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THE RELIABILITY OF THE WEBSTER METHOD FOR MEASUREMENT OF TNT

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of requirements for the degree of Masters in Public Health – Occupational Hygiene

Johannesburg, September 2018

DECLARATION

I, Joyce Kgasoane, declare that the research report is my own work. It is being submitted for the degree of Master of Public Health in the field of Occupational Hygiene at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

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Joyce Kgasoane

…….. day of ……., 2018

ABSTRACT

Background: Organic nitro aromatic compounds are the most frequently used constituents of explosives. The most widely distributed and best-known nitro aromatic compound used in explosives is probably 2,4,6-trinitrotoluene. Though it is relatively safe to handle during the manufacturing process, its toxicity has been widely known for many years. Numerous studies have indicated that chronic exposure to TNT can lead to chronic toxicity in humans. Given the numerous toxic effects of TNT it is important to monitor exposure, by means of biological monitoring, since it is a more accurate estimation of systemic absorption then air monitoring (occupational hygiene surveys). The Webster urine test and GC-MS analytical technique for TNT metabolites were used in the munitions facility to quantify worker exposure to TNT. The Webster urine test in general presented negative results, suggesting that control measures were in place and were effective against TNT, during biological monitoring of exposed workers. However, test conducted using the GC-MS method indicated the presence of TNT metabolites in the urine samples of some of the exposed workers. Given that the two methods yielded different results, the purpose of the study was to determine, whether the Webster method was a reliable method to determine TNT exposure in workers.

Objectives: This research report focused on the reliability of the Webster method to determine TNT exposure in workers.

The objectives of the study were as follows:

- To describe the concentration of TNT in the urine of munitions workers exposed to TNT by using the Webster method for the years 2001 to 2007.
- To compare TNT concentration in the Webster method with the concentration of TNT metabolites in the GC-MS analytical technique.

Methods: A retrospective descriptive study was conducted, using historical data collected between the years 2001 to 2007. For the first objective historical data from the years 2001 to 2007 were entered and tabulated on a Microsoft Excel spread sheet,

to demonstrate the distribution of positive Webster urine results by means of a table and figure. For the second objective the GC-MS method for TNT urine metabolite analysis was considered to be the "gold standard" and the following steps were conducted. First, urine sample data were presented in a XY scatter diagram. Second, paired urine sample data were presented in a box and whisker plots. Third, paired urine samples were presented in a Bland-Altman scatter plot. Fourth, the association between the Webster test and the GC-MS analytical technique for TNT metabolites was examined by using Chi-square statistics. Fifth, the Webster test and the GC-MS analytical technique were measured in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)

Results: The results for the first objective indicate that only 1.9% of the 426 samples collected for the years 2001 to 2007 tested positive ((≥2.0mg/ℓ) i.e. indicated significant exposure. Urine samples that tested negative over this period were 98.1% i.e. all readings between 0mg/ℓ and 1.5mg/ℓ. The results of the statistical analysis for the second objective applying sensitivity and specificity indicated that the Webster test is more specific and not sensitive. The Webster test had a sensitivity of 27.2%; this is poor, indicating that a large proportion of over-exposed subjects were not identified by the Webster test. The specificity for the Webster test was 97.8%. A chi-square test showed that there were statistically significant differences between the Webster and the GC-MS methods: P value = 0.0000.

The PPV was 94% for the Webster test. This indicated that 94% of workers who tested positive with Webster were truly positive. The NPV was 50% for the Webster test, thus indicating that 50% of the Webster test that were negative was truly negative. The area under the ROC (AUC) equalled to 0.80, this basically stated that the Webster test performed well in general. However it should be noted that only a small proportion of values was positive in this population and that false negatives were particularly problematic, so the AUC of 80% is a bit misleading.

Discussion and conclusion: The Webster test failed to identify workers that were over-exposed even when it was zero. The sensitivity of the test was very low (27%), this is too low for a test that has serious health implications if over-exposure is missed. This study indicates that the Webster method cannot be used as applied in the munitions plant to determine TNT exposure in workers due to the toxicity of TNT exposure.

Recommendations: If the munitions facility continues to use the Webster urine test for determination of 2,4,6-trinitrotoluene exposure it is recommended that Webster test should be checked against the GC-MS method more frequently or against another recognised standard. If the test is used the workplace laboratory should introduce quality control for the test.

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ABBREVIATIONS

TNT	Trinitrotoluene
TWA	Time Weighted Average
RDX	Royal Demolition Explosive
IARC	International Agency for Research in Cancer
EPA	Environmental protection Agency
BEI	Biological Exposure Index
DFG	Deutsche Forschungsgemeinschaft
BAT	Biologischer Arbeitsstoff-Toleranz-Wert
4-ADNT	4-amino-2,6-dinitrotoluene
2-ADNT	2-amino-2,6-dinitrotoluene
OEL	Occupational Exposure Limit
TLV	Threshold Limit Value
AGIH	American Conference of Governmental Industrial Hygienist
NIOSH	National Institute of Occupational Safety and Health
AOAC	Official Analytical Chemist
APHA	American Public Health Association
HCS	Hazardous Chemical Substance
OHS Act	Occupational Health and Safety Act

CHAPTER 1: INTRODUCTION

This chapter provides background information on the essential concerns relating to this study. These include concise information on the chemical, 2,4,6-trinitrotoluene, the application of the chemical in the industry of munitions, the routes of exposure, health impacts, and the different methods to measure 2,4,6-trinitrotoluene exposure in body fluid.

1. BACKGROUND INFORMATION AND LITERATURE REVIEW

1.1 2,4,6-trinitrotoluene

Organic nitro aromatic compounds are the most frequently used constituents of explosives. The most widely distributed and best known nitro aromatic compound used in explosives is probably 2,4,6-trinitrotoluene, which, since the beginning of the century has been used in the manufacture of munitions [1]. In 1863 trinitrotoluene was discovered by Joseph Wilbrand and in 1902 it began to find important applications in the German military industry as the main explosive ingredient in shells, bombs, and grenades. It was soon adopted by other countries and during the two World Wars, millions of tonnes of 2,4,6-trinitrotoluene were produced and used mainly as an ingredient in binary explosives [2].

With the development of organic chemistry around 1830, chemists synthesised many new compounds by nitration and over the course of several years the explosive properties of some of these nitrated compounds were recognised and ways of using them for industrial and military purposes were devised. By the late nineteenth century the nitro aromatic explosives 2,4,6-trinitrotoluene and 2,4,6-trinitrophenol (piric acid) had been synthesised and as high explosives with inherent stability until detonation, their advantages over the more shock sensitive nitro glycerine were recognised particularly for military purposes [3].

Manufacturing plants use a standard process to produce 2,4,6-trinitrotoluene (Figure 1). 2,4,6-trinitrotoluene manufacturing involves nitration of toluene ($C_6H_5CH_3$) in a three-stage process going from mo-, di- and finally trinitrotoluene. Nitric acid (HNO_3)

and sulphuric acid (H_2SO_4) is used as a nitrating medium. Numerous other compounds are formed during the nitration of toluene including asymmetrical isomers of 2,4,6-trinitrotoluene and oxidation products such as tetranitemethane, nitrobenzoic acid, nitrocresol and partially nitrated toluenes. The 2,4,6-trinitrotoluene resulting from the nitration process is then purified by treating it with an aqueous sodium sulphite (sellite) solution to remove the less stable isomers of 2,4,6-trinitrotoluene and the other undesired reaction products [4]. In the finishing process, 2,4,6-trinitrotoluene, is then dried, flaked and packaged.

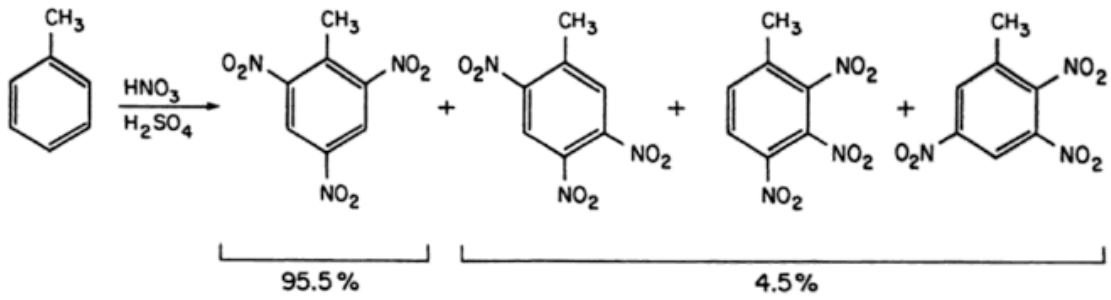


Figure 1: Manufacturing of TNT

2,4,6-trinitrotoluene is solid manufactured, synthetic, yellow, odourless nitro aromatic compound. It is an isomer of $C_6H_2(NO_2)_3CH_3$ (Figure 2) and is also known by other names such as symtrinitrotoluene, 1–methyl-2,4,6 trinitrobenzene and trinitrotoluene (TNT) [4]. Additional information regarding the chemical and physical properties is summarized in Table 1.

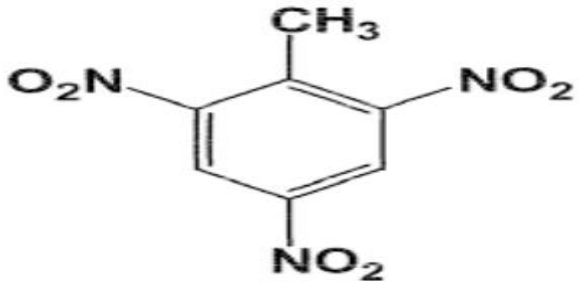


Figure 2: Two dimensional chemical structure of 2,4,6-trinitrotoluene

Table 1: Physical and Chemical Properties of 2,4,6-trinitrotoluene

PROPERTY	VALUE
CAS Number	118-96-7
Physical Description (physical state at room temperature)	Yellow, odourless, solid
Molecular weight (g/mol)	227
Water solubility (mg/L at 20°C)	130
Octanol-water partition coefficient (K_{ow})	1.6
Soil organic carbon-water coefficient (K_{oc})	300
Boiling point (°C)	240 (Explodes)
Melting point (°C)	80.1
Vapour pressure at 25°C (mmHg)	$199x10^{-4}$
Specific gravity	1.654
Henry's Law Constant (atm-m^3/mol at 20°C	$4.57x10^{-7}$

Abbreviations: g/mol – grams per mole; mg/L milligrams per litre, °C – degrees Celsius; mmHg – millimetres of mercury; atm-m^3/mol – atmosphere time cubic metre per mole

1.2 Application of 2,4,6-trinitrotoluene

2,4,6-trinitrotoluene is classified as a high explosive and has a detonation velocity greater than 22,000 ft/sec. It is the most widely used military compound, having a wide range of applications in military shells, bombs, grenades, demolition explosives and propellant compositions. 2,4,6-trinitrotoluene is considered an insensitive explosive. Insensitive explosives are characterised by their resistance to detonation by impact or friction. These properties of 2,4,6-trinitrotoluene, unlike nitro glycerine for example, allows it to be handled by workers in a relatively safe manner [5].

The importance of using 2,4,6-trinitrotoluene as a military compound is based upon its explosive properties and relative safety in manufacturing, loading, transportation and storage. Its relatively simple and economical production, its stability and its ability to be melted and cast, as well as press moulded into shells has also supported its wide spread use in weapons [6].

In commercial applications, it has been used in coal and mineral mining, deep-well and underwater blasting, and building demolitions. In chemistry, TNT is used as an

intermediate to generate charge transfer salts. Other uses include being used as a chemical intermediate in the manufacture of dyes and photographic chemicals [4].

Although valued for its safety, it is a potential health hazard, with a deadly track record of deaths secondary to aplastic anemia as well as hepatic necrosis noted during World War I [5].

1.3 Sources and routes of exposure to 2,4,6-trinitrotoluene

Human exposure occurs primarily in the manufacturing of 2,4,6-trinitrotoluene, in munitions plants and from disposal sites (from drinking contaminated water, or eating contaminated fruits or vegetables that may have been cultivated in contaminated soil) [4]. This research report focuses primarily on occupational exposure in the munitions environment.

Occupational exposure in a munitions environment may occur during the manufacturing and loading of munitions. Several studies have indicated exposure in the occupational environment. In a study of 533 exposed munitions workers, their TWA personal exposure ranged from not detected to 1.8mg/m^3, with 12% of workers exposed to more than 0.5mg/m^3 [7]. In a series of Czechoslovakian studies of a plant manufacturing ammunition, mean workroom air concentrations of 2,4,6-trinitrotoluene were ranging from 0.22 to 9.6mg/m^3 in the 1950s, 0.03 to 4.2 mg/m^3 in the 1960s and 0.04 to 0.76mg/m^3 in the 1970s. The highest air concentrations were found in pressing and filling operations. In another plant producing powdered explosives for mines and quarries, mean workroom air concentrations of 2,4,6-trinitrotoluene ranged from 0.05 to 6.3 mg/m^3 in the 1960s and 1970s. The highest air concentrations were found during cartridge- and sack-filling operations [7].

In a munitions plant in the United States, 2,4,6-trinitrotoluene and RDX was monitored in several areas during the manufacturing process, in the kettle area where it was transferred from boxes to kettles air concentrations averaged 0.02mg/m^3. In the incorporation area where 2,4,6-trinitrotoluene was melted and transferred to kettles for combination with RDX, 2,4,6-trinitrotoluene air concentrations averaged 0.207 mg/m^3 [7].

Exposures during intermittent 2,4,6-trinitrotoluene bagging operations ranged from 0.62 mg/m^3 to 4.00 mg/m^3 in a study at a United States military munitions washout plant. After engineering controls were introduced, personal 8 hour TWA exposures ranged from 0.08 mg/m^3 to 0.59 mg/m^3 [7]. An occupational study conducted in the shell loading plant also indicated exposure ranging from 0.3 to 0.8mg/m^3 [4].

Munition workers are exposed to 2,4,6-trinitrotoluene through inhalation, skin absorption and by way of the gastrointestinal tract. Inadequate or lack of controls from any of these types of exposures may increase the risk of developing adverse health effects. When 2,4,6-trinitrotoluene dust particles and vapour are inhaled the chemical is readily absorbed by the lungs. Following the inhalation of dust particles some of the particles can settle in the nasopharynx and in the upper airways. Subsequently the mucociliary escalator may transport particles to the nasopharynx where they can be swallowed and absorbed by the gastrointestinal system [8].

2,4,6-trinitrotoluene is readily absorbed through the skin, especially when the skin is exposed and wet with sweat [9].

1.4 Adverse health impacts of 2,4,6-trinitrotoluene

Nitro compounds of the dinitrobenzene and 2,4,6-trinitrotoluene type have a history of prominent hepatoxic effects often associated with aplastic anaemia. Earlier studies from the British munitions industry in both world wars have contributed [9]. Initially it was thought that 2,4,6-trinitrotoluene was a non-toxic substance, but during World War I, more than four hundred and seventy five persons died due to anaemia and hepatitis caused by 2,4,6-trinitrotoluene poisoning [8]. In the early part of the 20th century exposure to 2,4,6-trinitrotoluene in the munitions plants resulted in many, and occasionally fatal, cases of aplastic anaemia and toxic hepatitis [4].

The literature review also indicated that 2,4,6-trinitrotoluene has several types of effect on the haematological system. Sievers indicated in their study that exposure to 2,4,6-trinitrotoluene may cause methaemoglobinaemia, with cyanosis. In the bone marrow hypercellularity and hypocellularity have been reported, the latter resulting in a

reduction of red and white blood cells and platelets and, in severe cases, aplastic anaemia, which has features such as pallor, fatigue, bleeding and infection [7].
In late 2004, a plant occupational medicine physician noticed an increase in anaemia cases at McAlester Army Ammunition Plant, to investigate the outbreak they conducted a retrospective, cross-sectional investigation of anaemia cases and personal air sampling to target corrective engineering and administrative changes needed to reduce TNT levels and anaemia cases. They noted anaemia cases when exposure levels were above 0.31mg/m^3 . In their conclusion they found that the change in the ventilations system decreased exposure, but anaemia cases continued to rise with exposure to TNT and this was attributed to workers not wearing personal protective equipment (PPE) provided to them [10].

Sub clinical effects appear to be dose related, with destruction of red blood cells first appearing at exposure exceeding 0.5mg/m^3. At exposures exceeding 1.0mg/m^3 significant shortening of red blood cell survival occurs, although marked aneamia is unlikely because of increased reticulocytosis of the bone marrow. At 2,4,6-trinitrotoluene exposure between 0.5 and 1.0mg/m^3 elevation of liver function enzymes may also occur, particularly in new employees or those recently exposed to higher 2,4,6-trinitrotoluene concentrations [11].

Liver damage is the second main toxic effect of exposure to 2,4,6-trinitrotoluene. Initial features of acute poisoning include jaundice, excretion of bile pigments in urine, epigastric pain, nausea and, in some cases, eventual coma and death. In ten acute fatal cases, pathological examination revealed reduced liver weight, destruction of parenchymal cells, haemorrhagic areas, perivascular infiltration of lymphocytes and polymorphononuclear lymphocytes, and fat infiltration of cells [7]. Toxic hepatitis has been the principle clinical manifestation of acute morbidity and mortality, historically associated with 2,4,6-trinitrotoluene exposure [11]. Hepatic injury is manifested by jaundice, which often is demonstrable only in the conjunctivae since the skin of workers with this degree of exposure may already be dyed yellow from the 2,4,6-trinitrotoluene. Unfortunately this clinical manifestation is a late sign of injury and is not satisfactory in the prevention of acute yellow atrophy. Earlier symptoms are non-specific and not very useful in prevention [9].

The carcinogenicity of 2,4,6-trinitrotoluene has also been reviewed by cancer associations. The World Health Organization's IARC classified 2,4,6-trinitrotoluene as a Group 3 carcinogen which means it is not classifiable as to its carcinogenicity in humans [12]. EPA assigned to 2,4,6-trinitrotoluene a weight-of-evidence carcinogenic classification of C i.e. a possible carcinogen. The assessment done by the EPA was based on a study in which rats exposed to 2,4,6-trinitrotoluene by eating it for long periods developed tumours of the urinary bladder [13]. In Germany 2,4,6-trinitrotoluene is classified as a substance that is suspected of being potentially carcinogenic to humans [1]. The Office of Environmental Health Hazard Assessment of the California USEPA also listed 2,4,6-trinitrotoluene as a carcinogen in December 19, 2008 [14].

With a retrospective study, of male workers exposed to 2,4,6-trinitrotoluene over a period of one year from eight Chinese military factories, from 1970 to 1995 found a higher relative risk for malignant tumours, especially liver cancer. The morbidity of total malignant tumours in male 2,4,6-trinitrotoluene exposed workers was markedly higher than that of controls; the relative risk (RR) was 2.3. Liver cancer morbididty was 31.9% of the total malignant tumor, and its mortality was 3.97 times of the controls. The average death age of the 2,4,6-trinitrotoluene exposed workers (51.7 years old) was younger than that of the same factory control (54.1 years old) and male populations (55.6 years old) in large and medium cities. The incidence of liver cancer was closely related to the length of service, the kinds of job in a factory and the level of exposure to 2,4,6-trinitrotoluene, and alcohol consumption was synergistic with 2,4,6-trinitrotoluene carcinogenesis [15].

A preliminary study of a German population living near the sites of two World War II munitions plants, indicated an association between increased rates of some types of leukemia and living in a town near 2,4,6-trinitrotoluene waste from these plants. The study showed increased relative risk of acute myelogenous leukemia for adult males and females living near the former explosives plants when compared with adults in a neighboring county. The relative risk was particularly high for individuals >65 years of age. However, the study case numbers were very small [15].

A case report has supported these findings; in 1988 Garfinkel reported a case of liver cancer in a 61 year old engineer who had been exposed daily to 2,4,6-trinitrotoluene for 35 years, and the engineer had no past history of infectious hepatitis or alcohol abuse, which are known risk factors for liver cancer [14].

Some workers may also show increased sensitivity to 2,4,6-trinitrotoluene and they are those with impaired liver function, alcoholics', impaired kidney function, those who are anaemic**,** and individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency [4]. Acute hemolytic disease was described in three ammunition plant workers who were also glucose-6-phosphate dehydrogenase (G6PD) deficient (an enzyme that catalyzes the oxidation of glucose 6-phosphate to 6-phosphoglucono-lactone) onset of the disease was within two to four days after exposure [4]. There have also been deaths reported from massive hemolysis in those with glucose-6-phosphate deficiency [5].

Limited information is available on the reproductive or developmental toxicity of 2,4,6-trinitrotoluene in animals or humans following inhalation exposures. Information from occupational exposure studies suggested that 2,4,6-trinitrotoluene may cause menstrual disorders and male impotency [4].

The Ames assay is a well-validated assay for mutagenicity that has been used for the approximately 20 years [16]. Ames assay, a basic toxicological tool, is an in vitro test for mutagenicity and according to this tool 2,4,6-trinitrotoluene might be genotoxic. It has given positive results in the Ames assay both with and without metabolic activation [4].

Another potential adverse effect of 2,4,6-trinitrotoluene exposure is the formation of cataracts. It is believed to be specific to 2,4,6-trinitrotoluene and is often associated with chronic low-level exposure [4]. Cataracts resulting from exposure to 2,4,6-trinitrotoluene have also been reported in China and in Finland. A case series was reported in which 6 of 12 workers had cataracts. Changes occurred in a bilateral and symmetric fashion [17]. In China it was found that chronic occupational exposure to

2,4,6-trinitrotoluene caused mainly hepatomegaly and cataract. The incidence of hepatomegaly was 41% and cataract 79% in 2,4,6-trinitrotoluene workers with prolonged exposure [15]. A study was conducted in a Danish factory to compare the prevalence of cataract in workers exposed to trinitrotoluene to the prevalence in a group of unexposed workers, matched on age and sex, using Tiukina's description and grading of TNT-induced cataract. They found through eye examinations performed by an ophthalmologist four cases of TNT-induced cataract among the 23 TNT- exposed workers and none in the unexposed group. The difference in prevalence between the two groups was statistically significant ($p<0.01$;Mantel-Haenszel's chi square test). The strength of their study as compared to previous studies relied on the use of a truly unexposed control group and a well described TNT cataract grading scale [18].

Acute exposure to 2,4,6-trinitrotoluene can cause irritation of the upper respiratory tract and skin; symptoms and signs include sneezing, coughing, rhinitis and erythematous dermatitis. The onset of acute systemic toxicity is frequently heralded by gastrointestinal symptoms such as nausea, anorexia and epigastric pain. Systemic symptoms may progress to include headache, fatigue, malaise, palpitations, loss of memory and cyanosis [19]. A study examined 38 workers in a 2,4,6-trinitrotoluene production and shell loading plant and 20 unexposed control workers. The 2,4,6-trinitrotoluene exposure ranged from 0.1 to 1.2mg/m^3 with peaks of up to 10mg/m^3. The 2,4,6-trinitrotoluene exposed workers had higher prevalence's of respiratory (sneezing, sore throat and cough) and gastro intestinal (stomach ache, anorexia, constipation, flatulence, nausea and vomiting) complaints than the controls. However, there were no significant differences in the liver tests and no cases of cataract were recorded [7]. Other reported effects of 2,4,6-trinitrotoluene exposure include dermatitis, leukocytosis, neurological disorders (neurasthenia, nystagmus, irregularities in muscle reflexes and adiadochokinesia), and nephrotoxicity [20].

1.5. Methods to measure 2,4,6-trinitrotoluene exposure in exposed subjects

Given the numerous and the severe health effects of over exposure to 2,4,6-trinitrotoluene it is important to monitor exposure in at risk munitions workers. To

estimate individual exposure and the health risk of individuals occupationally exposed to 2,4,6-trinitrotoluene, biological monitoring is a suitable tool.
Biological monitoring can be defined as a systematic continuous or repeated measurement and assessment of workplace agents or their metabolites (biomarker) either in tissues, secreta, excreta or any combination of these to evaluate exposure and health risk compared to an appropriate reference [21] for example the BEIs proposed by the ACGIH, [22] or the DFG, biological tolerance value BAT for occupational exposures [23].

As previously specified nitro toluene's are readily absorbed through the skin, resulting in a higher uptake than would be expected from the inhalation route alone; therefore ambient air measurements are inadequate for the estimation of health risks. For example, although the respiratory exposure may be well below the OEL of 0.5mg/m^3 as stipulated by the Hazardous Chemical Substances Regulations of the OHS Act (Act 85 of 1993) [24], the total amount of 2,4,6-trinitrotoluene absorbed by the body may be excessive. It should also be noted that the TLV of the AGIH for 2,4,6-trinitrotoluene is 0.1mg/m^3 [25].

To assess internal burden, determination of both the unchanged compounds and their metabolites has to be considered. Biological monitoring is therefore a more reliable estimation of total exposure than air monitoring and should be done when deemed necessary on munitions workers exposed to 2,4,6-trinitrotoluene. Although biological monitoring is a more accurate estimation of total exposure to 2,4,6-trinitrotoluene it is important to note that there is currently no BEI or BAT reference value for 2,4,6-trinitrotoluene in South Africa or internationally. Given the consequences of over-exposure a reliable test method should be used to determine 2,4,6-trinitrotoluene exposure in munitions workers. A reliable test method will also further assist in research to set a biological threshold limit for 2,4,6-trinitrotoluene and its metabolites.

The major metabolic pathway of 2,4,6-trinitrotoluene as well as its ultimate toxic principle is the formation of aromatic amines by reduction of one or more nitro groups (Figure 3). 4-amino-2,6-dinitrotoluene (4-ADNT) and 2-amino-4,6-dinitrotoluene (2-ADNT) are the main metabolites of 2,4,6-trinitrotoluene and they are eliminated in

urine after conjunction to acid-labile glucuronides [1]. Since 2,4,6-trinitrotoluene is rapidly metabolised it may be difficult to determine trace amounts of unchanged compound in either blood or urine. Therefore, the presence of 2,4,6-trinitrotoluene metabolites such as 4-ADNT and 2-ADNT, which are present in the urine for over two weeks after acute exposure, can be used to indicate recent and past exposures [26]

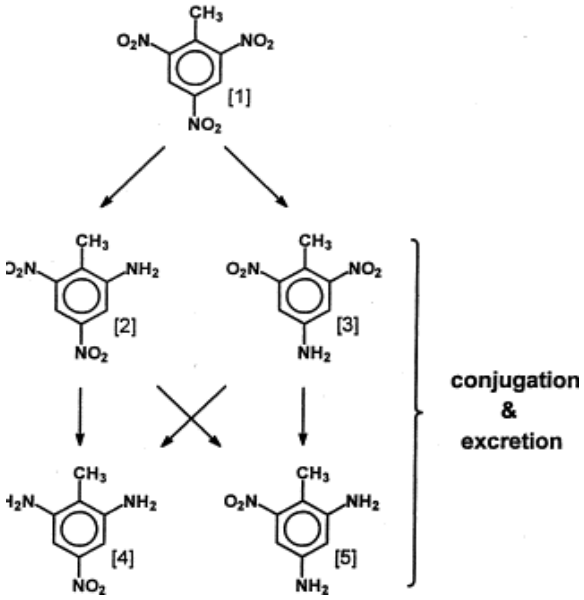


Figure 3: Basic metabolism of 2,4,6-trinitrotoluene *(*1) Trinitrotoluene, (2) 2-amino-4,6-dinitrotoluene, (3) 4-amino-2,6-dinitrotoluene, (4) 2,6-diamino-4-nitrotoluene and (5) 2,4-diamino-6-nitrotoluene.

There are several biological monitoring methods to determine exposure to TNT. These methods measure TNT and/or its metabolites in urine samples of exposed workers. Some of them are discussed below; the intent is not to provide an exhaustive list of analytical methods, but rather to describe the well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect 2,4,6-trinitrotoluene in environmental samples are the methods approved by federal organizations such as EPA and NIOSH. Other methods presented in this chapter are those that are approved by groups such as the AOAC and APHA. Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision [4]. .

Webster urine test

The Webster urine test is a simple qualitative test to determine TNT uptake [4]. . Webster developed this test in 1917 and it was mainly devised for a derivative of TNT [27], namely 2-6 dinitro hydroxylamino-toluene that is excreted in urine. This test is based on the reaction of alcoholic potassium hydroxide with an ether extract of acidified urine wherein colours are produced due to the presence of TNT breakdown products [4]. The Webster urine test is defined according to the British Medical Journal as a transient colour reaction indicating, when positive, that absorbed TNT has been acted on during metabolism and has been excreted in an altered form. The Webster test in no way denotes systemic poisoning; positive test results can be graded from "trace" to "intense" and this standard helps in monitoring of potential over-exposure since the intensity of a positive result can be considered in relation to the known exposure to TNT [28].

There are other more complex methods that need expensive equipment and are performed in specialised laboratories to analyse TNT and/or it metabolites in urine, and they are as follows:

The High-Performance Liquid Chromatography - Mass Spectrometry, (HPLC-MS) method.

The HPLC-MS method is known to be a highly selective and sensitive method for determining TNT in urine. The limited data on this method of analysing TNT in urine show a high recovery of TNT (90%) but a much lower recovery for its metabolites (30%). It has a sample detection limit of 0.1µg/L [4].

The Gas Chromatography - Electron Capture Detection, (GC-ECD).

GC-ECD accurately determines the TNT metabolite 4-ADNT in a toluene extract of hydrolyzed human urine. The limit of detection for this method is in the low-ppb range with high recovery of the analyte (90%) [4].

Thin-Layer Chromatography – Densitometry (TLC) methods.

The modified TLC method that employed a computer-linked densitometer for the detection and quantification of TNT or its metabolite in hydrolyzed human urine is about 3 – 4 orders of magnitude less sensitive than HPLC-MS [4].

The Gas Chromatography - Mass Spectrometry (GC-MS) and the Liquid Chromatography – Mass Spectrometry, (LC-MS) methods.

A number of fairly recent papers were available for the GC-MS and LC-MS methods. Interest in using MS in industrial hygiene was driven by its value in understanding basic physical, chemical and biological processes related to workers' exposure to occupational hazards [29].

The GC-MS and LC-MS analytical techniques have developed as some of the most sensitive and selective analytic techniques for the separation, identification and quantification of components of complex mixtures, these techniques have been used for the identification and quantification of TNT metabolites [29].

The application of the GS-MS for the quantification of organic compounds and biomarkers has grown spectacularly in environmental health and occupational exposure studies. GC/MS has been widely accepted as a "gold standard" for chemical identification of volatile and semi-volatile organic compounds in mixtures, drug detection, environmental analysis, explosives investigation, and identification of unknown samples. Additionally, it can identify trace elements in materials that were previously thought go undetected by other technologies [30]. The cost of GC-MS has fallen significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies as cost is a major consideration in this field [31].

2. APPLICATION OF TNT MONITORING BY A MUNITIONS FACILITY IN SOUTH AFRICA

TNT was used in the production of bombs by a munitions facility in South Africa. TNT is listed as a Hazardous Chemical Substance (HCS), in Table 2 of the Hazardous Chemical Substances Regulations, of the Occupational Health and Safety Act, 85 of 1993, of South Africa, thus labelling it a HCS for regulatory purposes.

The Webster urine test and GC-MS analytical technique for TNT metabolites were used in the munitions facility to quantify worker exposure to TNT. Webster's qualitative urine test was used in combination with a scale, developed in the laboratory, using an ultra violet spectrophometer. This qualitative test became a semi quantitative test in this setting. The scale read as follows: white – 0.0 mg/ L – nil contamination with TNT, light pink – 0.5 mg/L, pink – 1.0 mg/L, light red – 1.5 mg/ℓ, red – 2.0 mg/L. A step-by-step written procedure was available for this method and was utilised by the laboratory analysts. A laboratory analyst visually read the change of colour of urine and then compared it to the scale. The test was performed by two laboratory analysts in the laboratory.

With the GC-MS analytical technique, the analysis of urine was done by an external laboratory to determine TNT metabolites in urine. The laboratory determined the amount of 4-ADNT,2-ADNT and the total DNAT present. The metabolites were detected in milligrams per litre (mg/L). 0.0 mg/L was interpreted as no contamination with TNT by the external laboratory. The reference value for total DNAT was set at < 9.7mg/L for end of shift and < 10mg/L for end of a five day shift. The GC-MS method had a continuous scale to indicate uptake.

The Webster urine test in general presented negative results, suggesting that control measures were in place and were effective against TNT, during biological monitoring of exposed workers. However, tests conducted using the GC-MS method indicated the presence of TNT metabolites in the urine samples of some of the exposed workers. Given that the two methods yielded different results, the purpose of the study was to

determine, whether the Webster method was a reliable method to determine TNT exposure in workers.

3. PURPOSE OF THE STUDY

The purpose of this study was to determine the reliability of the Webster urine test. It is essential to have a reliable method to monitor human exposure to TNT due to its toxicity.

4. OBJECTIVES

- **First Objective**

To describe the concentration of TNT in the urine of ammunition workers exposed to TNT by using the Webster method for the years 2001 till 2007.

- **Second Objective**

To compare TNT concentrations using the Webster method with the concentration of TNT metabolites by the GC-MS analytical technique.

CHAPTER TWO: MATERIALS AND METHODS

This chapter begins with an outline of the study design, then presents the methodology, data collection and data analysis methods applied in the study and concludes with the ethical considerations taken into account when the study was conducted.

1. STUDY DESIGNS AND METHODS

1.1 Study Design

The design of the study was retrospective descriptive. This study was conducted to determine the reliability of the Webster method for measurement of 2,4,6-trinitrotoluene in urine. Historical data collected between the years 2001 and 2007 were used. The data collected were part of the biological monitoring programme conducted on exposed workers to comply with the Hazardous Chemical Substances Regulations promulgated in terms of OHS Act, 85 of 1993.

1.2 Study Setting

The setting was a large munitions facility in South Africa well-known for the quality of its medium and large calibre ammunition. The facility was established in 1979.

The facility consisted of the following plants:

- Manufacturing plant, for the manufacturing of explosive ordinances and equipment for local and export purposes.
- Detonator plant, for the manufacturing of primary explosives (lead nitrate, lead azide etc.) and detonators.
- Ammunitions plants (which consisted of four plants), for the filling and assembly of medium and large calibre ammunitions
- Recovery plant where 2,4,6-trinitrotoluene was recovered from faulty shells.
- Other sections in the munitions facility included the following, testing and development, training, maintenance, construction, security, paint shop, laboratory facilities, engineering workshop and research operations.

For the purpose of this study, the four ammunitions plants (AO1, AO2, AO3 and GO1) for large calibre ordinance filling and assembly and the explosive recovery plant (DO2) were selected. These plants were selected because they provided a work environment in which workers were potentially exposed to TNT dust or vapour. In the large calibre ordinance filling 250mm, 155mm and 120mm large calibre ammunitions were filled with the high explosives TNT or TNT/RDX. In the recovery plant TNT was recovered from faulty shells. The process was as follows; shells were first steam cleaned in a bath to melt the TNT and then subsequently followed by spraying with a high pressure water-jet to recover the TNT.

1.3 Study Population

The study population consisted of primarily male workers exposed to TNT in the ordinance, filling and recovery plants. These plants employed workers who were permanent and temporary contract workers, the age for both ranged between 20 – 65 years. The years of service for the permanent workers ranged between 15 – 27 years in the plant. The employment of temporary contract workers depended on external order requests, there contracts ranged from a one month to a one year contract. Temporary contract workers did not have continuous service within the plants. Permanent and temporary workers were rotated within the ammunitions manufacturing plant and the recovery section. These plants would accommodate about 60 workers at a time, again depending on external order requests.

For the purpose of this study only TNT exposed subjects, with biological monitoring data between the years 2001 and 2007 were selected. These workers were employed as production foremen, senior and flow line operators in ammunitions plants (AO1, AO2, AO3, GO1) and recovery section (DO2). Senior and flow line operators were allocated to various areas of responsibilities within the ammunitions plants to complete the processes.

The processes in these ammunitions plants (AO1, AO2, AO3, GO1) were as follows: receiving, sorting, melting - casting, probing and canning. In the receiving area the TNT flakes or TNT/RDX formed balls were received in sealed bags from the magazine storage facility. Thereafter it departed to the sorting area where the bag was scanned

by a metal detector to detect any metal and thereafter the operator would manually sort the TNT or TNT/RDX on a sorting table to detect any unwanted products. Afterwards the TNT or TNT/RDX flakes or balls were then emptied into a rotary melting machine for melting of the TNT. During the casting process, the operator manually operated this process, the shells were filled with a layer of TNT and a layer of TNT/RDX balls according to customer specification. The probing face was automated and the flow line operator only operated the conveyer belt. During the canning process the bombs were manually sealed by the flow line operator.

Health assessments (medical surveillance) on exposed workers were conducted at the Occupational Health Centre at the munitions facility as well as biological monitoring for exposure assessment of hazardous chemical substances. For biological monitoring purposes urine sample bottles were prepared for workers. Two urine samples were obtained from workers at the end of a five day work shift, usually on a Friday morning before workers entered the ammunitions plants. Samples were obtained prior to a weekend as TNT metabolises rapidly. After collection of the samples from the workers, the samples were taken to the onsite laboratory for analysis. The samples that were analysed externally were packed in ice and collected at the occupational health clinic by the external laboratory. Subsequently, monitoring was followed by a medical assessment by the occupational medical practitioner after results were obtained from the laboratories.

1.4 Data collection

Historical biological monitoring data were the primary source of data. All existing biological monitoring records for the years 2001 to 2007 available in the occupational health clinic of the munitions facility were retrieved for the study. The data included the Webster monitoring results recorded on hand-tabulated reports from the onsite laboratory and computer generated reports from the external laboratory archived in the TNT file.

For the first objective approximately 400 Webster urine sample results available for the years 2001 to 2007 were retrieved from the occupational health clinic. For the second objective 100 paired urine sample results available from the years 2006 to

2007 were retrieved. Paired samples defined for the purpose of this study were one urine sample for the Webster and one urine sample for the GC-MS method collected from the same worker at the same time. Paired samples were only available for the years 2006 to 2007 as active comparison between the two methods only commenced in 2006.

1.5 Quality control

To ensure no contamination, samples were collected from exposed workers in the morning (pre shift), prior to entering the ammunitions plants and wearing their personal protective equipment.

A standard written procedure for testing TNT exposure through the Webster urine test was available at the laboratory and the laboratory analysts were trained in this methodology (see Appendix 1).

The external laboratory performing the GC-MS analytical technique for TNT metabolites was ISO 9001 and SANS 17025 accredited.

2. DATA ANALYSIS

For the first objective, data from 2001 to 2007 were captured and tabulated on a Microsoft Excel spread sheet, to demonstrate the distribution of positive Webster test results.

For the second objective, the GC-MS analytical technique for urine metabolites was considered to be the “gold standard” and the following steps were conducted.

- First, urine sample data were presented in a XY scatter diagram.
- Second, paired urine sample data were presented in box and whisker plots.
- Third, paired urine sample data were presented in a Bland-Altman scatter plot.
- Fourth, the association between the Webster test and the GC-MS analytical technique for TNT metabolites was examined using Chi-square statistics.
- Fifth, the Webster test and the GC-MS analytical technique were measured in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and the receiver operating curve.

Also note that the Webster and GC-MS concentrations are in mg/L, but the cut points for exposure are ≥2 mg/L and 10 mg/L for the Webster and GC-MS respectively.

3. ETHICAL CONSIDERATIONS

As per the requirements of the Faculty of Health Sciences, the study was submitted to the Human Research Ethics Committee (HREC) of the University of the Witwatersrand for consideration. The study was reviewed and ethical clearance was granted, R14/49 Kgasoane (see Appendix 2).

Consent for the use of biological monitoring data was obtained through discussion in the health and safety meeting. The occupational medical practitioner, management and plant supervisors of the plant were informed as to the purpose and the objectives of the study. No medical records were accessed, only urine results collected to measure TNT exposure.

Workers were given an information sheet to ensure that the participants understood the purpose and the objectives of the study. Workers were also informed of their rights, such as withdrawal of the use of their results for the purpose of the study, without any negative consequences. The aims were to ensure informed consent and to ensure voluntary consent (i.e. no coercion)

CHAPTER THREE: RESULTS

This chapter will focus on the presentation of the concentration of TNT in the urine samples measured by the Webster urine test and the GC-MS analytical technique. First, the results of the Webster urine test will be presented by year and then by location within the ammunitions plants. Then the Webster and the GC-MS results are compared using scatter plots, box and whisker plots, sensitivity, specificity, PPV, NPV and ROC using the GC-MS as the "gold standard".

1. WEBSTER URINE RESULTS

The first objective was to describe the concentration of TNT in the urine of exposed munitions workers, using the Webster method to analyse exposure for the years 2001 to 2007.

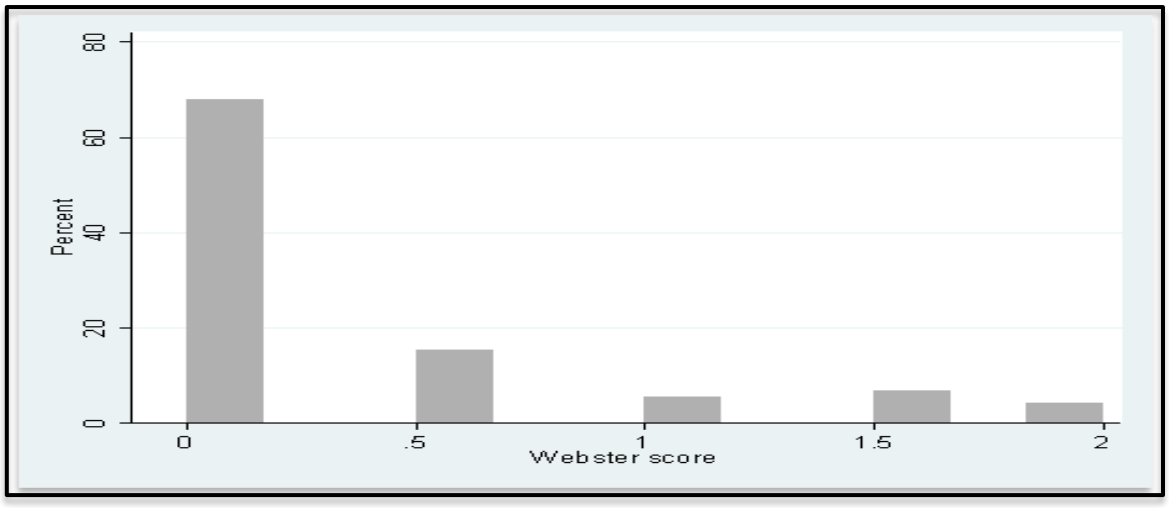


Figure 4: Bar graph of the percentage of Webster urine test results in each exposure category in mg/L

Figure 4 presents the data in the form of a bar graph, which shows the distribution of the percentage of the Webster urine results for the years 2001 to 2007. If applying purely the Webster scale in the munitions laboratory which is as follows: white – 0mg/ℓ (nil contamination), light pink – 0.5mg/L, pink – 1.0mg/L, light red – 1.5mg/L and red -

2.0mg/L. This graph indicates that the proportion of nil contamination was 86.3% with over-exposure only 1.9%

Table 2: The number and percentage of the Webster urine test results by categories for the years 2001 - 2007

Webster	2001		2002		2003		2004		2005		2006		2007		Total	
(mg/ℓ)	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
0	138	90.2	37	88.1	28	75.6	32	88.8	34	91.8	73	82.9	26	78.7	368	86.3
0.5	14	9.1	1	2.3	2	5.4	3	8.3	3	8.1	9	10.2	4	12.1	36	8.4
1	-	-	-	-	-	-	1	2.2	-	-	4	4.6	2	6	7	1.7
1.5	-	-	-	-	5	13.5	-	-	-	-	2	2.3	-	-	7	1.7
≥2	1	0.6	4	9.5	2	5.4	-	-	-	-	-	-	1	3	8	1.9
Total	153	35.9	42	9.8	37	8.68	36	8.45	37	8.68	88	20.6	33	7.7	426	

A summary of the distribution of the Webster urine test results over a seven year period is shown in Table 2. A total of 426 single urine samples were collected from munitions workers during this period. Urine samples that tested negative over this period were 98.1%, i.e. all readings between 0mg/L and 1.5mg/L. Only 1.9% of the 426 samples collected tested positive (≥2.0mg/L) i.e. indicated significant exposure. The number of urine samples collected over the years varied. The percent change over time in the proportion of samples with no exposure varied, with the highest percentage of 90.2% in 2001 and lowest percentage of 75.6% in 2003, a 15% difference, this could have been attributed to production demand (high production demand in 2003 and a low production demand in 2001). There was no specific trend in terms of the percent change over time in the proportion of samples with no exposure. There was an increase in the number of samples collected in the years 2001 and 2006 and this variation can be attributed to the production demand of large calibre ammunitions, which depended on external demand.

Table 3: Webster urine test results per plant

Plant	0	0.5	1	1.5	≥2	%	Total
A01	40	3	1	2	-	-	46
A02	61	5	4	5	3	3.8	78
A03	188	28	2	-	5	2.2	223
D02	74	-	-	-	-	-	74
G01	5	-	-	-	-	-	5
Total	368	36	7	7	8	1.9	426

Table 3 provides a summary of the distribution of the Webster urine test results per plant. A total of 426 samples were collected from exposed workers in these plants. Only in two plants was over-exposure demonstrated, namely in AO2 (3.8% of measurements were ≥2.0mg/L) and AO3 2.2% of measurements were ≥2.0mg/L). No exposure was demonstrated in DO2 and GO1, this could have been attributed to the following; the use of small quantities of trinitrotoluene in GO1, for DO2 the strict use of personal protective equipment (PPE) was observed in this plant, secondly the recovery of trinitrotoluene was also depended on the production demand.

2. COMPARISON OF THE WEBSTER URINE RESULTS TO THE GC-MS METHOD

For the second objective, the Webster urine results were compared to the GC-MS analytical technique for TNT urine metabolites considered to be the “gold standard”. The following steps were conducted:

- First, GC-MS urine sample data are presented in a histogram.
- Second, paired urine sample data are presented in scatter diagram and box and whisker plots.
- Third, paired urine sample data were presented in a Bland-Altman scatter plot
- Fourth, the association between the Webster test and the GC-MS analytical technique for TNT metabolites was examined using Chi-square statistics.
- Fifth, the Webster test and the GC-MS method were measured in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

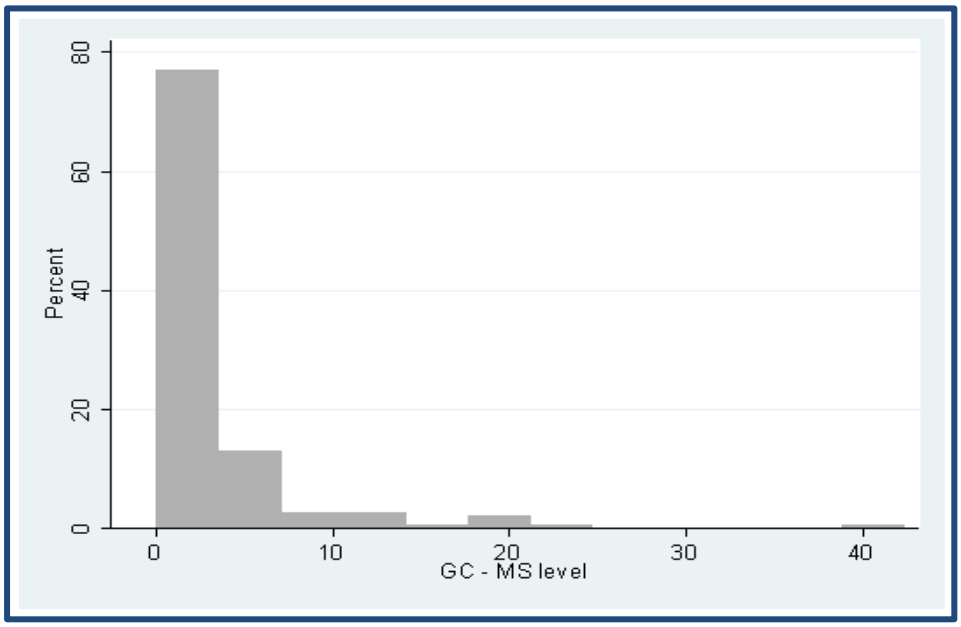


Figure 5: Histogram of the percentage of GC-MS test results by urine concentration in mg/L

Figure 5 presents data in the form of a histogram which shows the distribution of the percentage of the GC-MS urine results by concentration for the years 2006 to 2007. It can be noted that 93% of the measurements were <10mg/L and the positive results levels, ≥10mg/L, were 7% (n=10), applying the cut-off point of ≥10mg/L.

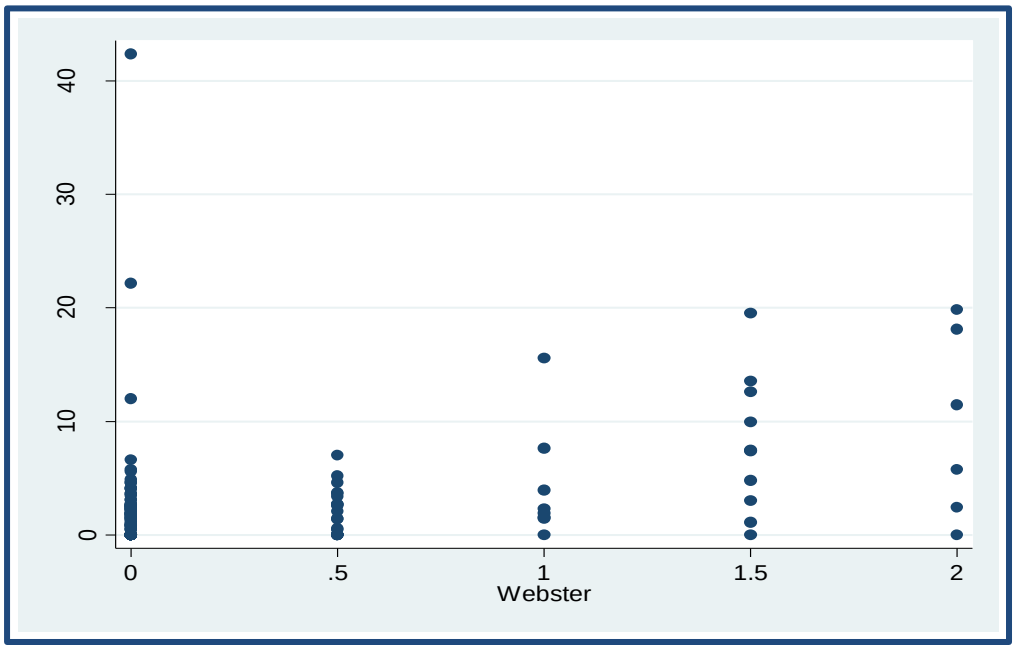


Figure 6: Scatter diagram of the Webster mg/L and GC-MS mg/L

Figure 6 presents the data in the form of a scatter diagram. As can be seen, the GC-MS index of exposure of ≥10mg/L was exceeded in all categories of the Webster except for category 0.5mg/L. Surprisingly the two highest values were in samples categorised as zero by the Webster test. Also, three of the measurements indicating over-exposure by the Webster (2.0mg/L) were <10mg/L as per the GC-MS method. The total positive tests that the Webster test misclassified was 70%, only 30% was correctly classified by the Webster test.

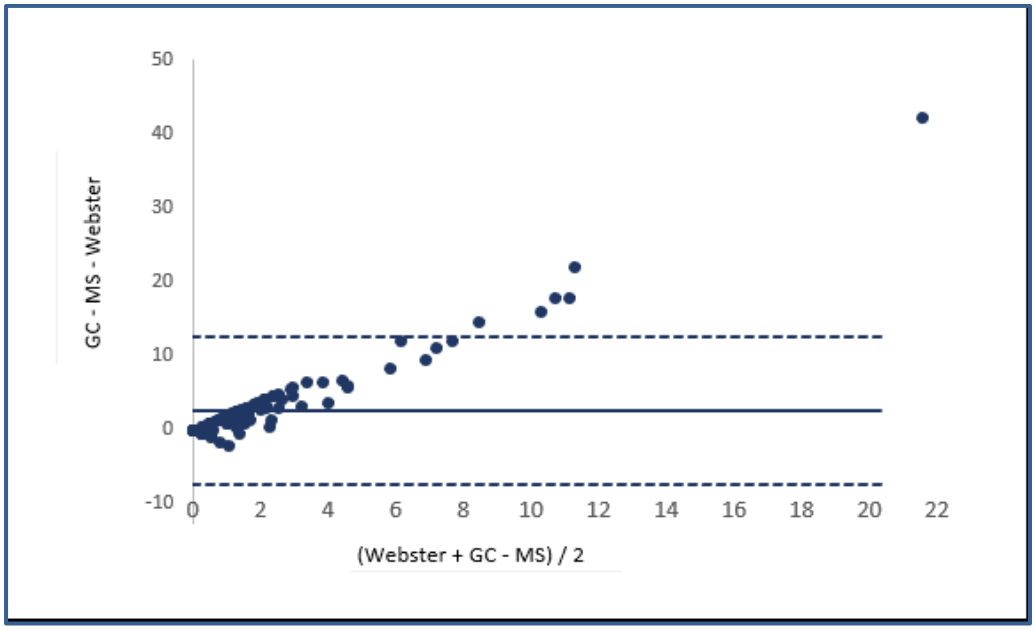


Figure 7: Bland-Altman scatter plot of the Webster mg/L and GC-MS mg/L

N	**144**

	Minimum	**Maximum**
Webster	0.000	2.000
GC-MS	0.000	42.370
(Webster+GC-MS) / 2	0.000	21.185

Parameter	**Estimate**	**95% CI**	**SE**
(Mean Difference)	2.590	1.7499 to 3.4308	0.4252
95% Lower LoA	-7.410	-8.8498 to -5.9704	0.7283
95% Upper LoA	12.591	11.1511 to 14.0305	0.7283

SD	5.102

Figure 7 presents the data in the form of a Bland-Altman scatter plot. As can be seen the data points are not scattered uniformly around the horizontal axis indicating a proportional error between the GC-MS and the Webster urine test. The difference between the two methods first tend to narrow down and then increase as the value of measurements increase, it indicates the existence of a proportional bias which means that the methods do not agree equally through the range of measurements. The

Bland-Altman plot shows poor agreement between the two methods, the 95% upper and lower limits of agreement are very large.

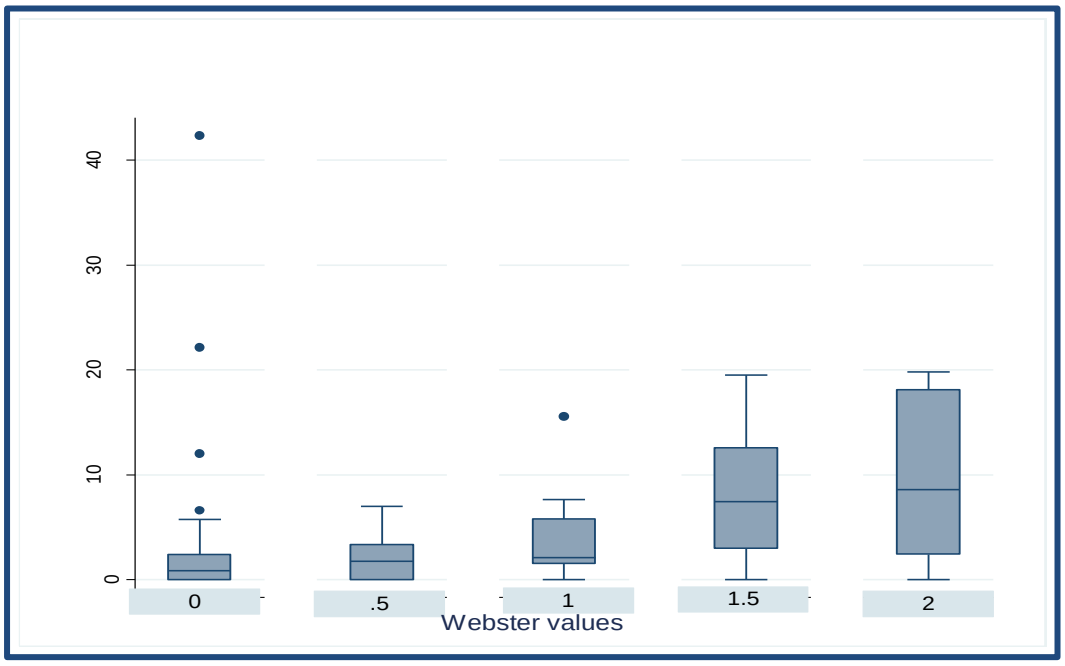


Figure 8: Box and whiskers plots of the Webster mg/L and GC-MS mg/L measurements

Figure 8 presents the data in the form of box and whisker plots. As can be seen there were substantial outliers in categories 0 and 1 of the Webster test. Nevertheless the median values across the Webster categories showed an increasing trend, as the categories increased so did the median GC-MS values which were 0.9, 1.8, 2.1, 7.5 and 8.6 for the Webster categories 0, 0.5, 1, 1.5 and 2 respectfully.

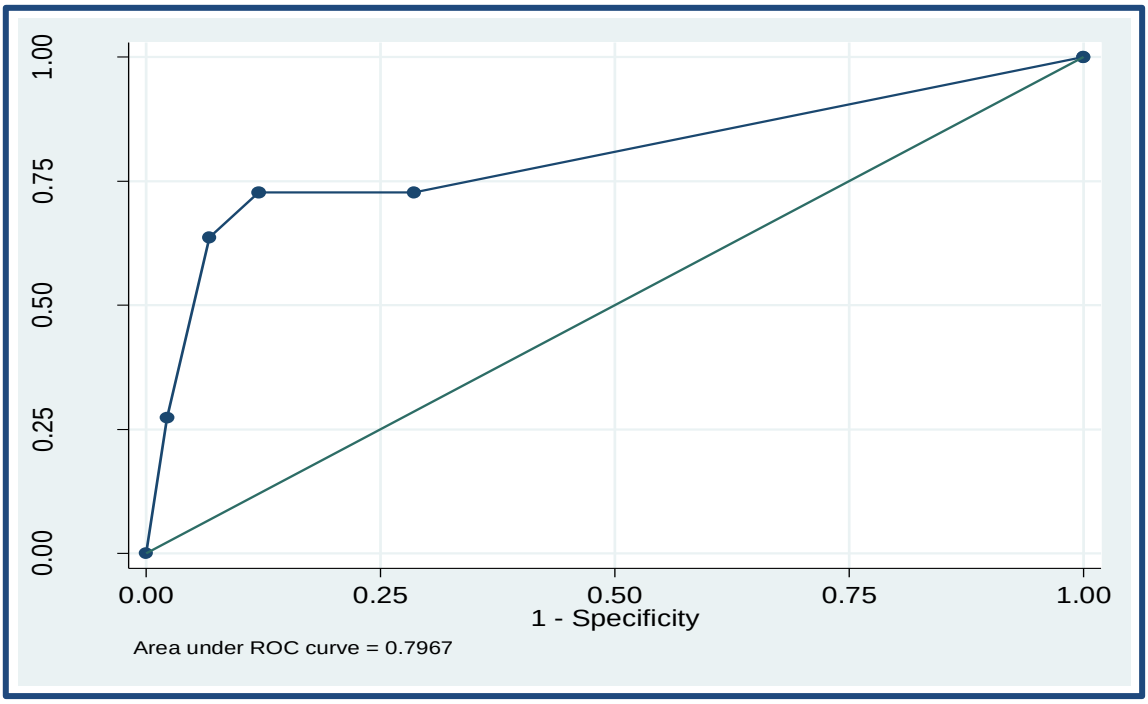
Table 4: Two by two table of GC-MS measurements and the Webster measurements

		GC – MS B		
		0	**1**	**Total**
Webster A	**0**	131 a	7 b	138
	1	3 c	3 d	6
Total		**134**	**10**	**144**

Table 4 presents a 2x2 table of the GC-MS and the Webster measurements, where A) 0 represents <2mg/L and 1 represents ≥2mg/L and B) 0 represents <10mg/L and 1 represents ≥10mg/L.

The sensitivity of a test is the proportion of truly positive subjects' labelled positive by the test. It is calculated by a/[a+c]*100. Table 3 shows that the Webster test has a poor sensitivity of 27.2% i.e. a large proportion of over-exposed subjects were not identified by the Webster test. Specificity is the proportion of truly negative subjects labelled negative by the test. It is calculated by d/[b+d]*100. Table 3 shows specificity for the Webster test of 97.8%. The specificity is high, indicating that a high proportion without exposure was correctly classified. The Webster test is thus more specific, but not sensitive in this study setting.

The positive predictive value (PPV), a/[a+b]*100, is the ability of the test to correctly label as positive individuals who are positive. The PPV was 95% for the Webster test. This indicated that 95% of workers who tested positive with Webster were truly positive. The negative predictive value (NPV), d/[c+d]*100, is the ability of a test to correctly label as negative individuals who are negative. The NPV was 50% for the Webster test, thus indicating that only 50% of the Webster tests that were negative were truly negative.



Obs	Area	Std. Err	[95% Conf. Interval]
144	0.7967	0.0863	0.62755 0.96575

Cut point	Sensitivity	Specificity	Classified	LR+ LR-
>= 0	100.00%	0.00%	7.64%	**1.0000**
>= 0.5	72.73%	71.43%	71.53%	**2.5455 0.3818**
>= 1	72.73%	87.97%	86.81%	**6.0455 0.3100**
>= 1.5	63.64%	93.23%	90.97%	**9.4040 0.3900**
>= 2	27.27%	97.74%	92.36%	**12.0909 0.7441**
> 2	**0.00%**	**100.00%**	**92.36%**	**1.0000**

Figure 9: Receiver operating curve (ROC) for the Webster using the GC-MS test as gold standard

Figure 9 shows the data in the form of a receiver operating curve (ROC) for the Webster test, by using the GC-MS test as the gold standard. The total area of the grid presented by an ROC curve is 1, since both the true positive rate (sensitivity) and false positive rate range from 0 to 1. The area under the ROC (AUC) = 0.80, this AUC is quite good, it is close to the ideal value of 1,this basically states that the Webster test performed well in general. However it should be noted that only a small proportion of values was positive in this population and that false negatives were particularly problematic, so the AUC of 0.80 is a bit misleading.

CHAPTER FOUR: DISCUSSION AND CONCLUSION

The focus of this chapter is a discussion of the reliability of the Webster urine test compared to the GC-MS analytical technique. The aim of the study was to determine the reliability of the Webster test to monitor human exposure to TNT. The study was conducted using historical urine test results collected between the years 2001 to 2007.

A reading of red (≥2.0mg/L) on the Webster scale indicated a positive result (i.e. over exposure) and based on this reading the occupational medical practitioner would remove workers from the plant who were over exposed. Only 1.9% of the 426 samples that were taken indicated significant exposure to 2,4,6-trinitrotoluene. Applying the cut-off point of ≥10mg/L for the GC-MS method only 7% of the144 samples that were taken tested positive for the years 2006 to 2007.

In interpreting the findings of the results reported in the research report it should be noted that there are currently no statutory BEIs for 2,4,6-trinitrotoluene in South Africa and internationally. However, guideline values have been published, for example an end of shift value of 2.5mg/L for total 2,4,6-trinitrotoluene (sum of 2ADNT and 4ADNT) and end of working week, 6.5mg/L total 2,4,6-trinitrotoluene (sum of 2ADNT and 4ADNT).

Coombs and Shillack (1998), conducted a study to determine the levels of 2,4,6-trinitrotoluene and its metabolite excretion using the GC-MS analytical technique. 2ADNT and 4ADNT metabolites and total 2,4,6-trinitrotoluene (sum of 2ADNT and 4ADNT), in the urine of exposed workers were used to determine absorption and excretion following 2,4,6-trinitrotoluene exposure. The results indicated an increased absorption of 2,4,6-trinitrotoluene during the working week as compared to international published studies. They found that post shift day four values exceeded post shift day one values in all samples and they concluded that reliable and specific research is needed to set a biological threshold limit for 2,4,6-trinitrotoluene and its metabolites since monitoring did not indicate definite symptoms, signs or biological effects due to increased exposure [32]. Based on this the laboratory determined their

reference values as follows: total DNAT as <9.7mg/L for end of shift and 10mg/L for end of a five day shift. The GC-MS has a continuous scale to indicate uptake of 2,4,6-trinitrotoluene. A cut-off point of 10mg/L was used in this research report.

The first objective was to examine the distribution of the Webster test results. The distribution of the concentrations of 2,4,6-trinitrotoluene for the period 2001 to 2007 and for the ammunition plants (AO1, AO2, AO3, DO2 and GO1) showed that only a few workers (1.9%) were over exposed to 2,4,6-trinitrotoluene during this period, but the Webster test may be unreliable. Two studies show that the Webster test can be falsely negative at times. Dobbin Crawford (1954) reported two cases where the Webster reaction was negative throughout, but the workers exposed to 2,4,6-trinitrotoluene developed toxic anaemia and one of the workers eventually died [33].

Steward and Witts (1945), collected 31 samples from students exposed to 2,4,6-trinitrotoluene, before, during and immediately after exposure. They found that the Webster test tested positive in 29 individuals, but they state that they needed to repeat the test about four to five times on the same individual, since it tested positive on one occasion and negative on another frequently [34].

Assuming that the GC-MS method was reliable and that it produced the same results over all the years as it did in 2006 – 2007, the likely number of tests that would have been positive on the GC-MS method over the years instead of the Webster method can be estimated using Figure 6. It can be seen that 50% of the Webster test >2mg/L were not >10mg/L, thus it is possible that only 50% of the positive Webster test, were truly positive over the years. Referring to Table 1, the truly positive Webster urine results were eight adding the total of ten positive GC-MS to this it can be concluded that the number of over exposed workers could have been 18. Over exposure over the years could have been 6.7% instead of 1.9% as indicated in Table 1.

For the second objective the concentration of 2,4,6-trinitrotoluene in the Webster urine test was compared with the concentration of 2,4,6-trinitrotoluene metabolites by the GC-MS method. Figure 6 indicates that the two highest GC-MS results (43,37mg/L

and 22.18mg/L) were categorised as zero by the Webster test. The Webster indicated no exposure whilst substantial over-exposure was experienced by these employees.

The Webster urine test may be useful to test a group of workers, but it is an inadequate test to identify individual over-exposed workers. The Wester urine test can be used to determine whether there is exposure to trinitrotoluene in a production area, but this test should be followed up with the GC-MS method to indicate individual uptake in over exposed workers.

To test agreement between to GC-MS and the Webster test, the Bland-Altman scatter plot was used. The Bland-Altman plot, Figure 7 indicated the existence of a proportional bias, which meant that the two methods did not agree equally through the range of measurements. The very large 95% upper and lower limit showed poor agreement between the two tests.

The usefulness of a test, in this context its ability to accurately classify individuals into low exposure and high exposure against a gold standard, is typically described by sensitivity, specificity, positive predicted value (PPV) and negative predictive value (NPV). The Webster test performed poorly in that it had a sensitivity of only 27.2% and a NPV of only 50%. Consequently, the test had high levels of false negative results, missing individuals who were over-exposed according to the GC-MS, the gold standard. False negatives are important in occupational exposure assessment as they would result in a lack of the necessary action to control exposure. In this setting of TNT exposure associated with serious health consequences following over-exposure, a sensitive test with high NPV is necessary. The Webster test performed much better with respect to specificity and PPV, being 97.8% and 95% respectively. False positives were thus infrequent, also important, in occupational health practice as false positives may lead to unnecessary interventions at a workplace or individual worker level. In summary, in this setting when the Webster test was positive the practitioner could confidently accept that over-exposure had occurred, but when negative, over-exposure could not be confidently rejected; an unsatisfactory situation. It should be noted, though, that the prevalence of over-exposure was low and prevalence has an influence on NPV and PPV, consequently the Webster test may have performed better in a higher prevalence setting.

The area under the ROC (AUC) = 0.80, this AUC is reasonable; it is fairly close to the ideal value of 1. This basically states that the Webster test performed fairly well in general. As the cut-off point increased separating unexposed from exposed, sensitivity was reduced, but specificity increased. The highest sensitivity and specificity was noted at a cut-off point of ≥1mg/L where sensitivity equated to 72.73% and specificity equated to 87.97%. A test with perfect discrimination has 100% sensitivity and 100% specificity.

LIMITATIONS OF THE STUDY

The following limitations may have affected the study. The laboratory facility at the munitions plant was not ISO 9001 or SANS 17025 accredited, but a standard written procedure on the Webster test was available in the facility and the laboratory analyst received training on this procedure. No quality control procedures were in place to monitor the validity of the Webster test performed. One of the clauses of SANS 17025 is the inclusion of inter-laboratories comparison or proficiency testing programmes, an arrangement not applied in the study setting. Based on this the validity of the Webster results are questionable and this might have affected the urine results which were used for the purpose of this study. Nevertheless, it is valuable to study a test performance under actual workplace conditions, as was done in this study.

Second, although a standard written procedure on the Webster test was available and the laboratory analysts were trained on it, the method involved visual reading of the change in colour of the urine then comparing it to the Webster scale. The visual interpretation of the colour by the laboratory analyst could have influenced the urine results of the Webster test, since there was no colour reference available for the analyst to compare to. A red reading equal to 2.0mg/L, which indicated exposure, could have been interpreted by the laboratory analyst as light red (1.5mg/L) that indicated no over-exposure.

Third, although many consider GC/MS to be the "gold standard" in scientific analysis, GC/MS does have some limitations. Because great faith is maintained in GC/MS analysis, erroneous results are not expected and hard to dispute. However, false

positives and false negatives are possible (35). Even though the analysis of urine by GC-MS analytical technique was done by a SANS 17025 accredited laboratory, sample preparation (that involves pre-treatment, dialysis after homogenization to remove free monomeric sugars and amino acids, derivatization to increase volatility of sample) that is vital part for successful analysis could have had an impact on the results (29). Maintenance and calibration of GC-MS analytical technique also play a vital role in ensuring reliable results.

Fourth, although precautionary measures were taken, such as instructing the workers to report to the clinic on a Friday morning before entering the plant or locker rooms, the risk of contamination in collecting the urine sample, may remain from clothing or hands contaminated with TNT.

The Webster and GC-MS urine results obtained could also not be linked to acute or chronic symptoms experienced by the workers in the plant; neither could it be linked to any occupational hygiene monitoring results. Further no documentation could be found on the exact allocation of the worker at that specific time. Since exposure could not be linked to medical records, surveys and task performed the value of the biomarkers are questionable in this setting.

CONCLUSION

A problematic situation in occupational health is when a test fails to identify over-exposed individuals. The Webster test failed to identify workers who were over-exposed, even recording zero in some instances. The sensitivity of the test was very low (27%), this is too low for a test that has serious health implications if over-exposure is missed. This study indicates that the Webster method cannot be used as applied in the munitions plant to determine 2,4,6-trinitrotoluene exposure in workers due to the toxicity of 2,4,6-trinitrotoluene exposure.

RECOMMENDATIONS

If the munitions facility continues to use the Webster urine test for determination of 2,4,6-trinitrotoluene exposure it is recommended that Webster test should be checked against the GC-MS method more frequently or against another recognised standard. If the test is used the workplace laboratory should introduce quality control for the test.

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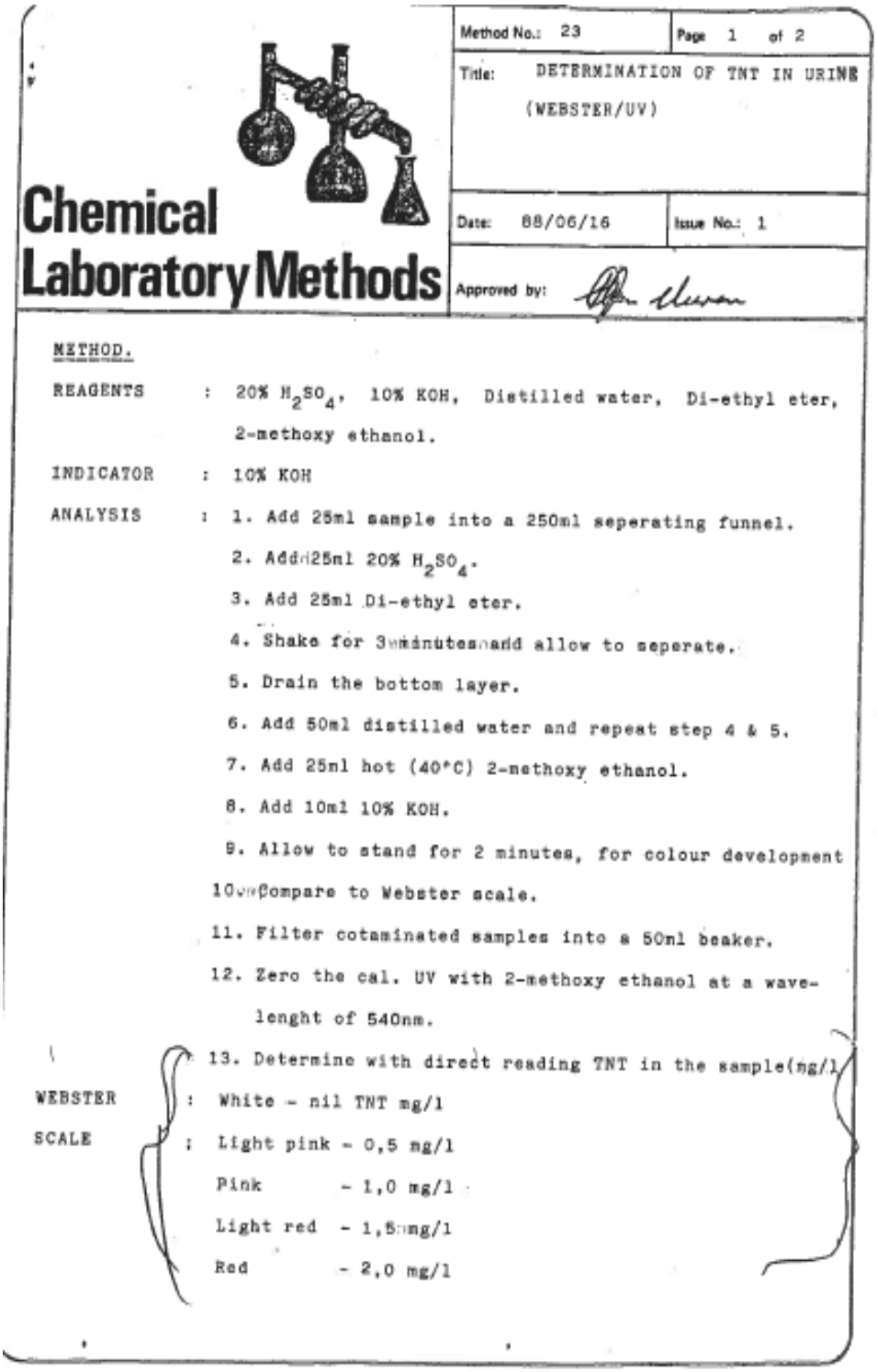
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APPENDIX 1: LABORATORY METHODODOLOGY



Chemical Laboratory Methods

Method No.: 23 | Page 1 of 2

Title: DETERMINATION OF TNT IN URINE (WEBSTER/UV)

Date: 88/06/16 | Issue No.: 1

Approved by:

METHOD.

REAGENTS : 20% H_2SO_4, 10% KOH, Distilled water, Di-ethyl eter, 2-methoxy ethanol.

INDICATOR : 10% KOH

ANALYSIS :
1. Add 25ml sample into a 250ml seperating funnel.
2. Add 25ml 20% H_2SO_4.
3. Add 25ml Di-ethyl eter.
4. Shake for 3 minutes and allow to seperate.
5. Drain the bottom layer.
6. Add 50ml distilled water and repeat step 4 & 5.
7. Add 25ml hot (40°C) 2-methoxy ethanol.
8. Add 10ml 10% KOH.
9. Allow to stand for 2 minutes, for colour development
10. Compare to Webster scale.
11. Filter cotaminated samples into a 50ml beaker.
12. Zero the cal. UV with 2-methoxy ethanol at a wave-lenght of 540nm.
13. Determine with direct reading TNT in the sample(mg/l)

WEBSTER SCALE :
White - nil TNT mg/l
Light pink - 0,5 mg/l
Pink - 1,0 mg/l
Light red - 1,5 mg/l
Red - 2,0 mg/l



Chemical Laboratory Methods

Method No.: 23	Page 2 of 2
Title: DETERMINATION OF TNT IN URINE (WEBSTER/UV)	
Date: 88/06/16	Issue No.: 1
Approved by:	

NOTE : UV spectrophotometer calibration. Calibrate UV with standards:

1 mg/l

2 ng/l

3 ng/l

4 mg/l

5 ng/l

APPENDIX 2: ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Kgasoane

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080335**
PROJECT	The reliability of the Webster method for measurement of TNT in urine
INVESTIGATORS	Ms J Kgasoane
DEPARTMENT	School of Public Health
DATE CONSIDERED	08.03.25
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.11.26 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof D Rees

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...
.....