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# Safety Evaluation of Wood–Based Activated Carbon Fibers in a 28–day Feeding Study in Sprague–Dawley Rats

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As a possible food moisture–proof material, wood–based activated carbon fibers (WACFs), prepared from one type of wood pulp: Laubholz Unbleached Kraft Pulp (LUKP) by the physical activation method with steam at an 850°C activation temperature with 60 min of activation time (850 LUKP WACFs), were evaluated in a 28–day feeding study of Sprague–Dawley rats (SD rats) after the Ames test had been carried out in a previous paper. SD rats were randomly distributed into five groups, including a blank group (reverse osmosis water), a control group (saline), and groups with low, moderate, and high (1.0, 2.5, and 5.0 g/kg/day, respectively) doses of 850 LUKP WACFs. All SD rats were sacrificed after a 28–day feeding toxicity study. Blood samples were analyzed for hematological and serum biochemical values, and histopathological examinations of the organs were investigated. There were insignificant differences from the clinical pathology; that is, no observable adverse effect levels (NOAEL) of 850 LUKP WACFs greater than 5.0 g/kg/day. It is suggested that WACFs prepared from wood pulp can be a potential type of food moisture–proof material and a potential type of material for water quality improvement due to their biological safety results.

**Key words**: Wood–Based Activated Carbon Fibers (WACFs), Wood Pulp, 28–Day Feeding Toxicity Study, Food Moisture–Proof Material

## INTRODUCTION

Activated carbon (AC) is a member of a family of carbons ranging from carbon blacks to nuclear graphite, from carbon fibers and composites to electrode graphite, and many more. All come from organic parent sources but with different carbonization and preparing processes. AC is a good adsorbent for gaseous and liquid adsorption and is widely applied in purification, de–colorization, removal of toxic substances, and treatment of waste water (Liu, 1998; Manocha, 2003; Yorgun *et al*., 2009; Sun and Jiang, 2010). This is because AC is regarded as a porous material that has a large specific surface area, a high adsorption capacity, chemical stability, and the ability to regenerate through desorption (Lu, 1994; Hsieh, 1998; Wu and Tseng, 2000; Huang, 2001). AC is classified into 5 types by size (dimension), shape (appearance), and purpose (Liu, 1998). Among all, activated carbon fibers (ACFs) are regarded as fibrous and porous material with a large specific surface area, a high adsorption capacity, and the ability to regenerate through absorption/desorption, as well as provide better characteristics such as a low–pressure drop to mass transfer and high contact efficiency due to their fibrous shape with a high aspect ratio (Asakura *et al*., 2004).

Previous work (Lin, *et al*., 2015) established that to develop Wood–Based Activated Carbon Fibers Paperboard (WACFP) as a moisture–proof material, Nadelholz/Laubholz Unbleached Kraft Pulp and cardboard from recycled cartons could be prepared into wood–based activated carbon fibers (WACFs) as part of WACFP. The biological action of WACFs was evaluated by *Salmonella* mutagenesis assay (Ames test), indicating that the WACFs had no cytotoxicity or mutagenicity in the test range (1.0–5.0 mg/plate of WACFs). The antimutagenicity against strains for the WACFs also suggested the safety of the WACFs used primarily as a material for food use. However, WACFs will probably be in contact with food directly or indirectly, and could even be eaten. It is necessary to undergo a mammalian test (biological safety assessment) after the Ames test, in accordance with Item 2, Article 3 of the Health Food Control Act and the Health Food Safety Assessment Method specified in the W.S.S.Z. No. 88037803 announcement from the Ministry of Health and Welfare, 1999.

The biological safety assessment contains an acute toxicity test and subacute toxicity test (28–day feeding toxicity study). The feeding dose for acute and subacute toxicity tests was 5–10 times the maximum dose level for the subacute toxicity test (1.0 g/kg/day) specified by the Ministry of Health and Welfare (Ministry of Health and Welfare, 1999); meanwhile, the maximum dose as an antidote (1.0 g/kg) in the prevention and response manual (Emergency Response Information Center, 2006) writ-

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ten by the Emergency Response Information Center was referred to, and this test was carried out referring to the dose. This study used WACFs prepared with the conditions of physical activation with steam at an 850oC activation temperature with 60 min of activation time from Laubholz Unbleached Kraft Pulp (850 LUKP WACFs), to evaluate a 28–day feeding study for Sprague–Dawley rats (SD rats) because the 850 LUKP WACFs have better porosity and adsorptivity, as seen from previous results (Lin *et al*., 2015). The purpose was to evaluate the safety of WACFs for organisms, in order to apply the feasibility of adding it to various foods as a diet, or using it as part of a food moisture–proof material, such as within WACFP.

## MATERIALS AND METHODS

### Precursor

Laubholz Unbleached Kraft Pulps (LUKP), a wood pulp, paperboard was provided by the Hou–li Mill, Cheng Loong Corporation in Taiwan. The LUKP specimen, with dimensions of 20 mm × 20 mm, was prepared and then defibrillated by a grinder to be used as the precursor. The basic properties and morphological characteristics of the specimens refer to (Huang, *et al*., 2010; Lin *et al*., 2015).

### Sprague–Dawley rat

Sprague–Dawley rats (SD rats) were bought from the BioLASCO Taiwan Co., Ltd. in Taiwan. There were 20 SD rats with five–week–old males and females to be used for acute animal testing, and there were 50 SD rats with six–week–old males and females to be used for subacute animal testing.

### Preparation of wood–based activated carbon fibers (WACFs) and characteristics

The precursor, 20 g of absolute dried LUKP specimen, was prepared in a closed container of super–high temperature vacuum carbonization activation equipment (Chi–How Heating Co., Ltd.). Nitrogen ($N_2$ gas) at a flow rate of 200 mL/min was added to make the container oxygen free. The heating rate was set at 10°C/min, and the specimen was heated to the carbonization temperature of 850°C. The activation temperature was carried out at 850°C with 60 min of activation duration. The steam was maintained at a flow rate of 500 mL/h. The WACFs were then cooled by $N_2$ gas at 200 mL/min to a normal temperature and taken out. The prepared WACFs code was 850 LUKP WACFs. Its yield was 14.31%, the iodine value was about 1007.43 mg/g, and the specific surface area was about 775 $m^2$/g. The aforesaid preparation conditions and the characteristics of 850 LUKP WACFs refer to (Lin *et al*., 2015).

### Biological Safety Assessment

*Feeding and management*

The experiment passed through the Approval of Animal Use Protocol, National Chiayi University in Taiwan by identity No. 101015. The ambient temperature of feeding was 23±2°C, the relative humidity was 40–60%, lighting and darkness lasted 12 h each and adequate chow diet and water were provided in accordance with general experimental animal feeding and management. The SD rats were raised in Macrolon Cages in stainless steel covered for 5–7 days before testing. The bedding was Northern White Maple (Bedding Company). The test materials were given at a fixed time every day during the test period, and the dose depended on weight. The feed was delivered by a stomach tube through the method of gavage.

*Acute animal test*

There was a control group and a test group; each group had 20 SD rats with an equal number of males and females (half and half). The test group took the 850 LUKP WACFs of the maximum feeding dose (10.0 g/kg) for the preliminary test, and the control group was fed with only physiological saline solution. The SD rats were observed, and their average body weight and the feed and drinking water consumption were measured during the test period for 14 days.

*Subacute animal test (a 28–day feeding toxicity study)*

The SD rats were randomly divided into five groups. Each group had 20 rats, 10 males and 10 females per group with 100 rats in all. The dose was 5–10 times the maximum dose level (1.0 g/kg/day) for the subacute toxicity test, as specified by the Ministry of Health and Welfare in Taiwan (1999). According to the results of the acute animal test, the maximum dose level for this test was set at 5.0 g/kg/day. The five groups were a blank group (reverse osmosis water, $H_2O$), a physiological saline solution (control group), a low dose group (1.0 g/kg/day), a moderate dose group (2.5 g/kg/day), and a high dose group (5.0 g/kg/day). The $H_2O$, saline solution and 850 LUKP WACFs were fed through feeding tubes for 28 days. When the test period ended, feeding was stopped for 12 hours on the 27$^{th}$ day, then, the SD rats were anaesthetized with carbon dioxide, and their organs were taken and weighed and their blood was taken from the vena portae hepatica for clinical pathology analysis.

*Observation of pathological tissues and weighing*

Following sacrifice a thorough necropsy was performed on all SD rats. The following organs were weighed (paired organs together) after dissection, including heart, kidneys, liver, ovaries, spleen and testes. The organ–to–body weight ratios (relative organ weights) were calculated from the absolute organ weights and the terminal body weight of the SD rats. Samples of the weighed organs were preserved in a neutral aqueous phosphate buffered 10% solution of formaldehyde. Histopathologic analysis was conducted on 5–m sections of paraffin–embedded tissues, stained with haematoxylin and eosin (Hematoxylin and Eosin, H&E), of the preserved organs from all control and high–dose animals by light microscopy.

*Blood examination*

The blood was collected in an anticoagulant test tube

containing heparin (Lithium Heparin Plasma tube, Becton, Dickinson and Company, NJ, USA) The white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet (PLT) were measured by full automatic hemocytometer (SYSMEX K1000).

*Blood serum biochemical analysis*

The blood was collected in the test tube without an anticoagulant, when the blood was agglutinated. The blood serum was separated centrifugally, including alkaline phosphatase (ALP), alanine aminotransferase (ALT), asparate aminotransferase (AST), blood urea nitrogen (BUN), albumin (ALB), globulin (GLO), A/G ratio: albumin/globulin (A/G) ratio, creatinine (CRE), glucose (GLU), total bilirubin (T–BIL), total protein (T–PRO), and some ion concentration from inorganic elements, such as: $Ca^{2+}$, $Na^{+}$, $K^{+}$, $Cl^{-}$ and inorganic phosphate (P) were determined by blood serum biochemical autoanalyzer (CIBA–CORNING).

*Statistical analysis*

The test results of average body weight, relative weight of organs, blood examination and blood serum analysis of the SD rats were represented by mean (standard deviation). The control group and test group were compared by Duncan's multiple analysis. If the $\rho$ value is smaller than 0.05, meaning a significant difference between the test group and control group, it is represented by different letters (n=10).

## RESULTS AND DISCUSSION

According to the aforesaid results (Lin, *et al*., 2015) from the Ames Test and antimutagenic activity of WACFs, the Survival for *Salmonella Typhimurium* TA98 and TA100, with or without the S9 mix in the test range (1.0–5.0 mg/plate of WACFs), were all higher than those of the control group by more than 80%, and the WACFs for TA98 and TA100 with or without S9 did not exceed spontaneous revertants by more than two times, indicating the WACFs had no cytotoxicity or mutagenicity. It is suggested that the mammalian test (biological safety assessment) of the WACFs is able to be carried out with consideration of the material for food use. To avoid unnecessary sacrifice of animals, a preliminary test was carried out first. The clinical conditions, average body weight, and food and water consumption of the SD rats were observed in the preliminary test, after 850 LUKP WACF was fed at doses of 0.1, 0.5, 1.0 and 5.0 g/kg. The results showed that there was no adverse effect on the fed animals after 7 days compared to $H_2O$ (blank group, reverse osmosis water). The subacute and acute animal tests for the animal body and the pathological observation on organs and tissues, blood examination and blood serum biochemical analysis were then carried out to evaluate the effects of feeding on the in vivo organ functions and metabolism of SD rats.

**Acute toxicity**

The average body weight, average food consumption, and average drinking water consumption of the male and female SD rats fed with 850 LUKP WACFs at 10.0 g/kg from the first day to the 14$^{th}$ day were investigated. The average body weight of male/female rats was similar to the results of the control group (0 g/kg), shown in Table 1, after testing by Duncan's multiple analysis. The 850 LUKP WACFs also had no adverse effects on feed intake and water intake of the SD rats in the test group on the 1$^{st}$ and 7$^{th}$ days (results not shown in Table); therefore, it is indicated that the 850 LUKP WACFs had no effect on the test groups of SD rats. The result is the dose reference for the subacute toxicity test. However, the feeding dose at 10.0 g/kg was not able to dissolve completely to become the feed for feeding SD rats. The dose for the subacute toxicity test was 5.0 g/kg in this study.

**Subacute toxicity**

According to the effects of different doses of 850 LUKP WACFs on the food consumption and drinking water consumption of SD rats (results not shown in Table), there was no difference between the control groups ($H_2O$, saline solution) and dose group of male SD rats at 1.0, 2.5, and 5.0 g/kg/day in feed intake and water intake for the 1$^{st}$ to 28$^{th}$ day, and there was also no significant difference between the groups and the female rats fed with different doses of 850 LUKP WACFs. The effect of feeding different doses of 850 LUKP WACFs on average body weight of SD rats is shown in Table 2. There was no significant difference between the various groups

**Table 1.** Effects of feeding 850°C LUKP WACFs in 10.0 g/kg to SD rats on average body weight in acute toxicity test

| Sex | Treatment | Average body weight (g)[1)] – days | | |
|---|---|---|---|---|
| | | 0 | 7 | 14 |
| Male | Control group | 140.4 ± 11.1[a] | 188.6 ± 17.1[a] | 263.8 ± 17.7[a] |
| | Test group | 140.0 ± 11.0[a] | 176.6 ± 8.9[a] | 247.2 ± 15.1[a] |
| Female | Control group | 125.4 ± 14.1[a] | 150.2 ± 12.8[a] | 201.0 ± 12.7[a] |
| | Test group | 118.5 ± 11.9[a] | 142.4 ± 8.3[a] | 184.6 ± 10.3[a] |

[1)] The results show mean ± S.D. by Duncan's multiple analysis, significant differences in data are represented by $\rho < 0.05$ between row sited by different alphabets (n=10).

of male SD rats on the 28th day according to Duncan's multiple analysis. There was a difference among female SD rats fed with $H_2O$ and various doses after the 24th day, but there was no significant difference to the control group with saline and doses at 1.0 to 5.0 g/kg/day. Therefore, our study showed that feeding different doses of 850 LUKP WACFs had no adverse effects on average body weight of SD rats.

*Observation of organs*

The effect of feeding different doses of 850 LUKP WACFs in the subacute test on the relative organ weight to body weight of SD rats is shown in Table 3. There was no significant difference between the dose groups and blank/control groups of SD rats according to Duncan's multiple analysis. The relative organ weight to body weight of various groups was described as follows: the weight percentage of heart of male SD rats was 0.35–

**Table 2.** Average body weight of SD rats during feeding period of 850°C LUKP WACFs in 28–day feeding toxicity study

| Sex | Time (days) | Average body weight (g) – Dose (g/kg/day) | | | | |
|---|---|---|---|---|---|---|
| | | $H_2O$[1)] | Saline | 1.0 | 2.5 | 5.0 |
| Male | 0 | 220.8 ± 16.2[a 2)] | 227.6 ± 20.6[a] | 219.2 ± 19.8[a] | 218.4 ± 19.8[a] | 219.7 ± 11.6[a] |
| | 4 | 247.0 ± 18.8[ab] | 257.7 ± 24.2[a] | 287.5 ± 23.1[a] | 247.2 ± 27.8[ab] | 231.7 ± 21.8[b] |
| | 8 | 284.8 ± 20.8[a] | 288.9 ± 27.1 [a] | 253.9 ± 20.4[ab] | 278.6 ± 29.9[ab] | 260.2 ± 21.4[a] |
| | 12 | 306.7 ± 20.5[ab] | 313.6 ± 29.8 [a] | 307.4 ± 24.8[ab] | 297.0 ± 37.6[ab] | 283.4 ± 24.4[b] |
| | 16 | 323.6 ± 22.0[a] | 321.0 ± 38.3[a] | 323.7 ± 29.2[a] | 328.4 ± 30.8[a] | 309.1 ± 33.6[a] |
| | 20 | 343.4 ± 23.6[a] | 345.2 ± 37.2[a] | 350.8 ± 32.3[a] | 342.3 ± 32.6[a] | 336.9 ± 32.9[a] |
| | 24 | 359.7 ± 27.3[a] | 356.8 ± 38.8[a] | 355.2 ± 32.4[a] | 358.7 ± 35.2[a] | 351.7 ± 35.1[a] |
| | 28 | 343.2 ± 26.5[a] | 352.4 ± 41.2[a] | 346.6 ± 29.9[a] | 349.5 ± 28.1[a] | 334.7 ± 25.3[a] |
| Female | 0 | 175.9 ± 14.8[a] | 171.0 ± 9.6[a] | 172.1 ± 13.7[a] | 174.1 ± 9.9[a] | 164.4 ± 8.7[a] |
| | 4 | 186.2 ± 14.0[a] | 184.9 ± 11.6[a] | 185.4 ± 17.0[a] | 185.8 ± 12.5[a] | 165.6 ± 12.6[b] |
| | 8 | 201.3 ± 17.0[a] | 200.3 ± 10.9[a] | 193.9 ± 16.9[ab] | 195.0 ± 14.6[ab] | 178.6 ± 16.0[b] |
| | 12 | 219.4 ± 16.3[a] | 209.3 ± 14.6[a] | 206.1 ± 16.6[a] | 207.0 ± 17.0[a] | 187.0 ± 15.8[b] |
| | 16 | 223.1 ± 19.1[a] | 217.8 ± 14.0[ab] | 204.9 ± 15.7bc | 217.1 ± 14.0[ab] | 193.5 ± 17.5[c] |
| | 20 | 230.1 ± 13.2[a] | 223.5 ± 15.0[a] | 227.2 ± 18.76[a] | 229.4 ± 16.0[a] | 213.2 ± 16.0[a] |
| | 24 | 242.5 ± 16.0[a] | 231.1 ± 16.2[ab] | 230.5 ± 21.1[ab] | 228.4 ± 24.1[ab] | 210.6 ± 12.9[b] |
| | 28 | 225.0 ± 15.1[a] | 219.2 ± 16.5[ab] | 214.2 ± 16.9[ab] | 219.6 ± 15.3[ab] | 201.3 ± 9.6[b] |

[1)] Reverse osmosis water

[2)] Each value is expressed as mean ± S.D. (n=10) by Duncan's multiple analysis; Values within a transverse with the different superscripts are significantly different ( $\rho < 0.05$)

**Table 3.** Relative organ weight to body weight of SD rats after feeding 850°C LUKP WACFs in 28–day toxicity study

| Sex | Organ | Relative organ to body weight (%)[1)] – Dose (g/kg/day) | | | | |
|---|---|---|---|---|---|---|
| | | $H_2O$[1)] | Saline | 1.0 | 2.5 | 5.0 |
| Male | Heart | 0.39 ± 0.03[a3)] | 0.38 ± 0.06[a] | 0.35 ± 0.03[a] | 0.37 ± 0.05[a] | 0.36 ± 0.03[a] |
| | Liver | 3.46 ± 0.29[a] | 3.27 ± 0.22[a] | 3.30 ± 0.12[a] | 3.25 ± 0.29[a] | 3.18 ± 0.44[a] |
| | Spleen | 0.20 ± 0.03[a] | 0.18 ± 0.03[a] | 0.19 ± 0.03[a] | 0.18 ± 0.02[a] | 0.17 ± 0.01[a] |
| | Kidney | 0.99 ± 0.38[a] | 0.93 ± 0.05[bc] | 0.97 ± 0.08[ab] | 0.96 ± 0.05[ab] | 0.88 ± 0.04[c] |
| | Testes | 0.93 ± 0.13[a] | 0.93 ± 0.10[a] | 0.93 ± 0.13[a] | 0.92 ± 0.10[a] | 0.94 ± 0.10[a] |
| Female | Heart | 0.39 ± 0.03[a] | 0.36 ± 0.03[a] | 0.38 ± 0.04[a] | 0.38 ± 0.02[a] | 0.38 ± 0.04[a] |
| | Liver | 3.53 ± 0.41[a] | 3.43 ± 0.33[a] | 3.50 ± 0.27[a] | 3.48 ± 0.21[a] | 3.60 ± 0.37[a] |
| | Spleen | 0.23 ± 0.03[a] | 0.22 ± 0.02[a] | 0.25 ± 0.05[a] | 0.22 ± 0.03[a] | 0.23 ± 0.03[a] |
| | Kidney | 0.91 ± 0.06[a] | 0.87 ± 0.08[a] | 0.93 ± 0.05[a] | 0.88 ± 0.06[a] | 0.90 ± 0.10[a] |
| | Ovary | 0.07 ± 0.01[a] | 0.05 ± 0.01[a] | 0.06 ± 0.00[a] | 0.06 ± 0.02[a] | 0.06 ± 0.01[a] |

[1)] The percents (%) of relative weights of rat's organ equal to weights of organ divided by body weights then multiplied 100

[2)] Reverse osmosis water

[3)] Each value is expressed as mean ± S.D. (n=10) by Duncan's multiple analysis; Values within a transverse with the different superscripts are significantly different ( $\rho < 0.05$)

0.39% and 0.36–0.39% for female one; that of liver was 3.18–3.60% for both rats; that of spleen was 0.17–0.20% and 0.22–0.23% for female one; that of kidney was 0.87–0.99% for all rats; that of testes was 0.92–0.94% and that of ovaries 0.05–0.07% for female one. According to the aforesaid results of male SD and female SD rats found by testing Duncan's multiple analysis individually, the relative organ weight to body weight of each SD rat was similar, except for the weight percentage of the kidney for the male SD rats, but there was no significant difference between the saline and feeding of 850°C LUKP WACFs in 5.0 g/kg in the 28th day after testing by Duncan's multiple analysis. Therefore, it is inferred that the dose of 1.0–5.0 g/kg of for the test groups and the control groups had no toxicity in regard to the rats' organs.

The pathologic tissue slices of the items of the dose groups (1.0, 2.5, and 5.0 g/kg), control group and blank group of male SD rats included the heart, kidney, liver, spleen, and testes (male) / ovaries (female) results (Fig. 1 ×100). The myocardial cells of the dose, control, and blank groups of male SD rats had no ruptures and were not broken. The pattern was similar to the normal cardiac atlas of Histology and Cryobiology written by Abraham and Laura (2006). The renal corpuscles and renal tubule (including proximal and distal convoluted tubules) for all groups showed that they were complete without injury. It is indicated that 850°C LUKP WACFs do not cause a pathological change in the kidney. The hepatocyte at the liver and gall triad were not injured, and the blood cells in the vein were complete without hemolysis, as well, there were no fat and inflammatory reactions in the liver. The spleen's red pulp and white pulp were similar to the control/blank group, and there was no leukocytosis produced; therefore, there was no adverse effect on the spleen of the dose group fed with 850°C LUKP WACFs. The seminiferous tubules, including spermatogonia and sertoli cells, and the leydig cell among seminiferous tubules were complete and not ruptured as well. In terms of the ovarian mature follicle of female SD rats, the tissue observation results of the dose group and blank/control group were similar, as well; their ovarian cortex was a normal type of follicular. According to these results, feeding 850°C LUKP WACFs had no adverse effect on the organ functions of SD rats.

*Blood examination*

The results of male/female SD rats for the hematological value fed with different doses of 850°C LUKP WACFs are shown in Table 4. The WBC of male SD rats was 9.50–13.04 $10^3/\mu$L, RBC was 8.31–8.62 $10^6/\mu$L, HGB was 17.84–18.68 g/dL, HCT was 58.61–60.16%, MCV was 69.64–71.71 fL, MCH was 21.36–21.80 pg, MCHC was 30.24–31.14 g/dL, and PLT was 0.99–1.14 $10^3/\mu$L. For female SD rats, the WBC was 13.34–15.65 $10^3$/µL, RBC was 8.14–8.86 $10^6$/µL, HGB was 17.52–18.50 g/dL, HCT was 55.37–59.98%, MCV was 67.38–70.34 fL, MCH was 20.92–21.62 pg, MCHC was 30.77–31.69 g/dL and PLT was 0.93–1.18 $10^3/\mu$L. The WBC was about 13 $10^3/\mu$L, the RBC was in the range of 7.0–10.0 $10^6/\mu$L, the HGB was about 17.00 g/dL, the HCT was about 50%, the MCV was in the range of 53–73 fL, the MCH was about 5–10 pg, the MCHC was in the range of 29–34 g/dL, and the PLT was 0.5–1.3 $10^3/\mu$L, according to the hematological normal values of SD rats as indicated by the National Laboratory Animal Breeding and Research Center (1993). Compared with the values specified by the National Laboratory Animal Center, the WBC and MCH of the dose groups and blank/control groups of SD rats were a

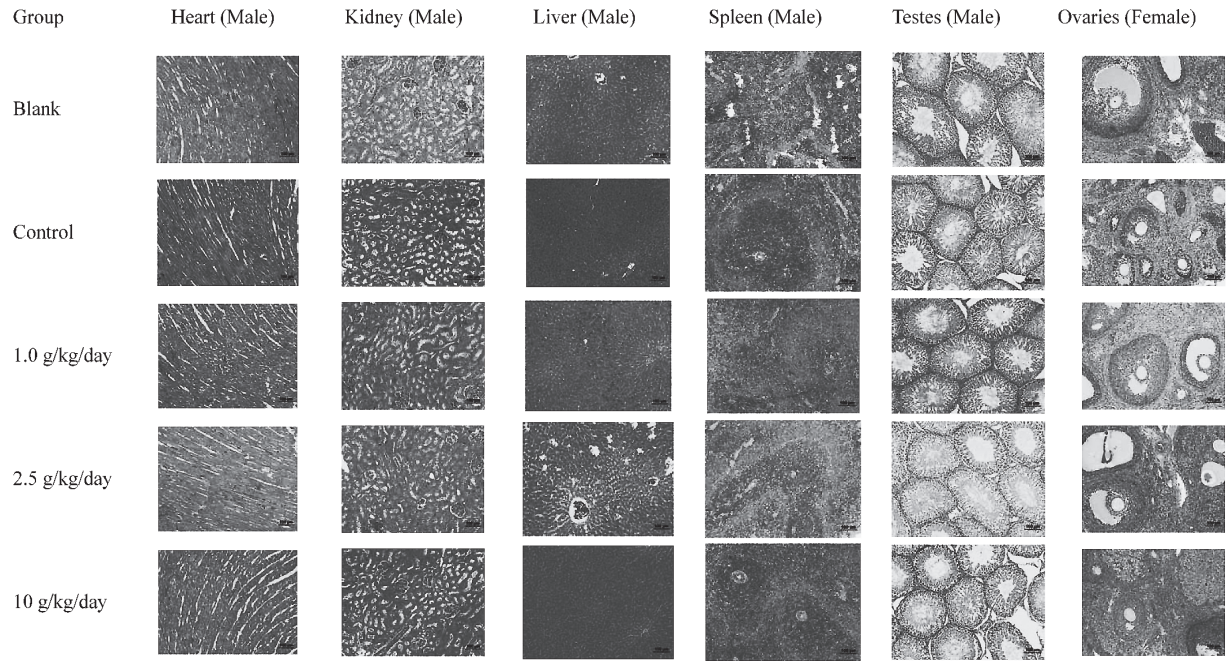


**Fig. 1.** Observations (× 100) on pathological tissue of SD rat by daily gavages for 850°C LUKP WACFs in a 28–day feeding toxicity study.

**Table 4.** Hematological value of SD rats after feeding 850°C LUKP WACFs in 28–day toxicity study

| Sex | Items[1)] | Hematological value – Dose (g/kg/day) | | | | |
|---|---|---|---|---|---|---|
| | | $H_2O$[2)] | Saline | 1.0 | 2.5 | 5.0 |
| Male | WBC ($10^3/\mu L$) | 13.04 ± 2.86[a3)] | 11.60 ± 2.47[ab] | 10.78 ± 4.06[ab] | 9.50 ± 1.46[b] | 13.03 ± 2.27[a] |
| | RBC ($10^6/\mu L$) | 8.49 ± 0.66[a] | 8.62 ± 0.51[a] | 8.42 ± 0.39[a] | 8.35 ± 0.23[a] | 8.31 ± 0.44[a] |
| | HGB (g/dL) | 18.19 ± 1.19[a] | 18.68 ± 0.80[a] | 18.24 ± 0.93[a] | 17.84 ± 0.62[a] | 18.11 ± 0.79[a] |
| | HCT (%) | 60.16 ± 4.55[a] | 60.08 ± 3.13[a] | 58.61 ± 2.54[a] | 58.61 ± 1.84[a] | 59.59 ± 3.50[a] |
| | MCV (fL) | 70.90 ± 1.98[a] | 69.73 ± 1.12[a] | 69.64 ± 2.25[a] | 70.23 ± 1.97[a] | 71.71 ± 2.42[a] |
| | MCH (pg) | 21.46 ± 0.62[a] | 21.70 ± 0.50[a] | 21.69 ± 1.05[a] | 21.36 ± 0.54[a] | 21.80 ± 0.53[a] |
| | MCHC (g/dL) | 30.24 ± 0.59[b] | 31.11 ± 0.53[a] | 31.14 ± 1.19[a] | 30.44 ± 0.52[ab] | 30.43 ± 0.89[ab] |
| | PLT ($10^3/\mu L$) | 1.02 ± 0.16[a] | 1.03 ± 0.08[a] | 1.00 ± 0.34[a] | 0.99 ± 0.29[a] | 1.14 ± 0.14[a] |
| Female | WBC ($10^3/\mu L$) | 13.34 ± 3.12[a1)] | 13.74 ± 2.62[a] | 15.65 ± 2.23[a] | 14.13 ± 3.51[a] | 14.31 ± 2.60[a] |
| | RBC ($10^6/\mu L$) | 8.14 ± 0.31b | 8.15 ± 0.45[b] | 8.27 ± 0.35b | 8.86 ± 0.38[a] | 8.29 ± 0.57[b] |
| | HGB (g/dL) | 17.61 ± 0.57[b] | 17.52 ± 0.74[b] | 17.89 ± 1.01[ab] | 18.50 ± 0.51[a] | 17.60 ± 1.08[b] |
| | HCT (%) | 57.30 ± 2.44[ab] | 55.37 ± 3.33[b] | 56.85 ± 2.34[b] | 59.98 ± 2.87[a] | 55.81 ± 3.80[b] |
| | MCV (fL) | 70.34 ± 1.86[a] | 67.94 ± 1.43[b] | 68.75 ± 1.35[b] | 67.74 ± 1.49[b] | 67.38 ± 1.38[b] |
| | MCH (pg) | 21.60 ± 0.43[a] | 21.52 ± 0.55[a] | 21.62 ± 0.67[a] | 20.92 ± 0.60[b] | 21.25 ± 0.70[ab] |
| | MCHC (g/dL) | 30.77 ± 0.58[b] | 31.69 ± 0.90[a] | 31.48 ± 0.88[ab] | 30.88 ± 0.89[b] | 31.55 ± 0.48[a]b[b] |
| | PLT ($10^3/\mu L$) | 1.18 ± 0.17[a] | 0.96 ± 0.26[a] | 1.15 ± 0.40[a] | 0.93 ± 0.28[a] | 1.04 ± 0.32[a] |

[1)] WBC: white blood cell count; RBC: red blood cell count; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet.

[2)] Reverse osmosis water

[3)] The results show mean ± S.D. by Duncan's multiple analysis, significant differences in data are represented by $\rho < 0.05$ between row sited by different alphabets (n=10).

higher value. However, Tsang's Veterinary Hematology of Chau–Loong Tsang indicates that the MCH varies with the MCHC (Tsang, 1994), and its clinical interpretation is less meaningful than the MCV and MCHC. The testing results of Duncan's multiple analysis showed no difference for the items of the hematological values between the values of all groups of SD rats with different feeding doses and blank/control groups. It is suggested that feeding different doses 1.0–5.0 g/kg/day of 850°C LUKP WACFs has no effect on the hematological value of SD rats.

*Blood serum biochemical analysis*

The results of the blood serum biochemical values are one of the examined safety indexes for biological safety assessment. For instance, the increase in the ALP resulted from the animal age (younger has higher values than usual) or hepatic disease (Jian, 1983). The ALT and AST are important indexes of hepatic necrosis examination; heart disease, injury, and tumors shall be identified before examination (Shen, 2002). The biochemical effects of different doses of 850oC LUKP WACFs on the blood serum of male/female SD rats is shown in Table 5. The ALP of male SD rats was 169.20–183.67 U/L, ALT was 40.57–48.00 U/L, AST was 204.60–257.60 U/L, BUN was 22.93–26.29 mg/dL, ALB was 4.92–5.08 g/dL, GLO was 1.73–2.05 mg/dL, A/G ratio was 2.48–2.91, CRE was 0.48–0.54 mg/dL, GLU was 101.50–171.00 mg/dL, T–BIL was 0.04–0.06 mg/dL, T–PRO was 6.79–7.09 mg/dL, and Na/K/CL was 151.87–153.06/7.21–7.68/93.72–96.66 mmol /L and Ca/P was 10.69–11.46/18.89–21.84 mg/dL. The results of various groups for male SD rats showed no significant differences in Duncan's multiple analysis. For female SD rats our study found: ALP: 107.70–128.30 U/L, ALT: 32.80–42.13 U/L, AST: 114.13–168.67 U/L, BUN: 23.74–26.82 mg/dL, ALB: 5.09–5.29 g/dL, GLO: 1.93–2.30 mg/dL, A/G ratio: 2.29–2.74, CRE: 0.50–0.56 mg/dL, GLU: 87.40–122.30 mg/dL, T–BIL: 0.07–0.08 mg/dL, T–PRO: 7.14–7.54 mg/dL, and Na/K/CL: 146.98–149.03/7.63–10.15/96.13–99.98 mmol/L and Ca/P: 9.79–11.07/13.58–15.44 mg/dL. These results show that they are in the range of the blood serum biochemical reference data of SD rats for the National Laboratory Animal Breeding and Research Center (1993), as well as similar to previous studies (Shen, 2006; Tsai *et al.*, 2005; Lin *et al.*, 2014).

## CONCLUSIONS

The observed body weight, tissue slice, and organ weight, as well as blood examination and blood serum biochemical analysis in the results, exhibited no significant differences to the SD rats in the control group. In terms of tissue pathology observation, there was no organopathy or inflammation in the maximum dose level group. These results indicate that the 850 LUKP WACFs produced no systematic poison for the experimental animals (a 28–day feeding study of Sprague–Dawley rats), and the atoxic dose was higher than 5.0 g/kg. It is suggested that the 850 LUKP WACFs is in the dose range (1.0, 2.5,

**Table 5.** Blood serum biochemical values of SD rats after feeding 850°C LUKP WACFs in 28–day toxicity study

| Sex | Items[1)] | The serum chemistry – Dose (g/kg/day) | | | | |
|---|---|---|---|---|---|---|
| | | $H_2O$[2)] | Saline | 1.0 | 2.5 | 5.0 |
| Male | ALP (U/L) | 183.67 ± 40.08[a3)] | 177.70 ± 42.49[a] | 169.20 ± 28.09[a] | 175.00 ± 29.61[a] | 171.4 ± 35.49[a] |
| | ALT (U/L) | 42.33 ± 5.07[ab] | 42.90 ± 5.00[ab] | 41.90 ± 4.61[b] | 40.57 ± 2.76[b] | 48.00 ± 9.20[a] |
| | AST (U/L) | 222.89 ± 69.54[a] | 257.60 ± 3.43[a] | 204.60 ± 30.70[a] | 206.00 ± 53.84[a] | 227.71 ± 63.67[a] |
| | BUN (mg/dL) | 22.93 ± 3.95[a] | 26.29 ± 3.43[a] | 25.24 ± 2.45[a] | 25.17 ± 0.18[a] | 25.96 ± 2.57[a] |
| | ALB (g/dL) | 5.08 ± 0.17[a] | 4.92 ± 0.30[a] | 5.07 ± 0.23[a] | 4.93 ± 0.18[a] | 5.06 ± 0.13[a] |
| | GLO (mg/dL) | 1.81 ± 0.22[ab] | 2.05 ± 0.32[a] | 2.02 ± 0.27[a] | 1.91 ± 0.23[ab] | 1.73 ± 0.13[b] |
| | A/G ratio | 2.84 ± 0.36[a] | 2.48 ± 0.55[a] | 2.54 ± 0.42[a] | 2.60 ± 0.36[a] | 2.91 ± 1.12[a] |
| | CRE (mg/dL) | 0.54 ± 0.05[a] | 0.54 ± 0.08[a] | 0.54 ± 0.06[a] | 0.49 ± 0.06[a] | 0.48 ± 0.03[a] |
| | GLU (mg/dL) | 109.11 ± 46.71[b] | 101.50 ± 43.57[b] | 135.40 ± 51.55[ab] | 171.00 ± 50.17[a] | 115.71 ± 44.10[b] |
| | T–BIL (mg/dL) | 0.06 ± 0.01[a] | 0.06 ± 0.02[a] | 0.07 ± 0.01[a] | 0.04 ± 0.02[b] | 0.05 ± 0.00[ab] |
| | T–PRO (mg/dL) | 6.89 ± 0.30[ab] | 6.97 ± 0.28[ab] | 7.09 ± 0.25[a] | 6.84 ± 0.21[ab] | 6.79 ± 0.18[b] |
| | Na (mmol/L) | 152.40 ± 1.50[a] | 152.45 ± 1.41[a] | 152.00 ± 1.47[a] | 153.06 ± 0.91[a] | 151.87 ± 1.42[a] |
| | K (mmol/L) | 7.23 ± 0.73[a] | 8.28 ± 4.16[a] | 7.68 ± 0.93[a] | 7.26 ± 0.87[a] | 7.21 ± 1.10[a] |
| | Cl (mmol/L) | 94.68 ± 1.37[b] | 94.49 ± 2.05[b] | 93.72 ± 2.30[b] | 95.20 ± 1.69[ab] | 96.66 ± 1.05[b] |
| | Ca (mg/dL) | 10.96 ± 1.47[a] | 11.46 ± 0.64[a] | 10.69 ± 2.20[a] | 11.69 ± 0.72[a] | 11.24 ± 0.24[a] |
| | P (mg/dL) | 21.40 ± 3.00[a] | 21.42 ± 2.90[a] | 21.84 ± 3.06[a] | 19.87 ± 2.74[a] | 18.89 ± 1.69[a] |
| Female | ALP (U/L) | 107.70 ± 25.24[a1)] | 121.20 ± 36.20[a] | 118.30 ± 27.68[a] | 112.10 ± 34.74[a] | 128.30 ± 39.40[a] |
| | ALT (U/L) | 33.89 ± 11.48[b] | 33.60 ± 4.35[b] | 32.80 ± 4.44[b] | 35.30 ± 5.87[ab] | 42.13 ± 11.04[a] |
| | AST (U/L) | 168.67 ± 54.96[a] | 148.80 ± 31.5[ab] | 150.70 ± 56.38[ab] | 121.80 ± 12.59[b] | 114.13 ± 25.20[b] |
| | BUN (mg/dL) | 25.30 ± 4.58[a] | 23.74 ± 2.48[a] | 26.82 ± 5.47[a] | 24.71 ± 4.78[a] | 25.00 ± 3.13[a] |
| | ALB (g/dL) | 5.29 ± 0.36[a] | 5.21 ± 0.24[a] | 5.09 ± 0.45[a] | 5.42 ± 0.28[a] | 5.21 ± 0.20[a] |
| | GLO (mg/dL) | 2.26 ± 0.26[ab] | 2.29 ± 0.21[ab] | 2.30 ± 0.57[a] | 2.19 ± 0.37[ab] | 1.93 ± 0.24[b] |
| | A/G ratio | 2.38 ± 0.37[a] | 2.29 ± 0.21[a] | 2.35 ± 0.62[a] | 2.56 ± 0.52[a] | 2.74 ± 0.35[a] |
| | CRE (mg/dL) | 0.53 ± 0.08[a] | 0.54 ± 0.06[a] | 0.50 ± 0.08[a] | 0.56 ± 0.07[a] | 0.50 ± 0.08[a] |
| | GLU (mg/dL) | 101.22 ± 39.71[a] | 87.40 ± 24.71[a] | 96.60 ± 54.22[a] | 122.30 ± 29.10[a] | 96.88 ± 40.17[a] |
| | T–BIL (mg/dL) | 0.07 ± 0.02[a] | 0.07 ± 0.02[a] | 0.08 ± 0.03[a] | 0.07 ± 0.02[a] | 0.07 ± 0.02[a] |
| | T–PRO (mg/dL) | 7.54 ± 0.25[a] | 7.50 ± 0.29[a] | 7.39 ± 0.41[ab] | 7.61 ± 0.37[a] | 7.14 ± 0.30[b] |
| | Na (mmol/L) | 149.03 ± 1.15[a] | 148.11 ± 1.69[ab] | 147.54 ± 2.16[ab] | 147.08 ± 2.14[b] | 146.98 ± 1.17[b] |
| | K (mmol/L) | 7.63 ± 1.29[b] | 8.23 ± 0.84[b] | 8.83 ± 1.70[ab] | 9.13 ± 2.64[ab] | 10.15 ± 1.50[a] |
| | Cl (mmol/L) | 96.23 ± 1.08[b] | 97.20 ± 1.92[b] | 96.13 ± 1.75[b] | 97.57 ± 1.53[b] | 99.98 ± 2.49[a] |
| | Ca (mg/dL) | 10.82 ± 1.27[a] | 11.07 ± 0.63[a] | 10.47 ± 1.51[a] | 10.86 ± 1.36[a] | 9.79 ± 2.15[a] |
| | P (mg/dL) | 14.99 ± 0.86[ab] | 15.44 ± 1.40[a] | 14.49 ± 1.57[ab] | 13.73 ± 1.86[b] | 1.04 ± 0.32[a] |

[1)] ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; ALB: albumin; GLO: globulin; A/G ratio: albumin/globulin ratio; CRE: creatinine GLU: glucose; T–BIL: total bilirubin; T–PRO: total protein; Na: sodium; K: potassium; Cl: chloride; Ca: calcium; P: phosphorus. [2)] Reverse osmosis water. [3)] The results show mean ± S.D. by Duncan's multiple analysis, significant differences in data are represented by $\rho < 0.05$ between row sited by different alphabets (n=10).

and 5.0 g/kg/day) of biological safety assessment. In other words, WACFs prepared from wood pulp can be a potential type of food moisture–proof material and a potential type of material for water purification for water quality improvement.

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