Anatomical and Histological Effects of Formaldehyde Inhalation on the Lung of Albino Rat

Asmaa M. T. Mohamed 1, Mamdouh El-Ashtokhy 1, Hanan M. Ahmed 2 and Osama Y. Ibrahim 3

Department of Human Anatomy and Embryology, Faculty of medicine, Zagazig University 44519, Egypt
Department of Clinical Toxicology and Forensic, Faculty of medicine, Zagazig University 44519, Egypt
Department of Histology and cell Biology, Faculty of medicine, Zagazig University 44519, Egypt

Abstract: Formaldehyde (FA) is a common indoor and outdoor pollutant found in many products. Formaldehyde-induces cytotoxicity in the respiratory tract, in the form of acute lung injury which is caused by respiratory epithelial cell damage and loss of function. Much attention to the effects of formaldehyde is paid on the upper airway, in particular the nose, much less concern is focused on the pulmonary toxicity. This study evaluated the cellular toxicity of formaldehyde gas on the lung of albino rats. For this purpose, sixty adult albino rats were divided into five groups. The rats in group (I) comprised the controls, while the rats in groups (II&III&IV and V) exposed to formaldehyde gas inhalation in a toxic dose (0.5 part per thousand) for four hours daily for four weeks. After exposure, lung samples prepared for light and electron microscopic examination every week. The anatomical results revealed gross morphological changes in the lungs such as congestion in most lobes and focal pulmonary organization. Light microscopic examination showed thickened alveolar septum, bronchial epithelial hyperplasia, proliferative capillary, pulmonary vasculitis, hyperplastic parabroncholar lymphocytic aggregations, pulmonary fibrosis and precancerous changes (goblet cell metaplasia and bronchiolar epithelial dysplasia). The ultrastructural histological results revealed new cell type development in the lung called Tunnel cell, destructed blood gas barrier, inflammatory exudates, dilatation of rough endoplasmic reticulum, thickened basement membrane, dilatation of interalveolar septal capillaries and dilatation of the pulmonary blood vessels. It was concluded that formaldehyde inhalation leads to an irritant toxic carcinogenic effect on the albino rat lungs related to the exposure durations.

Keywords: Formaldehyde inhalation; rat lung; Tunnel cell; Ultrastructural; Metaplasia.

1. Introduction

Formaldehyde is an organic carbon compound frequently used in occupational environments (hospitals, textiles, paper, resins and wood composites) and house indoor environments (insulating materials, fabrics, chipboard and cooking emissions) and is considered as one of the major components responsible for sick building syndrome (Kim et al., 2002; Kita et al., 2003; Nakazawa et al., 2005). Formaldehyde is colorless gas has a pungent odor and is irritating to the mucous membranes of the nose, throat and eyes (Nelson et al., 1986). It is recognized as toxic at certain doses and the chances of harmful effects are increased at room temperature due to its volatility (Songur et al., 2003). The toxicity of formaldehyde is of concern to all who work closely with it such as embalmers, anatomists, histology technicians and medical students are among the people who have high exposure to formaldehyde (Celik et al., 2001). Respiratory system is the major target of formaldehyde. It was reported that after rats inhale formaldehyde, the volume of formaldehyde is higher in the lung than in the blood, brain, liver and kidney (Cui et al., 1996). Acute lung injury is caused by respiratory epithelial cell damage and loss of function. The initial tissue injury triggers the production of growth factors and cytokines, which stimulate pulmonary epithelial cell inflammatory responses by activating a series of intracellular signaling pathways (Kheradmand et al., 1994; Geiser et al., 2000). Formaldehyde is a direct acting carcinogen and it is likely to pose a carcinogenic risk to human as it is classified as group 2A carcinogen by the International Agency for Research on Cancer (IARC) as a result of accumulated evidence in animals and to a limited extent in human (IARC, 1995). The expert working group of IARC determined that formaldehyde causes nasopharyngeal cancer in humans while in industrial workers, cumulative relative risk for lung cancer was marginally, but not significantly increased (IARC, 2006). The genotoxicity and cytotoxicity are considered to play important roles in the formaldehyde induced nasal carcinogenesis, in which cell proliferation due to cytotoxicity is considered to be a key element in the development of upper airway cancer (McGregor et al., 2006; SCOEL, 2008).
Because much attention to the effects of formaldehyde is paid on the upper airway, particularly the nose and much less concern is focused on the pulmonary toxicity, the present study investigated the cellular toxicity of inhalation of formaldehyde gas on the lungs and lower respiratory airways of albino rats.

2. Materials and methods

Experimental design

Sixty adult albino rats of both sexes weighing 200-250 g and aged 6-7 months were obtained from the Animal House, Faculty of Medicine, Zagazig University. They were caged in stainless steel mesh cages under normal conditions of temperature (22-24°C) and humidity with free access to water and food on balanced diet according to animal care in compliance with the European Community Guidelines on the care and use of laboratory animals. The animals were randomly divided into 5 groups, 12 rats each. Group I comprised the controls (distilled water exposed animals). Group II exposed for one week group III exposed for two weeks group IV exposed for three weeks and group V exposed for four weeks. All animals were exposed to the same concentration of formaldehyde in a toxic dose (0.5 part per thousand) four hours daily. This concentration was obtained by heating 1 gram of paraformaldehyde every exposure.

Calculation of formaldehyde concentration

Formaldehyde gas was obtained by heating its solid form, the paraformaldehyde, which is a formaldehyde polymer. Paraformaldehyde is a white substance which when exposed to heat, changes rapidly to the gaseous state (formaldehyde gas). To calculate the concentration of formaldehyde in the air of the exposure chamber, the laws of physical chemistry were applied. The molecular weight of any substance in gram gives a constant volume of gas in atmospheric pressure 1 and 273 kelvin temperature. This volume is 22.414 litres (Barrow, 1982).

The volume of air in the chamber = its length x its breadth x its height = 115x75x190= 1638750cm³. So the concentration of formaldehyde (liberated from one gram paraformaldehyde) in the air of the exposure chamber estimated as part per thousand =

\[
\frac{800}{16380750} = 0.5 \text{ part/1000}
\]

Histological investigation

At the time of sacrifice, the animals were anaesthetized with ether inhalation. Thoracotomy was performed, then the lungs were dissected out carefully and examined by naked eye for changes in shape, color, size and consistency. The gross morphological changes in lungs were photographed by digital camera. Half of animal lungs of each group were fixed in 10% formaldehyde for processing for light microscopy (Bancroft & Gamble, 2002) and the rest fixed in 4% glutaraldehyde for processing for electron microscopy (Glauret & Lewis, 1998).

3. Results

For proper comprehension of this study, the results were summarized according to the durations of exposure to formaldehyde into the following:

Anatomical results

The morphological appearance of the lungs of control group (I) revealed normal rosy pink color of the examined lobes. There is no changes in size and consistency. The lungs had smooth regular borders and normal shape of both the ventral and dorsal surfaces in all lobes with no air cystic changes or abnormal masses. Cut sections revealed no serous ooze with good differentiation between the different parts of the lung tissue. The examination of the conducting portion (trachea and bronchi) revealed normal appearance and color (Fig.1).

The gross morphological changes in the lungs of group II (one week FA exposed) animals revealed mild congestion in most lobes with no significant changes in the size. The lungs had normal shape and borders with no air cystic changes or abnormal masses, cut sections revealed no serous ooze with good differentiation between the different parts of the lung tissue. The examination of the conducting portion (trachea and bronchi) revealed normal appearance and color (Fig.2).

The gross morphological changes in the lungs of group III (two weeks FA exposure) lungs revealed irregular thin borders of most lobes specimens, sever congestion in all lobes characterized by dark red color including the pulmonary vessels in the hilum.
which showed congestion with clotted blood. There was smooth raised surfaces and increased size of the examined lung lobes suggesting edema. The cut surface showed serous ooze confirming the edema (Fig.3).

The gross morphological changes in the lungs of group IV (three weeks FA exposure) animals revealed irregular convoluted surfaces of most lungs. Increased congestion in all lobes with increased pulmonary vascular marking indicated prominent lung vasculature. The lungs had irregular borders, size and there were minute red spots in the upper lobes of both lungs (Fig.4).

The gross morphological changes in the lungs of group V (four weeks FA exposed) animals showed focal pneumatic organization of the pulmonary tissue which is characterized by developed fibrotic areas of pulmonary tissues. It is detected as evident three contracted pitting depressions in the ventral surface of the right upper lobe, also the left lobe is completely affected (Fig.5).

**Histological results**

**Light microscopic examination:**

Lung sections of hematoxylin and eosin stain of control group (I) revealed normal pulmonary tissue architecture with clear patent bronchial passages and alveolar cavities including the alveolar sacs, the alveolar ducts and the alveoli. The alveolar wall was thin and lined by two types of epithelial cells (pneumocytes); squamous epithelial cells (pneumocyte Type I) and large cuboidal cells (pneumocyte Type II) that present at the angular junctions of the alveolar walls. Pulmonary vessels was normally distributed within the pulmonary parenchyma. The alveolar septa had normal thickness with no abnormality in alveolar septal blood capillaries. Normal architecture of bronchiolar epithelium and normal parabronchiolar lymphoid aggregation was also observed (Fig.6).

Group II (one week FA exposure) animals showed acute pneumatic areas characterized by aggregations of inflammatory cell nodules with brown hemosiderin free aggregated particles accumulation in the pulmonary tissue. Also, there is increased cellularity of alveolar wall due to proliferation of pneumocytes resulting in thickened alveolar septa, emphysema evident from bulla formation of air spaces and dilated interalveolar septal capillaries (Fig.7).

After FA exposure for two weeks, group III lungs showed bronchiolar epithelial hyperplasia characterized by many newly formed abnormal epithelial cell layers that observed inside the bronchiolar lumen. Squamous metaplasia of the goblet cells characterized by abnormal proliferation of the goblet cells, hyperplastic parabronchiolar lymphocytic aggregations also were observed (Fig.8).

The lungs of group IV (three weeks FA exposure) animals showed dilatation of the pulmonary blood vessels accompanied by vasculitis, this vascular reactivity characterized by marked edema detected in the smooth muscle of pulmonary blood vessels which infiltrated with inflammatory cell aggregations. Some specimens showed signs of epithelial dysplasia which was found in more than one layer of bronchiolar epithelium characterized by appearance of multiple mitotic figures, cytoplasmic and vesicularity (Fig.9).

Group V (four weeks FA exposure) specimens confirmed the gross morphological fibrotic changes. There were focal pneumatic granulomas were observed characterized by spindle shaped cells fibroblasts (Fig.10).

**Transmission electron microscopic examination:**

Group (I) control animals showed normal epithelial arrangement with normal chromatin material in the nuclei of pneumocyte Type-I and Type-II cells. The pneumocyte Type-I appeared as squamous cells with attenuated cytoplasm. The interalveolar septum was formed of normal cuboidal shape pneumocyte Type-II cells with intact microvilli and lamellar bodies (Fig.11).

Group II (one week FA exposure) animals showed serofibrinous exudates containing aggregations of inflammatory cells and multinucleated macrophages with multiple pseudopodia inside the alveolar lumen. The interstitial tissue showed a relatively high electron density, suggestive of high protein content (edema). The edema led to compression of the interalveolar spaces which appeared as clefts. The alveolar lumen contained extravasated red blood cells. There was focal aggregates of macrophages with multiple pseudopodia containing numerous lysosomes and electron dense material corresponding to lipids or lipoprotein like material, that came from the damaged lining of pulmonary alveoli (Fig.12).

In group III (two weeks FA exposure), there was alveolar epithelial Type-II compressed between two non ciliated epithelial cells. The microvilli projecting from the apical surface of the cell were numerous, disoriented and projected in different directions. Some destroyed and detached microvilli were observed in alveolar lumen. The cell had irregular hyperchromatic nucleus with indented nuclear envelope with chromatin masses adjacent to this nuclear membrane (peripheral heterochromatin). Dilatation and vesiculation of the rough endoplasmic reticulum of epithelial cells were observed. The reticulum appeared as numerous rounded vesicles of...
variable size, that contained light electron dense granular material (matrix). Many ribosomes were attached on the membranes of the vesicles and also many free ribosomes were observed in-between the vesicles. Marked dilatation of interalveolar septal capillaries and free nuclei with prominent nucleoli without cell membrane boundaries of variable shape and size were observed (Fig.13).

The lungs of group IV (three weeks FA exposed animal) showed tunnel cells found in examined pulmonary tissue. The tunnel cell was characterized by large cavity (tunnel) occupying most of its cytoplasm (intracellular tunnel). The tunnel was surrounded by a single unilaminar electron dense membrane and contained light electron dense material especially adjacent to its wall (Fig.14).

Group V (four weeks exposed animals) showed increased thickened blood gas barrier facing the alveolar lumen, separation of the alveolar capillary membrane in some portions due to damaged discontinuous layer of squamous alveolar cells (type I pneumocyte), and separated from the underlying capillaries by an interrupted damaged basement membrane (Fig.15).

**Figure (1).** A Photomicrograph of control albino rat lungs and trachea (double arrow) showing rosy pink color. The smooth regular borders (arrows), normal size and shape of left lung (LT) and right lung (RT) upper lobe (UP), middle (M), lower (L) and cardiac (C) lobe.

**Figure (2).** A photomicrograph of one week FA exposed albino rat showing heart (*) and lungs, localized congested areas (arrows) in the left lung (T) ventral surface and the right lung dorsal surface in the upper (U), middle (M), cardiac (C) and lower (L) lobes. There is congested left internal thoracic wall (double arrow).

**Figure (3).** A photomicrograph of two weeks FA exposed albino rat showing irregular thin borders of lung lobes, severe congestion in the left lung (T) and the upper (U), middle (M), lower (L) and cardiac (C) lobes of the right lung. The pulmonary vessels in the hilum (H) congested with clotted blood.

**Figure (4).** A Photomicrograph of three weeks FA exposed albino rat lungs showing irregular convoluted surfaces. Congestion is seen in right (R) and left (L) lobes with prominent pulmonary vascular marking (arrow). There are minute red spots in the upper lobes of both lungs (arrow head).

**Figure (5).** A photomicrograph of four weeks FA exposed albino rat showing irregular thin borders of all lung lobes and irregular contracted surface of the left lung (T) (arrow), congestion in middle (M) and lower (L) lobes of right lung. The right lung upper lobe (U) showed three contracted pitting depressions (tailed arrow). There are many rosy pink spots with dark red congested spots indicated congested spots (arrow head).
Figure (6). A photomicrograph of control albino rat lung showing alveoli (A), bronchiole (B) and pulmonary blood vessels (BV).

Figure (7). A photomicrograph from the lung of one week FA exposed albino rat showing emphysematous bullae (EB), hemosiderin free aggregated particles (arrow) and dilated interalveolar capillaries (D).

Figure (8). A photomicrograph of two weeks FA exposed albino rat lung showing goblet cells metaplasia (M) mixed with bronchiolar hyperplasia (H), desquamated epithelium (DE) in the lumen.

Figure (9). A photomicrograph from the lung of three weeks FA exposed albino rat showing bronchiolar epithelial proliferation, cell vesicles (arrow head) and mitotic figures (arrow).

Figure (10). A photomicrograph of four weeks FA exposed albino rat lung showing spindle shaped fibroblast cells (arrow), narrowing of alveolar spaces and pulmonary atelectasis (A).

Figure (11). An electron photomicrograph of control albino rat lung showing the interalveolar septum that formed of normal cuboidal shape pneumocyte Type-II (PnII) cells with intact apical microvilli (arrow), lamellar bodies (lb) and normal large nucleus (N) with prominent nucleolus (n). Pneumocyte Type-I (PnI) appears as squamous cells with attenuated cytoplasm contains flat nucleus (Ni). There is elongated interstitial cells (I) between the pneumocyte Type-I and Type-II cells.

H & E X200

H & E X200

H & E X200

H & E X200

X 4000
Figure (12). An electron photomicrograph of one week FA exposed albino rat lung showing compression of the interalveolar spaces which appears as clefts (arrow). The alveolar lumen contains extravasated red blood cells (RBC), serofibrinous exudates (E) and multinucleated macrophages (M) with multiple prominent pseudopodia (P). The ruptured multinucleated macrophage contain three nuclei (n) and the cytoplasmic contents (thick arrow) escapes into the alveolar lumen(A).

Figure (13). An electron photomicrograph of two weeks FA exposed albino rat lung showing sever necrotic degenerative changes and ruptured cell membrane of (Type-II) alveolar epithelial cell (PnII). There is condensed nucleus (N) and dilated vesicles of rough endoplasmic reticulum (r). Free ribosomes are present (arrow). There is marked dilatation of interalveolar septal capillary (C).

Figure (14). An electron photomicrograph of three weeks FA exposed albino rat lung showing light electron dense material (F) adjacent to the unilaminar electron dense membrane of tunnel cells (T). Thickened blood gas barrier (BGB).

Figure (15). An electron photomicrograph of the lung of four weeks FA exposed albino rat showing thickened blood gas barrier facing alveolar lumen (A), formed of damaged discontinuous layer of squamous alveolar cells (type I) cytoplasm (ep). The fused basal laminae is interrupted (arrow) and the cytoplasm of the capillary endothelium is swollen (end). Two red blood cells are seen in the capillary lumen (RBC).

4. Discussion
The lung is the essential organ of respiration and the organ that receives the entire cardiac output. Also, the lung plays an important role in host defense and regulation of circulating levels of biologically
active materials by extensive surface of pulmonary vascular bed (Fishman et al., 1998). The study of the relation between atmospheric pollution and respiratory health needs to take into account the anatomical, histological and the toxicological effect. Inhalation is the main exposure pathway of these pollutants, which makes the respiratory tract the first target organ of airborne pollutants (Arts et al., 2006; Takahashi et al., 2007; Bernstein et al., 2008).

Formaldehyde inhalation inflicts various harms on many systems, not include respiratory system only but also exerted on variety of organs of living bodies such as testis (Zhou et al., 2006), brain (Lee et al., 2008), kidney (Golalipour et al., 2009) and liver (Cikmaz et al., 2010). Formaldehyde is a potent respiratory irritant, while the mechanism by which formaldehyde exerts its cytotoxic effects is not known. Formaldehyde reacts directly with tissue constituents, and cytotoxicity is presumably a function of this reactivity (Kimbell et al., 2001). The pulmonary changes pronounced in this work as hemorrhages, thickened alveolar wall, dilatation of the pulmonary blood vessels and inflammatory cells invasion were consistent with the findings in the rabbit lungs after exposure to 40% formaldehyde (Neelam et al., 2011), also detected in rats by Sheela & Sreedevi, (1991); Kamata et al. (1996) and Turkoglu et al. (2008), mice by YU et al. (2004) and in children by Casset et al. (2006). The mechanism of polymorphonuclear leukocytes inflammatory cells invasion induced by FA inhalation explained by Ryoko et al. (2010) who reported that inhaled formaldehyde rapidly increased vascular permeability in rat airway and produced microvascular leakage in the airway through stimulation of tachykinin NK1 receptors by tachykinsins released from sensory nerves. However, the lungs of rats exposed to 10 ppm formaldehyde vapor, for 6 hr/day over 4 consecutive days, either from an aqueous solution of formaldehyde or from heated paraformaldehyde did not show any signs of injury, even at the ultrastructural level (Dinsdale et al., 1993).

There was emphysisma evident from bulla formation of air spaces in all animal exposed groups due to rupture of inter alveolar septa. This finding obtained in the experiment of Njowa et al. (2009) who reported that the mechanism by which formaldehyde brought about the ulceration of the alveoli was by excavation and desquamation of the surface epithelium and derangement with distorted supporting tissues of alveolar wall. The ulceration was observed to be dependent on the days of exposure as the animals exposed for longer days presented a more sever ulceration. Massive cellular proliferation of bronchiolar epithelium were detected, the epithelial lining of bronchioles showed loss of mucosal folds and the cellular proliferation resulted in conversion of the epithelial lining of bronchi from pseudostratified columnar ciliated epithelium into thickened hyperplastic bronchiolar epithelium formed of many layers of cells. These results are consistent with that obtained by (Roemer et al., 1993) who reported that exposure to formaldehyde at 2.7, 8.0, 26.8 mg/m3 by inhalation for 6 hours per day for one or three consecutive days could induce lung cell proliferation of rats, which has a carcinogenic potential. Also, Monticello et al. (1996) who observed increases in cell proliferation in the respiratory tract and hyperplastic epithelial changes following repeated exposure to formaldehyde. Appearance of multiple mitotic figures, cell vescularity in bronchiolar epithelium indicated FA carcinogenicity. Naya & Nakanishi, (2005) reported that formaldehyde is carcinogenic at the site of contact as a consequence of epithelial cell regenerative proliferation resulting from cytotoxicity and mutation.

In the present study invasion of pulmonary tissues with spindle shaped fibroblast cells forming granulamatous pulmonary necronic areas was observed. The mechanism of fibrosis induced by FA explained by (Valérie et al., 2012) who reported that interleukin-11 (IL-11) is a molecular target of FA which could be involved in inflammatory and fibrogenic pulmonary effects in a dose-dependent manner in cultured lung epithelial cells. Squamous metaplasia had developed in more than one layer of bronchiolar epithelium, squamous metaplasia was identified as hyperplastic epithelium formed of large polygonal cells. These data are in agreement with (Ohtsuka et al., 1998) who had observed that after rats inhaled formaldehyde solution aerosol for 3 hours per day, for 10 days changes such as degeneration, necrosis stratification and squamous metaplasia were observed in bronchi of the lungs.

Formaldehyde exposure resulted in the appearance of tunnel cells which could not be found in the pulmonary epithelium of the non exposed rats. However, the tunnel cells were described in normal rabbit trachea (Tandler & Liedtke, 1981) and cat trachea (Tandler et al., 1983 I). Tunnel cell was observed in many pathological conditions, in the bronchioles of nitrogen dioxide exposed rats (Stephens et al., 1971 & Anderson et al., 1977) in tracheobronchial epithelium of hamster receiving intratracheal instillations of benzopyrene (Harris et al., 1974), in bronchi of hyper oxygenated guinea pig (Torikata et al., 1976), in nasal mucosa of workers employed in a nickel refinery (Boysen & Reith, 1980) and in tracheobronchial epithelium of colchicine treated rabbit (Ohashi et al., 1991). Although the tunnel cells was described by many

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authors, the genesis of these tunnels could not be determined until now. However, different authors described different explanations of tunnel cells origin. **Tandler et al. (1983 I)** had demonstrated the intracytoplasmic vacuoles to be extremely long tunnels that were connected to the epithelial surface. **Tandler & Liedtke (1981)** considered the tunnels to be formed by invagination of the epithelial surface. This consideration is confirmed by the results of the present study, the intracellular tunnel could be considered as a huge dilation of rough endoplasmic reticulum as the wall of tunnel cell was unilaminar and resembled the membranes of the adjacent rough endoplasmic reticulum. Moreover, the small clear dilated vesicles of the rough endoplasmic reticulum appeared to coalesce with the large tunnel.

When using "duration" as a factor, histological changes found in groups IV and V were more intensive than in groups II and III. The results obtained in this study, pertaining to the relationship between histological changes and exposure duration, are in agreement with those studies obtained by( **Kerns et al., 1983; Javedan et al., 1999** ) on rat nasal mucosa, and **Davarian et al. (2005)** who determined the histological changes of albino rat tracheal mucosa exposed to formaldehyde

**Conclusion:**

Formaldehyde inhalation leads to an irritant toxic carcinogenic effects on the albino rat lungs and the histological changes had direct relationship with formaldehyde vapor exposure duration.

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**Corresponding author**

Asmaa M. T. Mohamed
Department of Human Anatomy and Embryology, Faculty of Medicine, Zagazig University 44519, Egypt
a_tolba2009@yahoo.com

**References**


Avery HE, Shaw DJ. Basic physical chemistry calculations Butterworths1983.


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Abbreviations

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<th>FA</th>
<th>P</th>
<th>V</th>
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<tr>
<td>Formaldehyde</td>
<td>Atmospheric pressure</td>
<td>Volume of gas liberated.</td>
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<tr>
<th>IL-11</th>
<th>R</th>
<th>W</th>
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<tr>
<td>Interleukin-11</td>
<td>Gas constant = 0.082</td>
<td>Weight of the solid substance used, in grams.</td>
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M Molecular weight of the substance

T Temperature in Kelvin degree