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ANALYSIS OF PHOSGENE AND CYANIDE IN BLOOD SAMPLES AMONG SCIENCE AND MEDICAL LABORATORY USERS

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Abstract: This study was carried out to assess the level of exposure of some medical and school science laboratory workers in Yobe State to some dangerous organic solvents such as chlorinated hydrocarbons and acetonitrile whose resulting effect may lead to accumulation of Phosgene and cyanide in blood. Phosgene and cyanide are known to have toxic pulmonary effects, and are generated from chloroform and acetonitrile vapors respectively. Most laboratories, especially school laboratories in the research areas do not have enough safety facilities to protect students and other workers from being exposed to poisonous gases, therefore the need for proper investigation to ascertain the level of exposure and suggest possible measure to provide safety to laboratory users. The research employed the use of questionannire, UV spectrophotometer and Gas chromatography and other analytical instruments to analyze about 200 blood samples collected from various laboratory workers across the study area. The results of the analysis indicated that most of the workers were expose toxic organic solvents during their experimental works in the laboratory without any safety measures taken into consideration and about 75% of the laboratories (both teaching and medical) under the area of this study have inadequate safety measures. The highest concentration of the cyanide 0.068 mg/L in the blood samples is within the harmful level of 0.05 mg/L while phosgene, 1.210 mg/L is below the harmful level of 2.0 mg/L respectively for cyanide and phosgene in blood.

Introduction

Phosgene is commonly use as an industrial chemical in making plastics and pesticides (American Conference of Governmental Industrial Hygienists, 2018). The term “phos” means light in Greek and “gene” means born; hence the name “phosgene” (ACC, 2014). It is a poisonous gas at room temperature (70°F). Liquid phosgene quickly turns into a gas when released and rapidly spreads at a level closer to the ground. The gas is colorless with pleasant odor in most cases at low concentration. At high concentrations, it has a strong and irritating odor (NCEH, 2018). Depending on the level of exposure, common laboratory chemicals such as chlorinated hydrocarbons (e.g. chloroform) can lead to phosgene poisoning in blood, eye and throat irritation and severe lung damages. This substance is present in at least 10 of the 1,585 National Priorities List sites identified by the Environmental Protection Agency (EPA).

Exposure to low phosgene concentration may cause delayed effects that may not be notice for up to 48 hours post exposure. Common symptoms of phosgene poisoning within 48 hours include difficulty breathing, Coughing up white to pink-tinged fluid (a sign of pulmonary edema), Low blood pressure and Heart failure (NCEH, 2018). When released to soil, phosgene will evaporate into air or pass through the soil surface and contaminate groundwater. Most of the phosgene in soil will be broken down when it meets moisture (ATSDR, 2002).

Cyanide is a group of chemicals considered powerful poison that may occur naturally or artificially. Cyanide levels could be present in blood and urine in minute quantities due to natural processes, however, during cyanide poisoning, the blood levels increased. At levels higher than 0.05ppm in blood, a harmful effect may occur. workers exposed to 6.4–10.4 ppm cyanide for 5–15 years, which evolved from sodium cyanide and copper cyanide during electroplating, complained of primordial pain (Jessilynn, Nickolette, Carolyn, Margaret, & Steven, 2006).

Phosgene and cyanide have toxic pulmonary effects, and are generated from chloroform and acetonitrile vapors respectively. Most laboratories, especially school laboratories in the research areas do not have enough safety facilities to protect students and other workers from being exposed to poisonous gases, therefore the need for proper investigation to ascertain the level of exposure and suggest possible measure to provide safety to laboratory users.

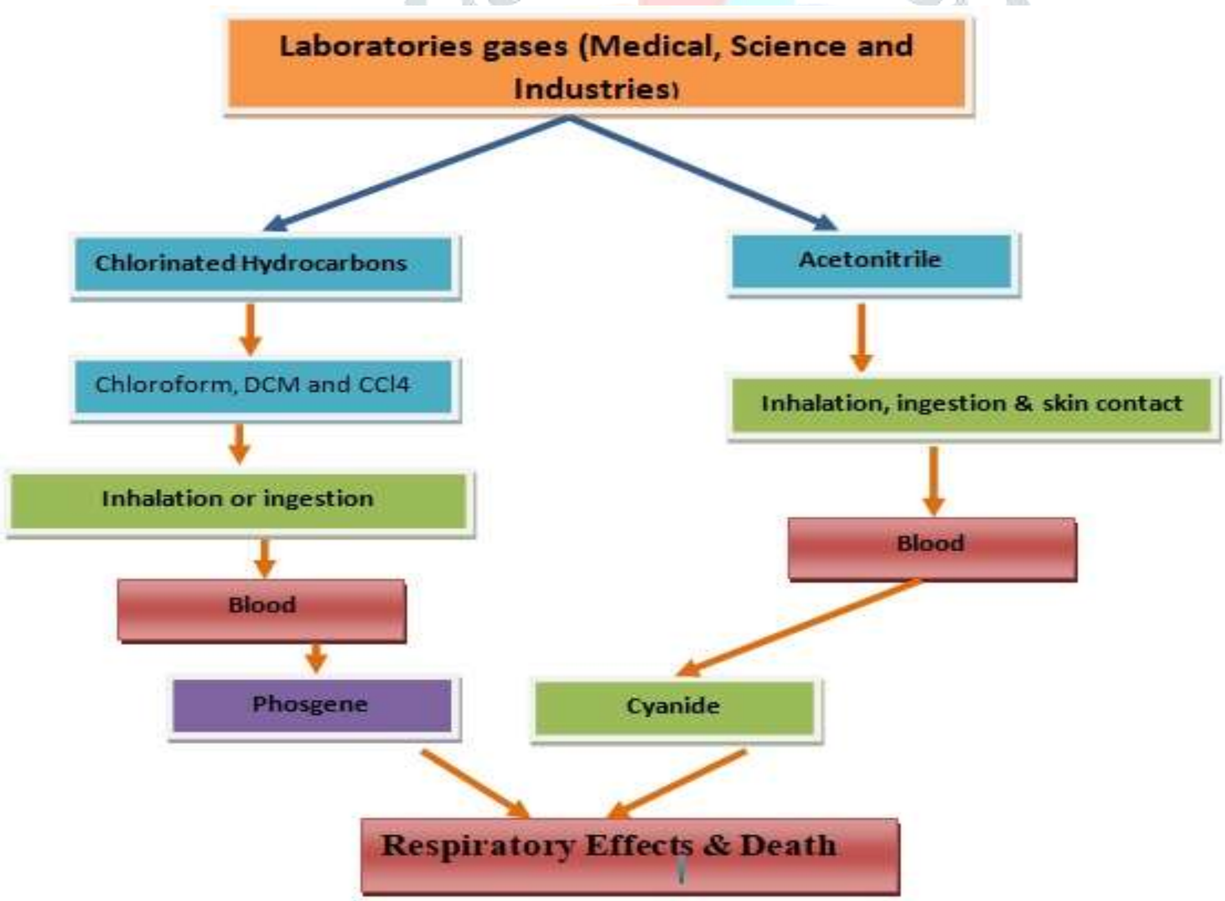

Figure 1: Study design and Background

3.0 Method

3.1 Study Area

The study area of this research covers only medical and science laboratories in higher institution and public hospitals within Damaturu ($11^0$44'49.09"N/$11^0$57'38.99E), Gashua (12.8765^{0}N, 11.3016^{0}E) and Potiskum (11.7072^{0}N, 11.0825^{0}E) Local Government areas in Yobe State. The laboratories include medical and research laboratories in Yobe State University, Federal Polytechnic Damaturu, Specialist Hospital Damaturu, General Hospital Potiskum, Federal College of Education Technical (FCET) Potiskum and Federal University Gashua. An ethical approval was obtain from Ethical Committee at Yobe State Specialist Hospital Damaturu

3.2 Questionnaire

A questionnaire was use to gather data from respondents about their attitudes, experiences, or opinions with respect to safety in school and medical laboratories.

3.3 Blood Samples Collection and Pre-Treatment

The blood samples were collected via venipuncture method (from superficial vein in the upper limb) (Neoteryx, 2017) and transferred into a sterile tube and stored in an ice cold box (4-8^{0}C) and then transported immediately to the laboratory (WHO, 2006). The samples were extracted with toluene and purified by solid-phase extraction (SPE) methods (Rosenfeld, Mureika-Russel, & Phatak, 1984) and (Stanisław & Zygfryd, 2009). The purified extracts were centrifuge at 3000rpm for 10 minutes and the supernatant were use for GCMS analysis.

3.4 GC-MS Analysis of Cyanide and Phosgene

Cyanide (Thiocyanate) and phosgene were extracted and derivatized using 1-propanol in 40% pyridine solution for GC-MS analysis (Marja, et al., 2020) n (Stephanie, Gary, John, & Brian, 2010). Agilent gas chromatography (GC 7890B) coupled with Mass Spectrometer detector (MSD 5977A) were used for the analysis. Samples were identified based on their retention time matching with standard chromatogram and NIST library comparison. **Chromatographic Condition;** Column 30m x 0.25 mm id x 0.25 µm; Helium carrier gas set at constant pressure mode. Oven program 45 °C (1 min); 10 °C/min to 280 °C; hold 15 min Splitless mode Injection Volume: 1 µL.

Blood samples were screened for cyanide using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) to convert cyanide to 2-cyano-4,6-dimethoxy-1,3,5-triazine This reaction proceeded in whole blood samples after treatment with trichloroacetic acid, and in basic aqueous solution samples.

3.5 Blood Samples Collection and Pre-Treatment for UV Spectroscopic Analysis of Cyanide

0.5 mL of sample solution was pipet into a 10 mL flask and then diluted to 50 mL with 0.25N sodium hydroxide solution. 15 mL of 1M sodium phosphate solution was added and mixed. This was followed by addition of 2 mL of chloramine-T. After a minute,5 mL of pyridine-barbituric acid solution was added and mixed. The mixture was the diluted to 100 mL with water and mix again, and the allowed to stand for 8 minutes. After a full color development, the absorbance of the mixture read at 578 nm in a 1-cm cell within 15 min. A series of standards were prepared by pipetting suitable volumes of working standard potassium cyanide solution into 250-mL volumetric flasks. To each flask, 50 mL of 1.25N sodium hydroxide were added and diluted to 250 mL with water (SW-846, 2014)

4.0 Results and Discussion

Table 1: Responses to Questionnaire

S/No.	Age	Exposure to Chlorimated Hydrocarbons	Exposure to acetonitrile	Duration of Exposure (hours/day)	Level of Safety Measures taken (%)
1.	15-20	6	4	1-3	5
2.	20-25	45	28	2-5	12
3.	25-30	58	29	1-2	11.5
4.	35 and above	18	12	3-4	14

About 200 blood samples were collected and analyze during the research. From the results of the analysis, most of the sample donors were expose to chlorinated hydrocarbons, acetonitrile and other toxic organic solvents during their experimental works in the laboratory.

Eighty percent (80%) of the respondents are within the age of 20 to 35 years, 5% are below 20 years while 15% are above 35 years. Among the respondents, 45% are female, and about 73.25% of the total respondents were in one way or the other exposed to chlorinated hydrocarbons such as chloroform, dichloromethane and tetrachloromethane as well as acetonitriles during experimental work in the laboratories without any safety measures taken into consideration. Only few workers are using safety kits likes hand glove, laboratory coats, safety goggles and fume cupboard.

About 75% of the laboratories (both teaching and medical) under the area of this study have inadequate safety measures. In most cases, Fume hood/bio-safety cabinets were either missing or not functioning.

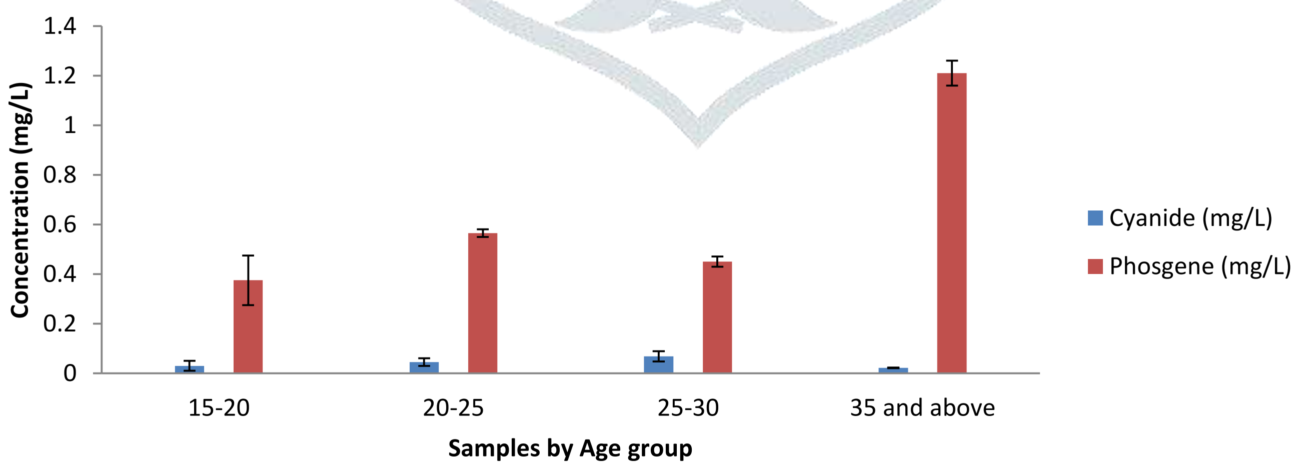

Figure 2: Average Concentrations of Cyanide and Phosgene in blood samples of Science and Medical Laboratory Users

From figure 2 above, average cyanide concentration in blood samples used were found to be 0.03±0.02, 0.045, 0.068 and 0.022 mg/L for age group 15-20, 20-25, 25-30 and 35 years above respectively. From the analysis results the concentration increases gradually with age. However, above 35 years the concentration decline. This may b connected to the level of their experience, safety aware and possible safety measure taken into consideration.

Figure 2 above also revealed the average concentrations of phosgene in blood samples used were approximately 0.375, 0.365, 0.450 and 1.210 mg/L for age group 15-20, 20-25, 25-30 and 35 years above respectively. The analysis results show an increasing concentration with age. Unlike cyanide, the concentration does not decline even above 35 years.

Figure 3 and 4 indicated the GCMS chromatograms of Cyanide and phosgene in the blood samples, confirming the UV spectroscopy. The results show that most of the blood samples collected from the laboratory workers and students were contaminated with either phosgene, cyanide or both.

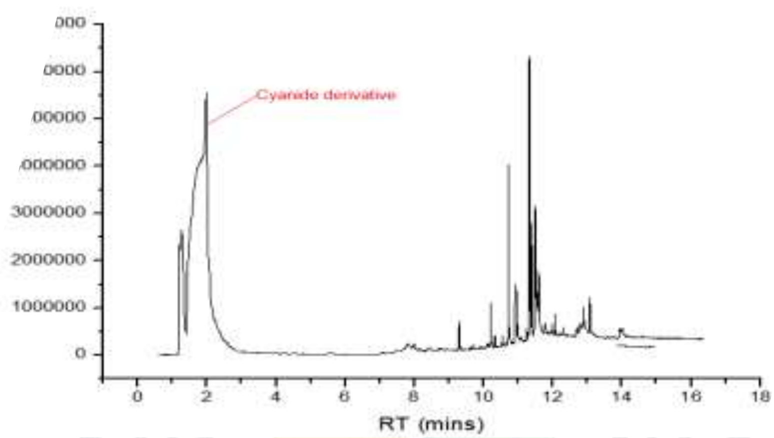

Figure 3: GCMS Chromatogram showing cyanide derivative in blood

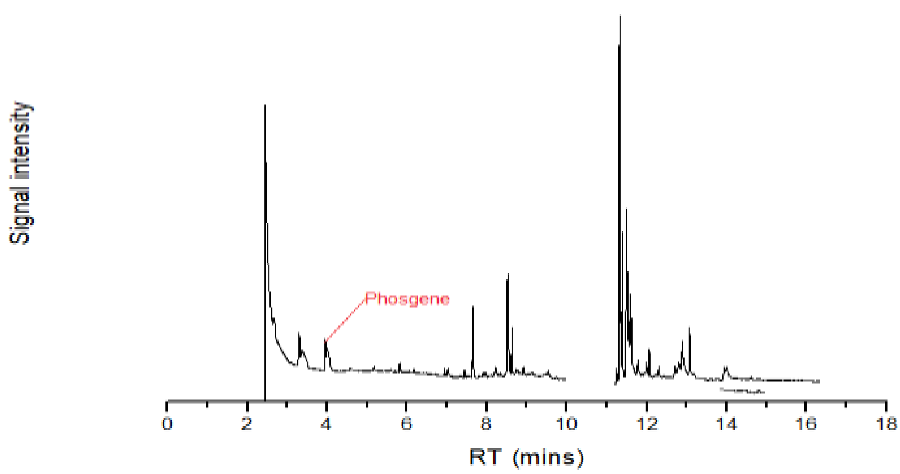

Figure 4: GCMS Chromatogram showing the presence of phosgene in blood samples

According to ATSDR public health statement 2002, when blood levels of cyanide is higher than 0.05 mg/L, it could have harmful effects (ATSDR, Cyanide, Public Health). In stored food and water EPA sets the highest permissible limit to 0.2 mg/L. While citrus fruit is 50 mg/L (ASTDR, 2022) (OSOBAMIRO, 2012) .

Harmful effects can occur when blood levels of cyanide are higher than 0.05 parts per million (ppm), but some effects can occur at lower levels (ATSDR 2022) In one reported fatal case, a 14-year-old boy was given nitroprusside had a post-mortem blood cyanide concentration of 5 mg/l. Normal blood cyanide level is less than

0.2 mg/L, above 2 mg/L can produce severe toxicity, and a level above 5 mg/L may be lethal if not treated immediately (Amitava & Amer, 2014)

According to the Occupational Safety and Health Administration permissible, exposure limit (OSHA PEL) for the workplace is **0.1** mg/L **as an 8-hour time weighted average**. The level immediately dangerous to life or health (IDLH) is 2 mg/L. Even a short exposure to 50 mg/L may result in rapid fatality. (Paul, 2021)

Phosgene toxicity depends on dose and time of exposure. At 3-5 mg/L, phosgene causes eyes and throat irritation with coughing. Exposure to about 25 mg/L of phosgene for 30-60 min is dangerous. It is rapidly fatal when a person is exposed to phosgene at 50 mg/L. For acute inhalation exposure, Phosgene is extremely toxic; having s**evere respiratory effects, including pulmonary edema and death** in humans. It also causes severe dermal burns and ocular irritation when in contact with skin or eye. Permissible exposure limit at averaged of over 8-hour work shift is 0.1-0.2 mg/L and immediately dangerous to life at 2 mg/L and above. (NRCCT, 1984)

Conclusion

This research findings, revealed that most of the sample donors were in one way or the other exposed to chlorinated hydrocarbons such as chloroform, dichloromethane and tetrachloromethane as well as acetonitriles during experimental work in the laboratories and about 73.25% of them work without any safety measures taken into consideration. This may be due to lack of adequate safety facilities in most of the laboratories. The average concentration of the cyanide and phosgene in the samples are high in most of the blood samples, as 0.05 mg/L and 2.0 mg/L respectively for cyanide and phosgene in blood. It was observe however that, only those that work without safety adherence and long-term exposure are mostly affected.

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