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A TOXICOLOGICAL STUDY INTO NEW MOLECULES FOR TREATING RAW WATER TO MAKE IT POTABLE

UNIVERSITY OF GHANA SCHOOL OF PHARMACY

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INTRODUCTION

- Chemical based disinfectant of the polymeric guanidine family.
  - Potent virucide and *in vitro* bactericide.
  - Odorless, non-corrosive, and has been shown to be non-toxic in an *in vitro* cytotoxicity study involving low concentrations (0.04 and 0.005 % w/v).

INTRODUCTION

- Highly soluble in water.
- PHMGH is also used as an effective sporicidal disinfectant.
- In an in vitro study
  - killed all spores at a concentration corresponding to 0.52 % (w/v) within 90 s of contact and 0.36 % (w/v) for 3 min.

INTRODUCTION

- Current recommendation of PHMGH
  - as bactericidal and fungicidal disinfectant for the treatment of harvested cocoa beans
  - cooling systems
  - for the treatment of raw water to make it potable (ie good for drinking without fear of poisoning or disease).

INTRODUCTION

- Current water treatment in Ghana.
- Regulatory concerns .
  - Administration of PHMGH in both rats and humans have been associated with the potential for hepatic, renal gastro-intestinal tract (GIT) and lung effects.
  - August 2006 to May 2007 , more than 12,500 patients were admitted to hospital with a history of drinking illegal cheap “vodka” in 44 different regions in Russia, of whom 9.4% died.
  - ethanol (≈93%), diethyl phthalate, and 0.1–0.14% PHMGH (“Extrasept-1”)[9]

ACUTE TOXICITY STUDIES

- Initial administration of 3000 mg/kg in one rat and 400 mg/kg in another rat.

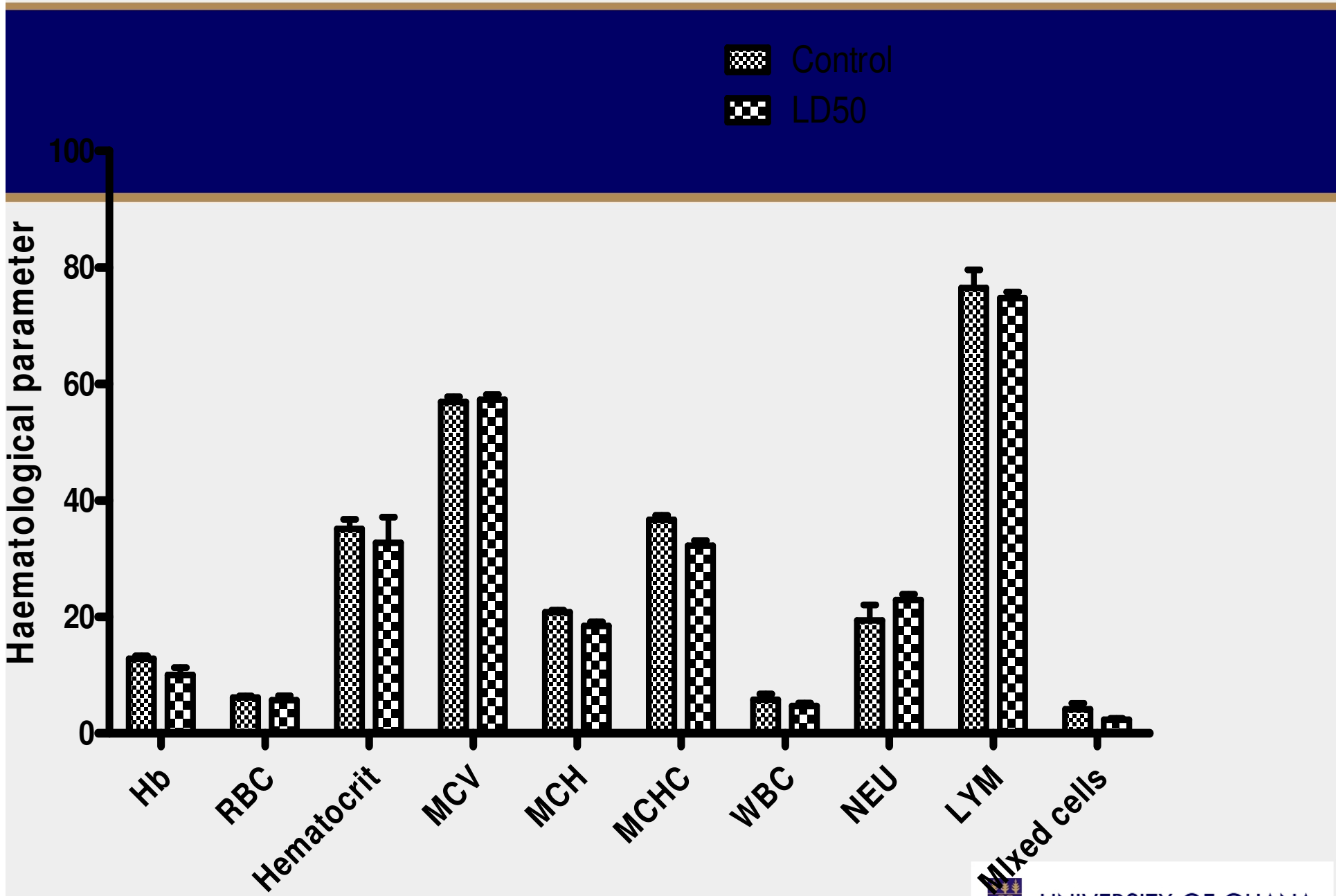
- Thereafter, doses of 8.0 mg/kg, 400 mg/kg, 600 mg/kg, 2000 mg/kg and 3000 mg/kg were administered in order to determine 50% death of the animals at the doses tested.

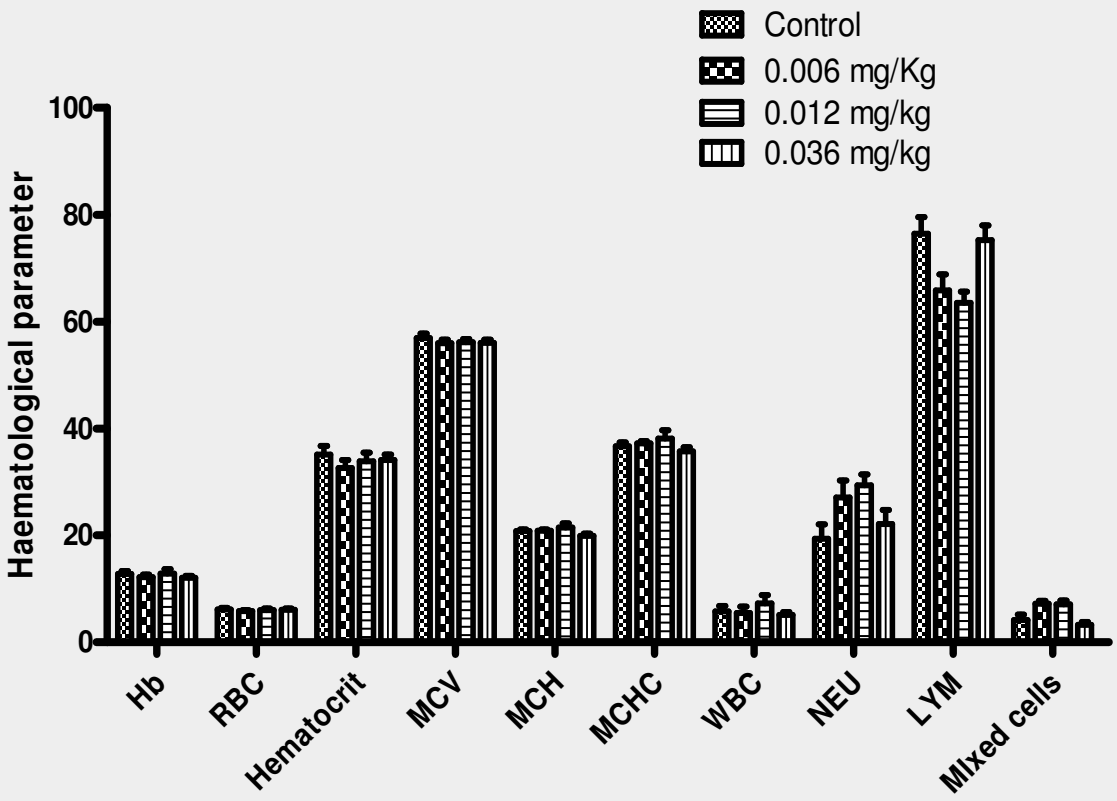
SUB-CHRONIC TOXICITY STUDIES

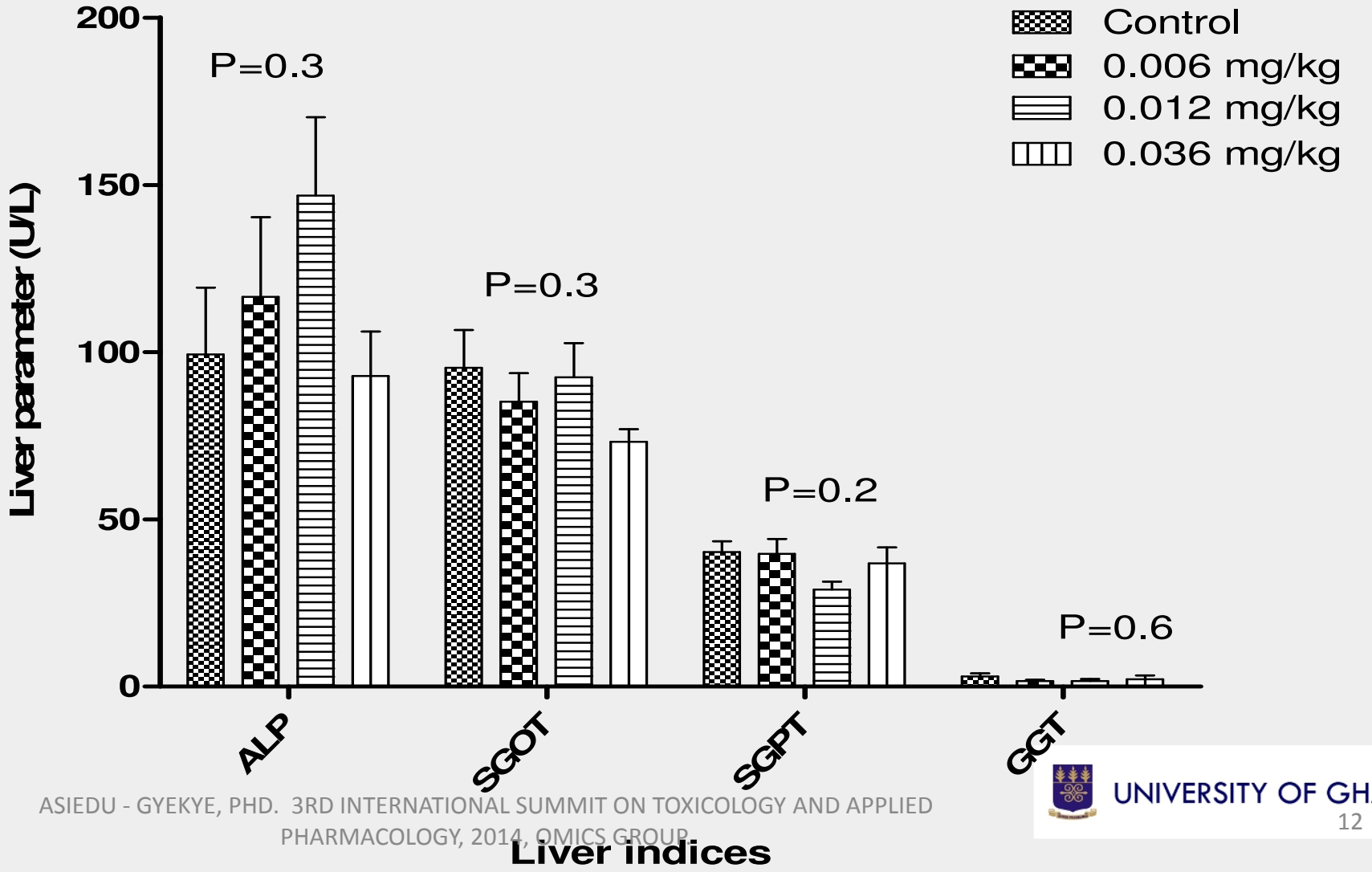
- **Group 1:** 0.006 mg/kg (ie 1.5 mg/L)
- **Group 2:** 0.012 mg/kg (ie 3.0 mg/L)
- **Group 3:** 0.036 mg/kg (ie 9.0 mg/L)
- **Control:** Deionized water
- Automated hematology analyzer; KX-2IN, Sysmex Corporation, Japan)
- 2 mg/kg, 8 mg/kg, 32 mg/kg and 40 mg/kg of PHMB.

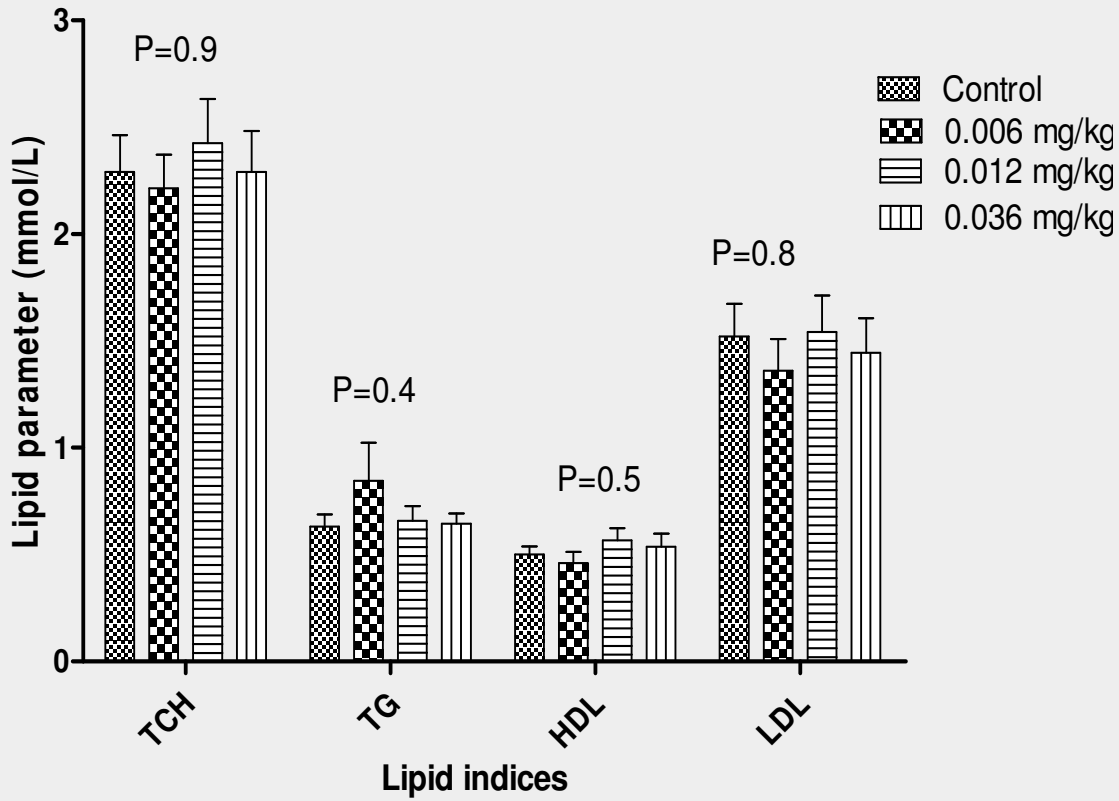
Statistical Analysis

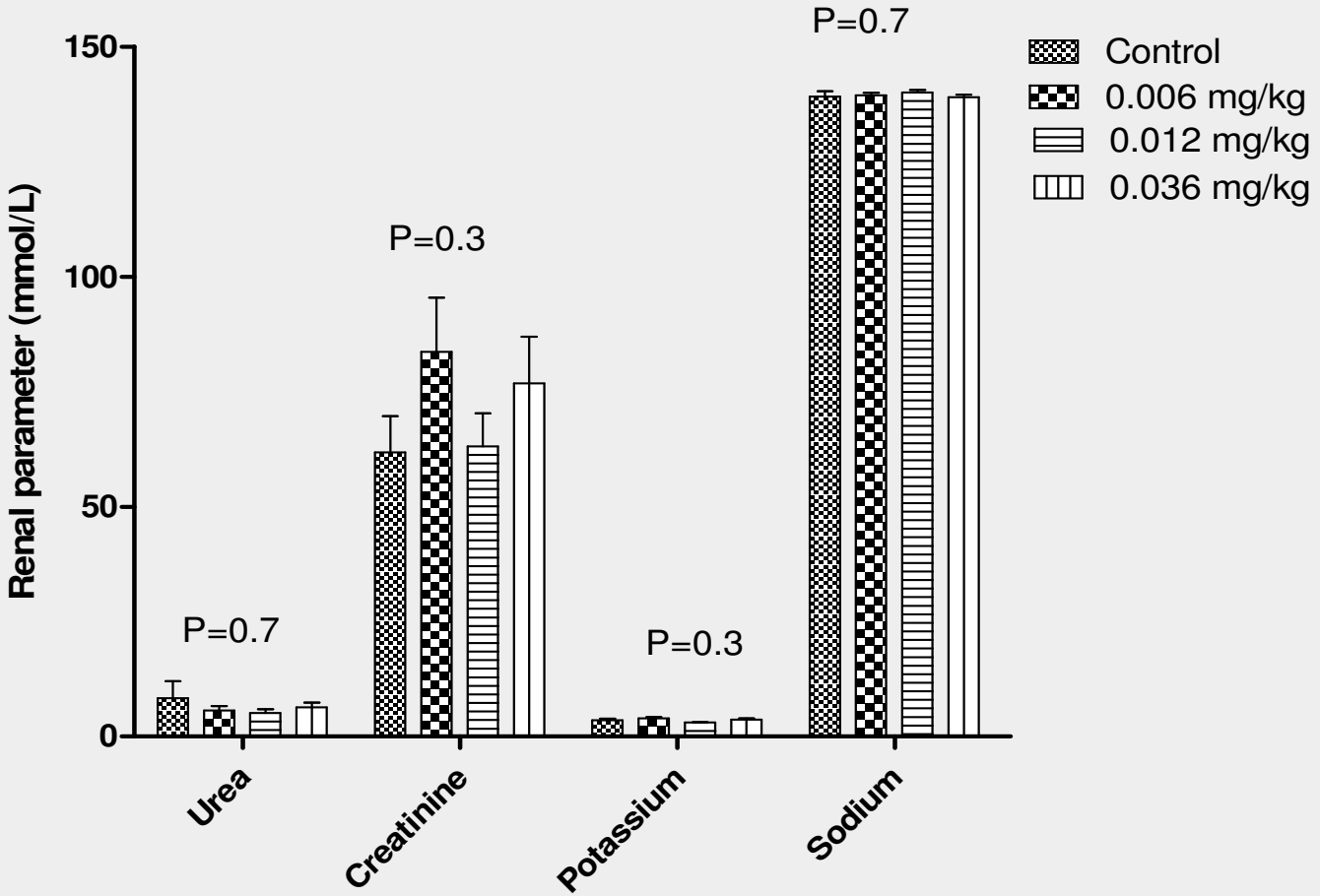
- Graphpad Prism 5. Means ± SEM were determined for quantitative variables.
- Analysis of variance (ANOVA)
  - determine statistical significance invariables among the groups at *p*-values ≤0.05. This was used for the subchronic studies
  - unpaired t-test was used for the analysis of the acute toxicity study results.


Control
LD50
Haematological parameter
100
80
60
40
20
0
Hb
RBC
Hematocrit
MCV
MCH
MCHC
WBC
NEU
LYM
Mixed cells


Control
0.006 mg/Kg
0.012 mg/kg
0.036 mg/kg
Haematological parameter
100
80
60
40
20
0
Hb
RBC
Hematocrit
MCV
MCH
MCHC
WBC
NEU
LYM
Mlxed cells


Control
0.006 mg/kg
0.012 mg/kg
0.036 mg/kg
Liver parameter (U/L)
200
150
100
50
0
P=0.3
P=0.3
P=0.2
P=0.6
ALP
SGOT
SGPT
GGT
Liver indices


P=0.9
P=0.4
P=0.5
P=0.8
Control
0.006 mg/kg
0.012 mg/kg
0.036 mg/kg
Lipid parameter (mmol/L)
0
1
2
3
TCH
TG
HDL
LDL
Lipid indices

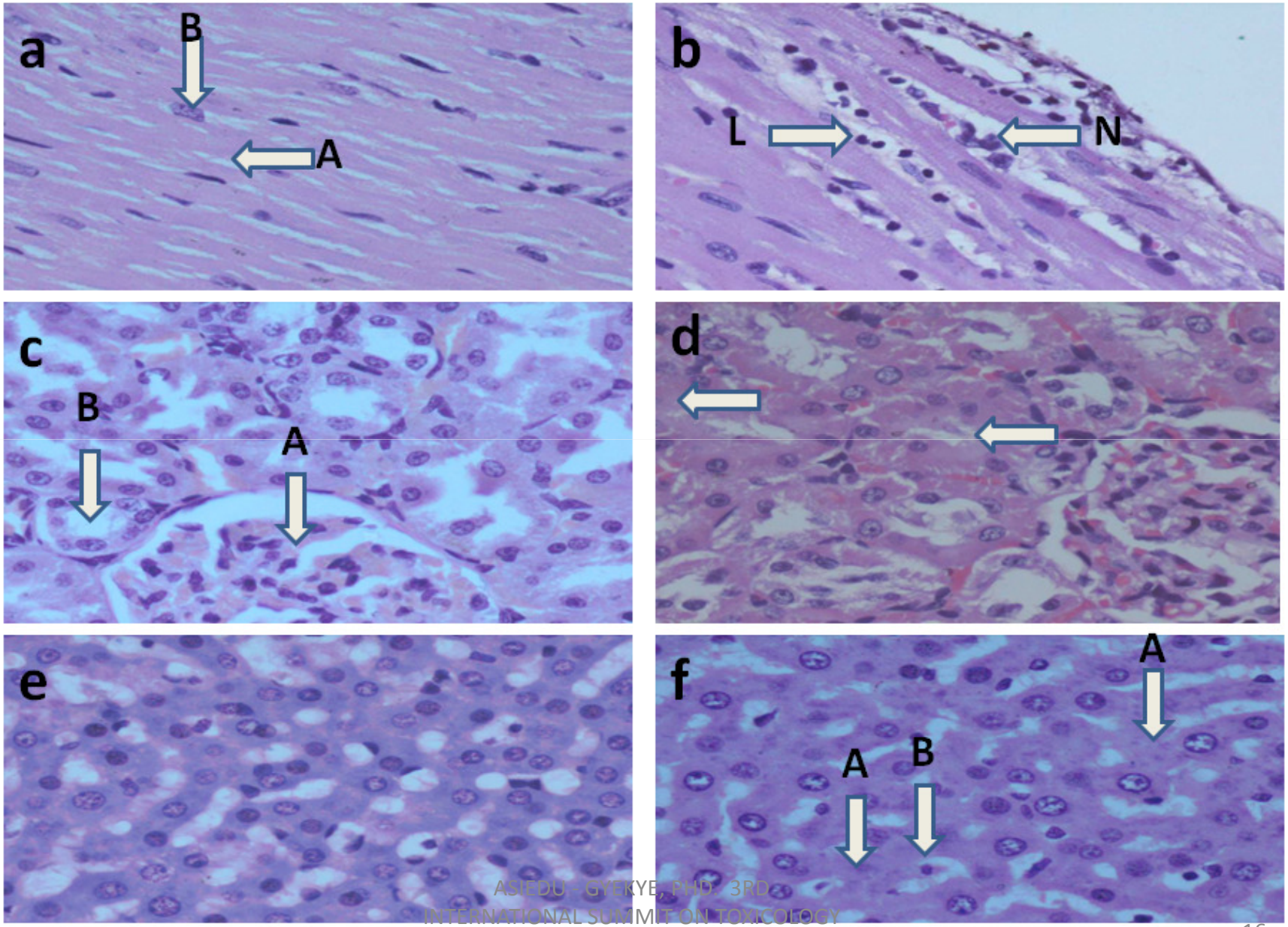

P=0.7
150
100
50
0
Renal parameter (mmol/L)
Control
0.006 mg/kg
0.012 mg/kg
0.036 mg/kg
P=0.3
P=0.7
P=0.3
Urea
Creatinine
Potassium
Sodium
Renal function indices

HISTOPATHOLOGICAL STUDIES

- Microscopic evidence showed
  - mild hepatocellular necrosis in 10% of animals at all dose levels administered.
  - mild tubular damage in 20% of animals (0.012 mg/kg and 0.036 mg/kg).
  - mild myocarditis in 10% of animals (0.006 mg/kg).

Manufacturer recommends a dose of 0.012 mg/kg bwt (3.0 mg/L) to be used for water treatment process.

FIGURE 5:



CONCLUSION

- According to other studies conducted, the LD_{50} to minimal working dose ratio of PHMGH lies between 50-126
  - —average cumulative toxicity.
- Our acute study results also did not confirm such a claim since our LD_{50} of 600 mg/kg to the manufacturers working dose of 0.012 mg/kg lies beyond this range.

Conclusion

- Median lethal dose (LD_{50}) to be 600 mg/kg of PHMGH and 16 mg/kg PHMB.
- Subchronic toxicological studies not associated with mortality or visible clinical signs of toxicity.
- No observable anomalies in the hematological and biochemical parameters utilized to evaluate liver function, kidney function, and lipid profiles.

REFERENCES

- Oulé MK, Azinwi R, Bernier AM, Kablan T, Maupertuis AM, Mauler S, Nevry RK, Dembélé K, Forbes L, Diop L. Polyhexamethylene guanidine hydrochloride-based disinfectant: a novel tool to fight meticillin-resistant Staphylococcus aureus and nosocomial infections. *J Med Microbiol*. 2008; 57(Pt 12):1523-8. doi: 10.1099/jmm.0.2008/003350-0.

- Oulé MK, Quinn K, Dickman M, Bernier AM, Rondeau S, De Moissac D, Boisvert A, Diop L. Akwaton, polyhexamethylene-guanidine hydrochloride-based sporicidal disinfectant: a novel tool to fight bacterial spores and nosocomial infections. *J Med Microbiol*. 2012; 61(Pt 10):1421-7. doi: 10.1099/jmm.0.047514-0. Epub 2012 Aug 7.

REFERENCES

- Kusnetsov JM, Tulkki AI, Ahonen HE, Martikainen PJ. Efficacy of three prevention strategies against legionella in cooling water systems. *J Appl Microbiol.* 1997; 82: 763–768. CrossRefMedline.

- Mathurin YK, Koffi-Nevry R, Guéhi ST, Tano K, Oulé MK. Antimicrobial activities of polyhexamethylene guanidine hydrochloride-based disinfectant against fungi isolated from cocoa beans and reference strains of bacteria. *J Food Prot*. 2012; 75(6):1167-71. doi: 10.4315/0362-028X.JFP-11-361.

- Frayne Colin. The Selection and Application of Nonoxidizing Biocides for Cooling Water Systems. The Analyst, the voice of the water treatment industry, 2001; 5.

REFERENCES

- Background Document to the opinion proposing harmonised classification and labelling at Community level of Polyhexamethylene biguanide or Poly(hexamethylene) biguanide hydrochloride or PHMB ECHA/RAC/CLH-O-0000001973-68-01/A1, September 2011. http://echa.europa.eu/documents/10162/2125cf0b-8320-48fc-b213-2f4fe29e3d38

- Ostapenko YN, Brusin KM, Zobnin YV, Shchupak AY, Vishnevetskiy MK, Sentsov VG, Novikova OV, Alekseenko SA, Lebed'Ko OA, Puchkov YB. Acute cholestatic liver injury caused by polyhexamethyleneguanidine hydrochloride admixed to ethyl alcohol, *Critical care toxicology,*2011; **49**;6:471-477. doi:10.3109/15563650.2011.592837.

- Ministry Of Health of Russian Federation. Report on Research Work "Experimental Estimation of Maximal Permitted Concentration of Polyhexamethylene Guanidine Hydrochloride (PHMG) In Aquatiq Environment" Sechenov Moscow Medical Academy, Moscow, 1993; 3-5. *www.teflexvissac.com/shop/board/download.php?id=report&no*.

REFERENCES

- Greaves P. Histopathology of preclinical toxicity studies: Interpretation and Relevance in Drug Safety Evaluation. 2007. 3rd ed. New York Academic Press.

- Kim JY, Kim HH, Cho KH. Acute cardiovascular toxicity of sterilizers, PHMG, and PGH: severe inflammation in human cells and heart failure in zebrafish. *Cardiovasc Toxicol.* 2013; **13**(2):148-60. doi: 10.1007/s12012-012-9193-8.

- Jung HN, Zerin T, Podder B, Song HY, Kim YS. Cytotoxicity and gene expression profiling of polyhexamethylene guanidine hydrochloride in human alveolar A549 cells. *Toxicol. in Vitro*. 2014; 28: 684–692.

THANK YOU

FOR YOUR ATTENTION