exposure assembly for smoke of fentanyl and analogs.

and standardization of all the physical parameters were carried out prior to animal exposure. No sampling was performed during animal exposure, as it was a static exposure system.

Recording of respiratory variables

The inspiration and expiration of mouse caused small changes in the air pressure inside the plethysmographs and these changes were sensed by a differential volumetric pressure transducer (Grass model PT5, Rhode Island, USA) connected to the plethysmograph with a silicone tube. The transducer was connected to an amplifier (Grass model 7P1G, USA) of the polygraph (Grass model 7D) to amplify, condition and record respiratory signals. The respiratory signals were recorded intermittently on an oscilloscope as upward deflection (inspiration) and downward deflection (expiration) to obtain typical respiratory pattern. The amplified signals were fed to (i) a digital oscilloscope (L & T Gould, Model 1425, India) for continuous monitoring of respiration and (ii) a computer through analog to digital converter card (Keithley MetraByte, Model Das 16, USA) for computing the physiological (respiratory) parameters. The respiratory parameters were computed by a computer program capable of characterizing the respiratory variables, specifically developed for this purpose (18). The program recorded the signals at 15 sec interval. With the help of other computer programs, the following parameters were derived: viz., % of normal respiration (N), airway limitation (A), pulmonary irritation (P), sensory irritation (S), tidal volume (VT), time of inspiration (TI), time of expiration (TE), frequency (f) etc.

Results

LC_{50} determination

The LC_{50} of fentanyl and its analogs was determined for 15 min static exposure. On the basis of LC_{50}, analog **5** was found to be most toxic (2820.0 mg/m^3) and analog **2** was found to be least toxic (>8000 mg/m^3) (Table 1). The toxicity of the compounds was in the following order, **5** > fentanyl > **6** > **2**. During exposure, most of the animals died within 10 min, while no death was recorded thereafter. The animals surviving after exposure did not show any significant change in the body weight, behavior, and the amount of food and water intake during the 14 d post exposure period (data not shown).

Characterization of test atmosphere

Various physical parameters of smoke of fentanyl and its analogs are shown in Table 2. These parameters reveal that percentage of suspended particles of 0.65-1.1 µm size was maximum (26.95) and 4.7-5.8 µm size

Table 1. LC_{50} of smoke of fentanyl and its analogs in mice.

Compounds	Concentration (mg/m^3)	14th day mortality (died/treated)	LC_{50} (mg/m^3) for 15 min exposure	LCt_{50} (g/min/m^3)
Fentanyl	2500 5000 10000	0/4 1/4 3/4	7070 (3170 - 15720)	106.05 (47.55 - 235.8)
2	4000 8000	0/4 0/4	>8000	>120
5	2000 4000	1/4 3/4	2820 (1290 - 6160)	42.3 (19.35 - 92.40)
6	4000 8000	1/4 2/4	8000	120

Values in parentheses are 95% confidence limits.

Table 2. Particle size (aerodynamic diameter) distribution of smoke of fentanyl and its analogs.

Stage No.*	Aerodynamic diameter (μm)	% in size range			
		Fentanyl	2	5	6
0	9.0 – 10.0	3.531 ± 1.77	2.42 ± 0.82	0.989 ± 0.46	2.597 ± 1.84
1	5.8 – 9.0	5.999 ± 2.19	5.584 ± 1.68	1.948 ± 1.23	3.897 ± 1.25
2	4.7 – 5.8	1.791 ± 1.27	6.179 ± 0.99	6.296 ± 1.94	3.279 ± 1.58
3	3.3 – 4.7	5.169 ± 1.46	12.484 ± 3.63	8.519 ± 2.58	4.718 ± 1.39
4	2.1 – 3.3	9.779 ± 4.60	20.584 ± 4.62	24.605 ± 3.45	10.952 ± 2.20
5	1.1 – 2.1	22.720 ± 7.12	28.502 ± 4.43	32.365 ± 3.45	29.562 ± 2.65
6	0.65 – 1.1	26.951 ± 3.84	15.517 ± 4.61	18.191 ± 2.89	27.363 ± 2.71
7	0.43 – 0.65	24.054 ± 8.49	8.723 ± 3.08	7.081 ± 2.10	17.630 ± 1.24

Values are mean ± SEM of five experiments.

*Stage number corresponds to different stages of cascade impactor Andersen Sampler Mark II, (Andersen Samplers Inc., Georgia).

Table 3. Physical parameters of smoke of fentanyl and its analogs.

Parameters	Compounds			
	Fentanyl	2	5	6
Aerodynamic median diameter (AMD) (μm)	1.07	2.05	1.85	1.22
Geometric Standard deviation (σg) (μm)	3.17	2.04	1.94	2.78
Residue after smoke generation (%)	8.0 ± 1.83	6.0 ± 1.69	5.5 ± 2.0	6.0 ± 2.03
Percent of the suspended particles (0.43-10.0 μm) to the quantity of the material	82.4 ± 3.86	84.4 ± 2.46	61.25 ± 2.38	40.15 ± 0.56
Percent of the gases and vapor generated	9.6 ± 2.86	9.6 ± 2.43	33.25 ± 4.0	53.85 ± 2.33
Percent of total smoke generated to the material	92.0 ± 1.83	94.0 ± 1.69	94.5 ± 2.0	94.0 ± 2.03

Values are mean ± SEM of five experiments. Quantity of the materials taken for the smoke generation = 4.0 mg.

was minimum (1.791) in case of fentanyl. In case of **2**, **5**, and **6**, the percentage of suspended particles of 1.1-2.1 μm size was maximum and was 28.502, 32.365, and 29.562, respectively. The minimum suspended particles were found in 9.0-10.0 μm size with percentage of 2.42, 0.989, and 2.597 for **2**, **5**, and **6**, respectively. Table 3 shows the summary of physical parameters of fentanyl and its analogs. The AMD (μm) of fentanyl, **2**, **5**, and **6** was found to be 1.07, 2.05, 1.85, and 1.22, respectively, and σg (μm) was 3.17, 2.04, 1.94, and 2.78, respectively. The calculated AMD with σg of fentanyl and its analogs was found to be within the respirable range (21). The percentage of suspended particles i.e., particulate matter (0.43-10.0 μm) was maximum in case of analog **2** (84.4) followed by fentanyl (82.4) while for analogs **5** and **6**, it was 61.25 and 40.15, respectively. Fentanyl yielded 92.0% total smoke (9.6% gases and vapor, and 82.4% particulate matter) and 8.0% residue. Analog **2** yielded 94% total smoke (9.6% gases and vapor, and 84.4% particulate matter) and 6% residue, analog **5** yielded 94.5% total smoke (33.25% gases and vapor, and 61.25% particulate matter) and 5.5% residue, and analog **6** generated 94% total smoke (53.85% gases and vapor, and 40.15% particulate matter) and 6% residue.

Recording of respiratory variables

Typical oscillographic tracings of respiratory pattern of animals exposed to fresh air (control) and smoke of fentanyl are shown in Figure 2A and 2B, respectively. The animals exhibited normal breathing pattern before exposure. However, all the animals showed irregular breathing pattern immediately after exposure to test atmosphere of various compounds. Figure 3, 4, 5, 6, and 7 show the typical time-dependent changes in the respiratory variables before and after exposure to fresh air (control), and smoke of fentanyl, **2**, **5** and **6**, respectively. After exposure to smoke of fentanyl, decrease in normal breath and respiratory frequency, with concomitant increase in tidal volume, time of inspiration, and time of expiration were observed. These changes were not completely resolved even after termination of the exposure. In case of **2,** there was a decrease in normal breath and respiratory frequency, with an increase in tidal volume, time of inspiration, and time of expiration, which was almost recovered after discontinuation of exposure in recovery period. In case of **5** and **6**, there was a decrease in normal breath and respiratory frequency, which persisted after discontinuation of exposure. In general, all the compounds decreased the respiratory frequency or caused persistent respiratory depression.

Discussion

Drug delivery through inhalation route is more advantageous owing to large absorptive surface area of the lung alveoli, the thinness and permeability of the barrier separating the alveolar airspace from the pulmonary capillary bed, and the direct passage of absorbed

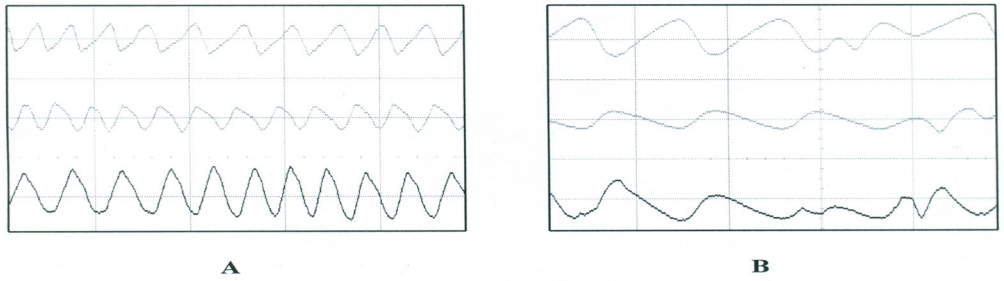


Figure 2. Typical oscillographic tracings of respiration of three mice. The upward deflection represents inspiration and downwards deflection represents expiration. **(A):** Control (the exposure chamber air) and **(B):** changes in respiration induced by fentanyl.

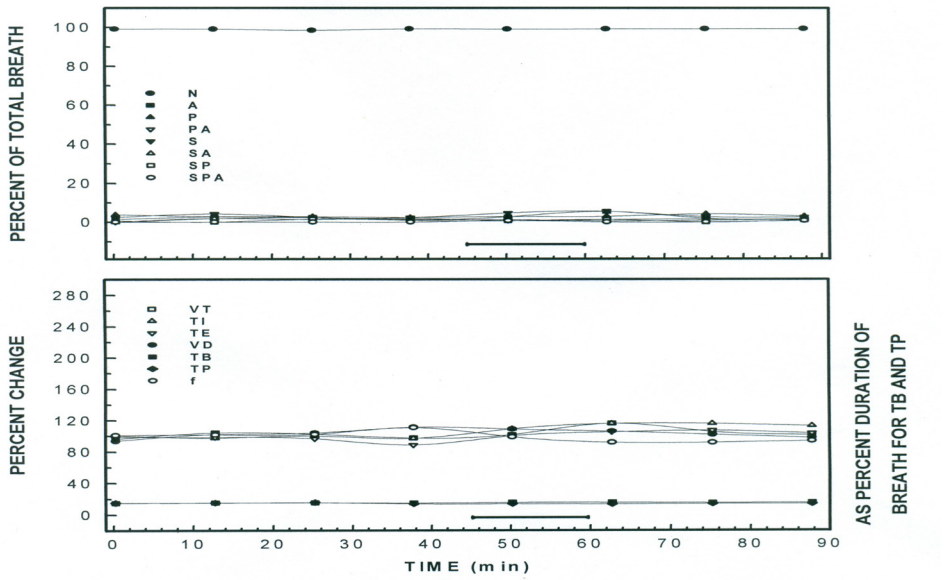


Figure 3. Typical time response profile of inhalation exposure to control atmosphere (heating without chemical).
N, normal respiration; A, airway limitation; P, pulmonary irritation; PA, pulmonary irritation + airway limitation; S, sensory irritation; SA, sensory irritation + airway limitation; SP, sensory irritation + pulmonary irritation; SPA, sensory irritation + pulmonary irritation + airway limitation; VT, tidal volume; f, respiratory frequency; VD, air flow at 0.5 VT during expiration; TI, inspiratory time; TE, expiratory time; TB, time of brake and TP, time of pause. Exposure from 45-60 min indicated by a bold line. Each point is mean of three animals.

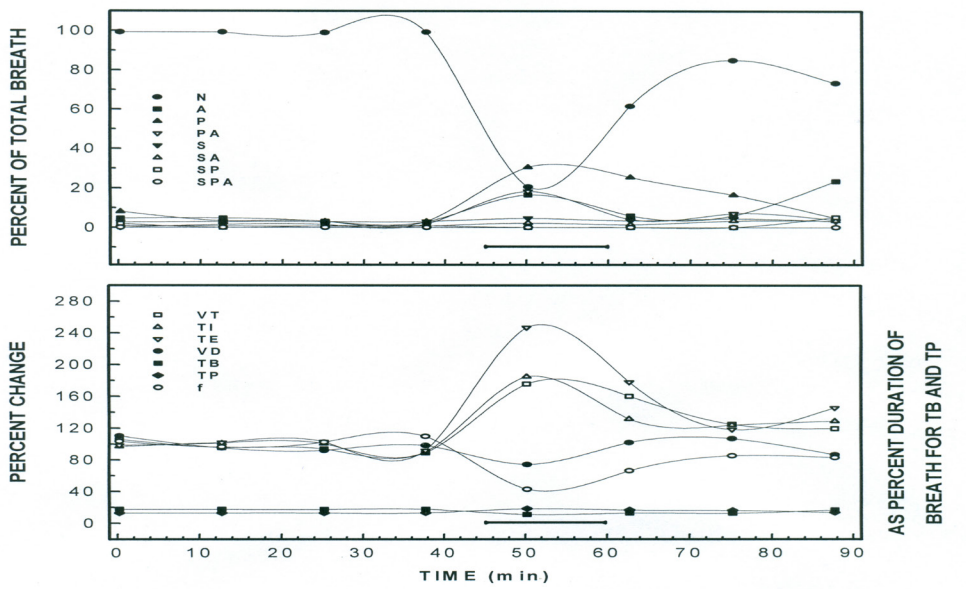


Figure 4. Typical time response profile of inhalation exposure to smoke of fentanyl (5000 mg/m^3).
N, normal respiration; A, airway limitation; P, pulmonary irritation; PA, pulmonary irritation + airway limitation; S, sensory irritation; SA, sensory irritation + airway limitation; SP, sensory irritation + pulmonary irritation; SPA, sensory irritation + pulmonary irritation + airway limitation; VT, tidal volume; f, respiratory frequency; VD, air flow at 0.5 VT during expiration; TI, inspiratory time; TE, expiratory time; TB, time of brake and TP, time of pause. Exposure from 45-60 min indicated by a bold line. Each point is mean of three animals.

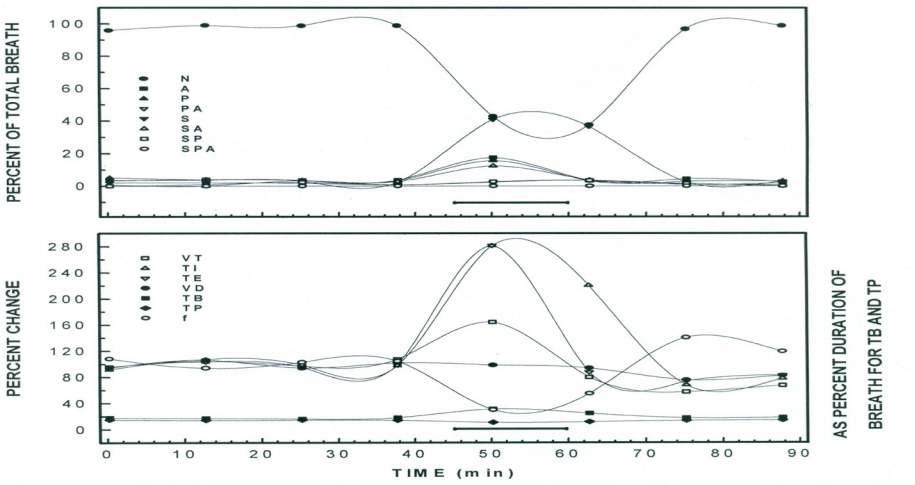


Figure 5. Typical time response profile of inhalation exposure to smoke of fentanyl analog **2** (4000 mg/m^3).
N, normal respiration; A, airway limitation; P, pulmonary irritation; PA, pulmonary irritation + airway limitation; S, sensory irritation; SA, sensory irritation + airway limitation; SP, sensory irritation + pulmonary irritation; SPA, sensory irritation + pulmonary irritation + airway limitation; VT, tidal volume; f, respiratory frequency; VD, air flow at 0.5 VT during expiration; TI, inspiratory time; TE, expiratory time; TB, time of brake and TP, time of pause. Exposure from 45-60 min indicated by a bold line. Each point is mean of three animals.

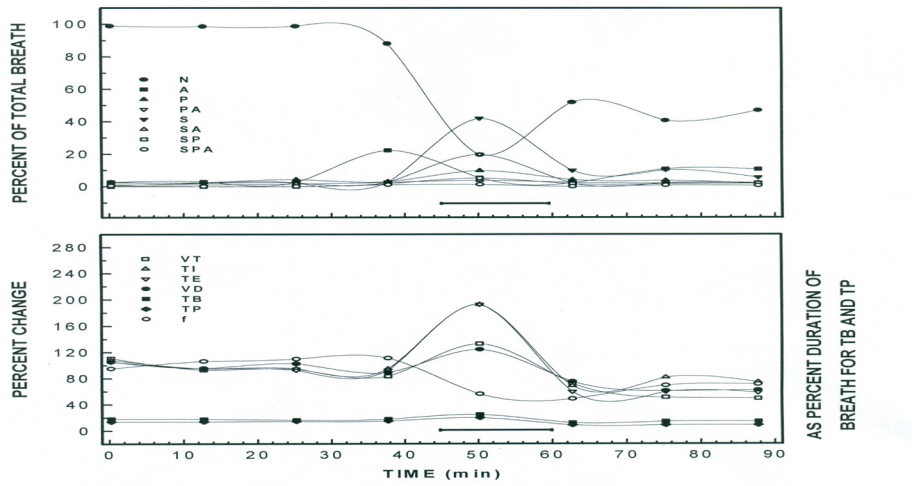


Figure 6. Typical time response profile of inhalation exposure to smoke of fentanyl analog **5** (2000 mg/m^3).
N, normal respiration; A, airway limitation; P, pulmonary irritation; PA, pulmonary irritation + airway limitation; S, sensory irritation; SA, sensory irritation + airway limitation; SP, sensory irritation + pulmonary irritation; SPA, sensory irritation + pulmonary irritation + airway limitation; VT, tidal volume; f, respiratory frequency; VD, air flow at 0.5 VT during expiration; TI, inspiratory time; TE, expiratory time; TB, time of brake and TP, time of pause. Exposure from 45-60 min indicated by a bold line. Each point is mean of three animals.

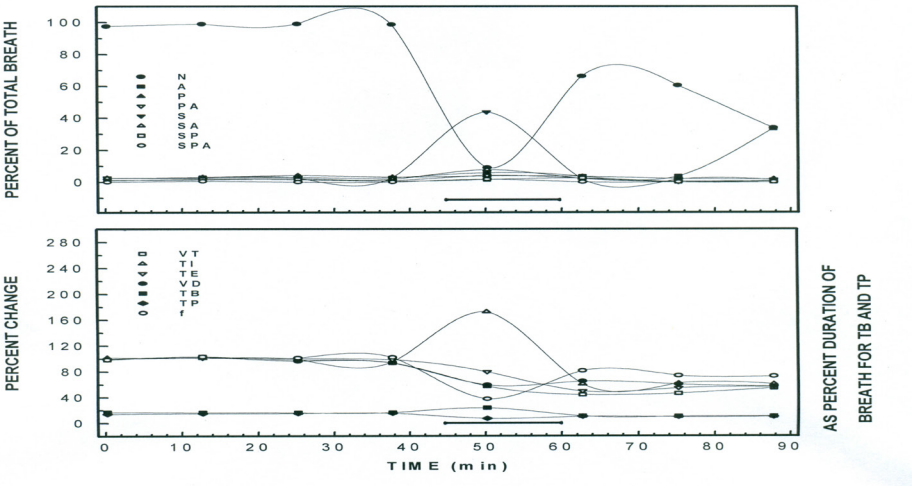


Figure 7. Typical time response profile of inhalation exposure to smoke of fentanyl analog **6** (4000 mg/m^3).
N, normal respiration; A, airway limitation; P, pulmonary irritation; PA, pulmonary irritation + airway limitation; S, sensory irritation; SA, sensory irritation + airway limitation; SP, sensory irritation + pulmonary irritation; SPA, sensory irritation + pulmonary irritation + airway limitation; VT, tidal volume; f, respiratory frequency; VD, air flow at 0.5 VT during expiration; TI, inspiratory time; TE, expiratory time; TB, time of brake and TP, time of pause. Exposure from 45-60 min indicated by a bold line. Each point is mean of three animals.

drug from the pulmonary circulation to the arterial circulation (9). If the aerosolized particles of the drug are of appropriate aerodynamic size (<3 μm in diameter), sufficient to reach the gas exchange region, their systemic entry can occur within a few minutes (22). The drug delivery for medical management is not usually fatal because the dose can be precisely controlled. However, purported misuse of inhalation technologies to generate gases of such compounds could have potentially fatal consequences (23). In the present study, smoke of fentanyl and its new analogs was generated by heating the compounds, and the LC_{50} in mice was determined using a static exposure assembly. Analog **5** was found to be most toxic while analog **2** was least toxic. The LC_{50} of all the compounds determined by this method was found to be very high compared to corresponding median lethal dose (LD_{50}) determined by p.o., intraperitoneal (i.p.) and i.v. routes in mice (10, 14). The LD_{50} of fentanyl was found to be 27.8, 17.5 and 6.9 mg/kg by p.o., i.p., and i.v. routes, respectively, whereas the corresponding LD_{50} for analog **2** was found to be 453.0, 112.2 and 22.1 mg/kg, respectively (10). The LD_{50} of analog **5** was found to be 285.8, 113.8 and 57.0 mg/kg, and that for analog **6** was 220.7, 107.3 and 45.3 mg/kg for p.o., i.p., and i.v., routes respectively (14). Route-to-route extrapolation from acute p.o. to acute inhalation toxicity has been proposed earlier (24). The data demonstrated that when the LD_{50} was approximately 100 mg/kg, the LC_{50} varied by 57-fold, and when the LD_{50} was around 1000 mg/kg the LC_{50} varied by 133-fold. Due to this wide variation, extrapolation from one route to the other warrants caution, and the same applies to our present study as well. Usually, in a static exposure setup, the decrease in concentrations of test compounds and oxygen is accompanied by an increase in carbon dioxide concentration, humidity, and chamber temperature. Therefore, as the study progresses, a non-uniform smoke concentration is observed. In the present study, the loading complement (ratio of body weight and volume of the exposure chamber) was within the recommended limits (25). To maintain the above limit of 0.2% for a static exposure system, in the present study we reduced the animal exposure time to 15 min only. Also, before and after exposure, continuous flow of fresh air was maintained in the exposure chamber. Further, spherical all glass exposure chamber was used to create uniform dispersal of smoke, because any other shape of exposure chamber may give rise to uneven distribution of smoke (16). Therefore, observations made in the present study cannot be construed as the negative effect of the static exposure system on animals, but the true effect of the generated smoke. This is also validated by the fact that a separate control animal group was also exposed under similar test conditions as shown in Figure 3. In the present study, relatively low toxicity of the compounds by inhalation route could possibly be due to decomposition of compounds by heating or poor absorption of smoke by the alveolar surface.

Particle-size distribution determines the site of initial deposition and retention in the respiratory tract and also allows for exposure of all the relevant regions of the respiratory tract. In case of fentanyl, the percentage of suspended particles of 0.65-1.1 μm size was maximum. Thus, the maximum suspended particles entered in the respiratory bronchioles and alveoli with the minimum being in the oropharynx. In case of **2**, **5**, and **6**, the percentage of suspended particles of 1.1-2.1 μm size was maximum, so the maximum particles could enter in the terminal bronchi with the minimum being in the oropharynx. Particles of 1-3 μm in diameter are ideal for deep lung delivery, because such particles are small enough to cross the mouth, larynx, and branching airways without inertial impaction, while being large enough to settle onto alveolar surfaces due to gravity (26). The main mechanism of particle deposition in the respiratory tract is by impaction, sedimentation and diffusion (27). Impaction occurs mainly in the extrathoracic airways and in the tracheobronchial tree, where the airflow velocity is high and abrupt, leading to rapid changes in airflow direction (28). However, deposition by diffusion and sedimentation depends on the long residence time of the inspired air (29). The pattern and efficiency of deposition of respirable particles in the respiratory tract mainly depend on the aerodynamic or thermodynamic diameter of the inhaled particles (30). It is not possible to predict the most responsive region of the respiratory tract or the most harmful particle-size range that is deposited throughout the entire respiratory tract. However, deposition of particulate matter in any region of the respiratory tract can produce lethality (31). In the present study, the generated particles of smoke of fentanyl and its analogs was found to be within the respirable range, the percentage of particulate matter (0.43-10 μm) was maximum for analog **2** (84.4) followed by fentanyl (82.4), while the same for analog **5** and **6** was 61.25 and 40.15, respectively. The order of toxicity of the compounds was: **5** > fentanyl > **6** > **2**. Therefore, it is clear that only particulate matters are not responsible for toxicity or lethality in short-term exposure studies, but some other factors like gases and vapors etc. may also be involved.

Fentanyl is known to have undesirable side effects including respiratory depression. Similarly, no opioid is free from such side effects. However, the degree of respiratory depression varies with different compounds (32). In the present study, fentanyl and its analogs caused a decrease in normal breath and respiratory frequency, with an increase in tidal volume, time of inspiration, and time of expiration. Previous studies also reported that fentanyl administration caused increase in tidal volume, time of inspiration, and time of expiration in female patient (33). Larger doses of opioids can affect the motor output of the respiratory centre, and activate expiratory muscles (34). Therefore, it is possible that increased tidal volume could be partly due to expiratory muscle action and part of the inspired volume could be the result of relaxation of the expiratory muscles (33).

The present study showed that inhalation of the smoke of fentanyl and its analogs caused respiratory depression in a dose- dependent manner in mice. The smoke induced a decrease in the respiratory rate, which did not completely recover after discontinuation of exposure. The acute toxicity of the compounds by inhalation route was found to be very low compared to parenteral routes of administration. Possibly, it could be due to decomposition of the compounds by heating or their poor absorption by the alveolar surface, as AMD was found to be within the respirable range. The study

concludes that thermally generated smoke of fentanyl and its analogs may not be an ideal technique for any medical or surreptitious use.

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