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ITJ

ISSN 1812-5638

INFORMATION TECHNOLOGY JOURNAL

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Effect of Formaldehyde Releasing from Wood-based Products on Physiological Characteristic of the Mice

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Abstract: In this study, the middle density fiberboard and particle board were used to imitate the real living environment and discussed the effect of the different dose of formaldehyde emission of artificial board on physiological function of mice. The results show that there is no significant effect on the body weight of the mice. It also show that exposure to high-dose artificial board make the liver and ovary organ index reduced corresponding to the low-dose artificial board, but it has no any significant effect on the other organs. In addition, all of the experimental data of blood routine demonstrates that the formaldehyde emission of the artificial board has no significant differences about the physiological function of the mice. The reproductive organs sections of the mice indicate that exposure to the artificial board make a difference to the corpus luteum, seminiferous tubule and vas deferens.

Key words: Formaldehyde, artificial board, body weight, organ index, blood routine

INTRODUCTION

Nowadays, the furniture seems to become an indispensable part in modern life and most of the furniture is made from the artificial board. As know to all, adhesive is an important part of artificial board, but it disadvantages in releasing formaldehyde which had been identified as a carcinogen (WHO, 2004). Formaldehyde (FA) a ubiquitous environmental pollutant is extensively used in hospitals, laboratories and many industrial setting, also can react with many molecules in the human body (Zhou *et al.*, 2011; Matsuoka *et al.*, 2010).

There were a fairly large number of papers about the effect of formaldehyde on the immune, nervous and reproductive system of mice. In the humoral immune system, Ye *et al.* (2005) reported that the peripheral lymphocytes can be affected in relation to long-term FA exposure and the increased ratio of B cells and decreased ratio of T lymphocytes, especially the decrease of CD8 subset that caused a higher ratio of T-helper-inducer cells to T-cytotoxic-suppressor cells (CD4/CD8) were observed in the group that long-term exposed to FA (Ye *et al.*, 2005). And Jakab *et al.* also demonstrated that occupational exposure to FA can induce apoptosis and chromosomal aberrations, thus indicating a possible excess cancer risk among exposed subjects (Jakab *et al.*, 2010). In the nervous system, Usanmaz *et al.* found that acute and subacute exposures of FA produce a significant

behavioral depression on mice and acute FA exposures at low concentrations may increase the excitability of central nervous system (Usanmaz *et al.*, 2002). In addition, results of several animal experiments demonstrate that inhaled formaldehyde starts to negatively affect

learning and memory Lu *et al.* (2008), Boja *et al.* (1985) and Pitten *et al.* (2000). Furthermore, Pitten *et al.* found that the animals exposed to formaldehyde made more mistakes in the maze and needed more time to reach the goal compared to the control group that was exposed to water steam (Pitten *et al.*, 2000). And Malek considered that the single inhalative exposure to formaldehyde in the used concentrations affects the open field behavior of the experimental mice and that these effects are traceable even one day after exposure withdrawal (Malek *et al.*, 2004). In the reproductive system, Kiteava *et al.* suggest that the harmful effect of formaldehyde on germ cells is noted under the dose 1.5 mg m^{-3} only, while the reliable clastogenic and cytogenetic effects on the marrow cells were revealed even in the dose 0.5 mg m^{-3} . (Kiteava *et al.*, 1990) Additionally, Zhou D *et al.* consider that the level of 0.5 mg m^{-3} can be considered as a safe level for FA exposure, but long-term FA exposure at a dose of 2.46 mg m^{-3} has a harmful effect on male reproduction by inducing oxidative stress in male rats (Zhou *et al.*, 2011).

Although, the symptoms and impairments of immune, nervous and reproductive system have been reported in mice which were exposed to FA and other solvents

together, there was no information about the effects on mice which long-term exposed to low levels of formaldehyde releasing by the artificial board.

In the present study, Automatic Blood Cell Analyzer, spin-drier of biologic tissue and microtome were applied to evaluate the blood routine and the reproductive organs sections to examine the effects of long-term exposed to low levels of formaldehyde releasing by the artificial board on the mice.

MATERIALS AND METHODS

Materials: There were 2 kinds of wood-based panel to make used for the mice cages. As shown in Table 1, the formaldehyde emission of group A was 8 times high as that of group B. A total of 16 mice were divided into two experimental groups, each group consisting of 4 female and 4 male animals. The inside measurement of wooden cages is 500×500×30 mm.

Both Particle Board (PB) and Medium Density Fiberboard (MDF) were purchased from the wood market and the production date is in the last six months. Before the experiment, PB and MDF were placed in the temperature and humidity chamber at the condition of (20±1) and the relative humidity of (65±3)% for 2 weeks. The formaldehyde emission of PB and MDF were, respectively 2.900 and 0.333 mg L⁻¹ according to GB/T17657-1999 standards. Test the formaldehyde content of wooden cages daily during the experiment and then average the data.

Animals: Mice (*Mus musculus*) were purchased from college of veterinary medicine South China Agriculture University (Guangzhou, China); 2-week-old mice were used in all experiments. There were 2 kinds of feed, one of them was purchased from college of veterinary medicine South China Agriculture University (Guangzhou, China), the other one was purchased from Southern Medical University. All animals were randomly divided into four groups and were housed in wooden cages which the formaldehyde release was different. All experiments were performed in the same animal facility for five months range from May 31st, 2011 to October 31st, 2011.

Experimental design: In this study, effect on human of exposure to low levels of formaldehyde releasing by the

artificial board was evaluated in mice. Male and female mice were kept individually and housed in PB and MDF cages. All of them were fed with standard diet and tap water ad libitum. For comparing the effects of formaldehyde emission on the reproduction, growth, immunization and so on, the mice were used for the following experiments.

Body weight: Replaced tap water and sawdust every 3 days, weighed the body weight of mice each week and observed the behavior characteristics of mice regularly. The method to weigh the mice was to make the mouse placed in the beaker and use the centesimal balance to weigh it.

Organ index: The definition of Organ Index (OI) is the per body weight of mice corresponding to the mass of specified organ and the unit of organ index is mg g⁻¹. Measured the body weight respectively before dissecting the mice, then collected and weighted the specified organ after anatomy.

Where M is the organ mass (mg) of the mice and W is the body weight (g) of the mice.

Blood routine: The method to examine the blood routine was blood sampling after extracting the eyeball and then been analyzed with automated hematology analyzer. We focused on the differences of white Blood Cell (WBC), lymphocyte (LYM) and platelet (PLT) between the high and low doses when analyzing the experimental data.

Section form of reproductive organization: Fix tissues with 10% formalin for 48 h at room temperature. Make sure it has enough fixative to cover tissues and fixative volume should be 5-10 times of tissue volume. Then, trim fixed tissues into appropriate size and shape and place in embedding cassettes. And process for paraffin embedding schedule in total 16 h. Trim paraffin blocks as necessary and cut at 5 μm. And then place paraffin ribbon in water bath at about 40-45°C. Mount sections onto slides. Allow sections to air dry for 30 min and then bake in 45-50°C oven overnight. Never allow baking temperature go higher than 50°C for sections thicker than 25 μm. Otherwise sections may crack and result in sections falling off slides during staining. The

Table 1: Materials

Group	Materials	Specification (mm)	Formaldehyde emission (mg L ⁻¹)	Formaldehyde content (mg m ⁻³)	Remarks
A	Particle board	1220×2440×18	2.900	A-1: 1.12±0.18	Group A-1 kept 4 male mice Group A-2 kept 4 female mice
				A-2: 1.10±0.15	
B	Medium density fiberboard	1220×2440×18	0.333	B-1: 0.05±0.02	Group B-1 kept 4 male mice Group B-2 kept 4 female mice
				B-2: 0.04±0.01	

deparaffinize sections in 2-3 changes of xylene, 10 min each. Hydrate in 2 changes of 100% ethanol for 3 min each, 95 and 80% ethanol for 1 minute each. Then rinse in distilled water.

RESULTS

Effect of formaldehyde emission on the body weight: The body weight of mice is bound up with the age, strain and environment factors. In this study, the age and strain of mice were the same. In addition, the content of Volatile Organic Compounds (VOCs) indoors was not beyond the national standard, therefore it could be considered that the formaldehyde emission was the only deciding factor to influence the reproduction, growth and body weight of the mice.

Figure 1 shows that no significant differences were found in body weight. Prolonged exposure to artificial board did not affect body weight gain of mice during the experiment. The body weights of the mice of group A and group B gradually increased in a time-dependent manner. Obviously, group A-1 and group B-1 were slightly higher than group A-2 and group B-2. But there was no significant difference between high and low doses.

Effect of formaldehyde emission on the organ index: A certain dosage of formaldehyde is toxic to the liver and ovary of mice, which not only destroy and inhibit the

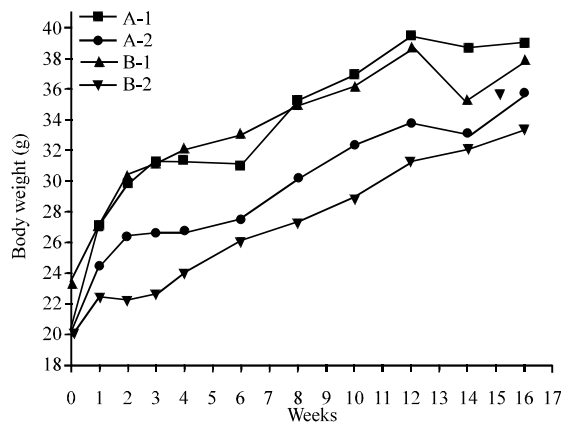


Fig. 1: Body weight of the mice

Table 2: Compare the organ index among the male mice

Group	Cardiac (mg g ⁻¹)	Liver (mg g ⁻¹)	Spleen (mg g ⁻¹)	Lung (mg g ⁻¹)	Kidney (mg g ⁻¹)	Thyroid (mg g ⁻¹)	Testis (mg g ⁻¹)	Ovary (mg g ⁻¹)	Thymus (mg g ⁻¹)
A-1	3.97±0.44	48.57±3.20	1.77±0.74	5.00±0.28	10.96±1.10	6.33±0.70	5.37±0.88	-	1.55±1.24
A-2	4.47±0.52	49.74±3.44	3.61±0.46	5.67±0.45	9.75±0.63	5.21±1.39	-	0.53±0.21	2.27±0.47
B-1	4.51±0.37	53.79±3.07	1.96±0.35	5.08±0.93	10.80±1.85	6.01±1.00	5.72±0.61	-	1.85±0.81
B-2	4.18±0.46	54.71±3.97	4.25±1.89	6.23±0.63	10.40±0.79	5.45±0.73	-	1.27±0.19	2.19±0.83

maturation process of oocyte, but also reduce the fertilization ability and survival rate of oocyte. Therefore, women of childbearing age long-term exposes to a certain dosage of formaldehyde, it may cause to decrease the reproductive ability or, in some cases, infertility. As shown in Table 2, the liver and ovary organ index of group A were, respectively only 90 and 47% corresponding to group B. Formaldehyde not only results in ovarian atrophy, but also seriously reduces ovarian function, meanwhile, it reduces endocrine function. Formaldehyde damages the growth and development of follicle, ultimately leading to the depletion of follicle and the irreversible damage of ovary which includes the failure of ovulation and endocrine (Peng *et al.*, 2010). The other organ index shows that long-term exposure to artificial board has no significant effects on the other organs.

Effect of formaldehyde emission on the blood routine: All of the test items are controlled within the bounds of reference range, which demonstrates that the formaldehyde emission has no significant differences about the physiological function of the mice. However, it also showed the effects of blood routine on formaldehyde emission from the difference among white blood cell (WBC), lymphocyte (LYM) and platelet (PLT).

White Blood Cell count (WBC) of Group B is 10% to 30% higher than Group A, as well as lymphocyte rate (LYM), Group B-1 is higher than 17.5% shown in Group A-1 and Group B-2 higher than Group A-2 7%. However, Mean Platelet Volume (MPV) of Group A is obviously higher than Group B. The reason why WBC was slight high may be the inflammation of the mice.

Effect of the SFRO on formaldehyde emission: As Fig. 2, 3 shows that seminiferous tubule has a slight exfoliation phenomenon and vas deferens slightly dilated among the male mice. Furthermore, vas deferens of Group A-1 dilated more seriously than Group B-1, rest others had no obvious lesions.

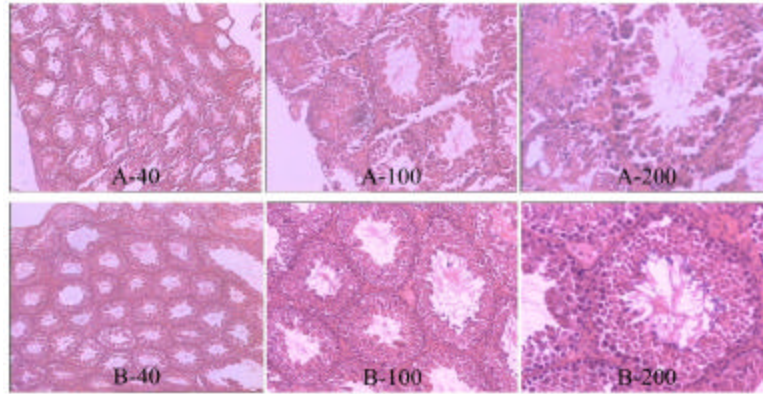


Fig. 2: Section form of testicle organization. A-40, A-100 and A-200 are the testicular section of Group A under 40, 100, 200x magnification, respectively. B-40, B-100 and B-200 are testicular the section of Group B under 40, 100 and 200x magnification, respectively

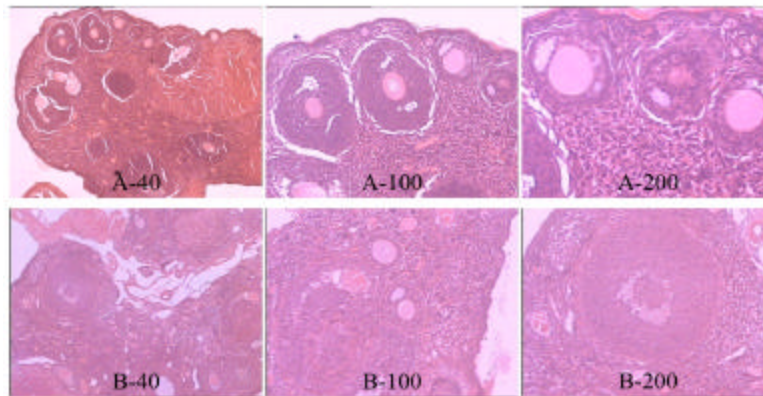


Fig. 3: Section form of ovary organization. A-40, A-100 and A-200 are the ovarian section of Group A under 40, 100, 200x magnifications, respectively. B-40, B-100 and B-200 are the ovarian section of Group B under 40, 100, 200x magnifications, respectively

DISCUSSION

Researches have shown that formaldehyde contains a variety of toxic effects on biological systems, such as the pathological changes and carcinogenicity of testicle and ovary tissue.

The main pathological changes in testicle tissue have been observed in experimental animals (Tang *et al.*, 2003). It has been reported that, the sperm quantity was decreased and the sperm heads deformation ratio was increased in all formaldehyde groups. Formaldehyde could induce genetic materials in spermatogone, primary spermatocyte and caused degeneration and necrosis in secondary spermatocyte, spermatogenic cell, sperm. In additional, testicular sperm head counts were significantly increased at the 200mg/kg levels of FA which was

administered orally to 10 week old male Wistar rats (Cassidy *et al.*, 1983).

Majumder also reported that when administered to rats at a dose of 10 mg kg⁻¹ formaldehyde body weight/day for a period of 30 days, resulted in a significant fall in sperm motility, viability and count. (Mojumder and Kumar, 1995) In addition, the DNA content was significantly lower in testis and prostate while the tissue protein content of prostate and epididymis had decreased in the treated rats. In vitro exposure to formaldehyde also inhibited the sperm motility and viability.

In the present study, evaluated the inhaled toxicity of formaldehyde releasing from the artificial board by analyzing the section form of reproductive organization. The results demonstrated seminiferous tubule had a slight

exfoliation phenomenon and vas deferens slightly dilated among the male mice. Furthermore, vas deferens of Group A-1 dilated more seriously than Group B-1, rest others had no obvious lesions. In short, the formaldehyde leads to the pathological changes in male mice and makes the metabolism increased. But the toxicity mechanisms of long-term exposure to formaldehyde need to study further.

In former researches, the ovarian reserve function of female rats can be impaired after subchronically exposed to formaldehyde for 14 days (Peng *et al.*, 2010). And formaldehyde has adverse effects on estrous cycle and ovary of female mice (Wang *et al.*, 2002). Additionally, Liu suggested that among the concentrations investigated in the present study, the form of DNA damage induced by gaseous FA on reproductive cell of female mice was DPC and DPC was increased with the increasing of FA concentrations, indicating that gaseous FA has obvious genetic toxicity on the reproductive cells of female mice since DPC is a grievous damage of DNA (Liu and Wang, 2006).

The health corpus luteum is cystic structures and slightly swells the ovary. It will lead to ovarian corpus luteum cyst, if the corpus luteum continues to exist and grow. And the experiment showed that formaldehyde leads to oxidative damage in ovary tissue, characterized by enlarging the corpus luteum among the mice. This was no less than the results of precious study.

In conclusion, several results can be obtained from this study: (1) Long-term exposure to low levels of formaldehyde of artificial board affects the development of liver and ovary; however, there is no obvious difference among the other organ index, (2) Pass thorough research toward the blood routine test, prolonged exposure to artificial board have little influence on the mice, but anatomized the blood routine data, it can be found that indexes of high dose group are weaker than low dose group, (3) Long-term exposure to artificial board leads to enlarge the corpus luteum among the female and seminiferous tubule has a slight exfoliation phenomenon and vas deferens slightly dilated among the male mice.

ACKNOWLEDGMENTS

Research special funds for public welfare projects(2012104006-8).

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