

## Effects of 1,4- Dioxane on Some Blood Parameters of the Swiss Albino Mice

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### **Abstract:**

Maximum tolerated dose (MTD) of 1,4-dioxane in distilled water (5.7mg/kg) forced fed into male mice twice a week for 8 weeks (total 16 doses). Total red blood cells (RBCs), hemoglobin content (Hb), haematocrite value (Hct,%), mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), in addition to the total and differential white blood cells (WBCs) counts were studied as compared to control animals. Multiple exposure of the mice to this specified dose found to express a significant influence in most of blood parameters. Toxicity with 1,4-Dioxane (5.7 mg/kg) induced: 1)- decreases in total RBCs count ,Hb , Hct,% and all investigated blood indices, 2)- The total WBCs was increased significantly and 3) - The differential leucocytes count revealed that a decreased in lymphocytes % and unlike to neutrophils, and monocytes were increased while eosinophil and basophile showed non-significant effects when compared with control group.

**Keywords:** Dioxane, blood parameters & mice.

### **Introduction:**

1,4-dioxane is a colorless liquid with a mild ether-like odor. It is used as a solvent and in textile processing, printing processes and detergent preparations. 1-4-Dioxane is also present in ordinary household products like shampoos (50,000-300,000 ppb), liquid/dishwashing soap(2,000-65,000 ppb), baby lotion(11,000 ppb), hair lotions(47,000-108,000 ppb), bath foam (22,000-41,000 ppb) and other cosmetic products (6,000-160,000 ppb). Also , 1,4-Dioxane is found in manufactured food additives (at 10,000ppb level ) and in shrimp, chicken, tomatoes,coffee and some condiments (Sack and Steele,1989). Dioxane reported that, it has been frequently emitting by carpets and draperies (Rogozen et al.,1987; Bayer, and Papanicolopoulos, 1990; CTCP,1990 and HSDB, 1990).

Dioxane is absorbed by all routes of administration (HSDB,1995). Barber(1934) described dioxane exposed factory workers ,some of them exhibited signs of liver changes, increased urinary protein and increased white blood cell counts, and some of them died from apparent acute exposures. Due to accidental or voluntary ingestion of Roundup (Glyphosate) contaminated with dioxane were induced red blood cell destruction, low blood pressure and kidney failure or damage(EPA,1999; Meister,2000 and Williams et al,2000).

1-4,dioxane has been reported to cause reproductive toxicity, teratogenic and mutagenic effect in animals or humans. Inhalation exposure of higher dose level (>5000 ppm), can cause irregular heart beat, kidney and liver damage, fall in blood pressure and even death. Ingestion of 1,4-dioxane may cause moderate decrease in Hemoglobin and red blood cell counts(MSDS,1997).

1, 4-dioxane listed at the technical reports as one of the twelve chemicals inducing nasal tumors to male and female rat but the nasal carcinogenicity test found to be negative when tested in male and female mice (Haseman et al., 1990 and Haseman and Hailey,1997). In a mortality study of 165 workers who had been exposed to low concentrations of 1,4-dioxane since 1954, seven deaths had occurred by 1975, two of which were from cancer (Buffler, et al, 1978). EPA (1995) classifies 1,4-dioxane as B2, a probable human carcinogen, based on the induction of nasal cavity and liver carcinomas in multiple strains of rats, liver carcinomas in mice, and gall bladder carcinomas in guinea pigs. Also IRAC (1999) has classified 1,4-Dioxane in group 2B(possibly carcinogenic to humans).

Administration of 1,4-dioxane in drinking water at several dose levels to rats and male guinea-pigs produced adenomas and carcinomas of the liver in rats of each sex, hepatomas in guinea-pigs, carcinomas of the nasal cavity in male and female rats and carcinomas of the gall-bladder in guinea-pigs. It increased the incidence of skin tumors in mice when applied after 7,12-dimethylbenz[a]anthracene (IARC, 1976).In a mouse-lung adenoma assay, 1,4-dioxane produced a statistically significant increase in the incidence of tumors in males given an intermediate intraperitoneal dose (Stoner et al; 1986). It induced DNA strand breaks in rat hepatocytes in vitro. It induced chromosomal aberrations in plants (IARC,1987a). Micronucleus assay in

mouse bone marrow cells revealed a significant increase in the percentages of polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) over control group (Moussa and El-Habit,2005). This study aims to investigate effects of 1,4-dioxane on some hematological parameters of the Swiss albino mice.

## **Materials and Methods**

### **Dioxane:**

The chemical formula of 1,4-dioxane is  $C_4H_8O_2$ , (Merck, 1989). 1,4-dioxane (Sigma) dissolved in distilled water, 5.7 mg/ kg is the maximum tolerated dose (MTD) NTP, (1985). Synonyms of Dioxane as written in (IRAC,1987b) was Diethylene dioxide, 1,4-Diethylene dioxide, Diethylene ether, Di(ethylene oxide) 1,4-Dioxacyclohexane, Dioxan, 1,4-Dioxan, p-Dioxan, Dioxane, p-Dioxan, Dioxyethylene ether, Glycol ethylene ether, Tetrahydro-1,4-dioxin and Tetrahydro-p-dioxin.

### **Animals:**

Male albino mice, *Mus musculus*, (2 months old, 20-25 g) were obtained from an inbred strain in the college of Veterinary Medicine, King Faisal University (Al-Ahssa, Saudi Arabia). Mice housed at room temperature (20-22 C<sup>0</sup>), with light regime of 14 h light and 10 h darkness, in different stainless steel cages containing hard wood chips and five animals/ cage.

Animals in all groups were given a basal diet composed of 60 % of ground corn meal, 15% ground beans, 10% wheat bran, 10% corn oil, 3% casein, 1% mineral mixture, and 1% vitamin mixture. Water was given at libitum.

### **Experimental animals:**

This group of animals (60 male mice) given 5.7mg/kg 1,4-dioxane which is the maximum tolerated dose (MTD) forced fed to mouse stomach by gavages twice a week for 8 weeks.

### **Control animals:**

Another group of 20 male mice served as control forced fed the same amount of distilled water in the experimental animals twice a week for 8 weeks.

**Hematological studies:**

Blood collection using the saphenous puncture (Hem et., al. 1998) for blood sampling of the mouse (superficial veins of the hind limb) using heparinized tubes .As a general rule, a blood volume equivalent to about 0.5% of the animal's body weight may be safely drawn, as a single sample (Wolfensohn & Lloyd 1994), and this can usually be repeated at fortnightly intervals without disturbances to the animal's hematological status.

Counting of RBCs and WBCs was carried out manually by Neubaur chamber, using Hayem's solution for RBCs count and Turkey's solution for WBCs count (Sheirley, 1975, Krieg, 1979, Freshney , 1987; and Brecher et. al., 1992). The haematocrite value (Hct,%) was determined according to (Dacie and Lewis,1991) and the haemoglobin (Hb) was measured as described by Lucky (1977). Also , mean corpuscular volume (MCV), mean cell haemoglobin concentration(MCHC), mean cell haemoglobin (MCH) and total and differential leucocytes were calculated according to Dacie and Lewis (1991). The results were analyzed statistically using Student's two tailed- test (Flower and Cohen,1997).

**Results**

All investigated blood parameters are recorded in (Table 1 & 2). The total count of RBCs in the experimental mice showed a fluctuated results in the first time of experiments. At the end of the experiment, the present data showed very highly significant decrease in RBCs (Table1) There was highly significant decrease in Hb ( $P<0.01$ ) and Hct,% ( $P<0.001$ ) in experimental group compared the control.

The calculations of the three absolute values of the erythrocyte indices, MCV, MCH and MCHC were carried out. MCV showed fluctuated results initially, then their data showed a non significant decrease followed by very high significant decrease( $P<0.001$ ) at the end of the experiment. MCH showed a similar pattern to MCV. MCHC showed a fluctuated results with significant increase( $P<0.05$ ) at the third week , followed by nearly stable values and finally showed highly significant decrease ( $P<0.01$ ) at the end of the experiment.

**Table (1)**  
Changes in RBCs, Hct, % Hb and blood indices (MCV, MCH & MCHC) in control and 1,4-dioxane treated male mice

Test	C	1 <sup>st</sup> W	2 <sup>nd</sup> W	3 <sup>rd</sup> W	4 <sup>th</sup> W	5 <sup>th</sup> W	6 <sup>th</sup> W	7 <sup>th</sup> W	8 <sup>th</sup> W
		1,4-dioxane treated group							
RBCs 10 <sup>9</sup> /μl	10.6±0.43	9.4±0.96	10.2±0.98	10.9±0.98	10.7±1.2	9.4±0.26 *	8.8±0.2 **	8.1±0.12 ***	7.8±0.08 ***
Hct,%	51.5±0.35	49.6±1.54	52±0.86	51±0.6	50±1.42	46±0.74 **	43±0.29 ***	38.5±0.28 ***	37±0.23 ***
Hb	14.2±0.67	13±1.26	14±0.42	15±0.86	14.6±0.92	12.6±0.44	11.5±0.23 **	10.4±0.64 *	10±0.48 **
MCV μ <sup>3</sup>	49±1.20	48.5±0.76	50±0.28	51±0.62	50±0.54	47±0.52	47.1±0.29 *	44.2±0.82 *	43.6±0.3 ***
MCH pg	13.4±0.78	12.3±0.34	13.3±0.46	14±0.28	13.7±0.8	12.4±0.08 **	12±0.09 **	11.7±0.38 *	11.6±0.09 ***
MCHC g/dl	28±0.14	26±1.2	28±1.04	28.5±0.08 *	29±0.36	26.3±1.2	25.5±0.64	27±0.22 *	26.2±0.2 **

W= week

C= control

Results = Mean ± SD / n=5

\*= significant ( $P < 0.05$ ).

\*\* = Highly significant ( $P < 0.01$ ).

\*\*\* = very highly significant ( $P < 0.001$ )

Significance was calculated between control and treated groups

**Table ( 2 )**  
Changes in the total and differential leucocytic (WBCs) count in control and 1,4-dioxane treated male mice.

Test	C	1,4-dioxane treated group							
		1 <sup>st</sup> w	2 <sup>nd</sup> w	3 <sup>rd</sup> w	4 <sup>th</sup> w	5 <sup>th</sup> w	6 <sup>th</sup> w	7 <sup>th</sup> w	8 <sup>th</sup> w
WBCs 10 <sup>3</sup> /μl	7.6±0.54	7.2±0.34	7.9±0.42	8.1±0.45	8.4±1.26 *	8.3±0.09 *	8.5±0.12 **	8.9±0.02 ***	9.8±0.07 ***
L	67±0.62	65±0.68	64±0.84	61.2±0.62 *	58.3±0.78 **	54.7±1.67 *	53.7±0.6 ***	50.6±0.51 ***	46±0.62 ***
M	2±0.34	5.1±0.23 **	5.6±0.45 **	7.1±0.89 *	9±0.82 **	8±1.1 *	10±0.26 **	11.3±0.76 **	13.6±0.1 ***
N	29±0.68	27.5±0.6	29 ±0.63	30.6±1.2	31±0.64	34±0.74 **	34±0.08 *	35.1±0.48 **	38±0.35 ***
E	2±0.46	1.8±0.46	1.2±0.26	0.7±0.61	1.4±0.28	2.5±0.36	1.8±0.32	2.3±0.6	1.9±0.4
B	0±0.35	0.6±0.34	0.2±0.1	0.4±0.41	0.3±0.2	0.8±1.1	0.5±0.4	0.7±0.64	0.5±1.01

W= week

C= control

Results = Mean ± SD / n=5

\*= significant ( $P < 0.05$ )

\*\* = Highly significant ( $P < 0.01$ )

\*\*\* = very highly significant ( $P < 0.001$ )

Significance was calculated between control and treated groups

L = Lymphocyte

M = Monocyte

N = Neutrophil

E = Eosinophil

B = Basophil

The total WBCs increased non-significantly in the first 3 weeks as compared to control group( Table 2) , then a continuous and gradual increase was occurred till the end of the experiment( $P<0.001$ ). The differential leucocytes count (Table 2) revealed that a decreased in lymphocytes ( $P<0.001$ ) in the experimental group with dioxane as compared with the control one, while monocytes and neutrophils were increased significantly( $P<0.001$ ). There was a continuous increase in monocytes from the first week till the end of the experiment while neutrophils were increased significantly from the fifth week till the end of the experiment. The eosinophil and basophil showed non-significant effects between the experimental and control groups.

### **Discussion:**

The present study revealed that toxicity of mice with 1,4-dioxane exhibited practically many changes in the investigated blood parameters. Values of measured blood parameters of treated mice deviated from control mice. Such results became more sever in the mice toxicity with dioxane. The reported normal mean value of these blood parameters in mice in this experiment in accordance with the normal value reported before (,Mitruka and Rawnsley,1977 and Haeba et al, 2002).

Mice treated group with 1,4-dioxane showed very highly significant reduction of erythrocytes counts, accompanied by significant decrease in Hb as well as Hct %, compared with control values(Table 1), which indicate the progression toward anemia. The reduction in Hb may be resulted from the decrease in the formation of the globulin. Also the reduction in the Hct, and Hb may be due to the decreased RBCs resulting from the toxicity by Dioxane. This decrease in the total RBCs may be due to the destructive effect of the toxic 1,4-dioxane as supported by Linman (1975) or may be due to the circulating failure as a result of inability to maintain circulatory blood volume due to the decrease in the developing stages of RBCs in haemopoitic tissues ( El-Feki ; 1987). Linman (1975) and Zahran, (1997) postulated that the destructive effect of the toxic substances on the erythrocytes increased directly the catabolism of hemoglobin. The blood indices MCV,MCH were decreased compared with control, which may be due to the failure in blood osmoregulation and plasma osmolarity (Wong and Davidson,1983).

In contrast, Total WBCs count in the Dioxane treated group (Table2) showed a significantly gradual elevated values associated with progression of toxicity. The total leucocytes number in blood of treated mice was manifested by an obvious increase in neutrophils followed by monocytes, paralleled with a marked drop in lymphocytes percentage (Table 2). This changes could explain by some researchers which found that the increase of WBCs was mainly pointed to the elevated neutrophils (Zahran;1997) and monocytes showed significant increase in their percentage due to their increase phagocytic activity towards destructive RBCs, or may be due to innate defense capability of mice against toxins(dioxane) ( Uhlir,1991). Harding and Hogland, (1984) concluded that, the changes in monocyte number may be due to the engagement of these cells in the phagocytic process against different antigens. The increase monocyte number may be explained by the increase in haemopoietic activity after exocytosis which was done by monocytes after the discharge of its granules to lyse the antigens extracellularly (Norman et. al, 1973; Roitt et al., 1982 and Dolen et al., 1992). The changes in the differential leucocytes counts may be due to the changes in the immunological parameters during toxicity with Dioxane. This increase may be due to the increase in hemopoietic activity stimulated by loss of blood cells (Awad, 1992) as a result of haemolysis of RBCs.

Otherwise, the increase of neutrophils was accompanied with a highly significant decrease in lymphocytes percentage in this treated group of mice. Zahran, et al., (1995) reported that lymphocytes play the key role in all immune reactions and is always directed against the specific foreign antigens (toxins). lymphocytes were significantly decreased in number in response to stressful condition after antigen (Dioxane) entrance as reported by Sovenyl et. al.,(1990) or may be due to the production of specific or non specific antibodies against different antigens, since lymphocytes are responsible for achieving the defense mechanism introduced into the body (El-Feki,1987). Additionally, lymphocytes migrate to the site of inflammations which may be resulted due to toxic effect of dioxane, as reported by Mahmoud,(1995). Therefore, its percentage in blood stream and population in the thymus, spleen and lymph node were decreased (Zahran, 1997), because the circulation is not the essential site of WBCs, but only for their passage to infected organs and their circulation with lymph by filtration via blood capillaries.



In conclusion, there were general changes in most of blood parameters in the toxicity with 1,4-dioxane under the test conditions.

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### الملخص :

تناول هذا البحث تأثير سمية مركب ٤,١ - ديوكسان على ذكور الفئران السويسرية البيضاء وقد تم استخدام أقصى جرعة تحت مميتة من الديوكسان (٧٥مجم/كجم) وذلك إجباريا عن طريق الحقن في المعدة مرتين أسبوعيا ولمدة ٨ أسابيع . وقد تمت دراسة العد الكلي لكريات الدم الحمراء RBCs ، نسبة الهيموجلوبين Hb ، النسبة المئوية للهيماتوكريت (حجم الكريات المثقلة) % Hct ، متوسط حجم كريات الدم الحمراء MCV ، متوسط الهيموجلوبين في كرية الدم الحمراء MCH ، متوسط تركيز الهيموجلوبين في كرية الدم الحمراء MCHC بالإضافة إلى العد الكلي والنوعي لكريات الدم البيضاء WBCs ومقارنتها بالحيوانات الضابطة. هذا وقد أظهرت النتائج أن تعرض ذكور الفئران لهذه الجرعات المتكررة من الديوكسان تأثير معنوي ملحوظ في الغالبية العظمى من قياسات الدم التي تم دراستها . فقد أحدثت سمية الديوكسان: (١) انخفاض معنوي ملحوظ في العد الكلي لكريات الدم الحمراء و HB و %Hct و MCV ، MCH ، MCHC. (٢) زيادة معنوية ملحوظة في العد الكلي لكريات الدم البيضاء . (٣) أظهر العد النوعي لكريات الدم البيضاء انخفاض معنوي ملحوظ في عدد الخلايا الليمفاوية على عكس الخلايا البلعمية Monocytes والخلايا متعادلة الصبغ حيث ازدادت نسبتها زيادة معنوية ملحوظة كما انه لم يلاحظ تغيير معنوي لنسبة الخلايا حمضية Acidophils وقاعدية الصبغ Basophils وذلك بالمقارنة بالحيوانات الضابطة.