A prospective study to assess the value of liquid chromatography-tandem mass spectrometry in the management of paediatric poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa

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Declaration

I, Norbertta Nzwisisayi Washaya, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date: 11 January 2021

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Abbreviations

DALYs	Disability adjusted life years
HICs	High income countries
IQR	Interquartile range
LC – MS/MS	Liquid chromatography tandem mass spectrometry
LMICs	Low- and middle-income countries
POCUDS	Point-of-care urine drug screen
PSS	Poisoning severity score
RCWMCH	Red Cross War Memorial Children's Hospital
SST	System suitability test
TAT	Turnaround time
$\chi 2$ test	Chi-square test

Chapter One:
Published manuscript

Abstract

Background

Paediatric poisoning is a common presentation to emergency departments worldwide. There is a paucity of data on the role of liquid chromatography-tandem mass spectrometry (LC-MS/MS), in the management of paediatric poisoning in low-and middle-income countries (LMICs). In high-income countries, most studies are retrospective, and few include children.

Objective

The study describes the prevalence of liquid chromatography-tandem mass spectrometry confirmed paediatric poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa.

Methods

Children admitted with suspected poisoning between 1 January 2017 and 31 December 2017, were recruited. All patients had a urine and/or blood sample sent for LC-MS/MS toxicology. Data collected included demographic data, clinical features, investigations, management, outcome and social interventions.

Results

152 children, with median age of 39 (IQR 25 -61) months were enrolled of which 128 (84%) were poisoning cases. Of the 128 poisoning cases, 88 (69%) presented with a history of ingesting a known substance, 16(12%) an unknown substance and 24(19%) were cases of occult poisoning. LC-MS/MS was able to identify a substance in 92% of the cases of occult poisoning. In those who had presented with a seemingly known substance, LC-MS/MS found a different substance in 15 cases. LC-MS/MS was also able to detect multiple drugs in 40 patients. Of the poisoning cases, six (5%) cases were attempted homicide cases and 5 (4%) cases were attempted suicide cases. No children died. Individualized social interventions were instituted in poisoning cases. Emergency placement safety reasons was required in 6 children.

Conclusion

When the limitations are known, LC-MS/MS is useful in identifying cases of occult poisoning; identifying patients who have ingested multiple substances and/or an unknown substance and when targeted towards child protection. As LC-MS/MS is an expensive test, it should be used judiciously in LMICs.

Key words: Poisoning, Africa, children, mass spectrometry, LC-MS/MS toxicology results in poisoning cases

The prevalence of liquid chromatography-tandem mass spectrometry confirmed paediatric poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa

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Background

Paediatric poisoning is a common presentation to emergency departments worldwide.(1, 2)Though it has a good prognosis, it is an important cause of both morbidity and mortality.(1, 3) In 2016 it was responsible for 6 268 554 disability adjusted life years (DALYs) globally, with children less than 15 years accounting for 45% of these DALYs.(4) In a study done in South Africa, poisoning was responsible for 5.7% of all hospital admissions.(2) While, a retrospective patient folder review carried out at a hospital in Johannesburg, indicated that toxin ingestion was responsible for 17% of the admissions in to the paediatric intensive care unit.(5)

In 2016, poisoning resulted in 31 400 unintentional deaths globally in children less than 15 years of age.(6) The death rate of poisoning was higher in low-and middle income countries (LMICs), with LMICs accounting for 69% of the deaths that year.(6) Despite the higher death rates in LMICs, data on the incidence of paediatric poisoning is more accurate in high-income countries (HICs) where poison control centers have been established and poisoning registries are kept.(1)

Risk factors for poisoning include age, sex and environmental factors such as neglect.(3, 7) Child abuse, in particular neglect, is a big problem in low resource settings such as in Africa, especially in the under-5 population.(8-11) This under-5 population is the age group with the highest incidence of poisoning.(1-3, 7, 12-14) In LMICs, neglect may present as accidental poisoning as children are often left unsupervised, while child abuse may present as intentional or occult

poisoning.(15-17)

The role of investigations in poisoning is controversial but may be of benefit in occult poisoning, where it is difficult to confirm the presence and cause of poisoning. Point-of-care urine drug screen (POCUDS) testing is cost effective and readily available, able to give immediate results but has several disadvantages, such as, a high false positive rate; can only screen for a limited number of drugs; inability to quantify the drug; inability to name the drug, as it can only identify the drug class and the risk of false negatives if the drug in question is below the threshold cut-off for detection.(18-22)

Liquid chromatography tandem mass spectrometry (LC-MS/MS) on the other hand, is a good confirmatory test.(18, 20) Unlike POCUDS, it has a higher sensitivity and specificity and has other advantages, such as, the increased breadth of substances that it can detect and its ability to identify and quantify drugs and their metabolites by name, and not just by the drug class.(18, 21, 23, 24) The main problem with LC-MS/MS, however, is that it is expensive and may have a long turnaround time.(18, 19, 21)

Most of the studies done on the use of LC-MS/MS in poisoning have been done in a retrospective manner and in high-income settings.(21) Additionally, few of these studies have included children. The role of LC-MS/MS in LMICs, where the number of cases of child abuse and neglect are high and the resources to manage poisoned children are severely constrained, is not clear. Its

use may be able to assist in identifying high-risk children in households that need social (child protection) interventions.

This study aims to describe the prevalence of LC-MS/MS confirmed poisoning in children who presented to a LMIC paediatric tertiary hospital over a period of a year, with an emphasis on the value that LC-MS/MS adds in LMICs.

Methods

Setting

The study was done at Red Cross War Memorial Children's Hospital (RCWMCH), a public children's hospital that provides secondary and tertiary health care services to children less than 13 years, living in urban, peri-urban and informal settlements. The hospital manages approximately 35 000 non-trauma emergency care patient-visits each year. A substantial proportion of the patients come from extremely poor and marginalized communities.(25) The children in the catchment area of RCWMCH are not only vulnerable because of poverty but also because of the increase in substance abuse in formal and informal settlements in the Western Cape Province of South Africa.(8, 26, 27)

Study design

The study prospectively enrolled patients with suspected poisoning admitted to the RCWMCH

from the 1st of January 2017 to the 31st of December 2017, in a cross-sectional design.

Participants

All patients admitted at RCWMCH with suspected poisoning were eligible for recruitment into the study if their legal guardians were willing to sign consent for them to be included. Patients who ingested corrosives requiring surgical intervention were excluded from the study.

Data collection and procedures

After consent, data on demographic information, clinical presentation and results of investigations done by the attending clinician were taken, history was taken from the caregiver to establish possible causes of poisoning. The patient was followed up over the period of admission and management including clinical outcomes was recorded. The Poisoning Severity Score (PSS) was used to grade the severity of poisoning at admission (**Table 1**).(28)

Table 1: PSS grading

Grade	Description
None	No symptoms or signs related to poisoning
Minor	Mild, transient and spontaneously resolving symptoms
Moderate	Pronounced or prolonged symptoms
Severe	Severe or life-threatening symptoms
Fatal	Death

PSS: poisoning severity score. From Hans E Persson et al, 1998, Poisoning Severity Score. Grading of Acute Poisoning

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156 ## Toxicology investigation

157 A urine sample from eligible participants was sent to the laboratory for LC-MS/MS to establish the
158 cause of poisoning. In addition, the attending clinician and laboratory were consulted for any
159 leftover blood specimen after laboratory tests ordered by the attending clinician were completed
160 that could likewise be tested on LC-MS/MS. Study participants were not bled solely for the study.

161

162 The LC-MS/MS unit used for this study was the, AB Sciex 3200 QTRAP (© 2013 AB Sciex Pty. Ltd.,
163 AB Sciex, 500 Old Connecticut Path, Framingham MA 01701-4574) unit, housed in the Division of

Clinical Pharmacology, University of Cape Town, Groote Schuur Hospital, Cape Town. At the time of the study it had a library of 120 prescription drugs, over the counter medicines, illicit drugs and some of their metabolites. The library did not include pesticides or herbal compounds used in traditional medicines.

Due to the limited availability of the LC-MS/MS unit, samples were tested in batches. Once collected, samples were registered and transported to the laboratory where they were stored at 4 °C until analysis. The median turnaround time (TAT) for obtaining a result was 5 (interquartile range, (IQR) 3 – 7) days for urine LC-MS/MS and 6 (IQR 4 -7) days for blood LC-MS/MS. A total of five patients had LC-MS/MS results within 24 hours.

Trained personnel ran the samples and interpreted the results. For quality control, internal standards were added to each sample as part of the sample preparation. Each run included blanks, as well as positive and negative controls to ensure accurate results.(29, 30)

In order to observe for possible substance degradation, compound stability tests were done on the LC-MS/MS unit. A commercially obtained control, a system suitability test (SST) (Restek® Corporation) was run daily. The kit contains 8 compounds of known concentrations. The peak areas of each compound were observed to confirm that the instrument performance and sensitivity were optimal and at the same time to observe for possible compound degradation, by

comparing these areas to previously acquired data.

As poisoning is defined by the presence of clinical (somatic and/or mental) manifestations, or laboratory and/or electrocardiographic abnormalities resulting from exposure to a substance that can lead to harmful clinical effects(31), once all the toxicology investigations and clinical presentations were analysed, the authors classified the cases into one of three groups: substance-intake-unlikely, substance-intake-likely or substance-intake- unclear. The substance-intake-unlikely group were patients whose clinical presentation could be explained by an alternative medical diagnosis and were, therefore, not considered poisoning cases even though they were admitted as cases of suspected poisoning. The substance-intake-unclear group were patients whose clinical presentation could not be explained by a medical diagnosis and whose toxicology investigation results were not indicative of poisoning. The substance-intake-likely group were those patients whose clinical presentation could be explained by a toxic substance (even in the absence of an LC-MS/MS identified substance) and were therefore considered poisoning cases, even in the absence of symptoms.

Irrespective of laboratory results, for the purposes of our study, poisoning cases were also clinically divided by the authors into three groups: substance known (history of exposure to a known substance), substance unknown (history of exposure to an unknown substance) and occult poisoning (no history of poisoning, but clinical presentation in keeping with poisoning).

Data analysis

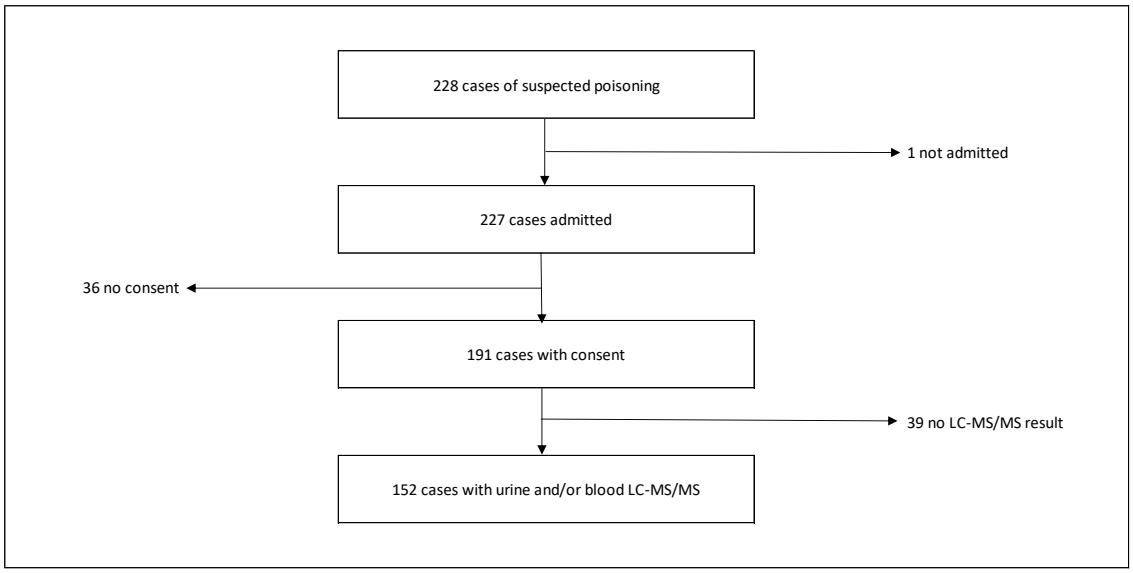
Statistical analyses were done using STATA® 14.0 (StataCorp LLC, College Station, Texas, USA). The demographic characteristics and clinical findings at presentation were tabulated to provide a background description of the study population. All substances that tested positive with LC-MS/MS were described. Percentages and their 95% confidence intervals in outcomes of interest were used to depict proportions of categorical variables while medians with interquartile ranges were used to summarise continuous variables. The $\chi 2$ test or Fisher's exact test were used to assess the strength of association between two categorical variables as appropriate. A significance level at a two-tailed $P<0.05$ was used for all analyses.

Results

Demographic Data

A total of 228 cases of suspected poisoning were screened of which 152 were included (**Figure 1**). The median age of the included children was 39 (IQR 25-61) months, of whom 86 (56%) were male and 113 (74%) were below 5-years-of age (**Table 2**).

Figure 1: Study participant flow chart



Legend: LC-MS/MS- liquid chromatography tandem mass spectrometry

Table 2: Baseline characteristics of the study population (N =152)

Variable		n (%)
Male		86 (56%)
Age	< 1 year	14 (9%)
	1- 5 years	99 (65%)
	>5 – 12 years	31 (21%)
	> 12 years	8 (5%)
Housing	Formal	96 (63%)
	Informal	39 (26%)
	Unknown	17 (11%)

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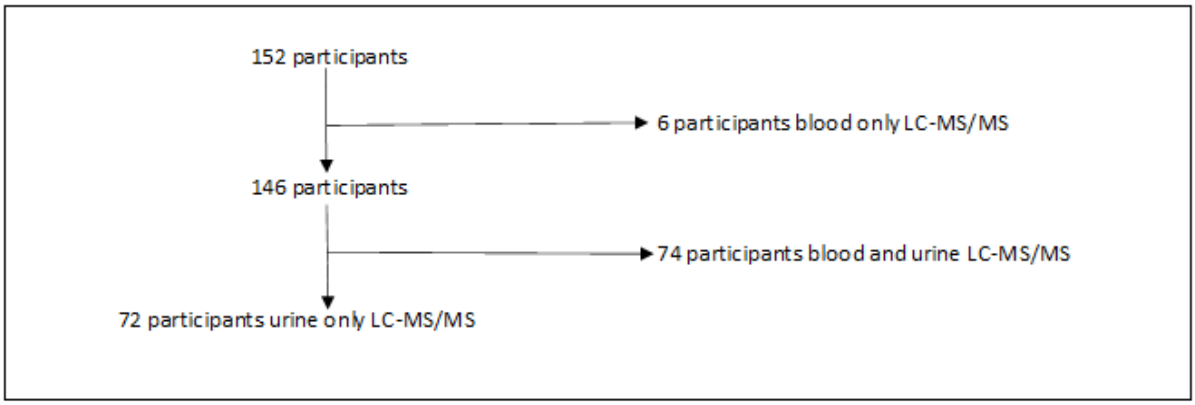
228 ## Toxicology results

229 A total of 146 (96%) urine samples from the 152 study participants were analysed by LC-MS/MS

230 after six samples were lost due to leakage in transit **(figure 2)**. For 80 (53%) participants, there

was sufficient left-over blood specimen in the laboratory for LC-MS/MS testing. This included the six participants whose urine samples had been lost to leakage.

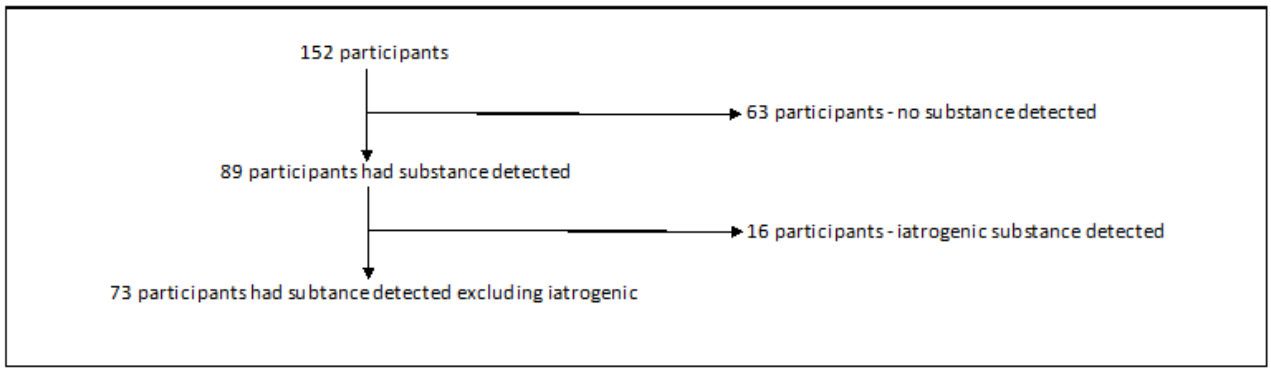
Figure 2: LC-MS/MS samples tested



Legend: LC-MS/MS - liquid chromatography tandem mass spectrometry

Altogether, in 89/152 (59%) participants a substance was detected **(Figure 3)**. In 16 (18%) of these the detected substances were iatrogenic secondary to administration of in-hospital care or therapy given at home. After discounting the iatrogenic substances or medicines given at home 73 of 152 (48%, 95% CI 40 – 56%) participants had a substance detected by LC-MS/MS.

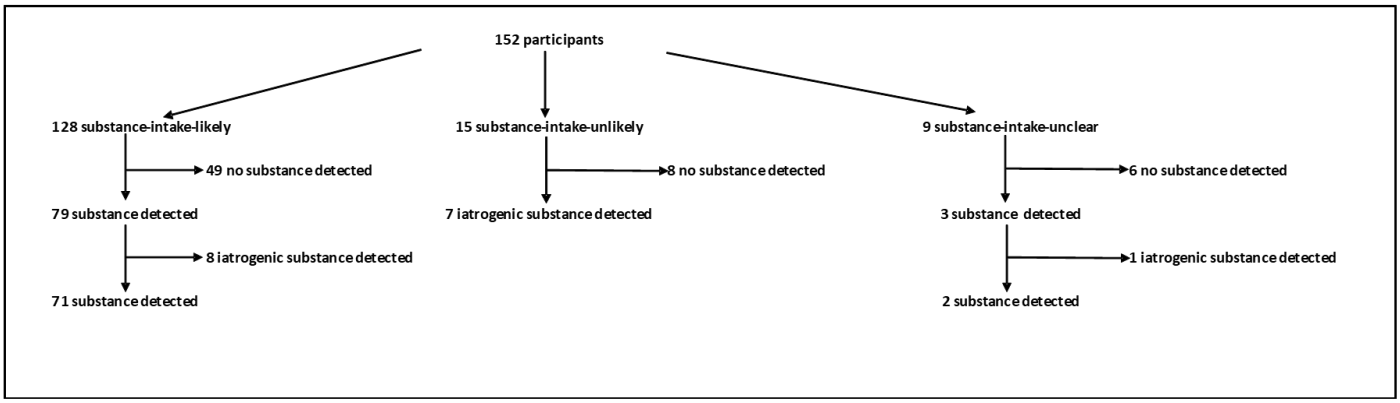
Figure 3: Substance detection by LC-MS/MS



Legend: LC-MS/MS – liquid chromatography tandem mass spectrometry

In total, 128 (84%) of the children, 71 (55%) in whom a substance was detected on LC-MS/MS, were classified as genuine cases of poisoning (substance-intake-likely), while 15 (10%) of the 152 were classified as unlikely to have been poisoned (substance-intake-unlikely) **(Figure 4).** In nine (6%) of the children it was not clear whether poisoning had taken place or not (substance-intake-unclear).

Figure 4: Substance detection by LC-MS/MS according to classification



Legend: LC-MS/MS – liquid chromatography tandem mass spectrometry

Despite being classified as genuine cases of poisoning, 57 (45%) of the 128 substance-intake-likely children did not have a causative substance identified via LC-MS/MS and 49 (38%) children had no substance identified and eight (6%) had iatrogenic substances identified (**Figure 4)**. The median TAT for the 57, who were substance-intake-likely cases but in whom the LC-MS/MS was negative, was 5 (IQR 3- 9) days for urine LC-MS/MS and 5 (IQR 4 – 9) days for blood. TAT of the 71 poisoning cases that had positive LC-MS/MS was 5 (IQR 2 – 7) days for urine and 6 (IQR 4-7) days for blood.

In 26 (20%) of the substance-intake-likely group in whom no substance was detected, the suspected substance was not in the LC-MS/MS reference library used. Of these, 17/26 (65%) were pesticides (11 rat 'poison', 4 'cockroach poison', 1 'tick poison' and 1 undefined pesticide).

There were eight organophosphate poisonings cases in the substance-intake-likely group. In two of the eight organophosphate poisonings, LC-MS/MS detected other substances (bromazepam and diphenhydramine), ingested by the same patients. Likewise, in one of the four cases of iron poisonings, trimethoprim was concomitantly identified by LC-MS/MS. Eight patients who had ingested hydrocarbons, three ethanol ingestions, two turpentine, and one each of petrol, eucalyptus oil and paraffin ingestion, had no additional substances detected by LC-MS/MS.

Five (4%) patients in the substance-intake-likely group presented with a history of ingesting an unknown substance, and the identity of the unknown substance was not identified via LC-MS/MS. Cannabis was detected via LC-MS/MS in a tablet brought by one of these patients but could not be confirmed in the patient's samples.

Nine patients in the substance-intake-likely group presented after ingesting a substance found in the LC-MS/MS library and yet the substance was not detected by LC-MS/MS, despite seven patients being symptomatic from the suspected substance. Four of the nine patients had both blood and urine LC-MS/MS done, while five had only urine LC-MS/MS done .The drugs that were not detected were the following, clonazepam, diazepam, lorazepam, phenytoin, alprazolam, cannabis, antiretrovirals (tenofovir/emtricitabine/efavirenz), chlorpromazine and tricyclic antidepressant. Six of the patients had vomiting induced by the care giver in an attempt to decontaminate. Furthermore, two of these patients received charcoal before the LC-MS/MS was done (one case of tricyclic antidepressant toxicity and one case of chlorpromazine ingestion). The

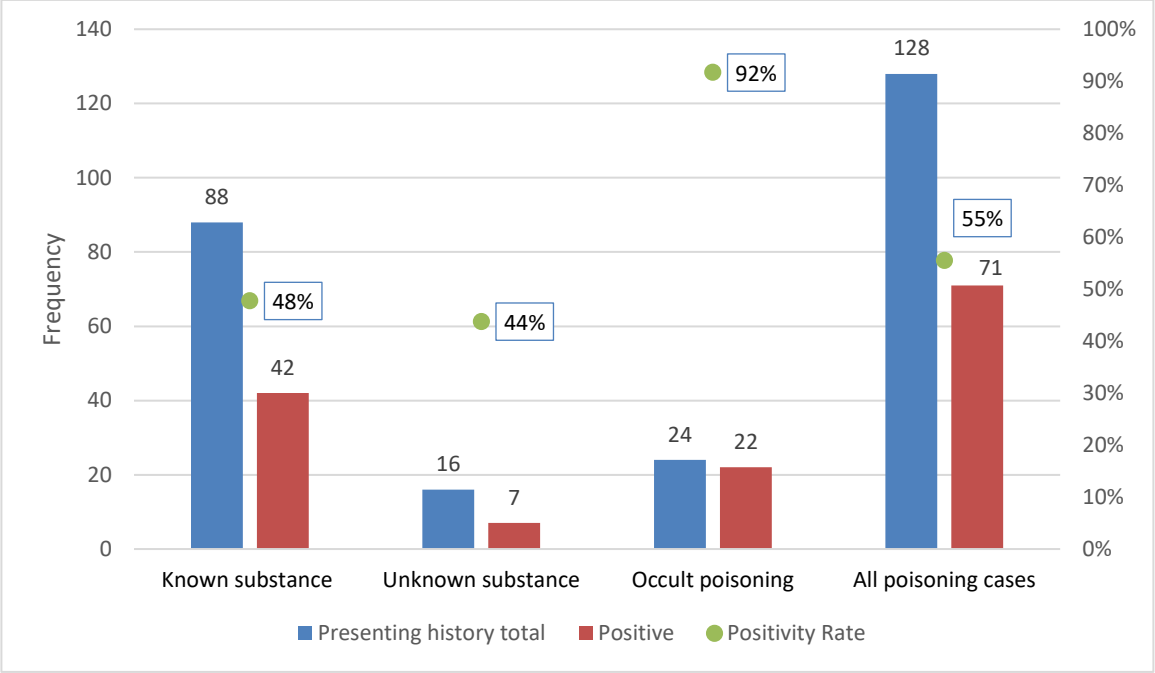
median TAT for these nine patients was seven days with a range of 1 – 13 days.

Of the 15 patients, in the substance-intake-unlikely group, LC-MS/MS detected no substances in eight (53%) and identified iatrogenic medicines in seven (47%) **Figure 4**). Of the nine substance-intake-unclear patients, one patient had a positive result due to iatrogenic medicines and two had positive results, but the drugs identified could not explain the clinical presentation.

Presenting history versus LC-MS/MS results in poisoning cases (substance-intake-likely)

When the 128 children in the substance-intake-likely group was further analysed according to the history obtained from the caregiver, 24 (19%) participants had no history of exposure to a substance (occult poisoning). (**Figure 5**) In those who had occult poisoning, the suspicion of poisoning came from the clinician's examination findings, and/or investigations done by the attending clinician. The substance detection rate of LC-MS/MS, after removing iatrogenic medicines, was then analysed in three different groups, known substance, unknown substance and occult poisoning. (**Figure 5**)

Figure 5: Number and proportion of substance detection rates on LC-MS/MS in substance-intake-likely group



Legend: LC-MS/MS liquid chromatography tandem mass spectrometry

In children with occult poisoning, LC-MS/MS was able to identify the substance in 22/24 (92%) compared to 42/88 (48%) when a guardian reported ingestion of a known substance (p=<0.0001), and 7/16 (44%) when a guardian reported ingestion of an unknown substance (p value = 0.003) (**Figure 5**).

In the 22 (92%) cases of occult poisoning, in which LC-MS/MS identified a substance, the substance identified was in keeping with the clinical presentation in 20/22 (91%). The two

patients, in whom LC-MS/MS identified a substance not in keeping with the clinical presentation, concomitant organophosphate poisoning was identified by alternative means. In these two organophosphate cases LC-MS/MS identified a substance that would have otherwise been missed. All 15 patients who had presented with an unknown substance and 23 (96%) of the 24 cases of occult poisoning had neurological symptoms.

In the patients who reported ingesting a seemingly 'known' substance, the substance found on LC-MS/MS was different in 15/88 (17%) patients. In these 15 cases, six were asymptomatic, while four had symptoms consistent with the substance found on LC-MS/MS.

Overall, 18/128 (14%) cases of poisoning would have been missed had LC-MS/MS not been used in this study.

Causes of poisoning

In 106/128 (83%) of the cases, poisoning was unintentional. There were however 6/128 (5%) cases of attempted homicide and 5/128 (4%) of attempted suicide (**Table 3**).

Table 3: Causes of poisoning (Intent), n=128

Intention		**Frequency (N=128)**
Unintentional	Self	99 (77.3%)
	Caregiver medication error	1 (0.8%)
	Traditional medicine	3 (2.3%)
	Iatrogenic	3 (2.3%)
Intentional	Attempted homicide	6 (4.7%)
	Caregiver/adult but not attempted homicide	6 (4.7%)
	Attempted suicide	5 (3.9%)
	Self but not suicide attempt	1 (0.8%)
Undetermined		4 (3.1%)

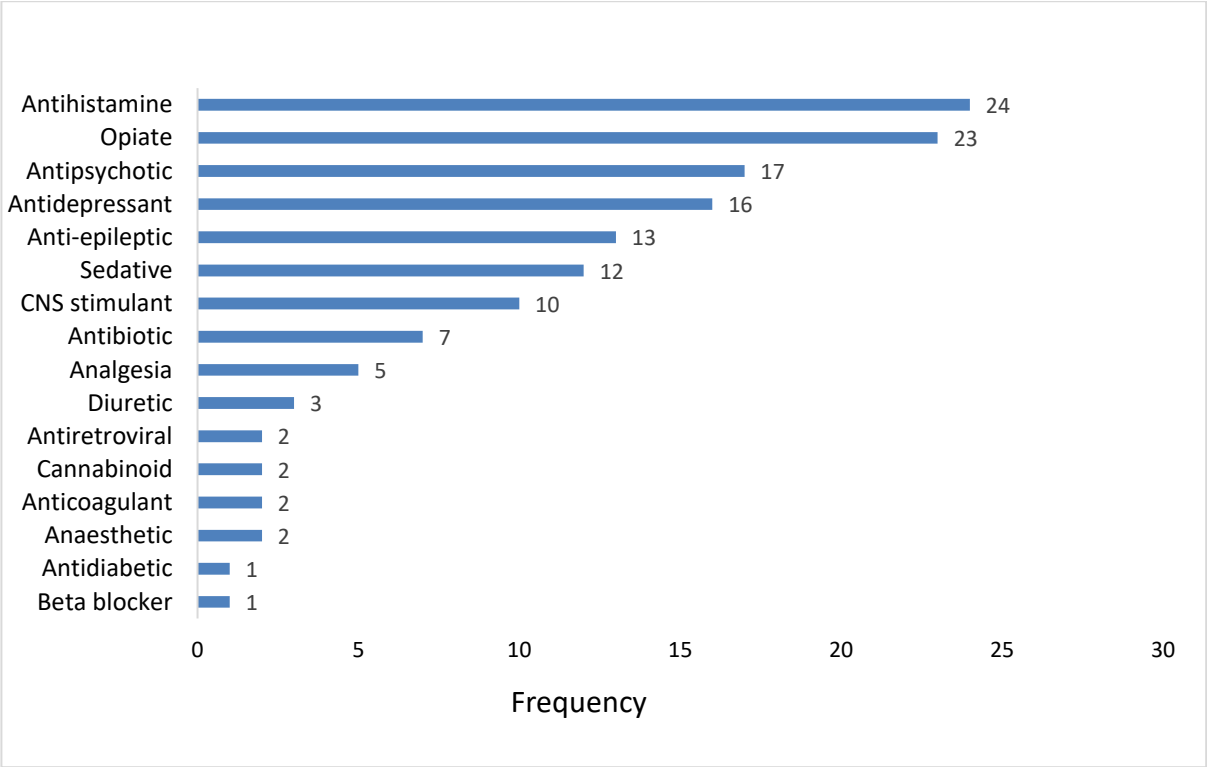
Of the six attempted homicides, two cases involved siblings from a family that had three deaths due to the same organophosphate poisoning event. In one of the patients who had been given traditional medicines, norfluoxetine, trimethoprim and diphenhydramine were detected by LC-MS/MS. Four of six children given substances intentionally by adults received drugs of abuse- two

received cannabis, one received methamphetamine and the other ethanol. The other two patients, presented with neurological symptoms, and the substances administered could not be identified.

Drugs identified by LC-MS/MS

LC-MS/MS was able to identify a total 45 different drugs after removal of iatrogenic medicines and medicines given at home (**Figure 6**). In the 128 substance-intake-likely cases, LC-MS/MS identified 140 substances. The most common causative group identified by LC-MS/MS was antihistamines found in 24 (19%) patients, followed by opiates in 23 (18%) and antipsychotics in 17 (13%). The most common drugs were chlorpheniramine and haloperidol found in 9 (7%) patients each. LC-MS/MS was able to identify multiple drugs in 40 (31%) of the substance-intake likely group.

Figure 6: Drug classes identified by LC-MS/MS



Legend: LC-MS/MS: liquid chromatography tandem mass spectrometry

Comparison of urine and blood LC-MS/MS results

Seventy-four (74) patients had both urine and blood samples analysed on LC-MS/MS. Urine and blood LC-MS/MS yielded the exact same result in 48 (65%) patients (**Table 4**). In 18 (24%) of the participants with paired samples, more substances were detected in urine but not in blood, while in 4 (5%) samples, more substances were detected in blood but not urine.

Table 4: Comparing urine and blood LC-MS/MS positivity rate (N = 74)

LC-MS/MS Result	Frequency (%)
No detected substance in urine and blood	27 (36%)
Same substance detected in urine and blood	21 (28%)
Different substance detected in urine and blood	4 (5%)
Substance detected in urine and blood, but more substances found in urine	7 (9%)
Substance detected in urine and blood, but more substances found in blood	3 (4%)
Substance detected in urine but not in blood	11 (15 %)
Substance detected in blood but not in urine	1(1%)
Total	**74 (100%)**

LC-MS/MS: Liquid chromatography tandem mass spectrometry

Clinical systems involved in the poisoning cases

Of the 71 positive LC-MS/MS results in the substance-intake-likely group, the substances identified by LC-MS/MS were in keeping with the clinical presentation in 55/71 (77%) participants. Nine (13%) of the 71 positive LC-MS/MS cases in the substance- likely-group were asymptomatic even though a substance was detected by LC-MS/MS.

The most common system involved was neurological, found in 88 (69%) of the substance-intake-likely cases followed by gastrointestinal found in 49 (38%), cardiovascular in 26 (20%) and 22 (17%) were asymptomatic. Of the 49 that had gastrointestinal symptoms 24 (49%) had the presence of the confounder of intentional induction of vomiting by the caregivers using manual induction, milk and/or saltwater. LC-MS/MS detected a substance in 58 (66%) out of 88 poisoning cases with neurological symptoms compared to 13 (33%) of the 40 without neurological symptoms ($p<0.0001$).

Substance-intake-likely management and outcome

According to the PSS, most cases were classified as moderate, 51 (40%), while 12 (9%) were classified as none and 42 (33%) were minor and therefore required minimal supportive care. Of the 23 (18%) children with a PSS severe grade, 10 (8%) required admission to the Paediatric Intensive Care Unit (PICU). Twenty-nine children (23%) were given an antidote and 6 (5%) received activated charcoal. There were no deaths.

Individualized social intervention was instituted in all the patients with removal and emergency placement occurring in six patients. All six attempted homicide cases were referred for forensic investigation. The mother was the perpetrator in four of the attempted homicide cases. LC-MS/MS detected a substance in three of the attempted homicides. A total of 22 (14%) patients

had an LC-MS/MS result prior to discharge.

Discussion

Our study describes the prevalence of LC-MS/MS-confirmed paediatric poisoning in a LMIC setting. LC-MS/MS was particularly helpful in occult poisoning where it was able to identify over 90% of the substances, as well as in identifying multiple substance ingestion. In addition, our study indicates the urine sample as having a higher detection rate for identifying potential ingested substances when compared to a blood specimen. According to the authors' knowledge, this study is the first prospective one of its kind done in a LMIC setting.

Similar to previous studies, most of the poisoning cases were males between the ages of one and five years.(1-3, 7, 12-14) This is likely due to the developmental stage toddlers are in, that involves curiosity about the world and a desire to explore it.(13, 14)

Previous literature has demonstrated shortfalls with urine point of care drug screen immunoassay, therefore, a positive point of care urine drug screen result requires a confirmatory test, such as mass spectrometry.(18, 20-22) After excluding iatrogenic medicines and therapy given prehospital, LC-MS/MS was able to detect substances in 48% of all study participants and 55% in the substance-intake-likely cases.

Twenty-six patients in this study reported ingesting a substance that was not in the library. This was the main reason for a negative LC-MS/MS result in poisoning cases, in this study. This indicates that the ability of LC-MS/MS to detect a substance is limited by the extent of the LC-MS/MS library available at the time. Notably, the LC-MS/MS library can be updated and additional drugs/substances added.(18, 19, 21) The LC-MS/MS used in this study could detect the presence of various drugs in concentrations as low as 20ng/ml. Despite this high sensitivity, nine poisoning cases who had ingested drugs in the LC-MS/MS library were not detected. The possible explanations are varied and include, that the concentration of these drugs in the analysed samples may have been below the limit of detection of the instrument, either due to rapid metabolism or elimination. Six of the nine patients had vomiting induced by the care givers which could have led to decontamination, before the patient could absorb the drug. Notably, two of these patients were given activated charcoal. Worryingly, the first was a tricyclic antidepressant overdose, that LC-MS/MS did not detect. The second was a symptomatic chlorpromazine ingestion. This ingestion was witnessed, and the patient was given activated charcoal before the LC-MS/MS was done. It is possible that LC-MS/MS may have been limited by failure to detect substances that are eliminated via the hepatobiliary system which may not have been detectable in urine, as well as substances with a short half-life that may have degraded before sampling or analysis. These reasons are limitations of LC-MS/MS that the clinician needs to be aware of when utilising LC-MS/MS. All nine drugs that the LC-MS/MS failed to identify, and yet were in the LC-MS/MS library, are excreted in urine except for tenofovir, which is mainly excreted in faeces. A study done on sample stability indicated that substance degradation was dependent upon the type of substance and the temperature at which a sample is stored.(32) Substances stored at 25°C, 4 °C and -20°C were later extracted and analysed at 15, 60 and 90 days and the average

relative peaks on these days were compared with the average relative peaks at baseline.(32) The study concluded that the best temperature to store samples is -20°C, although even at 4°C the substances could still be detected even if the peaks were lower.(32) The samples in our study were stored at 4°C, and therefore substance degradation cannot be ruled out.

There are other substances that the LC-MS/MS could not detect, and these included: volatile substances such as hydrocarbons, which require a different method i.e., gas mass spectrometry for detection; organophosphates because they metabolize fast and metals such as iron. As a result, though a positive LC-MS/MS result is beneficial, a negative LC-MS/MS result does not rule out poisoning.

Due to circumstantial evidence, such as an open bottle or missing tablets, the causative agent in paediatric poisoning is generally obtained from history, which means laboratory investigations to identify the cause of poisoning is often regarded as not necessary. However, in our study, LC-MS/MS found that of the 88 poisoning cases that had ingested a seemingly 'known' substance, almost a fifth (17%), of the patients had ingested a different drug from that reported by the caregiver. This has management implications as the wrong drug level can be requested from the laboratory and the wrong antidote given while the right one is delayed.

Most of the studies and reports that look at the causes of poisoning in children do not highlight multiple drug exposure as an important cause of poisoning. (1, 7, 13, 33) Veale et al., in a study

that included both adults and children, indicated that only 13.8% of the poisoning cases had been exposed to multiple drugs.(12) While a retrospective study done at the same children's hospital as our study setting, indicated that less than 10% of the children who presented with poisoning had been exposed to more than one toxin. (3) Contrary to the previous mentioned studies, that reported low rates of multiple drug ingestion in children, in our study, LC-MS/MS detected 40 (31%) cases of multiple drug ingestions further demonstrating the ability of LC-MS/MS in positively identifying multiple drug ingestions. The use of laboratory specific drug levels to detect multiple drugs requires the clinician to request different specific drug levels to be run, in contrast, LC-MS/MS requires only one sample to be run to identify multiple drugs and/or substances. Without LC-MS/MS multiple drug ingestions would have been missed in this cohort of children. However, it is important to note that LC-MS/MS was not able to differentiate between multiple drugs from a single medicine with two or more drugs, and that which involved ingestion of multiple separate drug formulations.

The most common drug classes found in our study were antihistamines (19%), opiates (18%), antipsychotics (13%) antidepressants (12%) and antiepileptics (10%), while the most common drugs detected on LC-MS/MS were chlorpheniramine and haloperidol. This may explain the high frequency (69%) of neurological symptoms in the cases with likely substance ingestion. Historically, agro-based and non-drug chemicals were the main causes of poisoning in LMICs.(1, 3, 7, 33-35) There is a need to strengthen preventative campaigns in LMICs as pharmaceuticals are becoming important causes of poisoning.(12, 34)

Traditional medicine use is not uncommon in LMICs, there have been previous reports of these medicines being adulterated, as was the case in one of our patients who ingested traditional medicine and LC-MS/MS identified norfluoxetine, trimethoprim and diphenhydramine.(24, 36-39)

While, both blood and urine samples can be analysed by LCMSMS, urine is usually readily available as a non-invasive specimen with minimal discomfort to children. Furthermore, unlike in blood, drugs and their metabolites are known to remain in urine for longer (up to one week) post last exposure depending on the drug.(20, 21, 40) This gives a greater window of opportunity to still identify a substance after ingestion, especially when this is unknown or occult.

It is important to note that the clinical outcome was not altered using LC-MS/MS, this corresponds to previous studies, and in our study was because of the long turnaround time, with a median of 5 (IQR 3 – 7) days for urine LC-MS/MS and 6 (IQR 4 -7) days for blood.(19, 21) In our study, the turnaround time was prolonged because the test samples were batched. The other major limitation of mass spectrometry is its expense.(18, 19) However, as technology has improved, mass spectrometers have become cheaper and faster.(18, 23, 29, 41-43) A study by Caspar et al., demonstrated its value in 24/7 toxicology by analysing 22 drugs and active metabolites in a qualitative and quantitative manner.(30) In the study done by Caspar et al., the total run time for a test was 11 minutes, extrapolated to the emergency care setting, such run times would enable the clinician to treat the patient accordingly and in a timely point-of-care manner.(30, 43) It would also avoid unnecessary treatment procedures in those that do not require them. The LC-MS/MS system may also be made more efficient using automation, this reduces the need for skilled personnel to run the equipment.(18, 21)

LC-MS/MS identified 92% of all cases of occult poisoning and the substance identified by LC-MS/MS were in keeping with the clinical presentation in 91% of the cases of occult poisoning. There is limited data on the prevalence of occult poisoning in children especially in LMICs, in our study, one in five (19%) of the poisoning cases were due to occult poisoning. Occult poisoning was more likely if the patient had acute unexplained neurological symptoms that were not due to an infection or trauma. This makes LC-MS/MS of value in the area of child protection, when children may be poisoned intentionally. Child protection is also required in all cases of unintentional poisoning that are due to neglect. In this study six children required removal from the adverse environment as well as further child protective measures.

Limitations

Our study is limited by a small sample size which reduced our ability to stratify the data further by causes of poisoning. Furthermore, we were not able to include unnatural home deaths that presented to the mortuary. As alluded to earlier, the LC-MS/MS library used did not contain an exhaustive list of possible substances.

Conclusion

In conclusion, the use of LC-MS/MS in toxicology screening is novel in the African paediatric population. It appears to be of greatest value in the paediatric patient who presents with occult poisoning or has ingested multiple and/or unknown substances. It was less helpful in those that

had ingested a known single substance unless the substance found on LC-MS/MS was found to be different. Though a robust test, clinicians need to be aware of its shortfalls. In high-risk settings, it can be utilized in community toxicovigilance and child protection. Finally, the authors could not find guidelines on the use of investigations, in particular LC-MS/MS, in LMICs, and because LC-MS/MS is an expensive test, we recommend that a protocol for its judicial use in LMICs be developed.

"Key messages" box

Section 1: What is already known on this subject

The use of investigations in paediatric poisoning is controversial. There is a paucity of prospective data on the use of LC-MS/MS in paediatric poisoning in LMICS, where resources are constrained and risk factors for poisoning such as neglect and child abuse are high.

Section 2: What this study adds.

LC-MS/MS is beneficial in the paediatric patient who presents with occult poisoning or has ingested multiple and/or unknown substances. Requesting clinicians need to be aware of its shortfalls. In high risk settings, it can be utilized in community toxicovigilance and child protection. Due to its expense, a protocol needs to be developed for its judicial use in LMICS.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town: REF: 742/2016. Written informed consent was sought from all participating children's parents or legal guardians. Assent was also obtained from children older than seven years.

Consent for publication

Not applicable

Availability of data and materials

All the data that support the findings of this study are contained within the manuscript. Any requests for additional data can be made available upon reasonable request from the corresponding author and with permission of The Human Research and Ethics Department of the University of Cape Town.

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Competing interests

The authors declare that they have no competing interests.

Author contributions

NW: developed study protocol, study material, collected the data, preliminary analysis of data and did the final write up. HB: supervisor, conceptualisation of study, assisted with data collection and reviewing data analysis and review of final write up; RM: supervisor, assisted with data analysis and review of final write up. AE: technical support, assisted with running the mass spectrometer and review of final write up. PS: assisted with conceptualisation of study and review of final write up. All authors have read and approved the manuscript.

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Appendices

Appendices

Appendix A: Study protocol

Appendix B: Informed consent English version

Appendix C: Informed consent Afrikaans version

Appendix D: Assent English version

Appendix E: Assent Afrikaans version

Appendix F: Data collection tool

Appendix G: Proposed protocol for use of LC-MS/MS at RCWMCH

Appendix H: BMC Pediatrics journal reviewer comments and rebuttal

Appendix I: Post-rebuttal BMC Pediatrics journal reviewer comments

Appendix J: Acceptance letter from BMC Pediatrics journal

Appendix K: Turnitin report

Appendix L: Author guidelines BMC Pediatrics journal

Appendix M: Manuscript formatting guidelines BMC Pediatrics journal

Appendix A: Study protocol

Protocol V11

A prospective cross-sectional study to assess the value of mass spectrometry in the management of paediatric poisoning at Red Cross War Memorial Children's Hospital

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Background

Statement of the problem

According to a World Health Organization report, poisoning is the 4th biggest cause of unintentional injury after road traffic injuries, fires and drowning[1]. It accounts for about 7% of all

accidents in children under 5 years and is implicated in about 2% of all childhood deaths in the developed world, and over 5% in developing countries[2]. As a cause of emergency unit visits, its incidence varies with each location with one study documenting an incidence of 0.28% and another that of 3%[3, 4].

Childhood poisoning is rarely fatal in developed nations, where good data collection and various public health preventative methods have been implemented [1, 5]. However, mortality is high in Africa and low

and middle income countries with the highest fatality rates noted in those less than 1 year of age[1]. Despite the higher fatality rates in developing nations, data on childhood poisoning remains poor in developing nations[5].

This burden of childhood poisoning goes beyond mortality as it puts a significant burden on the health system in terms of morbidity and costs incurred with case management[7]. In order to curb this negative effect on the health system, work is required to both identify and implement preventative strategies to decrease the number of cases of paediatric poisoning[7].

In South Africa a number of the cases of paediatric poisoning are managed at tertiary hospitals, Red Cross War Memorial Children's Hospital (RCWMCH) is an example of a tertiary hospital that manages paediatric poisoning. RCWMH is a public children's hospital that provides secondary and tertiary health care services to children living in peri-urban settlements. The hospital manages approximately 260 000 patient visits each year[8]. A significant percentage of the patients come from exceptionally poor and marginalized communities[8], namely informal settlements. It has been noted that 8% of all children under the age of 18 years in the Western Cape live in informal settlements, with children under the age of 5 contributing the largest percentage of 42%[9]. This under five age group is at the greatest risk of unintentional poisoning because of their innate

exploratory behavior[10].

The causative agents of poisoning in children varies with each location, where the causes in developed nations differ from the causative agents in developing nations[10]. A study done at RCWMCH illustrated a difference in the causative agents in patient's residing in different suburbs[6]. However, despite the differences in communities, the number one cause of paediatric poisoning was kerosene (paraffin) while drugs were the most common toxic group[6].

Despite the magnitude of the burden of paediatric poisoning the global trend shows a steady decline in the incidence of paediatric poisoning[4, 6]. Recent data from the Information Management Unit at RCWMCH illustrates a similar trend as there was a steady decline in the number of patients presenting with suspected poisoning to the medical emergency department from 224 cases in 2010 to 124 cases in 2014. This decline may be attributed to increased community awareness as well as initiatives and campaigns such as child safety. It also coincides with the opening of two district hospitals, Khayelitsha District Hospital and Mitchells Plain District Hospital. Despite the decline, data from the same source shows that the offending substance was not confirmed in 89, 5% of the cases. This not only has medical implications but potential deleterious social implications for the child who will return to the same home environment that predisposed them to unintentional poisoning.

Investigation of paediatric poisoning

The role of investigations in paediatric poisoning remains unclear as there is currently no agreed upon gold standard[11, 12]. This has led to various guidelines been drawn up to assist emergency departments on the laboratory tests to use in different poisoning scenarios[11].In a number of centers the need for a toxicology screen depends upon the clinical scenario and is rarely needed

in those who have an unintentional known substance ingestion and are asymptomatic or have clinical features consistent with the history[12]. However, a toxicology screen becomes valuable when[12]

- The clinical picture of the patient remains unexplained
- Antidote administration depends upon rapid identification of the toxic agent
- And when there is a suspicion of child abuse or Munchausen syndrome by proxy

Urine immunoassay has been identified as a cost effective toxicology screen. However, like all screening tests it has its limitations. The limitations of the urine immunoassay drug screens include the following: [13]

- Limited number of street drugs
- Inability to quantify drug
- False positives due to chemically similar compounds
- False negatives for example when drug level is below the threshold level
- Affected by the pharmacokinetics
- Positive result requires confirmatory test with mass spectrometry

Other modalities that have been proposed to aide investigation of poisoning include the use of Mass Spectrometry. Mass spectrometry has the advantage of detecting more poisons or toxins when compared to its immunoassay counterpart. In a review on paediatric poisoning, the gas chromatography/mass spectrometry was able to get positive results in 85% of the samples, while the immunoassay test was able to get positive results in only 16% of the samples[14].Despite the

significant difference, a number of studies, however, show conflicting evidence concerning the usefulness of mass spectrometry in toxicology. It has been argued that the use of mass spectrometry does not change the management, as management is based mainly on the clinical picture[15, 16]. One study done in 1999 noted that aside adding significant charges to the patient, the comprehensive drug screen, using High Performance Liquid Chromatography (HPLC), had no additional clinical benefit when compared to the limited component test[15]. Fabbri et al., on the other hand, concluded that a rapid comprehensive drug screen, such as mass spectrometry, is useful in the diagnosis of patients with suspected drug poisoning[16]. It identifies unsuspected drugs in symptomatic patients and excludes drugs in asymptomatic patients[16].This is of particular importance as patients with negative tests can be safely discharged within a few hours, thus avoiding unnecessary admissions and decontamination procedures[16]. In those with positive results their management can then be tailored to the toxin found as well as the clinical severity of the patient. Furthermore, there are compounds that can be detected by mass spectrometry that are not in any immunoassay kits, such as clozapine and citalopram, which change patient management considerably, as they would otherwise be reported as negative.

At RCWMCH the toxicology screen done on each patient rests on the clinician's clinical suspicion in requesting the appropriate test. However, in cases where the history is not clear, as is with a number of our cases, appropriate testing to identify the offending agent cannot be carried out. Point-of-care toxicology urine screening (Sure slip®) is available in the medical emergency department and tests for the following substances:

1. Methamphemine
2. Amphetamine

3. Cocaine
4. Cannabis
5. Morphine
6. Benzodiazepine

The National Health Laboratory Services (NHLS) is able to offer testing for the following substances at considerable cost:

1. Paracetamol
2. Salicylate
3. Lithium
4. Methotrexate
5. Tricyclics
6. Phenytoin
7. Phenobarbitone
8. Sodium Valproate
9. Carbamazepine
10. Digoxin
11. Theophylline
12. Urine mandrax
13. Cyclosporin
14. Amikacin
15. Gentamicin
16. Vancomycin

Because of the limited number of toxins or drugs that we are able to test for in the Medical Emergency Department at RCWMH, methods to increase the diagnostic ability, such a mass spectrometry, need to be evaluated. This method of mass spectrometry is available at the University of Cape Town's Division of Clinical Pharmacology based at Groote Schuur Hospital and offers considerable advantage in that it is able to identify, through targeted analysis, multiple substances, as well as prolonged exposure to a substance through the use of hair samples.

This study is aimed at evaluating the value of mass spectrometry in the management of paediatric poisoning at busy health care centers such as RCWMCH; as well as documenting and recording the toxins that children ingest, this will further direct appropriate preventative programs.

Research Question and Objectives of the Study

Study question

What is the value of mass spectrometry in the management of paediatric poisoning?

Research Hypothesis

Diagnostic mass spectrometry is of no value in the management of paediatric poisoning.

Aim

To assess the value of mass spectrometry in the management of paediatric poisoning

Research Objectives

1. To describe the demographic characteristics of children who present with paediatric poisoning to the RCWMCH
2. To describe the substances causing paediatric poisoning in patients presenting to the RCWMCH

3. To determine the proportion of positive tests that are identified by mass spectrometry in comparison to the proportion of positive tests that are identified by routine toxicology (urine immunoassay and NHLS laboratory tests)
4. To determine the clinical outcome of children with poisoning managed with the help of diagnostic mass spectrometry

Methodology

Characteristics of the study population

All children that present to the RCWMCH with suspected poisoning will be eligible. The list of patients will be complied from the Medical Emergency Unit patient register and Short Shay Ward register.

Inclusion criteria:

All children that present to Red Cross War Memorial Children's Hospital with any of the following:

1. Confirmed or suspected acute poisoning from history
2. Unexplained neurological symptoms such as unexplained altered level of consciousness; unexplained seizures
3. Atypical presentation of diarrhoea and vomiting
4. Unexplained metabolic derangements for example unexplained acute kidney injury and unexplained acute liver failure

Exclusion criteria:

1. Acute food poisoning and
2. Care- giver refusal to participate
3. Presentation of poisoning that would not routinely require laboratory investigation e.g paraffin poisoning

Recruitment and enrolment

Will be done through the Medical Emergency Department and the Short Stay Ward

Study design

Prospective cross-sectional study will be carried out for a period of 12 months.

When a patient presents with suspected poisoning clinical management and investigations will be carried out as per standard protocol. On the specimens collected for routine toxicology screening, urine and blood, some urine and blood from the same specimens will be sent for mass spectrometry. In relevant cases hair samples will also be collected.

Once results are available a comparison will be made between samples sent for routine toxicology screening and sample sent for mass spectrometry. Each mass spectrometry sample will be matched with a routine toxicology screen sample from the same patient specimen.

Research procedures and data collection methods

Each patient will be managed according to current best clinical practice with AfriTox® being used,

as is routine care, when the ingested substance is known or strongly suspected.

Investigations:

1. Urine samples will be collected simultaneously for routine toxicology as well as mass spectrometry and be sent off, where possible, at the same time
2. When required for appropriate management, blood samples will be collected as well for both routine toxicology and mass spectrometry
3. If parental consent is obtained, a small hair sample will be taken

A generic data collection sheet will be used to collect demographic data, clinical symptoms, initial diagnosis, final diagnosis, management, clinical course and outcome.

Comparison will then be made between the samples sent for routine toxicology screening and the samples sent for mass spectrometry.

ABSciex 3200 QTRAP

The mass spectrometer that will be used in the study is the ABSciex 3200 QTRAP unit used by the UCT Clinical Pharmacology division at Groote Schuur Hospital. It is described as a preliminary screening research tool and not a validated diagnostic system. Its library database includes 250 medicinal drugs or drugs of abuse and some of their metabolites. Sensitivity varies according to the compound but the specificity is high. Due to the current logistical framework, during the study the instrument will be run once every week.

Shortfalls of Mass Spectrometry

1. As indicated earlier it is a screening test and not a validated diagnostic system
2. Matrix effect, which is defined as the combined effect of all compounds of the sample other than the analyte. This interferes with the ionisation process in the Mass Spectrometer causing ionisation suppression or enhancement that negatively affects the measurement of quantity[17].
3. The cost of running the instrument is expensive and approximates R500 per sample

Data safety and monitoring plan

Each patient will be given a unique code for identification. The data will then be entered using a pass word protected and encrypted Excel spread sheet.

Data Analysis and Statistical methods

Sample size

Based on available data, we estimate that mass spectrometry would be able to confirm diagnosis in 50% to 85% of cases of poisoning while routine toxicology will only be able to confirm between 10% and 30% of the cases. [14,18]. Using a type 1 error rate of alpha = 0.05, paired sample sizes of 103 for each diagnostic method will be required to detect a statistically significant difference with 80% power using the upper limit (30%) and lower limit (50%) of detection for routine diagnostic and spectrometry respectively. Other sample sizes required to detect statistically significant difference in proportions of confirmed poisoning are shown in table 1. Data from RCWMCH Information Management Unit indicates that we can expect to collect between 150 and 200 paired samples over a one year period.

Table 1: Sample size for different proportions of confirmed drug poisoning

	Mass Spectrometry Diagnostic yield						
Routine Toxicology Diagnostic yield	**Percentage positive tests**	**40**	**50**	**60**	**70**	**80**	**90**
	5	27	19	14	11	8	7
	10	38	25	17	13	10	8
	15	57	33	22	15	11	9
	20	91	45	28	19	13	10
	25	165	66	36	23	16	11

	30	376	**103**	49	29	19	13

Data management and Analysis

Data will be entered into a password-protected computer on an encrypted Excel database after which it will be imported into Stata (StataCorp LP, College Station, TX) for cleaning up and analysis.

The demographic characteristics and clinical findings at presentation will be tabulated to provide a background description of the study population. All toxins that test positive for routine toxicology and/or mass spectrometry will be described. The proportion of patients testing positive for different toxins will be calculated for each diagnostic method. Percentages and their 95% confidence intervals in outcomes of interest will be used to depict proportions of categorical variables while medians with interquartile ranges will be used to summarise all continuous variables. Proportions of confirmed diagnosis will be compared between the two diagnostic methods as described below.

The χ^2 test or Fisher's exact test will be used to assess the strength of association between two categorical variables as appropriate. Continuous variables will be tested for normality and the appropriate statistical test used to test for association. To adjust for potential confounders, a multivariable model will be used to estimate effect measures using logistic regression or general

linear modelling as appropriate. A significance level at a two-tailed $P<0.05$ will be used for all analysis. A statistician will be consulted to help with the data analysis.

Dissemination of Information

The data will be prepared for presentation at several venues including a local presentation to the Department of Paediatrics and Child Health. A manuscript will be prepared and submitted for publication in a peer-reviewed journal.

Description of risks and benefits

Risks

Physical

- Urine collection – urine bags will be used for young children and infants and mid steam samples for older children. No suprapubic aspirates or in and out catheter specimens will be collected unless it is part of the routine management of the patient
- Blood Sampling - risk will be minimised in that the blood will be taken from the same sample used for routine toxicology and/or other relevant tests needed for their medical management
- Hair sampling- a very small sample of head hair will be taken by snipping with a pair of scissors and only taken if the care giver consents, or in the case of children aged 7-years or older, assents (See appendix 2,3 & 4).

Psychological

- Psychological effects of blood sampling and loss of a small snippet of hair in children

Social

- Identifying substances and or drugs of abuse would lead to a social investigation of the home environment

Benefits

- Identification of actual ingested substance and thus streamlining management
- Identification of multiple ingested substances
- Contact details of parents/guardians will be obtained such that feedback of positive results that come after discharge can be given to the family and appropriate social and/or clinical intervention will be instituted
- In the event of identification of a substance of abuse the clinician in charge will be notified who will refer the case to the Social Work Department as per our standard protocol. Currently the Department of Social Services is involved in all cases of ingestion via the hospital Social Work Department. The parents and family will be interviewed and social interventions and protective measures as deemed appropriate will be followed depending on the findings of the Social Work Department
- Ability to identify the causes of poisoning would lead to targeted preventative strategies
- There is limited data on the use of Mass Spectrometry in paediatric poisoning in the African context making this study beneficial in providing data in the African context
- The study will assist in drawing up recommendations on the use of investigations in resource limited settings

Informed Consent process

Legal guardians of children who present will be requested to give written consent in their language of choice for specimens to be sent for mass spectrometry.

In the event of a patient older than 7 years written assent shall be obtained except in cases where the mental status of the patient is impaired.

Limitations of study

1. The Mass Spectrometer will be run once a week resulting in a delay in access to valuable results, but as mentioned the guardian details will be obtained.
2. Inability of the Mass Spectrometer to identify volatile compounds
3. Traditional medicine ingestion is common in our setting however the Mass Spectrometer in use is unable to identify traditional medicines as we are not able to get reference standards to confirm findings

Table 2: Budget

Item	**Cost per patient (Rands)**	**Cost per 100 patients (Rands)**	**Cost per 200 patients (Rands)**
Data collection sheets	0,8	80	160
Stationary (miscellaneous)	5	500	1000
Patient Mass Spectrometry labels	4	400	800
Urine Mass Spectrometry	500	50 000	100 000
Blood Mass Spectrometry	500	50 000	100 000
Total cost	**1009,80**	**100 980**	**201 960**

There are no sponsors for the study. The cost of Mass Spectrometry and sample storage will be borne by the UCT Division of Pharmacology at Groote Schuur. The cost for stationary, patient labels and data collection sheets will be borne by the principal investigator. Routine toxicology will be done as routine clinical care and so the cost will be covered as per routine practice.

Study Time lines

The study is expected to take place over an 18 months period according to the milestones shown in table 3.

Table 3: Study Time line

Activity	Time frame
DRC submission	24 June 2016
Ethics submission	3 October 2016
Collection of data	1 November 2016 – 30 September 2017 (1year)
Cleaning Data	1 November 2017 – 30 November 2017 (1 month)
Data Analysis	1 December 2017 – 31 December 2017 (1 month)
Write up	1 January 2018 – 31 March 2018 (3 months)

Appendix

1. Data collection sheet
2. Informed Consent document
3. Assent document
4. Hair collection guidelines
5. Synopsis

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Appendix B: Informed consent English version

Part I: Informed Consent form for parents and guardians

A prospective study to assess the value of mass spectrometry in the management of paediatric poisoning

Your child is invited to participate in research aimed at identifying causes of unintended poisoning in children as well as finding better methods for testing children who come to hospital with signs and symptoms of unknown causes that could be due to poisoning.

Children are curious by nature. As a result, they are prone to accidents including, exposure to household substances and medications. Exposure to medicines or household products in a child may result in them falling sick. A number of times it is not clear what the child was exposed to. Often, poisoning in children is only suspected by health care givers if the guardian/parent suspects it, or if the signs and symptoms the child has are suggestive. In both instances the cause itself is usually unknown.

Our research aims to identify causes of poisoning in children admitted to the Red Cross War Memorial Children's Hospital. The study also aims to find out whether the use of a test called Mass Spectrometry could be useful in the treatment of children coming to hospital with signs and symptoms suggestive of poisoning.

We can confirm whether or not your child was exposed to a potential poison by sending urine or blood samples from your child for testing with Mass Spectrometry. This is not a test that we usually do, although it has been used in other hospitals outside of Africa.

All children that present to Red Cross War Memorial Children's Hospital with any of the following problem can participate in this research:

1. Confirmed or suspected recent poisoning
2. Unexplained mental signs such as drowsiness, confusion, irritability and unexplained fits
3. Unexplained or unusual forms of diarrhoea and vomiting
4. Unexplained blood tests that may show problems with the kidneys, liver or other organs

You and your child may choose to participate in this research or not. Your child will receive the usual care as per our protocols whether or not you choose to participate in this study. The study does not change the treatment that your child receives. However, if you decide to take part in the study and Mass Spectrometry identifies a possible poison that needs treatment or further referral your child may be able to benefit from this.

Study investigations

Your child will have extra tests done which will be sent for mass spectrometry:

- A small amount of blood (about a teaspoonful) will be collected in addition to the usual tests
- A small amount of urine
- Depending on the symptoms, a snippet of hair may be collected

With your permission some or all of leftover specimens will be stored for future tests

After discharge you and your family will be contacted via a phone call within 3 weeks to inform you of any results as well as further management if it is required.

Side Effects and risks

To minimise physical trauma and distress to the child, blood and urine sample collection will be done at the same time as the routine tests that your child will be having.

Benefits

If your child participates in this research, your child will have the benefit of extra tests to look for the cause of the illness he/she came in with. This may improve treatment as it may indicate a specific poison or drug that may need specific treatment.

Reimbursements

The research will not cost you anything. You will not be given any money or gifts to take part in this research.

Confidentiality

The information that we collect from this study will be kept confidential. Any study information about you and your child will have a number on it instead of a name. Only the researchers will know what your child's number is. However, there is a small risk that someone may identify your child, but this is unlikely to happen

What will happen to the information collected?

What we learn from this research will be used to make recommendations on how best to treat children who come in with suspected poisoning. It will be shared with other health care workers. The identity of your child will be kept confidential throughout.

Withdrawal from the study

You may choose to withdraw from the study at any time and your child will still receive the same treatment they are meant to receive.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact the following:

Dr Norbertta Washaya

Red Cross War Memorial Children's Hospital

Klipfontein Road

Rondebosch, 7700

Tel: 021 658 5111 E-mail: nnwashaya @ gmail.com

OR

Dr Heloise Buys

Red Cross War Memorial Children's Hospital

Klipfontein Road

Rondebosch, 7700

Tel: 021 658 5111 E-mail: Heloise.buys@uct.ac.za

This proposal has been reviewed and approved by the Human Research Ethics Committee of the University of Cape Town, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find about more about the ethics committee, contact

Mrs Lamees Emjedi

Research Ethics Committee

E 52 Room 24, Old Main Building, Groote Schuur Hospital, Observatory

Telephone: +27 21 406 6338

Fax: 27 21 406 6411

Email: nosi.tsama@uct.ac.za and shuretta.thomas@uct.ac.za

Print Name of Parent/Guardian_____

Signature of Parent/Guardian _____

Date _____

Day/month/year

Part II: Certificate of Consent for the storage of samples

The samples will be stored by the UCT Clinical Pharmacology division at Groote Schuur Hospital, who will be the primary custodians. The value of storage of the samples is that better research methods may become available in the future. We can then use the same samples to analyse these methods and so help improve the care of more children in the future.

Storage of samples

If any of the blood or urine or hair samples my child has provided for this research project is unused or leftover when the project is completed:

☐ I give my permission for my child's blood/urine/hair ***(circle what applies)*** samples to be stored and used in future research of any type, which has been properly approved

☐ I give permission for my child's blood/urine/hair ***(circle what applies)*** samples to be stored and used in future research but only for research in poisoning.

☐ I give permission for my child's samples to be stored and used in future research except for research about _____

OR

☐ I wish my child's samples to be destroyed immediately.

AND

☐ I want my child's identity to be removed from my child's samples.

☐ I want my child's identity to be kept with my child's samples.

AND

☐ I wish my child's specimens to be destroyed in the event of his/her death

☐ I give permission for the use of my child's specimens even in the event of his/her death

The stored samples will only be used for further research after approval from The Human Research Ethics Committee of the University of Cape Town, which is a committee whose task it is to make sure that research participants are protected from harm. Access will be limited to approved researchers.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for my child to participate in this study.

Print Name of Parent/Guardian_____

Signature of Parent/Guardian _____

Date _____

Day/month/year

If illiterate

A literate witness must sign (if possible, this person should be selected by the Parent/Guardian and should have no connection to the research team). Parents/Guardians who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ AND Thumbprint of Parent/Guardian

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Blood collection
2. Urine collection
3. Hair collection
4. Samples may be stored for possible future analysis.

I confirm that the Parent/Guardian was given an opportunity to ask questions about the study, and all the questions asked by the Parent/Guardian have been answered correctly and to the best of my ability. I confirm that the Parent/Guardian has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this information and consent form has been provided to the Parent/Guardian.

Name of Researcher/person taking the consent _____

Signature of Researcher/person taking the consent _____

Date _____

Day/month/year

Appendix C: Informed consent Afrikaans version

Deel i: Ingeligte Toestemming Vorm Vir Ouers En Voogde

'n Vooruitsigtige studie om die waarde te bepaal van Massaspektrometrie in die behandeling van pediatriese vergiftiging

Jou kind is genooi om deel te neem aan navorsing gemik op die identifisering van oorsake van onbedoelde vergiftiging in kinders. Ons wil ook daardeur beter metodes vind vir toetse in kinders wat na die hospitaal toe kom met tekens en simptome van onbekende oorsake, wat dalk as gevolg van vergiftiging kan wees.

Kinders is nuuskierig deur die natuur. Gevolglik is hulle geneig tot ongelukke, insluitend blootstelling aan huishoudelike stowwe en medikasie. Blootstelling aan medisyne of huishoudelike produkte in 'n kind mag hulle siek maak. Partykeer is dit nie duidelik wat die kind aan blootgestel is nie. Dikwels kan vergiftiging in kinders slegs vermoed word deur gesondheidsorg versorgers as die voog/ouer vermoed dit noem, of daar tekens en simptome is daarvan. In albei gevalle is die oorsaak gewoonlik onbekend.

Ons navorsing se doel is om die oorsake van vergiftigings van kinders wat by Rooi Kruis hospitaal opgeneem word te identifiseer. Die studie se doel is ook om vas te stel of die gebruik van 'n toets, naamlik ”Massaspektrometrie” nut het in die behandeling van kinders in die hospitaal met tekens en simptome van vergiftiging in die toekoms.

Ons kan bevestig of jou kind blootgestel is aan 'n potensiale gif deur urine of bloed monsters van jou kind te stuur vir toetse met Massaspektrometrie. Dit is nie 'n toets wat ons gewoonlik doen nie, alhoewel dit in gebruik is in ander hospitale buite Afrika.

Alle kinders wat Rooikruis Hospitaal toe kom met enige van die volgende probleme kan deelneem aan hierdie navorsing:

1. Bevestigde of vermoedelike onlangse vergiftiging
2. Onverklaarbare geestelike tekens soos lomerigheid, verwarring, prikkelbaarheid en onverklaarbare pas
3. Onverklaarbare of ongewone vorms van diarree en braking
4. Onverklaarbare bloed toetse wat probleme met die niere, lewer en ander organe kan wys

Jy en jou kind kan kies om aan hierdie navorsing deelteneem of nie. Jou kind sal die gewone sorg kry wat ons protokolle aanbied, al kies jy nie om deel te neem aan hierdie studie nie. Die studie verander nie die behandeling wat u kind ontvang nie. Egter, as jy besluit om deel te neem aan die studie en massaspektrometrie identifiseer 'n moontlike gif wat moet behandel word kan jou kind dalk ‘n voordeel hieruit kry.

Studie Ondersoeke

Jou kind sal ekstra toetse kry wat gestuur sal word vir Massaspektrometrie:

- ‘N klein hoeveelheid bloed (oor 'n teelepelvol) sal versamel word bykomend tot die gewone toetse
- 'N klein hoeveelheid urine
- Afhangende van die simptome, mag 'n stukkie hare versamel word

Met jou toestemming sal sommige of al die oorskiet monsters gestoor word vir toekomstige toetse

Na ontslag sal jy en jou gesin gekontak word deur ‘n foon oproep binne 3 weke, met inlig van enige resultate, asook verdere behandeling indien dit nodig is.

Newe-effekte en risiko's

Om fisiese trauma end nood aan jou kind te minimaliseer, sal bloed- en urine- monster versameling terselfdetyd gedoen word as die roetine toetse wat jou kind sal kry.

Voordele

As jou kind deelneem aan hierdie navorsing, sal jou kind die voordeel van ekstra toetse kry wat ook kyk vir die oorsaak van die siekte wat hy/sy het. Dit mag behandeling verbeter as dit aandui op 'n spesifieke gif of dwelm wat spesifieke behandeling het.

Vergoeding

Die navorsing sal nie geld kos nie. Jy sal nie enige geld gegee word nie en geen geskenke kry om aan hierdie navorsing deel te neem nie.

Vertroulikheid

Die inligting wat ons insamel in hierdie studie sal vertroulik behou word. Enige studie inligting oor jou en jou kind sal net 'n nommer op dit he, in plaas van 'n naam. Slegs die navorsers sal weet wat jou kind se nommer is. Daar is 'n klein risiko dat iemand jou kind kan identifiseer, maar dit is onwaarskynlik dat dit sal gebeur.

Wat sal gebeur met die inligting wat versamel is?

Wat ons leer uit hierdie navorsing sal gebruik word om aanbevelings te doen oor hoe beste kinders te behandel kinders wat inkom met vermoedelike vergiftiging. Die navorsing sal gedeel word met ander gesondheidsorg werkers. Die identiteit van jou kind word regdeur vertroulik behou.

Onttrekking van die studie

Jy kan enige tyd kies om te onttrek van die studie en jou kind sal steeds die dieselfde behandeling wat hulle veronderstel is om te ontvang kry.

Wie om te kontak

As jy enige vrae het mag jy hulle nou vra, of later, selfs nadat die studie begin het. As jy later wil vrae vrae kan jy die volgende kontak:

Dr Norbertta Washaya

Red Cross War Memorial Children's Hospital

Klipfontein Road

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Tel: 021 658 5111 E-mail: nnwashaya @ gmail.com

OF

Dr Heloise Buys

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Hierdie voorstel is hersien en goedgekeur deur die Menslike Navorsing Etiese Komitee van die Universiteit van Kaapstad, wat 'n komitee is wie se taak dit is om te sorg dat navorsing deelnemers teen skade beskerm word. As jy meer wil uitvind oor die etiese komitee, kontak:

Mrs Lamees Emjedi

Research Ethics Committee

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Telephone: +27 21 406 6338

Fax: 27 21 406 6411

Email: nosi.tsama@uct.ac.za and shuretta.thomas@uct.ac.za

Naam Van Ouer/Voog In Drukskrif _____

Handtekening Van Ouer/Voog _____

Datum _____

Dag/maand/jaar

Deel II: Sertifikaat Van Toestemming Vir Die Stoor Van Monsters

Die monsters sal bewaar word deur die UK kliniese Farmakologie afdeling by Groote Schuur Hospitaal, wat die primêre bewaarders sal wees. Die waarde van die stoor van die monsters is dat beter navorsing metodes in die toekoms beskikbaar kan word. Ons kan dan die dieselfde monsters gebruik om te ontleed met hierdie metodes en so help om die behandeling van meer kinders in die toekoms te verbeter.

Stoor van monsters

Indien enige van die bloed of urine of hare monsters van my kind vir hierdie navorsing projek ongebruikte is, of daar oorskiet wanneer die projek voltooi is:

☐ Ek gee toestemming vir my kind se bloed/urine/hare (**sirkel wat geld**) monsters om gestoor en gebruik te word in toekomstige navorsing van enige tipe, wat die nodige goedkeuring het

☐ Ek gee toestemming vir my kind se bloed/urine/hare (**sirkel wat geld**) monsters om gestoor en gebruik te word in toekomstige navorsing, maar net vir navorsing in vergiftiging.

☐ Ek gee toestemming vir my kind se monster om gestoor te word en gebruik te word in toekomstige navorsing , maar nie vir navorsing

oor:_____

OF

☐ Ek wil my kind se monsters onmiddellik vernietig laat word.

EN

☐ Ek wil my kind se identiteit verwyder he van my kind se monsters.

☐ Ek wil my kind se identiteit behou met my kind se monsters.

EN

☐ Ek wil my kind se monsters vernietig he in die geval van sy/haar dood

☐ Ek gee toestemming vir die gebruik van my kind se monsters selfs in die geval van sy/haar dood

Die gestoorde monsters sal slegs gebruik word vir verdere navorsing na goedkeuring van die Menslike Navorsing Etiese Komitee van die Universiteit van Kaapstad, wat 'n komitee is wie se taak dit is om te sorg dat navorsing deelnemers teen skade beskerm word. Toegang sal beperk word tot navorsers wat goedgekeur is.

Ek het die voorafgaande inligting gelees, of dit was vir my gelees. Ek het die geleentheid gehad om vrae te vra oor dit en enige vrae wat ek gevra het was tot my bevrediging beantwoord. Ek gee vrywillig toestemming vir my kind om deel te neem aan hierdie studie.

Naam van ouer/voog in drukskrif _____

Handtekening van ouer/voog _____

Datum _____

Dag/maand/jaar

As ongeletterde

'n Geletterd wat getuie was moet teken (indien moontlik, moet hierdie persoon gekies word deur die ouer/voog en moet geen verbinding met die navorsing span he nie). Ouers/voogde wat ongeletterd is moet hul duim-print ook insluit.

Ek het die akkurate lees van die toestemmings vorm vir die potensiële deelnemer gesien, en die individu het die geleentheid gehad om vrae te vra. Ek bevestig dat die individu vrylik toestemming gegee het.

Naam van getuie in drukskrif _____ EN Duim van ouer/voog

Handtekening van getuie _____

Datum _____

Dag/maand/jaar

Verklaring deur die navorser/persoon wat toestemming neem

Verklaring deur die navorser/person wat toestemming neem:

1. Bloed versameling
2. Urine versameling
3. Hare versameling
4. Monsters kan bewaar word vir moontlike toekomstige analise.

Ek bevestig dat die ouer/voog 'n geleentheid gegee was om vrae te vra oor die studie, en dat al die vrae korrek beantwoord is, en na die beste van my vermoë. Ek bevestig dat die individu nie gedwing is om hierdie toestemming te gee nie, en dat die toestemming vrywillig gegee is.

'N afskrif van hierdie inligting en toestemmings vorm is aan die ouer/voog voorsien.

Naam van navorser/person wat die toestemming neem _____

Handtekening van navorser/persoon wat die toestemming neem

_____

Datum _____

Dag/maand/jaar

Appendix D: Assent English version

Patient information and assent form for children older than seven years

Utility of Mass Spectrometry in paediatric poisoning

You are invited to take part in a study that is being done at this hospital. Please read this information and ask the study staff or doctor any questions you may have.

Background

You are in hospital because it is suspected that you may have come in to contact with a medication or poison, or maybe you have not, but the way you are feeling and the tests we have done make us think you may have come in to contact with some medicine or poison.

We can find out if you have some medicine or poison in your body by sending urine and/or blood samples from you for a special test called Mass Spectrometry. This is not a test that we normally do. But, we would like to know the causes of poisoning in children admitted to The Red Cross War Memorial Children's Hospital, as well as to see if this special test would be helpful in helping other children who will come to hospital in the future

What will happen to you if you agree to take part in the research?

If you agree to take part, then the following tests will be done to check for the presence of any poison or medicine in your body

- A small amount of blood (about a teaspoon full) will be taken from you
- We will ask you for a small amount of urine

- We may ask for a small piece of your hair

How will it help you to take part in the study?

If you agree to take part in the study we will be able to tell if you are not feeling well because of a medicine or poison in your body. However even if you choose not to have these tests done you will still be treated the way that you are supposed to, it will not change how you are taken care of.

Will the study hurt or make you feel bad?

You will feel some pain from the needle when blood is taken. We will put some special cream on you to make it less sore.

Do you have to be in the study?

Your parent/legal guardian has said it is fine for you to be in the study, but you can still make up your own mind and do not have to take part if you don't want to. Nobody will be angry or upset with you if you don't want to take part.

What do I do if I have any questions?

If you have any questions about this study, you can ask your parent/legal guardian or the study staff or doctor any questions.

I have read and understood this form. My questions have been answered. I am willing to take part in this study.

I, _____ agree to take part in this study.

Signed: _____ Witness: _____

Date: _____ Date: _____

Day/month/year

If illiterate:

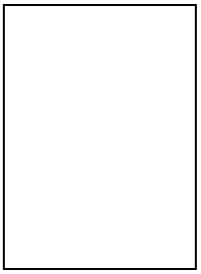
I have witnessed the accurate reading of the assent form to the child, and the child has had the opportunity to ask questions. I confirm that the child has given consent freely.

Print name of witness (not a parent)_____ AND Thumb print of child.

Signature of witness _____

Date _____

Day/month/year



Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the child understands that the following will be done:

1. Urine specimen
2. Blood specimen
3. Maybe a piece of hair

I confirm that the child was given an opportunity to ask questions about the study, and all the questions asked by him/her have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this assent form has been provided to the participant.

Print Name of Researcher/person taking the assent_____

Signature of Researcher /person taking the assent _____

Date _____

Day/month/year

Appendix E: Assent Afrikaans version

Pasiënt inligting en bekragtigings vorm vir kinders ouer as sewe jaar

Nut van Massaspektrometrie in pediatriese vergiftiging

Jy word uitgenooi om deel te neem aan 'n studie wat by hierdie hospitaal gedoen word. Lees asseblief hierdie inligting en vra die studie personeel of dokter enige vrae wat jy het.

Agtergrond

Jy is in die hospitaal opgeneem omdat dit vermoed is dat jy in kontak was met 'n medikasie of gif, of jy was dalk nie, maar die manier waarop jy voel en die toetse laat ons dink jy was in kontak met sekere medisyne of gif.

Ons kan uitvind of jy medisyne of vergiftiging in jou liggaam het deur urine en/of bloed toetse van jou te stuur vir 'n spesiale toets – dit word “Massaspektrometrie” genoem. Dit is nie 'n toets wat ons normaalweg doen nie, maar ons wil graag weet wat die oorsake van vergiftigings is in kinders in Rooi Kruis Hospitaal, sowel as om te sien of hierdie spesiale toets ander kinders ook kan help wat in die toekoms na die hospitaal toe sal kom.

Wat sal gebeur as jy instem om deel te neem aan die navorsing?

As jy instem om deel te neem, dan sal die volgende toetse gedoen word om te kyk vir die teenwoordigheid van enige gif of medisyne in jou liggaam:

- 'n Klein hoeveelheid bloed (ongeveer 'n teelepel vol) sal van jou geneem word
- Ons sal jou vra vir 'n klein hoeveelheid urine

- Ons kan jou vra vir 'n stukkie van jou hare

Hoe sal dit jou help om aan die studie deel te neem?
As jy instem om deel te neem in die studie sal ons in weet of jy siek gevoel het as gevolg van 'n medisyne of gif wat jou liggaam was. Selfs as jy kies om nie hierdie toetse te doen nie sal jy nog behandel word op die manier wat jy veronderstel is om te wees, dit sal nie verander hoe jy versorg word nie.

Sal die studie seermaak of maak laat jy sleg voel?

Jy sal 'n bietjie pyn van die naald voel wanneer bloed geneem word. Ons sal 'n spesiale room aansmeer voor die bloed geneem word om dit minder seer te maak.

Hoef ek in hierdie studie te wees?

Jou ouer/wettige voog het gesê dit is goed vir jou om in die studie te wees, maar jy kan steeds jou eie besluit neem om nie deel te neem nie, as jy nie wil nie. Niemand sal kwaad word of met jou ontsteld wees as jy nie wil deel neem nie.

Wat doen ek as ek enige vrae het?
As jy enige vrae het oor hierdie studie, kan jy jou ouer/wettige voog vra, of vra die studie personeel of dokter enige vrae.

Ek het gelees en verstaan hierdie vorm. My vrae is beantwoord. Ek is bereid om deel te neem in hierdie studie.

Ek, _____ stem in om deel te neem in hierdie studie.

Onderteken: _____ Getuie: _____

Datum: _____ Datum: _____

Dag/maand/jaar

As ongeletterde:

Ek het die akkurate lees van die bekragtigings vorm aan die kind gesien, en die kind het die geleentheid gehad om vrae te vra. Ek bevestig dat die kind vrywillig toestemming gegee het.

Naam van getuie (nie 'n ouer) in drukskrif_____ EN duim druk van kind.

Handtekening van getuie _____

Datum _____

Dag/maand/jaar

Verklaring deur die navorser/persoon wat toestemming neem

Ek het noukeurig gelees uit die INLIGTINGSBLAD vir die potensiële deelnemer, en het na die beste van my vermoë seker gemaak dat die kind verstaan dat die volgende gedoen sal word:

1. urine monster
2. bloed monster
3. miskien 'n stukkie hare

Ek bevestig dat die kind 'n geleentheid gegee was om vrae te vra oor die studie, en dat al die vrae korrek beantwoord is, na die beste van my vermoë. Ek bevestig dat die individu nie gedwing is om hierdie toestemming te gee nie, en dat die toestemming vrywillig gegee is.

'n Afskrif van hierdie bekragtig vorm is voorsien vir die deelnemer.

Naam van navorser/persoon wat bekragtig neem in drukskrif _____

Handtekening van navorser /persoon wat bekragting neem _____

Datum _____ (dag/maand/jaa)

Appendix F: Data collection tool

Value of Mass Spectrometry in Paediatric Poisoning Data Collection Sheet

A. Demographic Data

Date of Birth _____ Sex: Male□ Female□
Address: _____
_____
_____
Primary caregiver: Mother □ Father □ Grandmother □ Other (specify) _____
Historian: Patient □ Mother □ Father □ Neighbour □ Other (specify) _____
Type of housing: Formal □ Informal □ Number of Occupants: _____
Attends Crèche: Yes □ No □

B. Anthropometry

Weight: _____kg

C. Child survival

IUTD/INUTD RVD unexposed rapid -ve / RVD Exposed Rapid or -ve/ RVD exposed rapid or PCR +ve

D. TOXIN EXPOSURE

Suspicion of poisoning from: History □ Examination □ Investigations □
Witnessed Toxin ingestion: Yes □ No □
Toxin in the home: Yes □ No □
Toxin in the street: Yes □ No □
Toxin known: Yes □ No □
Toxin name: _____
Time and date exposed:
Name medications at home (in the case of drug ingestion)
1.
2.
3.
4.

Home remedy given:

_____

_____

Referral centre management:

_____

_____

_____

_____

_____

E. Clinical Details

Date of Admission: _____ Time of admission: _____

Referred: Regional Hospital/District Hospital/Day Hospital /CHC/Self

Presenting complaint _____

_____

_____

_____

_____

_____

Clinical Features

Scoring: done at admission and during admission/ done retrospectively

Central Nervous System Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Drowsiness/vertigo/tinnitus/ataxia Restlessness	Unconsciousness with appropriate response to pain	Deep coma with inappropriate response to pain or unresponsive to pain
	Mild extrapyramidal symptoms	Brief apnoea/bradypnoea	Respiratory depression with insufficiency
	Mild cholinergic/anticholinergic symptoms	Confusion/agitation/hallucinations/ delirium	Extreme agitation
	Paraesthesia	Infrequent, generalized or local seizures	Frequent/generalized seizures,/status
	Mild visual disturbances/auditory disturbances	Pronounced extrapyramidal symptoms	epilepticus/opisthotonus
		Pronounced cholinergic/anticholinergic symptoms	Generalized paralysis or paralysis affecting vital functions

		Localized paralysis not affecting vital functions	Blindness
		Visual/ auditory disturbances	deafness

Central Nervous System Other:

a) Level of consciousness E A M V V P Total U

b) Hyperactive Yes □ No □

c) abnormal movements Yes □ No □

d) nystagmus Yes □ No □

e) photophobia Yes □ No □

f) hallucinations Yes □ No □

g) dystonia Yes □ No □

h) muscle weakness Yes □ No □

i) pin point pupils Yes □ No □

j) dilated pupils Yes □ No □

k) other pupil defect Yes □ No □ Specify _____

GIT Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Vomiting/diarrhoea/pain/ Irritation/ 1st degree burns/ minimal ulcerations in the mouth Endoscopy: erythema/oedema	Vomiting/Diarrhoea/pain/ileus	Massive haemorrhage/perforation
		1st degree burns of critical localization/ 2nd & 3rd degree burns in restricted areas Dysphagia Endoscopy: ulcerative trans mucosal lesions	More wide spread 2nd &3rd degree burns/ Severe dysphagia Endoscopy: ulcerative transmural lesions/circumferential lesions/perforation

GIT Other:

a) nausea Yes □ No □

b) jaundice Yes □ No □

c) hepatomegaly Yes □ No □

Respiratory System Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Irritation/ coughing/ breathlessness/ mild dyspnea/ mild bronchospasm	Prolonged coughing/ bronchospasm/ dyspnea/ stridor/ hypoxemia requiring extra oxygen	Manifest respiratory insufficiency (due to e.g. severe bronchospasm, airway obstruction/glottal oedema/ pulmonary oedema/ ARDS, pneumonitis, pneumonia, pneumothorax)
CXR not done	Chest X-ray: abnormal with minor or no symptoms	Chest X-ray: abnormal with moderate symptoms	Chest X-ray: abnormal with severe symptoms

Respiratory system Other

a) tachypnoea Yes □ No □ RR _____BPM_
b) depressed effort Yes □ No □
c) increased secretions Yes □ No □
d) acidotic breathing Yes □ No □ Oxygen sats _____%

Cardiovascular System Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Isolated extrasystoles Mild and transient hypo/hypertension	Sinus bradycardia (HR ~40-50 in adults, 60-80 in infants and children, 80-90 in neonates) / Sinus tachycardia (HR ~140-180 in adults, 160-190 in infants and children, 160-200 in neonates) / Frequent extrasystoles, atrial fibrillation/flutter/ AV-block I-II, prolonged QRS and QTc-time/repolarization abnormalities Myocardial ischaemia More pronounced hypo/hypertension	Severe sinus bradycardia (HR ~<40 in adults, <60 in infants and children, <80 in neonates)/ Severe sinus tachycardia (HR ~>180 in adults, >190 in infants and children, >200 in neonates)/ Life-threatening ventricular dysrhythmias/ AV block III, asystole Myocardial infarction Shock/hypertensive crisis

Cardiovascular system other:

BP systolic:_____ BP diastolic:_____ Mean BP_____

HR: _____ BPM

Metabolic System Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Mild acid-base disturbances (HCO3 ~15-20 or 30-40 mmol/l; pH~7.25-7.32 or 7.50-7.59) • Mild electrolyte and fluid disturbances (K+ 3.0-3.4 or 5.2-5.9 mmol/l) • Mild hypoglycaemia (~50-70 mg/dl or 2.8-3.9 mmol/l in adults) • Hyperthermia of short duration	More pronounced acid-base disturbances (HCO3 ~10-14 or >40 mmol/l; pH ~7.15-7.24 or 7.60-7.69) More pronounced electrolyte and fluid disturbances (K+ 2.5-2.9 or 6.0-6.9 mmol/l) More pronounced hypoglycaemia (~3050 mg/dl or 1.7-2.8 mmol/l in adults) Hyperthermia of longer duration	Severe acid-base disturbances (HCO3 ~<10 mmol/l; pH ~<7.15 or >7.7) Severe electrolyte and fluid disturbances (K+ <2.5 or >7.0 mmol/l) Severe hypoglycaemia (~<30 mg/dl or 1.7 mmol/l in adults) Dangerous hypo- or hyperthermia

Liver Poisoning Severity Score

No symptoms or signs	Mild,transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Minimal rise in serum enzymes (AST, ALT ~2-5 x normal)	Rise in serum enzymes (AST, ALT ~5-50 x normal) but no diagnostic biochemical (e.g. ammonia, clotting factors) or clinical evidence of liver dysfunction	Rise in serum enzymes (~>50 x normal) or biochemical (e.g. ammonia, clotting factors) or clinical evidence of liver failure
	• Hyperthermia of short duration	Hyperthermia of longer duration	

Kidney Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild,transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Minimal proteinuria / haematuria	Massive proteinuria / haematuria	
		Renal dysfunction (e.g. oliguria, polyuria, serum creatinine of ~200-500 µmol/l)	Renal failure (e.g. anuria / serum creatinine of >500 µmol/l)

16. Renal other
    a) urinary retention Yes □ No □

Blood Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Mild haemolysis	Haemolysis	Massive haemolysis
	Mild methaemoglobinemia (metHb ~10-30%)	More pronounced methaemoglobinemia (metHb ~30-50%)	Severe methaemoglobinemia (metHb >50%)
		Anaemia, leukopenia, thrombocytopenia	Severe anaemia, leukopenia, thrombocytopenia
		Coagulation disturbances without bleeding	Coagulation disturbances with bleeding

Muscular Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Mild pain, tenderness/	Pain, rigidity, cramping and fasciculation/ Rhabdomyolysis,	Intense pain, extreme rigidity, extensive cramping and fasciculation / Rhabdomyolysis with complications
	CPK ~250-1,500 iu/l	CPK ~1,500-10,000 iu/l	, CPK ~>10,000 iu/l / Compartment syndrome

Local effects on skin Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Irritation, 1st degree burns (reddening) or 2nd degree burns in <10% of body surface area	2 nd degree burns in 10-50% of body surface (children: 10-30%) or 3rd	2 nd degree burns in >50% of body surface (children: >30%) or 3rd

		degree burns in <2% of body surface area	degree burns in >2% of body surface area

Skin Other

General

a) Fever Yes □ No □
b) Hypothermia Yes □ No □
c) Sweating Yes □ No □
d) Flushed skin Yes □ No □
e) Dry mouth Yes □ No □
f) Hyper-salivation Yes □ No □
g) Skin bruising Yes □ No □
h) Petechiae/ purpura Yes □ No □
i) Epistaxis Yes □ No □
j) Dehydrated Yes □ No □
   a. If yes level of dehydration Shock/ Severe / Some

Local effects on eye Poisoning severity score:

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Irritation, redness, lacrimation, mild palpebral oedema	Intense irritation/ corneal abrasion/ Minor (punctate) corneal ulcers	Corneal ulcers (other than punctate)/perforation /Permanent damage

Local effects from bites and stings

None	Minor	Moderate	Severe
0	1	2	3
No symptoms or signs	Mild, transient & spontaneously resolving symptoms or signs	Pronounced or prolonged symptoms or signs	Severe or life threatening symptoms or signs
	Local swelling, itching /Mild pain	Swelling involving the whole extremity/ local necrosis/Moderate pain	Swelling involving the whole extremity and significant parts of adjacent area, more extensive necrosis /Critical localization of swelling threatening the airways/Extreme pain

17. Other clinical features (Specify)

_____

F. Investigations

Date → **Test ↓**				
Na^+				
K^+				
Urea				
Creatinine				
Ca^+				
Mg^+				
PO_4^{2-}				
Ammonia				
ALT				
AST				
GGT				
ALP				
TSB				
CBIL				
Albumin				
Total Prot				
pH				
BE				
HCO_3				
pCO_2				
pO_2				
Glucose				
Lactate				
INR				
PT				
Fibrinogen				

G. Toxicology

17. SureSlip/POC test result (Immunoassay)
    DONE Yes □ No □
    Date and time _____
    Result _____
18. NHLS Toxicology test
    Date & time test done _____
    Date & time result available _____
    Result _____
19. Mass spectrometry Urine
    Date & time test done _____
    Date & time result available _____
    Result

1. _____
2. _____
3. _____
4. _____
5. _____

Mass spectrometry blood
Date & time test done _____
Date & time result available
Result

1. _____
2. _____
3. _____
4. _____
5. _____

Mass spectrometry Hair
Date & time test done _____
Date & time result available
Result

H. Management

20. Admitted □ Not admitted □
21. Admission ward: Short stay ward □ High Care □
Ward Floor □ PICU □
22. IV Fluids: Yes □ No □
Bolus required Yes □ No □
23. Inotropes: Yes □ No □
24. Respiratory Support: Yes □ No □
a) Nasal prongs □ b)HFNC □ c) CPAP □ d)IPPV □
25. Anticonvulsants: Yes □ No □
a) Lorazepam□ b) phenobarbitone □ c) phenytoin □ d) Other _____
26. Required resuscitation: Yes □ No □
27. Antidote given: Yes □ No □ If yes specify _____
28. Antidote given on: Basis of history □
Basis of examination □
Basis of investigation □
29. Date and Time antidote was administered _____

I. Outcome

30. Initial Diagnosis _____
31. Final Diagnosis _____
32. Died □ Discharged □

33. Date of death / discharge _____

J. Social services referral

In-patient referral: Yes □ No □
Community SS referral: Yes □ No □
Form 22 completed Yes □ No □ Unknown □
Specific SS intervention:

1. education
2. home visit
3. surveillance
4. removal and emergency placement order

35. Follow up required: Yes □ No □

Appendix G: Proposed protocol for use of LC-MS/MS at RCWMCH

Who can order the test?

Due to the cost of the test each test must be approved by the consultant on cover

Indications
• Unexplained acute neurological symptoms not due to trauma or infection • Known toxin or substance ingestion but with inconsistent clinical picture • Exposure or ingestion to an unknown/unidentifiable toxin or substance • First onset episode of psychosis not due to infection • Confirming positive point of care urine drug screen • Unexplained multiorgan failure
Relative indications
• History of drug abuse in family with supporting clinical symptoms
Not useful • Known toxin or substance ingestion with consistent clinical picture

What to include on the LC-MS/MS request form

- Any medicines or drugs given at home or in hospital as part of therapy
- Name of suspected toxin or drug

Reference

Norbertta Washaya, Alicia Evans, Rudzani Muloiwa et al. A Prospective Study to Assess the Value of Liquid Chromatography-Tandem Mass Spectrometry in the Management of Paediatric Poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa, 22 July 2020, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-45295/v

Appendix H: BMC Pediatrics journal reviewer comments and rebuttal

Rebuttal document: BPED-D-20-01221
A prospective study to assess the value of liquid chromatography-tandem mass spectrometry in the management of paediatric poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa
Norbertta Washaya; Alicia Evans; Rudzani Muloiwa; Peter Smith; Heloise Buys

Reviewer 1

	Reviewer 1 Comments		Responses to reviewer comments	
	Comment **The manuscript submitted by Washaya et al. evaluates the use of the LC-MS/MS methodology in the detection of intoxicants in children entering the emergency room with a suspected intoxication.** **The method described is potentially very useful especially, as the authors underline, in cases where the intoxicating substance is not known. Identification of the substance would be of great help in guiding therapy and the eventual administration of antidotes. Nevertheless, the manuscript needs to be modified and integrated under certain aspects.**		**Thank you for your kind words. The manuscript has been revised and amendments been made to address the issues raised by the reviewer.**	
	METHODS		**Page, lines**	
1.		It is not specified by which criteria tests were done on blood or on urine (or on both)		Thank you very much for the question. The study aimed to test for substances on a urine specimen and only opportunistically took a blood specimen. Patients were not bled solely for the purposes of the study. Only 6 patients did not have any urine samples and that was because the sample collected leaked. We have edited the text to clarify the above. The texts now read: "A urine sample of eligible participants was sent to the laboratory for LC-MS/MS to establish the cause of poisoning. In addition, the attending clinician and laboratory were consulted for any leftover blood specimen after laboratory tests ordered by the attending clinician were completed that could likewise be tested on LC-MS/MS. Study participants were not bled solely for the study." "A total of 146 (96%) urine samples from the 152 study participants were analysed by LC-MS/MS after six samples were lost due to leakage in transit. For 80(53%) participants, there was sufficient left-over blood specimen in the laboratory for LC-MS/MS testing. This included the six participants whose urine samples had been lost to leakage"
			Page 6, line 120-123 Page 11, line 199 - 202	

2.		Authors seem not to consider the half-life and elimination of the poisoning substances. I would not be surprised if some samples were negative because the toxic substance is eliminated via the bilio-fecal route, or if the time elapsed between the intake of the substance and the collection was not compatible with the half-life of the substance.	Page 22, line 382 - 389 Page 7, line 134 - 136 Page 12 line 215 - 218	Thank you very much for these valuable and valid points. We have considered these and agree that these points should have been reflected up in the discussion section as limitations of the study. We have made amendments that read: "It is possible that LC-MS/MS may have been limited by failure to detect substances that are eliminated via the hepatobiliary system which may not have been detectable in urine, as well as substances with a short half-life that may have degraded before sampling or analysis. These reasons are limitations of LC-MS/MS that the clinician needs to be aware of when utilising LC-MS/MS. All nine drugs that the LC-MS/MS failed to identify, and yet were in the LC-MS/MS library, are excreted in urine except for tenofovir, which is mainly excreted in faeces. A study done on sample stability indicated that substance degradation was dependent upon the type of substance and the temperature at which a sample is stored." "The median turnaround time (TAT) for obtaining a result was 5 (IQR 3 – 7) days for urine LC-MS/MS and 6 (IQR 4 -7) days for blood LC-MS/MS. A total of five patients had LC-MS/MS results within 24 hours." "The median TAT for the 57, that were substance-intake-likely cases and yet the LC-MS/MS was negative was 5 (IQR 3- 9) days for urine LC-MS/MS and 5(IQR 4 – 9) days for blood. TAT of the 71 poisoning cases that had positive LC-MS/MS was 5 (IQR 2 – 7) days for urine and 6 (IQR 4-7) days for blood."
3.		Moreover, since toxin is a poisonous substance that is a specific product of the metabolic activities of a living organism, this is not the right term to describe intoxicating substances all along the manuscript. Namely, bromazepam and diphenhydramine (lines 214 and 215) are not toxins, so please find an adequate definition.		Thank you very much for bringing up this point. We agree with your suggestion and we have changed the terminology from 'toxin' to 'substance' throughout the paper

4.	Objective	please clarify if the aim of the study is to describe the prevalence of LC-MS/MS, as written in the objective, or the value of LC-MS/MS, as written in the title	Page 1, line 1	Thank you for raising this point. We agree that the title should be clearly aligned with the aim of the study. The title has been clarified. The title now reads: ThThe prevalence of liquid chromatography-tandem mass spectrometry confirmed paediatric poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa"
5.		No information is provided on the time elapsed between sampling and testing nor on how samples were stored. The reader may think that some samples may have tested negative due to degradation of the poisoning substances.	Page 7, line 132-136 Page 7, line 138 - 140 Page 7 line 142 - 147 Page 7 lines 134 - 136 Page 12, lines 215 – 218 Page 22, lines 388 -	Thank you for noting the omission. LC-MS/MS was being used here as a research tool. As a result the samples were batched for testing. The turnaround time has been indicated and information on storage before testing has now been added to the methods section. The text now reads: "Due to the limited availability of the LC-MS/MS unit, samples were tested in batches. Once collected, samples were registered and transported to the laboratory where they were stored at 4 ºC until analysis. The median turnaround time (TAT) for obtaining a result was 5 (IQR 3 – 7) days for urine LC-MS/MS and 6 (IQR 4 -7) days for blood LC-MS/MS. A total of five patients had LC-MS/MS results within 24 hours. Trained personnel ran the samples and interpreted the results. For quality control, internal standards were added to each sample as part of the sample preparation. Each run included blanks, as well as positive and negative controls to ensure accurate results.(29, 30) In order to observe for possible substance degradation, compound stability tests were done on the LC-MS/MS unit. A commercially obtained control, a system suitability test (SST) (Restek® Corporation) was run daily. The kit contains 8 compounds of known concentrations. The peak areas of each compound were observed to confirm that the instrument performance and sensitivity were optimal and at the same time to observe for possible compound degradation, by comparing these areas to previously acquired data." "The median turnaround time (TAT) for obtaining a

			394	result was 5 (IQR 3 – 7) days for urine LC-MS/MS and 6 (IQR 4 -7) days for blood LC-MS/MS. A total of five patients had LC-MS/MS results within 24 hours." "The median TAT for the 57, that were substance-intake-likely cases and yet the LC-MS/MS was negative was 5 (IQR 3- 9) days for urine LC-MS/MS and 5(IQR 4 – 9) days for blood. TAT of the 71 poisoning cases that had positive LC-MS/MS was 5 (IQR 2 – 7) days for urine and 6(IQR 4-7) days for blood." "A study done on sample stability indicated that substance degradation was dependent upon the type of substance and the temperature at which a sample is stored.(31) Substances stored at 25ºC, 4 ºC and -20ºC were later extracted and analysed at 15, 60 and 90 days and the average relative peaks on these days were compared with the average relative peaks at baseline.(31) The study concluded that the best temperature to store samples is -20ºC, although even at 4ºC the substances could still be detected even if the peaks were lower.(31) The samples in our study were stored at 4ºC, and therefore substance degradation cannot be ruled out."
6.	Line 123-125	"The immunisation status was reviewed from the admission notes and/or the road to health card (immunisation status card) and noted as up to date if the child had received appropriate doses of vaccination for age as per the national immunisation schedule". What is the interest of this information in the context of the article?		Thank you for this question. We had intended to use health seeking behaviour as indicated by immunisation status as proxy tool for assessing the risk of poisoning. But we acknowledge your point and we realise that this statement may confuse readers and so we have removed it.
7.	Line 128	"urine specimen and/or blood sample" on the basis of what one or the other? There is a lot of difference, just consider the elimination route or the time elapsed since assumption.		We have addressed this question in our response to reviewer comment 1.

8.	line 143 and following	patient classification (line 143 and following) is not clear. Authors affirm that "The toxin-intake-unlikely group were patients whose clinical presentation could be explained by an alternative medical diagnosis and were, therefore, not considered poisoning cases." While when describing the participants, they state "All patients admitted at RCWMCH with suspected poisoning were eligible for recruitment into the study". Are therefore the toxin-intake-unlikely excluded from the study?	Page 8, Lines 149 - 161	Thank you for the comment and request for clarification. The classification was done after analysis and not when participants were recruited. Patients were admitted as suspected poisoning cases. And once the authors had analysed the results and clinical presentations, the patients were classified into one of three groups, for the purposes of our study. The section has been amended to bring clarity to the classification used. The section now reads, "As poisoning is defined by the presence of clinical (somatic and/or mental) manifestations, or laboratory and/or electrocardiographic abnormalities resulting from exposure to a substance that can lead to harmful clinical effects (31), once all the toxicology investigations and clinical presentations were analysed, the authors classified the cases into one of three groups: substance-intake-unlikely, substance-intake-likely or substance-intake- unclear. The substance-intake-unlikely group were patients whose clinical presentation could be explained by an alternative medical diagnosis and were, therefore, not considered poisoning cases even though they were admitted as cases of suspected poisoning. The substance-intake-unclear group were patients whose clinical presentation could not be explained by a medical diagnosis and whose toxicology investigation results were not indicative of poisoning. The substance-intake-likely group were those patients whose clinical presentation could be explained by a toxic substance (even in the absence of an LC-MS/MS identified substance) and were therefore considered poisoning cases, even in the absence of symptoms."
9.		The criteria for classification are very poorly described		Thank you for this comment. We have addressed this concern in the preceding related comment.
	RESULTS			
10	line 214	did you investigate the clinical history of the patients or if caregivers had any initiative to treat intoxication? Did you investigate if drugs detected by LC-MS/MS other than intoxicating	Page 13 line 242	Thank you for these questions which highlight the importance of a good clinical history in poisoning cases. We asked whether the caregivers attempted treating the poisoning cases. This is referred to in the text that reads: "Six of the patients had vomiting induced by the care giver in an attempt to decontaminate."

		substances, or in case of multiple drugs detected, were part of a normal therapy or were administered by the caregivers before hospital admission?	Page 20, line 331-333 Page 12, line 202 – 206	"Of the 49 that had gastrointestinal symptoms 24 (49%) had the presence of the confounder of intentional induction of vomiting by the caregivers using manual induction, milk and/or saltwater." We also assessed for therapeutic drugs given at home or in hospital and even if these were identified by LC-MS/MS, they were not included in the analysis as cases of intoxication but classified as iatrogenic. As such, we believe that the multiple drugs identified by LC-MS/MS constitute true cases of multiple drug ingestions. This is demonstrated in the text that reads: "Altogether, in 89/152 (59%) participants a substance was detected. In 16 (18%) of these the detected substances were iatrogenic secondary to administration of in-hospital care or therapy given at home. After discounting the iatrogenic substances or medicines given at home 73 of 152 (48%, 95% CI 40 – 56%) participants had a substance detected by LC-MS/MS."
11	Line 225	Is there any correlation between the source of isolation (blood/urine) and the test being positive or not? Did you consider the time after ingestion and the half-life of these drugs? Please provide these data.	Page 19, Table 4 and line 316 -319	Thank you for this comment. As previously mentioned (and now clarified in the manuscript), the testing of a blood sample was of secondary concern. It was however precisely for the reason stated in this comment that we embarked on this opportunistic pursuit. We wanted to know if there is any discrepancies between two different samples from the same individuals as would indicate value of metabolites that may have been excreted in urine that may aid identification of substances even when these are no longer detectable in blood. However since this was a secondary aim, the study was not powered to make formal correlations and we have refrained from claiming such in our discussion despite the noted higher yield in urine. We address our findings in a text that reads: "Seventy-four (74) patients had both urine and blood samples analysed on LC-MS/MS. Urine and blood LC-MS/MS yielded the exact same result in 48 (65%) patients (table 4). In 18(25%) of the participants with paired samples, more substances were detected in urine but not in blood, while in 4 (5%) samples, more substances were detected in

			Page 25, line 437 - 441	blood but not urine." We have highlighted the value of this in our discussion in a section that reads, "While, both blood and urine samples can be analysed by LCMSMS, urine is usually readily available as a non-invasive specimen with minimal discomfort to children. Furthermore, unlike in blood, drugs and their metabolites are known to remain in urine for longer (up to one week) post last exposure depending on the drug.(20, 21, 40) This gives a greater window of opportunity to still identify a substance after ingestion, especially when this is unknown or occult." An attempt was made to collect information on time of ingestion but because in a significant number of cases the time of ingestion was not known partly because the poisoning was occult.
12	Fig 2	In figure 2 each data is detailed twice (in addition to the graph) and data are repeated in the text. This is fairly redundant.	Page 15 figure 2	We apologise for this, thank you, we have removed the redundant data legend to get rid of the redundancy
13		Is there a correlation among the poisoning substance detected in case of occult poisoning and the clinical presentation? have you assessed whether the intoxicant revealed by the LC-MS/MS is likely to be responsible for the clinical presentation?	Page 15 , line 272-277 Page 19, line 324 - 327	Thank you for these questions which speak to clinical correlation with the substance identified. We have added the information and the text now reads: "In the 22(92%) cases of occult poisoning, in which LC-MS/MS identified a substance, the substance identified was in keeping with the clinical presentation in 20/22 (91%). The 2 patients, in whom LC-MS/MS identified a substance not in keeping with the clinical presentation, concomitant organophosphate poisoning was identified by alternative means. In these 2 organophosphate cases LC-MS/MS identified a substance that would have otherwise been missed." We have also added the information on whether the substance revealed by LC-MS/Ms is responsible for the symptoms. The text now reads: "Of the 71 positive LC-MS/MS results in the substance-intake-likely group, the substances identified by LC-MS/MS were in keeping with the

				clinical presentation in 55/71 (77%) participants. Nine (13%) of the 71 positive LC-MS/MS cases in the substance- likely-group were asymptomatic even though a substance was detected by LC-MS/MS."
13	Fig 3	In Fig 3 half of drug classes are missing	Page 18, figure 3	We apologize this was an error when the graph was being formatted, that has been rectified. Thank you for noting the error.
DISCUSSION				
14		I'm not convinced by the first sentence "Our study illustrates the value of LC-MS/MS in a LMIC setting, particularly in occult poisoning and in identifying multiple toxin ingestion." This statement would be more acceptable if a correlation between the substances found and the clinic were demonstrated. the work does not offer any data to show that the substance identified by LC-MS/MS is really responsible for the intoxication.	Page 21, line 351-353 Page 15 , line 272-277 Page 19, line 324 - 327 Page 12, line 202 – 206	Thank you for the comment. In line with your advice, we have modified the opening sentence and added the section explaining correlation between clinical presentation. The texts now read: "Our study describes the prevalence of LC-MS/MS-confirmed paediatric poisoning in a LMIC setting. LC-MS/MS was particularly helpful in occult poisoning where it was able to identify over 90% of substances, as well as in identifying multiple substance ingestion." "In the 22(92%) cases of occult poisoning, in which LC-MS/MS identified a substance, the substance identified was in keeping with the clinical presentation in 20/22 (91%). The 2 patients, in whom LC-MS/MS identified a substance not in keeping with the clinical presentation, concomitant organophosphate poisoning was identified by alternative means. In these 2 organophosphate cases LC-MS/MS identified a substance that would have otherwise been missed." "Of the 71 positive LC-MS/MS results in the substance-intake-likely group, the substances identified by LC-MS/MS were in keeping with the clinical presentation in 55/71 (77%) participants. Nine (13%) of the 71 positive LC-MS/MS cases in the substance- likely-group were asymptomatic even though a substance was detected by LC-MS/MS." Pertaining to multiple drugs identified we have indicated that we removed any drugs given in hospital or at home from the analysis. Such that multiple drugs identified were truly multiple drugs. The text reads: "Altogether, in 89/152 (59%) participants a substance was detected. In 16 (18%) of these the

				detected substances were iatrogenic secondary to administration of in-hospital care or therapy given at home. After discounting the iatrogenic substances or medicines given at home 73 of 152 (48%, 95% CI 40 – 56%) participants had a substance detected by LC-MS/MS."
15	Line 329:	"Twenty-six patients in this study ingested a substance that was not in the library, this was the main reason for a negative LC-MS/MS result in poisoning cases". I'm not convinced, the tests could be negative because they were done in the wrong time frame, or they could have revealed a substance that is not really responsible for the intoxication. The work as it is does not provide the useful elements to exclude these two possibilities.	Page 21 line 369 - 371 Page 21 , line 368 - 389	Thank you for this comment - we acknowledge this as a limitation and have incorporated your suggestion into the discussion. We have added the other possibilities for a negative result and more information on the substance identified being responsible for the clinical presentation. However, for these 26 whose reported ingested substances were not identified by LC-MS/MS in our study, specifically for them, it means absence from the library of these substances implies even if the substance was present in the sample in whatever quantities, it could not have been detected. Consequently, we find our conclusion that, "This indicates that the ability of LC-MS/MS to detect a substance is limited by the extent of the LC-MS/MS library available at the time.", inescapable. The text now reads: "Twenty-six patients in this study reported ingesting a substance that was not in the library. This was the main reason for a negative LC-MS/MS result in poisoning cases, in this study. This indicates that the ability of LC-MS/MS to detect a substance is limited by the extent of the LC-MS/MS library available at the time. Notably, the LC-MS/MS library can be updated and additional drugs/substances added.(18, 19, 21) The LC-MS/MS used in this study could detect the presence of various drugs in concentrations as low as 20ng/ml. Despite this high sensitivity, nine poisoning cases who had ingested drugs in the LC-MS/MS library were not detected. The possible explanations are varied and include, that the concentration of these drugs in the analysed samples may have been below the limit of detection of the instrument, either due to rapid metabolism or elimination. Six of the nine patients had vomiting induced by the care givers which could have led to decontamination, before the patient could absorb the drug. Notably, two of these patients were given activated charcoal. Worryingly, the first was a tricyclic antidepressant overdose, that

				LC-MS/MS did not detect. The second was a symptomatic chlorpromazine ingestion. This ingestion was witnessed, and the patient was given activated charcoal before the LC-MS/MS was done. It is possible that LC-MS/MS may have been limited by failure to detect substances that are eliminated via the hepatobiliary system which may not have been detectable in urine, as well as substances with a short half-life that may have degraded before sampling or analysis. These reasons are limitations of LC-MS/MS that the clinician needs to be aware of when utilising LC-MS/MS. All nine drugs that the LC-MS/MS failed to identify, and yet were in the LC-MS/MS library, are excreted in urine except for tenofovir, which is mainly excreted in faeces. A study done on sample stability indicated that substance degradation was dependent upon the type of substance and the temperature at which a sample is stored."
16	Please clarify line 386-387	"it is important to note that the clinical outcome was not altered using LC-MS/MS, this corresponds to previous studies, and in our study was a function of…	Page 25, line 443 - 446	Thank you for the comment, we have reworded the sentence to bring some clarity to this section. The statement we made only pertains to acute care as the results show that LC-MS/MS had an impact on considerations of child protection interventions as described. The text now reads: "It is important to note that the clinical outcome was not altered using LC-MS/MS, this corresponds to previous studies, and in our study was because of the long turnaround time, with a median of 5 (IQR 3 – 7) days for urine LC-MS/MS and 6 (IQR 4 -7) days for blood.(19, 21). In our study, the turnaround time was prolonged because the test samples were batched"

	Reviewer 2 Comments		Corrected/Revised dissertation	
1	This study understandably is focused only on drug poisonings and without consideration of non-drug poisoning.			Thank you, yes that is the main focus of the study.
2	As a Toxicologist, I will prefer the choice of toxic substance rather than toxin which skews more to substances of natural origin rather than synthetic substances like drugs.			Thank you for this valuable point we have changed the terminology from 'toxin' to 'substance' throughout the paper.
	Some expressions may have to be referenced by the authors eg:			
3	Page 7, line 143-145	Page 7, line 143-145 should have a reference		Thank you for the comment and request for reference. We opted not to group the cases as poisoning-likely, poisoning unclear and poisoning-unlikely, since the term poisoning would assume patient is symptomatic, whereas some patients ingested a substance but were not poisoned by it. We have added a reference explaining why we chose the terminology, and the text now reads: "As poisoning is defined by the presence of clinical (somatic and/or mental) manifestations, or laboratory and/or electrocardiographic abnormalities resulting from exposure to a substance that can lead to harmful clinical effects (31), once all the toxicology investigations and clinical presentations were analysed, the authors classified the cases into one of three groups: substance-intake-unlikely, substance-intake-likely or substance-intake- unclear. The substance-intake-unlikely group were patients whose clinical presentation could be explained by an alternative medical diagnosis and were, therefore, not considered poisoning cases even though they were admitted as cases of suspected poisoning. The substance-intake-unclear group were patients whose clinical presentation could not be explained by a medical diagnosis and whose toxicology investigation results were not indicative of poisoning. The substance-
			Page 8, Lines 149 - 161	

				intake-likely group were those patients whose clinical presentation could be explained by a toxic substance (even in the absence of an LC-MS/MS identified substance) and were therefore considered poisoning cases, even in the absence of symptoms."
4	Lines 153-156	lines 153-156 should be referenced except this is an original idea of authors.	Pages 8, lines 163 - 166	Thank you for your comment. This was done for the purposes of the study and was the original idea of the authors. We have clarified that and the text now reads: "Irrespective of laboratory results, for the purposes of our study ,poisoning cases were also clinically divided by the authors into three groups: substance known (history of exposure to a known substance), toxin substance unknown (history of exposure to an unknown substance) and occult poisoning (no history of poisoning, but clinical presentation in keeping with poisoning)."
5	Reference section			The whole reference section has been revised with the addition of DOI numbers in line with the journal's instructions to authors

PI signature: _Heloise Buys____ __ **Date:** __16. December 2020_____

Appendix I: Post-rebuttal BMC Pediatrics journal reviewer comments

BPED-D-20-01221R1
The prevalence of liquid chromatography-tandem mass spectrometry-confirmed paediatric poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa
Norbertta Washaya, MBChB, FCPaed; Alicia Evans; Rudzani Muloiwa; Peter Smith; Heloise Buys
BMC Pediatrics

Dear Dr Buys,

Your manuscript "The prevalence of liquid chromatography-tandem mass spectrometry-confirmed paediatric poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa" (BPED-D-20-01221R1) has been assessed by our reviewers. Based on these reports, and my own assessment as Editor, I am pleased to inform you that it is potentially acceptable for publication in BMC Pediatrics, once you have carried out some essential revisions listed below.

_____

1. Please upload the abstract as part of the main manuscript file.

2. Please confirm whether informed consent obtained from all participants was written or verbal, and clearly state this in your Ethics approval and consent to participate section. If verbal, please state the reason and whether the ethics committee approved this procedure.

3. At this stage, please upload your manuscript as a single, clean version that does not contain any tracked changes, comments, highlights, strikethroughs or text in different colours. All relevant tables/figures/additional files should also be clean versions. Figures (and additional files) should remain uploaded as separate files.

_____
Reviewer reports:

Reviewer 1: the authors have responded satisfactorily to all the observations that have been made, the manuscript is now ready for publication

Appendix J: Acceptance letter from BMC Pediatrics journal

BPED-D-20-01221R2
The prevalence of liquid chromatography-tandem mass spectrometry-confirmed paediatric poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa
Norbertta Washaya, MBChB, FCPaed; Alicia Evans; Rudzani Muloiwa; Peter Smith; Heloise Buys
BMC Pediatrics

Dear Dr Buys,

I am pleased to inform you that your manuscript "The prevalence of liquid chromatography-tandem mass spectrometry-confirmed paediatric poisoning at Red Cross War Memorial Children's Hospital, Cape Town, South Africa" (BPED-D-20-01221R2) has been accepted for publication in BMC Pediatrics.

If any final comments have been submitted from our reviewers or editors, these can be found at the foot of this email for your consideration.

Before publication, our production team will also check the format of your manuscript to ensure that it conforms to the standards of the journal. They will be in touch shortly to request any necessary changes, or to confirm that none are needed.

Articles in this journal may be held for a short period of time prior to publication.
If you have any concerns please contact the journal.

Please do not hesitate to contact us if you have any questions regarding your manuscript and I hope that you will consider BMC Pediatrics again in the future.

If you wish to co-submit a data note to be published in BMC Research Notes (https://bmcresnotes.biomedcentral.com/about/introducing-data-notes) you can do so by visiting our submission portal http://www.editorialmanager.com/resn/. Data notes support open data (https://www.springernature.com/gp/open-research/open-data) and help authors to comply with funder policies on data sharing. Please note that this additional service is entirely optional.

Best wishes,

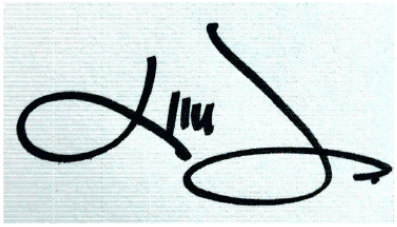
Lauren McMillan
BMC Pediatrics
https://bmcpediatr.biomedcentral.com/

Appendix K: Turnitin report

This Turnitin report has been verified and approved by supervisor A/Prof Heloise Buys

The high similarity index is attributable to the draft manuscript being submitted to a reputable registered preprint repository on 7th July 2020, when it was submitted for publication to the BMC Group of journals. It was assigned a DOI and the draft paper was posted on line by the preprint service; as such it is not considered prior publication and I affirm that the similarities detected are completely aligned with the draft paper and not due to plagiarism.

Signed:



Supervisor
Associate Professor Heloise Buys
11/01/2021

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by Norbertta Washaya

wshnor001:Mass_Spectrometry_Study_Final_manuscript_15_de.

ORIGINALITY REPORT

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3	J. Tyler Davidson, Zachary J. Sasiene, Glen P. Jackson. "The influence of chemical modifications on the fragmentation behavior of fentanyl and fentanyl-related compounds in electrospray ionization tandem mass spectrometry", Drug Testing and Analysis, 2020 Publication	1%
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Appendix L: Author Guidelines BMC Pediatrics Journal

Research article

Criteria

Research articles should report on original primary research, but may report on systematic reviews of published research provided they adhere to the appropriate reporting guidelines which are detailed in our editorial policies. Please note that non-commissioned pooled analyses of selected published research will not be considered. Studies reporting descriptive results from a single institution will only be considered if analogous data have not been previously published in a peer reviewed journal and the conclusions provide distinct insights that are of relevance to a regional or international audience.

BMC Pediatrics strongly encourages that all datasets on which the conclusions of the paper rely should be available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature's information on recommended repositories. Where a widely established research community expectation for data archiving in public repositories exists, submission to a community-endorsed, public repository is mandatory. A list of data where deposition is required, with the appropriate repositories, can be found on the Editorial Policies Page.

Preparing your manuscript

The information below details the section headings that you should include in your manuscript and what information should be within each section.

Please note that your manuscript must include a 'Declarations' section including all of the subheadings (please see below for more information).

Title page

The title page should:

- present a title that includes, if appropriate, the study design e.g.:
  - "A versus B in the treatment of C: a randomized controlled trial", "X is a risk factor for Y: a case control study", "What is the impact of factor X on subject Y: A systematic review"
  - or for non-clinical or non-research studies a description of what the article reports
- list the full names and institutional addresses for all authors

    - if a collaboration group should be listed as an author, please list the Group name as an author. If you would like the names of the individual members of the Group to be searchable through their individual PubMed records, please include this information in the "Acknowledgements" section in accordance with the instructions below
- indicate the corresponding author

Abstract

The Abstract should not exceed 350 words. Please minimize the use of abbreviations and do not cite references in the abstract. Reports of randomized controlled trials should follow the CONSORT extension for abstracts. The abstract must include the following separate sections:

- **Background:** the context and purpose of the study
- **Methods:** how the study was performed and statistical tests used
- **Results:** the main findings
- **Conclusions:** brief summary and potential implications
- **Trial registration:** If your article reports the results of a health care intervention on human participants, it must be registered in an appropriate registry and the registration number and date of registration should be in stated in this section. If it was not registered prospectively (before enrollment of the first participant), you should include the words 'retrospectively registered'. See our editorial policies for more information on trial registration

Keywords

Three to ten keywords representing the main content of the article.

Background

The Background section should explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

Methods

The methods section should include:

- the aim, design and setting of the study
- the characteristics of participants or description of materials
- a clear description of all processes, interventions and comparisons. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses
- the type of statistical analysis used, including a power calculation if appropriate

Results

This should include the findings of the study including, if appropriate, results of statistical analysis which must be included either in the text or as tables and figures.

Discussion

This section should discuss the implications of the findings in context of existing research and highlight limitations of the study.

Conclusions

This should state clearly the main conclusions and provide an explanation of the importance and relevance of the study reported.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations should be provided.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations':

- Ethics approval and consent to participate
- Consent for publication
- Availability of data and materials
- Competing interests
- Funding
- Authors' contributions
- Acknowledgements
- Authors' information (optional)

Please see below for details on the information to be included in these sections.

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- include the name of the ethics committee that approved the study and the committee's reference number if appropriate

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Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

- The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]
- The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
- All data generated or analysed during this study are included in this published article [and its supplementary information files].
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Acknowledgements

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Smith JJ. The world of science. Am J Sci. 1999;36:234-5.

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Article within a journal by DOI

Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. Dig J Mol Med. 2000; doi:10.1007/s801090000086.

Article within a journal supplement

Frumin AM, Nussbaum J, Esposito M. Functional asplenia: demonstration of splenic activity by bone marrow scan. Blood 1979;59 Suppl 1:26-32.

Book chapter, or an article within a book

Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. p. 251-306.

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Online document

Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999.

Online database

Healthwise Knowledgebase. US Pharmacopeia, Rockville. 1998. http://www.healthwise.org. Accessed 21 Sept 1998.

Supplementary material/private homepage

Doe J. Title of supplementary material. 2000. http://www.privatehomepage.com. Accessed 22 Feb 2000.

University site

Doe, J: Title of preprint. http://www.uni-heidelberg.de/mydata.html (1999). Accessed 25 Dec 1999.

FTP site

Doe, J: Trivial HTTP, RFC2169. ftp://ftp.isi.edu/in-notes/rfc2169.txt (1999). Accessed 12 Nov 1999.

Organization site

ISSN International Centre: The ISSN register. http://www.issn.org (2006). Accessed 20 Feb 2007.

Dataset with persistent identifier

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This section provides general style and formatting information only. Formatting guidelines for specific article types can be found below.

General formatting guidelines

Preparing figures

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- Figures should be numbered in the order they are first mentioned in the text, and uploaded in this order. Multi-panel figures (those with parts a, b, c, d etc.) should be submitted as a single composite file that contains all parts of the figure.
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- width of 85 mm for half page width figure
- width of 170 mm for full page width figure
- maximum height of 225 mm for figure and legend
- image resolution of approximately 300 dpi (dots per inch) at the final size

Figures should be designed such that all information, including text, is legible at these dimensions. All lines should be wider than 0.25 pt when constrained to standard figure widths. All fonts must be embedded.

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- Vector figures should if possible be submitted as PDF files, which are usually more compact than EPS files.
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- JPEG files should be saved at maximum quality.
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