Heamatotoxicity of Paint Effluent on Swiss Albino Mice.

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ABSTRACT

This study investigated the compositions and immunotoxic potentials of a paint effluent collected from Golden Key Paints along Lagos State University (LASU), road Akesan, Lagos. A total of 20 laboratory mice (about 30 days old) were obtained from Nigerian Institute of Medical Research, Yaba, Lagos. The mice were in four groups comprising of five mice each. The control group (group 1) was given distilled water, while groups 2 to 4 were given 25, 50, 75, and 100% v/v of the paint effluent, respectively, as the only sources of water. At the end of the 4th week, the control mice (given no effluents) had live birth of 15 young mice (babies) while the mice treated with paint effluent had no birth. At least one mouse died in each cage of treated group.

The relative growth rate (RGR) in percentage of the control mice was 2.1, while the RGR of the mice exposed to 25, 50, 75 and 100% v/v of the paint effluent were -18.2, -21.8, -28.9 and -33.9 respectively. A significant difference ($P < 0.05$) exists between the mean weight of the control mice and mice placed on the different concentrations of the paint effluent. Hematological examination reveals that the white blood cells, total proteins and differential counts were significantly high and different ($P < 0.05$) when compared with the control group. These indicated anaemic condition and the struggle to fight against the foreign substances by the treated mice. The constituents of the paints effluents probably aggravated the observed changes in the blood parameters. These results show that paint effluent had detrimental effects on treated mice and these accentuate the needs for proper handling and disposal of paint effluent.

(Keywords: packed cell volume, differential counts, anaemic, paint effluent)

INTRODUCTION

In recent years, there has been an increasing awareness of the genotoxic potential of a wide variety of wastes to which the human population is exposed to, whether environmentally or occupationally. The awareness is paralleled by the recent development of appropriate, sensitive and practical methods of detecting and estimating the effects of these substances (Odeigah *et al*., 1997).

Industrial waste/effluents are complex mixtures of several classes of pollutants including synthetic chemicals of various description, hydrocarbons and heavy metals as confirmed by Odiete (1999). Paint industries release effluents that can be solid, liquid or gas in the form of organic or inorganic substances. Bhaluro and Adeko (1981) and Lori (1991) recorded that of all the three forms of pollutants discharged by a paint industry, the liquid effluents are by far the most significant.

Most Industries discharge their untreated effluents through drains or canals into the nearest water body e.g. streams. In Nigeria, the major streams in the industrial areas of big cities like Lagos, Kano and Kaduna are seriously polluted by waste from industrial sources (Nriagu, 1980 and Ajayi and Osibanjo, 1981). Industrial chemicals had been strongly implicated in metal pollution of water bodies (Arms and camp, 1982). Arsenic acid poison comes from white Arsenic (arsenic trioxide), which is used in manufacturing Arsenical pesticides and herbicides.

The biological effects of heavy metals range from beneficial stimulation of health to harmful retardation and death. Bergquivist and Sundbom (1980) observed that at 1.0mg/L and higher concentration of copper, Allium root growth is strongly restricted and prolonged treatment causes root death. Haematological parameters are a sensitive index to changes in ecological

conditions and can constitute an important diagnostic tool in toxicological studies (Calstus *et al.,* 2002).

Much work has not been carried out on the effect of paint effluents on the weight and haematology of animals. Some industries release effluent such as paint effluent unto vegetation leading to high deposit of contaminants (bioaccumulation) in the plants or in the soil. When such plants are eaten by animals or humans, it will have adverse effect on them by the contaminants accumulating and biomagnifying in the vital organs leading to acute toxicity. It is against this background that this study was undertaken to investigate the deleterious effects of paint effluent on the weight and heamatology of mice. This work is an attempt to investigate the contaminants of paint effluents and also assess the heamatotoxic effect of this Paint Effluent on the Swiss albino mice, respectively.

MATERIALS AND METHODS

Sources and Collection of Test Effluent / Test Organism

The paint effluent used in this study was collected in plastic containers from main discharge point of Golden Key Paints, along Lagos State University (LASU) Road Akesan, Lagos, Nigeria. The test organism used were Albino mice collected from the Nigerian Medical Research (NIMR), Yaba Lagos, Nigeria. Swiss mice (about 30 days old) of either sex, bred in the Animal house, University of Lagos, Nigeria, were used for the research. They were housed in suspended, meshed- bottom cages and were left for about seven days to acclimatize before the commencement of the research. Pelletized feeds from FA Feeds, Lagos, were given to the mice *ad libitum.* F.A Feeds, Lagos) ***ad libitum*** in suspended, meshed-bottom cages.

Experimental Technique and Animal Exposure

The effluent samples were analyzed for a number of standard physical and chemical properties according to FEPA (1991) (Table 1). The procedures for physico-chemical analysis followed those described by Reish and Oshida (1986) and it includes pH, conductivity, alkalinity and total dissolved solids using pH meter and conductivity meter. The metal contents of the neat effluent were analyzed at the Environmental Analytical Laboratory of the Department of Chemistry, University of Lagos using Atomic Absorption Spectro-photometer (AAS) Model 969.

Four concentrations (25, 50, 75, and 100%v/v) of the paint effluent were prepared. The mice were grouped into four sets of 5 mice each after taken their initial weights. The control mice were resided in cage 1 and were given distilled water. The exposed mice in cages 2, 3, and 4 were given 25, 50, 75, and 100%v/v of the paint effluent, respectively, as the only sources of water. The experiments commenced a week after the purchase of the mice and were allowed to stay for about four weeks. At the end of the 4^{th} week, the final weights of the mice were taken, and their blood Parameters (PCV, HB, WBC, RBC and Differential counts) were examined in the laboratory.

The mice were sedated with chloroform in the laboratory. Total death was prevented to allow continuous flow of blood for proper blood collection. Each mouse was pegged down on a work bench, and held firmly with office pins. Surgical blades were used to cut through the chest region of the mice in a dorsal-ventral direction. The blood was then collected from a beating heart using a Na Heparinized capillary tube through capillary action into EDTA bottles. EDTA serves as anti-coagulant and also the Na heparin in the capillary tube. The blood parameters (PCV, HB, WBC, RBC and Differential counts) were determined using Sysmex auto-analyzer. Total counts were made with a bright- line Neubaeur haemocytometer.

Blood smears were made on cover slips and stained with Wright's stain. Differential counts were made by the 4-field meander method, then working back to the edge alternatively and finding the average percentage of cells counted in ten fields.

Calculation of Relative Growth Rate [RGR]

The relative growth rates of the mice were calculated using the formula below:

$$RGR\ [\%] = \frac{W_F - W_I}{T} \times 100$$

Where W_F =Final weight, W_I=Initial weight and T = Period of exposure.

Table 1: Physico-Chemical Characteristics of Golden Key Paint Effluent.

Parameter	Unit	Level Detected	FEPA'S Limit
pH	-	7.4	6-9
Color	-	Blue	NS
Conductivity	μscm^{-1}	780.0	NS
Alkalinity	mg/L	*150.0	20
Total Dissolved Solids	mg/L	390.0	2,000
Zinc	mg/L	35.6	< 1.0
Copper	mg/L	14.3	< 1.0
Lead	mg/L	2.1	< 1.0

NOTE: ND - Not Detected; NS - Not specified; * - With concentrations above statutory limits (Fepa's limit indicates the effluent limitation guide lines).

Statistical Analysis

All statistical analysis was carried out with the Microcal Origin 5.0. Comparison of data among exposed and control groups were calculated using Analysis of variance. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Table 2 showed that the different concentrations of the paint effluent had adverse effects on the weights of the mice. For example, the mean weight change of the control mice was 0.6g, while the mean weights change of the mice that were placed on 25, 50, 75 and 100% v/v solutions of the paint effluent were -5.1g, -6.1g, -8.1g and -9.5g, respectively. Also, the relative growth rate of the control mice in percentage was 2.1, while the relative growth rates of the mice exposed to 25, 50, 75, and 100% v/v of the paint effluent were -18.2, -21.8, -28.9, and -33.9, respectively.

It was observed that some of the mice treated with the paint effluents died and only the females in the control groups were pregnant. The hematology examinations of the exposed mice to the paint effluents showed marked reduction in the blood parameters. The mean PCV change of the control mice was 2.5%, whereas the mean PCV change (lost) by the mice placed on 25, 50, 75, and 100% v/v of the paint effluent were -9.7, -14.5, -17.4 and -21.5%, respectively (Table 3). However, at $P<0.05$ the mean change in the HB of the control mice was 0.7g/dl, while the mean HB change of the mice exposed to 25, 50, 75 and 100% v/v of the paint effluent were -3.8, -5.0,-5.6 and -6.0 g/dl, respectively (Table 4). Furthermore, the control mice showed mean WBC increase of $50mm^3$, whereas the mice treated with 25, 50, 75 and 100% v/v of the paint effluent showed mean WBC increase of 2380,2930, 3250 and $3800mm^3$, respectively (Table 5).

From Table 6, the mean RBC change for the control was 0.40×10^6, while the mean RBC lost by the mice exposed to 25, 50, 75 and 100% v/v of the paint effluent were -1.6, -2.3, -3.0 and -3.3 $x10^6$ respectively. The mean total protein change of the control mice at the end of the exposure was -0.02g/dl, while the mean total protein lost by the mice placed on 25, 50, 75 and 100% v/v of the paint effluent were -0.84, -0.90, 1.40 and 1.90g/dl, respectively (Table 7). In the analysis of differential count of the leucocytes, there was a statistically significant ($P< 0.05$) increase in peripheral blood neutrophils, monocytes, lymphocytes and eosinophils at all concentrations tested. However, there was a concentration dependent decrease in basophil count (statically significant at $P < 0.05$) when compared with the values of the control group.

Table 2: Effects of the Different Concentrations of the Paint Effluent on the Weights of the Mice.

Paint Effluent Concentrations/ Distilled water	Minimum Weight (g)	Maximum Weight(g)	0 day Mean Weight	4th week Mean Weight	Mean Weight Change (g)	Relative Growth Rate
Distilled water (control)	30.1	34.2	30.1 ± 0.04	30.70 ± 1.16	0.6	2.1
25%	21.3	29.2	29.4 ± 0.09	24.30 ± 0.89*	-5.1	-18.2
50%	17.2	28.3	29.1 ± 0.05	23.0 ± 1.38*	-6.1	-21.8
75%	14.4	28.1	28.9 ± 0.03	20.80± 0.73*	-8.1	-28.9
100% (neat)	12.8	27.7	28.5 ± 0.16	19.0 ± 0.66*	-9.5	-33.9

- Data were expressed as MEAN ± SEM
- When * P<0.05 = Significantly different from control and When P>0.05 = Not significantly different from control

Table 3: Effects of the Different Concentrations of the Paint Effluent on the PCV (%) of the Mice.

Paint Effluent Concentrations/Distilled Water	Mean PCV at 0 day	Mean PCV at 4th week	Mean PCV Change
Distilled water (control)	49.5 ±0.12 (38.0 – 54.0)	52.0 ±0.70	2.5
25%	47.9 ± 0.14	38.2 ±1.20*	-9.7
50%	49.0 ± 0.22	34.50 ± 1.17*	-14.5
75%	48.0 ± 0.38	30.60 ± 1.32*	-17.4
100%	49.5 ± 0.20	28.0 ± 1.39*	-21.5

- Data were expressed as MEAN **±** SEM
- When * P<0.05 = Significantly different from control and When P>0.05 = Not significantly different from control
- Values in bracket show the accepted International limit. (Kawasaki, 1994)

Table 4: Effects of the Different Concentrations of the Paint Effluent on the HB (g/dl) of the Mice.

Paint Effluent Concentrations/Distilled Water	Mean HB at 0 day	Mean HB at 4th week	Mean HB Change
Distilled water (control)	15.8 ±0.33	16.5 ±0.20	0.7
25%	14.8 ± 0.18	11.0 ±0.24*	-3.8
50%	15.8 ± 0.21	10.8 ± 0.34*	-5.0
75%	16.2 ± 0.10	10.6 ± 0.35*	-5.6
100%	16.2 ± 0.19	10.2 ± 0.23*	-6.0

- Data were expressed as MEAN ± SEM
- When * P<0.05 = Significantly different from control and When P>0.05 = Not significantly different from control

Table 5: Effects of the Different Concentrations of the Paint Effluent on the WBC (mm^3) of the Mice.

Paint Effluent Concentrations/Distilled Water	Mean WBC at 0 day	Mean WBC at 4th week	Mean WBC Change
Distilled water (control)	5150 ± 220	5200± 232	50
25%	5220 ± 217	7600 ± 250*	2380
50%	5270 ± 234	8,200 ± 340*	2930
75%	5350 ± 246	8,600 ± 352*	3250
100%	5400 ± 251	9,200 ± 376*	3800

- Data were expressed as MEAN ± SEM
- When * P<0.05 = Significantly different from control and When P>0.05 = Not significantly different from control

Table 6: Effects of the Different Concentrations of the Paint Effluent on the RBC(x $10^6/mm^3$) of the Mice.

Paint Effluent Concentrations/Distilled Water	Mean RBC at 0 day	Mean RBC at 4th week	Mean RBC Change
Distilled water (control)	7.00 ± 0.28	7.40 ± 0.30	0.40
25%	6.60 ± 0.31	5.00 ± 0.38*	-1.60
50%	6.80 ± 0.34	4.50 ± 0.41*	-2.30
75%	7.00 ± 0. 35	4.00± 0.45*	-3.00
100%	7.10 ± 0.38	3.80 ± 0.49*	-3.30

- Data were expressed as MEAN ± SEM
- When *P<0.05 = Significantly different from control and When P>0.05 = Not significantly different from control

Table 7: Effects of the Different Concentrations of the Paint Effluent on the Total Protein (mg/dl) of the Mice.

Paint Effluent Concentration/ Distilled Water	Mean Total Protein at 0 day	Mean Total Protein at 4th week	Mean Total Protein Change
Distilled water (control)	6.82 ± 0.03	6.80 ± 0.07	-0.02
25%	6.86 ± 0.08	7.40 ± 0.10*	-0.84
50%	6.90 ± 0.20	7.80 ± 0.21*	-0.90
75%	7.00 ± 0.27	8.40 ± 0.33*	1.40
100%	6.90 ± 0.29	8.80 ± 0.35*	1.90

- Data were expressed as MEAN ± SEM
- When * P<0.05 = Significantly different from control and When P>0.05 = Not significantly different from control

Table 8: Changes Observed in the Differential Counts in Rats Exposed to Different Concentrations of Paint Effluent.

Paint Effluent Concentrations/ Distilled water	Neutrophils (%)	Lymphocytes(%)	Monocytes (%)	Basophils (%)	Eosinophils (%)
Distilled water(control)	56± 2.20	32±3.40	2.5±1.90	1.0 ±0.5	2.6±2.60
25%	59±1.80*	38±3.12*	4.0±2.23*	1.2±3.21*	3.2±1.78*
50%	62±2.10*	39±2.18*	5.0±2.12*	0.70±2.76*	4.0±1.48*
75%	64±1.69*	40±2.15*	7.0±1.60*	0.50±2.89*	4.0±2.30*
100%	66±2.90*	44±1.70*	8.0±1.50*	0.24±2.20*	7.0±2.40*

NOTE: N = Neutrophil, L = Lymphocytes, M = Monocyte, B = Basophils, E = Eosinophils

- Data were expressed as MEAN **±** SEM
- When *P<0.05 = Significantly different from control and When P>0.05 = Not significantly different from control

One of the ways to establish the immunotoxic capabilities of an effluent, chemical or waste in animals is a change in the cellular components of the blood, especially in leucocytes counts (Bakare *et al*., 2009). This study exposed mice to different paint effluents concentrations. An assessment of the blood parameters including their weight change was made. The results obtained indicated that the paint effluents caused a remarkable change in the weights and blood parameters of the mice. Increased White blood cell counts and Differential counts show that there was no inhibitory effect on bone marrow production of white blood cells to fight against the foreign substance (paint effluent).

The values obtained from the blood parameters showed high rate of anaemia in the animals. Also, in order to defend their body system against the foreign substance, the level of white blood cells and differential counts tend to increase indicating an infection. The increase in the white blood cells (WBC) of the exposed mice was as a result of immune response of the mice to the toxic components of the paint effluent.

A concentration dependent increase in mean leucocyte count (WBC) observed is contrary to those reported in humans exposed to marijuana smoke (Oseni and Taiwo, 2006). The results of the differential counts showed neutrophilia (an increase in the number of circulating blood neutrophils). Bakare *et al.,* (2009) also observed similar results in rats exposed to municipal solid waste leachates. A significant decrease in basophils observed in this study is in agreement with the report on workers occupationally exposed to cement dust (Jude *et al*., 2002). The marked reduction in the blood parameters (PCV, HB, WBC, RBC and Total Protein) of the exposed mice compared to the control mice confirms the earlier report of Wadaan and Mubarak (2009), who reported blood parameters reduction in rabbit exposed to Sodium Dodecyl Sulfate (surfactant).

The toxic effect of detergent has also been reported by Dehelean *et al*. (2004) and Yahaya *et al*. (2011), to cause reduction in blood parameters and weight loss in mice. The remarkable change in the blood parameters in this study was probably as a result of the traces of heavy metals (Zn, Cu and Pb) found in the paint effluent. This is in corroboration with the report by (Abulude *et al.*, 2007) that the surfactant and traces amount of heavy metals found in the detergents must have led to the destruction of the cells of the mice.

The growth reduction observed in this study might have resulted from poor physiological conditions of the exposed mice which led to high metabolic demand. This is supported by the report made by Ogunbileje and Akinosun (2011) mice exposed to pollutants can develop certain physiological changes such as sluggishness and loss of appetite. The high mortality rate observed in the exposed mice was direct consequences of

the impaired physiological conditions of the mice by the effluent. Ogunbileje and Akinosun (2011) also reported low fecundity and high mortality in mice exposed to cement dust containing some heavy metals.

CONCLUSION AND RECOMMENDATION

The study has shown that exposure to industrial effluent is highly dangerous to human health. In the course of this study as stated earlier only untreated mice were pregnant and had live births. Some of the treated mice died as well. As a result, more work need to be done to evaluate the effects of paint effluents on the reproductive system and general body physiology on prolonged exposure.

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