Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 13 (1)

Histology of the Airways Following Chronic Exposure to Formaldehyde in Wistar Rats

Ebojele F and Iyawe V
Department of Physiology, School of Basic Medical Sciences,
College of Medical Sciences, University of Benin, Benin City, Nigeria.

Corresponding Author: Ebojele F
E-mail: frederick.ebojele@uniben.edu; +234 (0) 803 441 9399

ABSTRACT
Histology of the airways and tissues of the lungs following chronic exposure to formaldehyde was studied in wistar rats. The wistar rats were divided into three groups A, B and C with five animals in each group. Group A served as control with nil exposure while groups B and C were the test groups with 5 months exposure on non-dissection days and dissection days respectively. Formaldehyde air level was measured both at experimental and control sites. Histology of the nasal mucosa, tracheal mucosa, and lungs was carried out for each group. Statistical analysis on formaldehyde air level was done using Graph Pad Prism version 5.0. Results were presented as Mean ± SEM. Analysis of Variance was used to compare the means of test and control values while post hoc test was done using Student Newman Keul's test and a P-value of less than 0.05 was considered as statistically significant. Results from histology revealed severe erosion of the cilia and ulceration of the epithelium as well as infiltration of inflammatory cells, edema and dilation of glands. In conclusion, exposure to formaldehyde is shown to have deleterious effects on the airway epithelium of wistar rats and this may be a reflection of what could possibly happen in humans hence there is a need to seek ways of reducing formaldehyde exposure levels especially among people that work in places where formaldehyde is continuously utilized.

Keywords: epithelium, airways, formaldehyde, cilia, wistar rats

INTRODUCTION
Several claims abound with regard to formaldehyde in respect of carcinogenicity, neurotoxicity, embryotoxicity, teratogenicity, cytotoxicity, genotoxicity and allergenicity[1,2,3,4,5,6]. Some published reports in humans suggest that formaldehyde exposure has adverse effects on the respiratory health[7,8,9]. Some other researchers reported that formaldehyde inhalation could worsen allergic effects and asthma in adults and children[10,11]. In some studies carried out in rats formaldehyde was reported to produce oxidative stress in the liver[12,13]. The authors also reported evidence of lipid peroxidation among rats exposed to as high as 8ppm of formaldehyde. Some researchers have tried to look at possible effects of formaldehyde on the central nervous system. In another study the authors observed some alterations in brain structures of neonatal rats that were exposed to formaldehyde after birth for a duration of thirty days[14,15]. Impaired memory and learning as well as altered motor activity has also been reported in mice following acute exposure to formaldehyde[16]. Testicular toxicity following exposure to formaldehyde in male rats was studied by some other authors[17,18] and they reported decrease in testicular weight as well as some histopathological changes which include atrophy of the seminiferous tubules, a decrease in spermatogenic cells and edematous interstitial tissue with vascular dilation and hyperemia. They also reported decreased sperm motility as well as increased percentage of abnormal sperm. There is a report that the level of trace element in the lungs of neonatal rats can be altered after exposure to formaldehyde[19]. These authors reported decrease in iron and copper levels as well as increase in zinc levels which all occurred after the rats were exposed to formaldehyde for thirty days. However, there is paucity of information on the effect of formaldehyde exposure on the histology of the airways and lung tissues in wistar rats and this is what the present study seeks to address.

MATERIALS AND METHODS
Experimental animals: Fifteen male wistar rats of comparable age weighing between 180-220g were procured from the animal house of the Department of Anatomy, University of Benin. The animals were kept in plastic cages with wire mesh floor and allowed to acclimatize for a period of two weeks on normal feeds and water before the commencement of the experiments. Animal management and experimental protocols were carried out in accordance with the recommendations of the National Institutes of Health

Guide for Care and Use of Laboratory Animals[20].

Animal grouping: The rats were divided into three groups (A, B and C) with five animals in each group. Group A served as the control with nil exposure while groups B and C served as the test group. Group B animals were exposed to formaldehyde in the dissection room on non-dissection days for eight hours per week for a duration of five months while Group C animals were exposed to formaldehyde in the dissection room on dissection days for eight hours per week which was also equivalent to the period medical students spend in the anatomy laboratory during dissection and this was also carried out for a duration of five months.

Measurements of Formaldehyde Air Level: Formaldehyde air level was measured using Formaldehyde Gas Meter (EXTECH FM200). The meter is automated, calibrated and has an external probe that detects the air levels of formaldehyde. Five measurements were taken on five different occasions at the control site and in the dissection hall on dissection days and non-dissection days and the average was calculated and taken as the air exposure level. Within the dissection hall, the measurements were taken around the dissection table to get an idea of the personal exposure, and three meters away from the dissection table to get an idea of the area exposure. Measurements were also taken at the different corners of the laboratory. The formaldehyde air levels were measured in *part per million* (ppm). The meter also gave measurement of the room temperature and the relative humidity.

Sample Collection and Analysis: At the end of the experiment, the animals were anesthesized in chloroform after which they were sacrificed and sections of the nasal mucosa, trachea and lungs with bronchi and bronchioles were collected and fixed immediately in 10% formosaline for routine histopathological examination. They were later dehydrated by passing through varying (increasing) concentrations of alcohol, 70% to 100%, cleared in xylene and then the tissues were embedded in molten paraffin and then sectioned and stained with haematoxylin and eosin dye as described by Lillie[21]. The sections were examined under light microscope at high power magnification (x400) and photomicrographs taken. Histopathological evaluations were performed by a pathologist.

Statistical Analysis: Statistical analysis on formaldehyde air level was done using Graph pad prism version 5.0. Results was presented as Mean ± SEM. Analysis of Variance was used to compare the means of test and control values while post hoc test was done using Student Newman Keul's test and a p-value of less than 0.05 was considered as statistically significant.

RESULTS

Formaldehyde air level of control site and dissection room is shown in Table 1. There was a significant increase ($p<0.05$) in formaldehyde air level in the dissection room both on the dissection days and non-dissection days. Figures I-VII show the histology of the nasal mucosa, trachea and lungs (terminal bronchiole) of the animals in the control group and the test groups. There is cilia erosion or damage, epithelial ulceration, edema and infiltration of inflammatory cells, these are more evident in the group that was exposed on dissection days.

Table 1: Formaldehyde air level, room temperature and relative humidity of control site and dissection room

Parameters	Control site	Dissection room on non-dissection days	Dissection room on dissection days	P-value
Formaldehyde air level (ppm)	0.06±0.00	0.47±0.02*	1.95±0.02*	0.0001
Room temperature (°C)	30.32±0.08	29.42±0.10*	30.06±0.23	0.0039
Relative humidity (%)	73.40±0.71	76.36±0.12	76.14±1.27	0.0518

Significant values are Mean ± SEM compared to control (* = $P<0.05$)

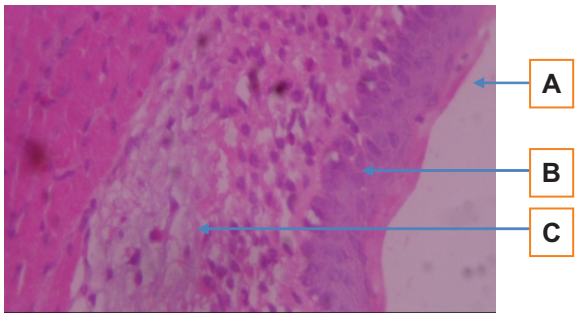

Figure I - Nasal mucosa of wistar rats with nil exposure to formaldehyde: nasal mucosa showing A, cilia, B, pseudostratified columnar epithelium and C, subepithelial zone (H&E x 400).

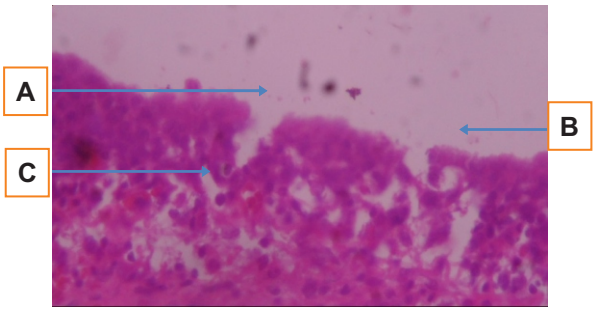

Figure II - Nasal mucosa of wistar rats following chronic exposure to formaldehyde on non-dissection days: nasal mucosa showing A, ulcerated epithelium and B, severely eroded cilia and C, oedematous epithelial lining (H&E x 400).

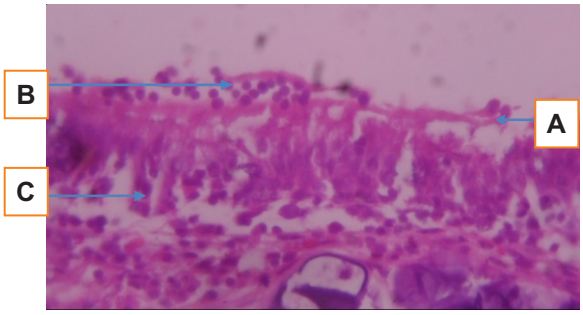

Figure III - Nasal mucosa of wistar rats following chronic exposure to formaldehyde on dissection days: nasal mucosa showing A, patchy ulcerated epithelium, B, infiltrated by inflammatory cells and C, oedematous mucosal lining (H&E x 400).

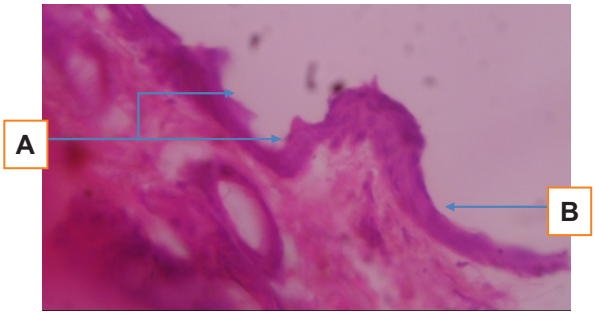

Figure IV - Tracheal mucosa of wistar rats following chronic exposure to formaldehyde on dissection days: tracheal mucosa showing A, patchy epithelia ulceration and B, severe cilia erosion (H&E x 400).

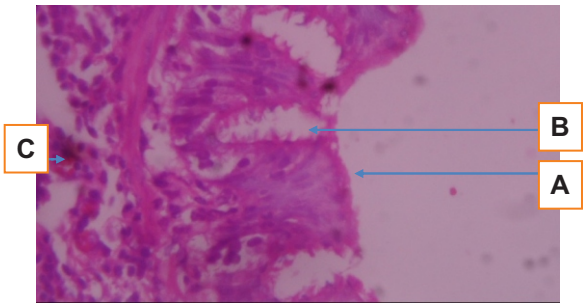

Figure V – Lung tissue (terminal bronchiole and alveoli) of wistar rats with nil exposure to formaldehyde: lungs showing A, terminal bronchiolar epithelium cilia, B, epithelia and C, alveolar space (H&E x 400).

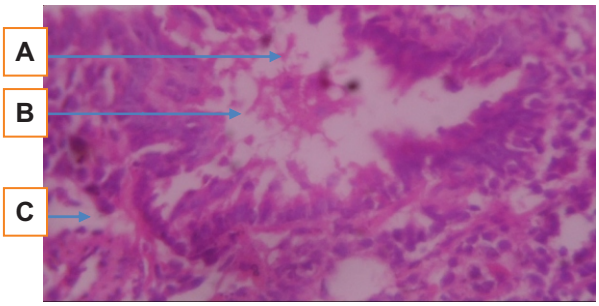

Figure VI – Lung tissue (terminal bronchiole and alveoli) of wistar rats following chronic exposure to formaldehyde on non-dissection days: lungs showing A, eroded cilia, B, ulcerated epithelium and C, alveolar space (H&E x 400).

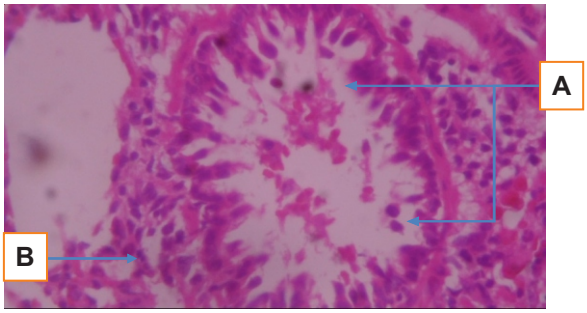

Figure VII – Lung tissue (terminal bronchiole and alveoli) of wistar rats following chronic exposure to formaldehyde on dissection days: lungs showing A, severe cilia erosion and B, alveolar space (H&E x 400).

DISCUSSION

The mucociliary apparatus presents a continuous layer of mucus, which flows over the surface of the respiratory epithelium which is typically pseudostratified ciliated columnar[22]. In this study, severe cilia erosion was observed both in group B (figure II and VI) which was exposed on non-dissection days and in group C (figure III, IV and VII) which was exposed on dissection days. There was also epithelial ulceration in both groups and this was worse in the group that was exposed on dissection days. Air exposure level to formaldehyde was more on dissection days (1.95±0.02ppm) when compared to non-dissection days (0.47±0.02ppm). There was also edema of the epithelial lining as well as infiltration of inflammatory cells. When activated, inflammatory cells are known to release certain chemical mediators like leucotrienes that can cause bronchoconstriction as well as increase mucus production; these are capable of clogging and narrowing the airways. The mucociliary clearance mechanism helps to remove excess mucus from the airways but when there is marked increase in inflammatory activity, the mucociliary clearance mechanism can be overwhelmed such that it is unable to cope with the clearance rate and there is a resultant clogging and narrowing of the airway by excess mucus. In the case where there is severe cilia damage and mucosa ulceration as observed in this study there could be a complete failure of the mucociliary clearance mechanism. This can result in severe airway obstruction. Chronic inflammatory cells are known to elaborate proteases that are capable of damaging bronchial tissues[23]. When there is obstruction of the airway arising from the possible mechanisms described above it can impact on airway function resulting in reduction in lung function parameters like PEFR, FVC and FEV_1 as reported in some human studies in which these parameters were measured[7,24,25]. Andersen and Molhave[26] reported that exposure to 0.3 ppm of formaldehyde for 4-5 hours in human volunteers inhibited nasal mucociliary function. This also lays credence to this present study on wistar rats which revealed a possible failure of the mucociliary function at an exposure level of 0.47ppm and 1.95ppm respectively. We conclude that formaldehyde has deleterious effects on airway epithelium of wistar rats. Therefore, it is recommended that formaldehyde exposure level should be reduced especially among persons that work in environments where formaldehyde is continuously utilized.

REFERENCES

1. US Environmental Protection Agency. Integrated Risk Information System (IRIS) on Formaldehyde. National Office of Research and Development, Washington DC, USA 1999.
2. Ohisuka R, Shutoh Y, Fujie H. Rat strain difference in histology and expression of Th1 and Th2 related cytokines in nasal mucosa after short term formaldehyde inhalation. Exp.Toxicol. Pathol. 2003; 54(4): 287-91.
3. IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 88. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. Geneva, Switzerland: International Agency for Research on Cancer, 2006;273:39-93.
4. Thomas R, Allen B, Nong A. A method to integrate benchmark dose estimates with genomic data to assess the functional effects of chemical exposure. Toxicol. Sci. 2007; 98(1): 240-48.
5. Costa S, Coelho P, Costa C. Genotoxic damage in pathology anatomy laboratory workers exposed to formaldehyde. Toxicology. 2008; 252: 40-48.
6. Gomaa MS, Elmesallamy GE, Sameer MM. Evaluation of genotoxic effects of formaldehyde in adult albino rats and its implication in case of human exposure. Life Science Journal. 2012; 9(4): 3085-93.
7. Pourmahabadian M, Azam K, Ghasemkhani M. Pulmonary function study between formaldehyde exposed and non-exposed staffs at some of the Tehran educational hospitals. J. Med. Sci. 2006; 6(4): 621-25.
8. Wei CN, Harada K, Ohmori S. Subjective symptoms of medical students exposed to formaldehyde during a gross anatomy dissection course. Int.J. Immunopathol. Pharmacol. 2007;

20(2): 23-25.
9. Mathur N, Rastogi SK. Respiratory effects due to occupational exposure to formaldehyde; systematic review with meta analysis. Indian J. Occup. and Environ. Med. 2007; 11: 26-31.
10. Sakamoto T, Doi S, Torri S. Effects of formaldehyde as an indoor air pollutant on the airway. Allergol. Int. 1999; 48(3): 151-60.
11. Mendell MJ. Indoor residential chemical emissions as risk factors for respiratory and allergic effects in children: A review. Indoor Air. 2007; 17(4): 259-77.
12. Petushok N. Activity of glutathione-related enzymes in rat tissues after formaldehyde exposure. Curr. Top. Biophys. 2000; 24(2): 167-69.
13. Sogut S, Songur A, Ozen O. Does the subacute (4-week) exposure to formaldehyde inhalation lead to oxidant/antioxidant imbalance in rat liver? Eur. J. Gen. Med. 2004; 1(3): 26-32.
14. Aslan H, Songur A, Tunc T. Effects of formaldehyde exposure on granule cell number and volume of dentate gyrus: A histopathological and sterological study. Brain Res. 2006; 1122(1): 191-200.
15. Sarsilmaz M, Kaplan S, Songur A. Effects of postnatal formaldehyde exposure on pyramidal cell number, volume of cell layer in hippocampus and hemisphere in the rat: A stereological study. Brain Res. 2007; 1145: 157-67.
16. Usanmaz S, Akarsu E, Vural N. Neurotoxic effects of acute and subacute formaldehyde exposure in mice. Environ. Toxicol. Pharmacol. 2002; 11(2): 93-100.
17. Ozen OA, Yaman M, Sarsilmaz M. Testicular zinc, copper and iron concentrations in male rats exposed to subacute and subchronic formaldehyde gas inhalation. J. Trace Elem. Med. Biol. 2002; 16(2):119-22.
18. Zhou D, Qiu S, Zhang J. The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats. Asian J. Androl. 2006; 8(5): 584-85.
19. Songur A, Kus I, Sahin S. The changes of zinc, copper, and iron levels in lung tissue after formaldehyde inhalation during the early postnatal period of rats. Eur. J. Gen. Med. 2005; 2(2): 62-68.
20. NIH Guide for the Care and Use of Laboratory Animals.a) DHEW Publication Number (NIH) 78-23, Revised 1978 and b) NIH Publication Number 85-23, Revised 1985, U.S. Department of Health, Education and Welfare. Research
21. Lillie RD. Histopathologic Technic and Practical Histochemistry. 3rd Ed. McGraw Hill Book Co. New York. 1965.
22. Autunes MB, Cohen NA. Mucociliary clearance – a critical upper airway host defence mechanism and methods of assessment. Current Opinion in Allergy and Clinical Immunology. 2007; 7(1): 5-10.
23. Mitchell RS, Kumar V, Abbas AK, Fausto N. Robbins Basic Pathology. 8th edn. Philadelphia.Saunders. 2007; P122.
24. Patil P, Hulke SM, Thakare A. Effect of formalin on pulmonary function: a nine months longitudinal study. Research J. of Pharmaceutical, Biol. and Chem. Sci. 2012; 3(1): 211-16.
25. Shrivastava A, Saxena Y. Effect of formalin vapour on pulmonary function of medical students in anatomy dissection hall over a period of one year. Indian J. Physiol. Pharmacol. 2013; 57(3): 255-60.
26. Andersen I, Molhave L. Controlled human studies with formaldehyde. In: Formaldehyde Toxicity (J. E. Gibson, Ed.), Hemisphere, New York. 1983; Pp 154-165.