行政院所屬各機關因公出國人員出國報告書
(出國報告類別:其他)

赴阿根廷參加2014年第52屆國際法醫毒物學者學會年會(TIAFT)會議報告

出國人員服務機關: 法務部法醫研究所
出國人員姓名/單位/職稱:
曹芸甄/ 毒物化學組/助理研究員
賴詠淳/ 毒物化學組/技士
出國地點: 阿根廷布宜諾斯艾利斯
出國期間: 民國一〇三年十一月七日
至民國一〇三年十一月十七日
報告日期: 民國一〇三年十二月十五日

出國報告名稱:赴阿根廷參加2014年第52屆國際法醫毒物學者學會年會(TIAFT)國際會議報告

頁數：16　含附件：否

出國計畫主辦機關/聯絡人/電話

法務部法醫研究所/陳忠福/22266555

出國人員姓名/服務機關/單位/職稱/電話

曹芸甄/法務部法醫研究所/毒物化學組/助理研究員/22266655

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出國類別：其他

出國期間：民國一○三年十一月七日至民國一○三年十一月十七日

出國地點：阿根廷布宜諾斯艾利斯

報告日期：民國一○三年十二月十五日

關鍵詞：法醫毒物、論文發表

內容摘要：

二○一四年十一月七日至十一月十七日期間赴阿根廷布宜諾斯艾利斯參加2014年第52屆國際法醫毒物學者學會年會（TIAFT）國際會議，為期十一日。

參加2014年第52屆國際法醫毒物學者學會年會（TIAFT）國際會議，會議內容包括專題演講、專業課程、口頭發表論文及電子壁報論文等，以及與鑑識科學研究與實務操作相關的商業展覽,並提供各級相關學位學程進修的資訊。來自世界各地與會代表約四百人參與，本所並於國際年會中公開發表有關法醫毒物分析之論文二篇。

與來自世界各地研究鑑識科學的學者權威、實務專家齊聚一堂，討論實務案例、相關議題的研究內容及研究方向並發表心得，可促進國際學術及鑑識技術之交流，並了解先進國家在鑑識科學各領域的具體作法，機會十分難得。一方面拓展視野，增加對鑑識科學領域多元化的認識，亦可體認先進國家與本國鑑識科學發展模式與人才培養途徑的不同,並瞭解世界鑑識科學研究的趨勢與近期關注的議題；另一方面，本所派員參加亦可促進學術交流，增加國際曝光度，提升本國及本所國際聲譽。

赴阿根廷參加2014年第52屆國際法醫毒物學者學會年會（TIAFT）會議報告

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摘　　要

二○一四年十一月七日至十一月十七日期間赴阿根廷參加2014年第52屆國際法醫毒物學者學會年會（TIAFT），為期十一日，會議採各學門分組及分項形式同時進行，包括專題演講、研討課程、口頭發表論文及壁報張貼論文等，以及與鑑識科學研究與實務操作相關的商業展覽，來自世界各地與會代表約四百人參與，本所並於年會中公開發表有關法醫毒物分析之論文二篇。

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壹、出國目的：

為促進國際學術交流、觀摩學習先進國家在鑑識科學領域之作法及研究現況，並由論文發表提升本所國際地位。本所於一○三年度內編列預算計畫派員至阿根廷參加2014年第52屆國際法醫毒物學者學會年會（TIAFT），並於會議中發表論文。

經向本屆會議投稿，獲評審委員團審核通過准予本屆年會中公開發表有關法醫毒物分析之論文二篇：「Simultaneous Determination and Quantitation of Paraquat, Diquat, Glyphosate, and Glufosinate in Postmortem Blood and Urine by LC-MS/MS（以液相層析串聯質譜分析法同時定量血液及尿液中巴拉刈、大刈、嘉磷塞及固殺草成分）」（曹芸甄、賴詠淳、劉秀娟、劉瑞厚、林棟樑）、「Zolpidem-Related Deaths in Taiwan 2004-2012: A Report on 117 Fatalities (2004-2012 年 Zolpidem 相關致死案例探討)」(賴詠淳、曹芸甄、劉秀娟、劉瑞厚、林棟樑）。

本所毒物化學組於每年申請之法務部科技計畫，均編有此項經費預算，本所能繼續赴國外接受專業訓練、發表論文及參與國際會議，是法醫毒物研究發展最大支柱，此要感謝法務部長官的持續鼓勵與支持，對本所法醫科學學術地位之提升，頗有助益，也藉此機會增加我國國際曝光度並促進本所與各國鑑識科學界之法醫毒物學的知名學者與教授在法醫毒藥物分析技術之交流，汲取法醫毒物新知，以充實本所未來研究發展實力。

在這裡要感謝本所周代理所長章欽及毒物化學組林組長棟樑之支持與指導，才有此次機會赴阿根廷參加年會並了解觀摩國外法醫鑑識之發展。

貳、過程：

11 月 7 日	自桃園機場搭乘班機前往阿根廷布宜諾斯艾利斯
11 月 9 日	至 Panamericano Hotel 辦理大會報到手續 第 10 屆區域 TIAFT 課程 年會開幕式
11 月 10 日	論文口頭報告、論文壁報展示 發表本所論文
11 月 11 日	論文口頭報告、論文壁報展示
11 月 12 日	論文口頭報告、論文壁報展示
11 月 13 日	論文口頭報告、論文壁報展示
11 月 16 日	自阿根廷布宜諾斯艾利斯回國
11 月 17 日	回程

參、會議內容：

一、 二○一四年十一月七日至十一月十七日期間赴阿根廷布宜諾斯艾利斯參加 2014 年第 52 屆國際法醫毒物學者學會年會（TIAFT），今年出席人數約 400 人，大會研討課程(Workshop) 3 門，口頭報告共 100 篇及壁報論文展示共 202 篇。領域橫跨法醫毒物學、臨床毒物學、藥物及新興藥物濫用、分析萃取確認方法探討、性侵案件酒精及藥物之角色、毒素及環境毒理學等主題。

二、 除了參加大會並參觀與大會同時進行的廠商展示、書籍販售，蒐集最新儀器及各式實驗室相關資料。世界各著名檢驗儀器、檢驗耗材廠商及鑑識書籍廠商，利用會場展覽及販售有關相關書籍、儀器及耗材，並展示最新可應用於鑑識科學之科技以及各式自動化及半自動化設備。

三、 於大會張貼壁報論文，今年度本所發表在法醫毒物類計有二篇「Simultaneous Determination and Quantitation of Paraquat, Diquat, Glyphosate, and Glufosinate in Postmortem Blood and Urine by LC-MS/MS(以液相層析串聯質譜分析法同時定量血液及尿液中巴拉刈、大刈、嘉磷塞及固殺草成分)」(曹芸甄、賴詠淳、劉秀娟、劉瑞厚、林棟樑)、「Zolpidem-Related Deaths in Taiwan 2004-2012: A Report on 117 Fatalities (2004-2012 年 Zolpidem 相關致死案例探討)」(賴詠淳、曹芸甄、劉秀娟、劉瑞厚、林棟樑)，期間與前來閱覽之與會學者討論，並與其他壁報論文作者們交換意見，了解最新研究情形。

四、研討課程內容

第 10 屆區域 TIAFT 課程

Course B：Postmortem biotransformation, toxigenomic& alternative matrices

此研討課程共有八堂課，由八位法醫毒物學領域之權威授課：一、Information sources and informatic technology in forensic toxicology；二、Marijuana from the street to the clinical for one hour?；三、Stability of drugs in postmortem specimens；四、Alternative matrices: Its usefulness in the medico legal and toxicological field；五、Predictive models for estimating time of cannabis use - blood & urine；六、Hair analysis for drugs of abuse. Application and pitfalls；七、Postmortem drug changes and redistribution: How to interpret forensic toxicology results；八、Toxicogenetics - A review。

大麻濫用是目前許多國家所需面對及正視之問題，如何檢測及研究其對人體之影響都是熱門探究領域。關於大麻的課程由來自美國的 Marilyn Huestis 及 Michael Smith 講授：大麻植物體內包含超過 530 種化合物，其作用於人體內 G 蛋白接受器(G-protein receptor)、CB-1 接受器、CB-2 接受器、Non-CB1 接受器及 Non-CB2 接受器等，因大腦內有高密度分布之 CB-1 接受器，故大麻對於腦的作用及影響甚大。研究發現，大麻吸食史與是否被檢出大麻及其代謝物有顯著關係，在 THC 與 11-OH-THC 方面，經常吸食大麻通常高於偶爾吸食大麻者，但在 THCCOOH

及 THCCOO-glucuronide 中，經常吸食大麻恆高於偶爾吸食大麻者。目前美國許多州是以血中 THC 濃度大於 5 μg/L 作為閾值，而研究顯示，在經常吸食大麻者中可驗出 THC 大於 5 μg/L 的時間中位數為 3.5 小時 (1.1-30 小時)，而在偶爾吸食大麻者中可驗出 THC 大於 5 μg/L 的時間中位數為 1.0 小時 (0-2.1 小時)，且發現有受試者未有 THC 大於 5 μg/L 之情形。因大麻之血中濃度對於吸食者之影響不甚相同，故以血中 THC 濃度大於 5 μg/L 作為閾值是否仍適當，是未來值得繼續商議及研究之議題。

關於法醫檢體方面，由來自義大利的 Donata Favretto、西班牙 Manuel Lopez Rivadulla 及 Carmen Jurado 講述：一般用來檢驗的法醫檢體有血液、眼球液、肝、胃內容物、膽汁、尿液、腦組織等，死後血液因有乳酸堆積，故 pH 值較低，且血中的 *E. Coli* 及 *Candida albicans* 為兩種主要產生酒精之菌相，主要影響檢體穩定之因素為溫度，若保存於 3℃ 中可維持兩年的穩定。收集檢體盡量不使用玻璃材質，如果是裝在試管內的血液通常會使用 1%氟化鈉保存，它的好處是可抑制某些藥物的分解，例如古柯鹼。另關於頭髮檢體，其好處為可檢測出受試者慢性或長期之施用，但缺點為檢出率較低，且較易遭受汙染及干擾，故頭髮檢體之檢驗結果應作為輔助工具，但未檢出不代表未施用。

毒物基因體學 (Toxicogenetics) 由來自瑞士的 Thomas Kraemer 主講：人體有約 2,5000 個基因，且普遍存在著基因多型性；因基因轉譯成蛋白質，而蛋白質產物影響生理機能，且影響藥物的代謝及效用，是故基因多型性可將人分群為反應者 (Responders)、不反應者 (Non-responders)、毒性反應者

(Toxic-responders)。在相同劑量的毒藥物作用下，不同基因多型性者會因代謝速率的不同而有不同的反應，致死量亦不相同，故在判讀法醫毒物檢驗報告時，需將毒物基因體學一併考慮，才可較審慎判斷死因，但因此領域仍在研究階段，且不同人種之基因多型性有差異，要推估至更大族群，需未來更大規模之合作研究。

肆、檢討建議及心得感想：

一、國際會議部分心得

今年的國際法醫毒物學者學會年會（TIAFT）第一次在拉丁美洲舉辦，故在會議及課程中除了英文報告之外，亦有西班牙文演講的部分，為因應語言之需求，這次會議啟用同步翻譯，可使用耳機轉換頻道，以聆聽適合自己的語言。另外，本次會議的壁報展示為「e-poster」的形式，不需紙本壁報，而使用 LCD 觸控螢幕，閱讀者可自行選取、滑動、放大所想閱讀的壁報部分，這項措施不僅減少紙張的使用，壁報投稿者也不需帶著厚重的壁報上下飛機或托運，且減少所需會場的空間，這應是未來的潮流，國內的壁報展示或許也可朝此方向前進與改善。

二、口頭論文、壁報論文閱讀摘要心得

本次會議的口頭發表論文及壁報論文主題分為：(一) 死後毒物學 (Post Mortem Toxicology)；(二) 藥物濫用及新興毒品 (Drugs of Abuse and New Psychoactive Substances)；(三)法醫毒物學之分析方法及確效 (Analytical Techniques, Validation and QA in Forensic Toxicology)；(四)臨床毒物學、藥物治療監控、行為毒物學及工作環境毒物檢測 (Clinical Toxicology and Therapeutic Drug Monitoring, Behavioral Toxicology and Workplace Drug Testing)；(五)酒精及性侵案件藥物 (Alcohol and Drugs in Sexual Assault)；(六)毒素、草藥治療、職業及環境毒物學 (Toxins, Herbal Remedies, Occupational and Environmental Toxicology) ；(七)法醫及臨床毒物學之替代檢體 (Alternative Matrices in Forensic and

Clinical Toxicology)等七大主題，茲就與毒物化學組業務相關摘要如下：

1. 三位年輕病患在經核磁共振掃描 (Magnetic resonance imaging, MRI) 含釓 (gadolinium) 顯影劑注射後死亡，解剖後除切片發現肺水腫外並無其他重大發現，而毒藥物檢測方面，所採檢之血液、尿液經氣相、液相層析質譜儀等篩驗後皆無發現濫用藥物、治療藥物、殺蟲除草劑及金屬等可致死毒藥物。以液相層析質譜法直接檢測血液中含釓顯影劑的主要成分 Gd-DTPA 及 Gd-DOPA，但僅驗出低於文獻中核磁共振掃描所需使用量的濃度；以感應耦合電漿質譜分析儀(ICP-MS)直接檢測血液中的釓離子 (Gd^{3+})，也只檢驗出低濃度。在警方的調查中發現，在核磁共振檢驗室中有一種特別的溶液—全氟碳化合物 (perfluorocarbon, FC-770, 3M)是用於生殖腺、軟組織等放射檢驗。以頂空氣相分析質譜儀檢測血液檢體中 FC-770，結果發現三位死者的 FC-770 血中濃度分別為 19.3 μg/mL、20.9 μg/mL、20.0 μg/mL，進而回推其被注射的劑量分別為至少 230 mg/kg、180 mg/kg、180 mg/kg，而在文獻中 FC-770 的推估致死劑量為 6.8 mg/kg，故三者的死亡原因應可推測為被誤注入不該使用的 FC-770。

2. 酒駕及藥駕是目前全世界面對的重要課題。美國針對使用大麻及低量酒精是否會影響開車，並以車輛的側位移 (Standard Deviation of Lateral Position, SDLP, lane weaving) 作為研究判斷依據。受試者為偶爾吸食大麻者 (最近三個月內至少吸食一次，一星期小於三天)，將其分為對照組及低量酒精組，駕車於不同的地區，例如鄉村、州間或城市，並以液相層析質

譜儀檢測血中四氫大麻酚 (THC) 濃度。共 19 位受試者 (13 位男性、6 位女性，21-38 歲) 完成所有部分的研究，研究發現：大麻及酒精顯著的增加 SDLP ($p < 0.05$)，濃度遞增 SDLP 亦顯著增加，且大麻所影響的程度較酒精來的大，特別是在轉彎時 SDLP 增加幅度較大，或許是因大麻影響腦部適應及反應的能力。

3. 氟西泮 (Flurazepam) 是一種苯二氮平類 (Benzodiazepines) 的鎮靜安眠藥，氟西泮食入後很快會代謝成 desalkylflurazepam (DAF)及 2-hydroxyethylflurazepam，目前在澳洲並未核准上市，但在台灣是合法的處方藥。在澳洲的法醫檢驗機構，自 2009 年起，有 13 個案子的尿液檢體中檢出 DAF，但並未檢出本體的氟西泮，且這 13 案皆同時檢出 midazolam；此 13 案尿液中的 DAF 平均濃度為 63 ng/mL (範圍為 13-250 ng/mL)，而其毒物學家皆判斷此 DAF 結果為檢驗的不純物，故無放入最後的毒物報告中。進一步研究得知，DAF 為製造 midazolam 的起始物，故在檢體中可檢驗出 midazolam 的存在；而在其他研究中也發現，在服用大量的 midazolam 後，病人的檢體中亦可發現 DAF 的存在，故作者提出，若檢出 DAF，可能並非是服用氟西泮，而是服用了 midazolam。

4. Propofol 於 1989 年問世，商品名為 Diprivan®，是一種短效性的催眠劑並常用於注射麻醉，且其引起暈眩嘔吐等副作用較低故使用普遍；近年來，propofol 在自殺案件中增加許多，特別是醫療相關人員。本篇作者檢測不同檢體的 propofol 濃度，例如大腿及心臟血、膽汁、尿液、腦及肝組織等，並使用氣

相及液相層析質譜法兩種分析法去比較檢測的效果。1 mL 或 1 g 的檢體先經 6N HCl 酸水解於 100 ℃ 作用 1 小時，氣相層析質譜法中採用 BSTFA 及 TMAH 衍生，液相層析質譜法採用 aryl-diazonium salt 的 azo 衍生法，而作者在比較結果後發現以液相層析質譜分析法的定量結果較氣相層析質譜法佳，而本組實驗室目前 propofol 的定量是使用氣相層析串聯質譜法，或許日後可進一步開發以液相層析質譜法做常規之定量檢驗。

5. Dextromethorphan 在低劑量時為止咳成分，但當使用高劑量時可成為解離性的迷幻劑，作用類似愷他命及天使塵 (phencyclidine, PCP)，但其鏡像異構物 Levomethorphan 與其有相當不同的功效，其為類鴉片止痛劑。世界衛生組織已發出安全警示，故現許多國家已報導關於 dextromethorphan 在製造時可能使用含 levomethorphan 的被汙染原料，而被服用後之副作用甚至死亡案件。本篇研究使用 LC/MS/MS 鑑定檢體中的 dextromethorphan、levomethorphan 及其代謝物，而檢體來源來自可能服用受汙染的 dextromethorphan 的病人，其血液、尿液檢體使用液相萃取法處理，而肝臟及腎臟檢體使用固相萃取法 (Oasis HLB cartridge)。使用 LC/MS/MS 方法成功定量出所有檢體種類中的 dextromethorphan、levomethorphan、dextrorphan、levorphanol、(+)-3-methoxymorphinan、(+)-3-hydroxymorphinan。利用 LC/MS/MS 進行藥物定量的研究仍是目前法醫毒物學的主流，本所以此為主的研究與實務經驗已臻至成熟，並與國際接軌。

三、建議：

1. 由於本次會議地點在阿根廷布宜諾斯艾利斯，時差為 11 個小時，到達隔天便要進行一聯串的研討課程，對於非英語系國家的參加者而言，實在有點吃力。為吸收更多相關知識，保持最佳精神與體力狀態參與課程以達到最大效益及最佳學習效果，建議可在不支領生活費的情況下，提前兩天到達會場，以適應當地的狀況。

2. 國際上大部份的實驗室及研究單位對於法醫毒物分析的品質要求越來越來高，分析儀器也追求至高解析度及高精密度，以求最精準之實驗數據，因此採購高精密度及高解析度之儀器，不僅可應用於未來司法鑑識案件，更有助於提升國內法醫毒物分析之品質，也能與國際法醫毒物分析或毒藥物分析之各研究單位或鑑識單位接軌，有利於提高國內鑑識品質及在國際上的曝光率。

3. 由於法醫毒化鑑識科技日新月異，一日千里，隨社會的變遷，犯罪案件與日俱增，千變萬化，因此專業人員的在職訓練相當重要，除了參加國際會議外，宜安排人員赴國外作短期進修，培養人力，藉此強化本所鑑識技術能力，汲取國外相關之專業知識和技能。

4. 希望以後在參與國際會議時，能順便安排參觀一、兩天的實驗室行程，除了增長見識外，亦可培養人脈，將來若有培訓的需要，才有與國外合作交流的機會。

Simultaneous Determination and Quantitation of Paraquat, Diquat, Glyphosate, and Glufosinate in Postmortem Blood and Urine by LC-MS/MS

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Aims: Paraquat (PQ), diquat (DQ), glyphosate (GLYP), and glufosinate (GLUF) are non-selective contact herbicides and widely used. Since these herbicides are often found in postmortem specimens collected from poisoning cases, we have developed a simple and sensitive LC-MS/MS method for the analysis of these herbicides in postmortem blood and urine samples. **Methods:** Deuterated analogs of PQ, DQ, GLYP, and GLUF were served as internal standards. Acetonitrile and dichloromethane were used for protein precipitation and organic solvent backwashing, respectively. The mobile phase of LC-MS consisted of 15 mM heptafluorobutyric acid and acetonitrile. Mass spectrometric analysis was performed under electrospray ionization in positive-ion multiple reaction monitoring (MRM) mode. The precursor ions and the two transition ions adopted for these four analytes were (m/e) PQ (185; 169 and 115); DQ (183; 157 and 78); GLYP (170; 88 and 60); and GLUF (182; 136 and 119). **Results:** Drug-free blood and urine, fortified with 1–20 μg/mL of the four analytes of interest, used for method validation yielded the following results: (a) average extraction recoveries ranged: 73.7–88.8% for urine, 43.4–102.9% for blood; (b) inter-day and intra-day precision ranges (percent CV): 0.7–8.7% and 1.0–11.5%; (c) inter-day and intra-day accuracy ranges: 93.4–108.1% and 89.8–112.3%; and (d) calibration linearity (r^2), detection limit, and quantitation limit: >0.999, 0.1–0.25 μg/mL, and 0.1–0.25 μg/mL, respectively. The observed ion suppression phenomenon was lower than 20% for PQ and DQ; 54% for GLYP; and 50% for GLUF. The validated protocols have been successfully utilized to the analysis in postmortem samples. During the 2013–JUN2014 period, 12 blood specimens were found to contain at least one of these four herbicides with the following means and concentration ranges (μg/mL): PQ (343.76; 2.95–1234.40) and GLYP (1027.81; 3.39–3654.85). **Conclusions:** Among 4726 toxicological cases during the 2013–JUN2014 period in our institute, in which at least one of these four herbicides was detected, these herbicides were ruled as the cause of death in 12 cases, clearly indicating the significance of herbicide analysis. For these samples, the means and concentration ranges (μg/mL) for blood samples were PQ (343.76; 2.95–1234.40) and GLYP (1027.81; 3.39–3654.85).

Simultaneous determination and quantitation of paraquat, diquat, glyphosate, and glufosinate in postmortem blood and urine by LC-MS/MS

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Introduction

Paraquat(PQ), Diquat(DQ), Glyphosate(GLYP), and Glufosinate(GLUF) are non-selective herbicides and widely used all over the world. Because these herbicides are relative cheap and accessible, we could find them in some poisoning and suicidal cases. In order to help medical examiners find the causes of death of victims, it is important to set up a method to detect and quantitate herbicides.

These herbicides are highly water-soluble, less volatile and different ion types in gaseous phase. Thus it is difficult to simultaneously determine the four herbicides. Our lab tried several organic solvent for protein precipitation and chemical extraction. We have developed a simple and sensitive LC-MS/MS method for the analysis of these herbicides in postmortem blood and urine samples. In real cases, we used this method and examine these herbicides sucoessfully. Therefore, it could assist medical examiners determining the cause of death of victims.

Table 1. Transitions and MS/MS conditions for each analyte and internal standard

Compound	Retention time(min)	Precursor ion (m/z)	Fragment (V)	Target ion(m/z)	CE	Qualifier ion(m/z)	CE
Parquat	3.07	185	131	115	48	169	28
Paraquat-d_6	3.06	193	114	121	56	176	32
Diquat	2.81	183	144	157	24	78	48
Diquat-d_4	2.81	187	163	159	24	158	24
Glyphosate	0.87	170	65	88	4	60	12
Glyphosate-$^{13}C_2$,^{15}N	0.87	173	92	91	4	62	16
Glufosinate	1.12	182	88	136	8	119	16
Glufosinate-d_3	1.12	185	94	139	8	122	16

Instrument Conditions

I. Liquid Chromatography: Agilent 1200 Series

Column : Zorbax SB-Aq (100 mm X 2.1 mm, 1.8 μm particle)

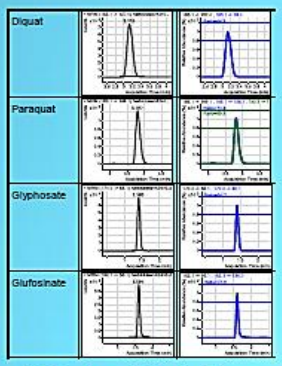
Column temperature : 40 ℃ Injection volume: 5 μL with needle wash

Flow rate : 0.30 mL/min

time (min)	A : % 15mM HFBA	B : % Acetonitrile
0	90	10
1.5	90	10
6.0	0	100
8.0	0	100
9.0	90	10
10.0	90	10

II. Agilent 6410 Triple Quadrupole

Ionization : ESI Positive	Gas temperature : 350 ℃	Drying gas : 10 L/min
Nebulizer pressure : 40 psi	Capillary voltage : 4000 V	Precursor isolation width : 4 amu
Spectra acquired : MS1 and MS2	Vcap : -3500 V Vend : -500 V	Capillary Exit Lens : 130.0 V
Skimmer1 : 40.0 V	Lens1 : -5.0 V Lens2 : -60.0 V	Acqusition Mode : Dynamic MRM Cycle time : 500 ms



Methods

I. Sample preparation

1, 2.5, 5, 10, 20 μg/mL spiked standards with 0.5 mL blank urine or drug-free blood and 50 μL internal standards (5 μg/mL)

↓

Add 1 mL acetonitrile and vortex for 5 minutes

↓

Centrifuge at 4000 rpm for 5 minutes and transfer the supernatant to new tubes

↓

Add 3 mL dichloromethane and vortex for 5 minutes

↓

Centrifuge at 4000 rpm for 5 minutes

↓

100 μL of the upper aqueous phase was injected into LC/MS/MS

II. Method validation

(A) calibration linearity (r^2); (B) Inter-day and Intra-day accuracy and precision; (C) limits of detection, LOD, and limits of quantitation LOQ; (D) matrix effects.

Results

i. Recovery

Table 2. Extraction recovery (%) and martix effect of paraquat、diquat、glyphosate、glufosinate in urine and blood, Mean ± SD (n=3)

Compound	Concentration (μg/ml)	Urine		Blood	
		Martix effect	Recovery(%)	Martix effect	Recovery(%)
Paraquat	2.500	94.08±6.23	82.01±4.05	152.42±19.72	58.80±6.21
	5.000	78.96±0.99	83.26±0.63	130.97±9.84	70.37±4.14
	10.000	75.50±0.48	88.83±0.82	135.18±14.48	69.79±4.98
Diquat	2.500	111.82±6.16	83.66±1.81	119.12±1.03	85.44±2.93
	5.000	96.26±1.31	76.67±0.98	93.09±1.33	95.69±1.88
	10.000	80.15±1.02	88.50±1.22	81.36±1.40	102.90±2.37
Glyphosate	2.500	52.86±1.65	79.63±3.35	35.31±2.12	43.42±1.13
	5.000	60.08±0.91	80.21±0.92	35.65±5.45	45.78±5.50
	10.000	44.07±1.30	78.10±1.97	40.60±2.35	49.36±1.91
Glufosinate	2.500	40.10±1.41	73.66±2.19	47.77±1.74	78.18±11.93
	5.000	40.64±0.98	78.27±2.84	59.00±2.83	77.96±3.79
	10.000	42.49±1.72	76.17±1.69	62.72±1.43	81.30±4.59

ii. Calibration

Table 3. Calibration results of paraquat、diquat、glyphosate、glufosinate in urine and blood

Sample	Compound	Regression equation	Correlation range (r^2)	Concentration range (μg/ml)	LOD (μg/ml)	LOQ (μg/ml)
Urine	Paraquat	y=2.4445x-0.01450	0.99972	1-20	0.1	0.25
	Diquat	y=42.8358x-1.1096	0.99943	1-20	0.1	0.1
	Glyphosate	y=1.4999x-0.0736	0.99948	1-20	0.25	0.25
	Glufosinate	y=1.1966x-0.0451	0.99969	1-20	0.1	0.25
Whole Blood	Paraquat	y=2.3737x-0.1220	0.99954	1-20	0.1	0.25
	Diquat	y=29.7737x-0.4204	0.99979	1-20	0.1	0.1
	Glyphosate	y=1.3722x-0.0071	0.99941	1-20	0.1	0.1
	Glufosinate	y=1.2028x-0.0479	0.99979	1-20	0.1	0.25

iii. Inter-day and Intra-day Accuracy and Precision

Table 4. Interday and intraday accuracy and precision(n = 5) for diquat in human urine and blood

sample	concentration (μg/ml)	Interday				Intraday			
		Mean (μg/ml)	SD	C.V.(%)	Accuracy(%)	Mean (μg/ml)	SD	C.V.(%)	Accuracy (%)
Urine	2.500	2.486	0.142	5.693	99.423	2.519	0.234	9.292	100.772
	5.000	4.927	0.309	6.277	98.541	5.141	0.302	5.864	102.827
	10.000	10.187	0.595	5.837	101.870	10.181	0.925	9.089	101.809
Blood	2.500	2.474	0.107	4.318	98.959	2.418	0.070	2.895	96.722
	5.000	4.846	0.340	7.013	96.927	4.802	0.237	4.932	96.047
	10.000	10.038	0.842	8.390	100.376	10.212	0.951	9.311	102.117

Table 5. Interday and intraday accuracy and precision (n = 5) for paraquat in human urine and blood

sample	concentration (μg/ml)	Interday				Intraday			
		Mean (μg/ml)	SD	C.V.(%)	Accuracy (%)	Mean (μg/ml)	SD	C.V.(%)	Accuracy (%)
Urine	2.500	2.408	0.088	3.667	96.317	2.429	0.126	5.191	97.144
	5.000	4.845	0.152	3.133	96.909	4.918	0.235	4.786	98.364
	10.000	9.841	0.402	4.089	98.410	9.891	0.686	6.934	98.913
Blood	2.500	2.394	0.137	5.726	95.753	2.399	0.035	1.473	95.967
	5.000	4.809	0.228	4.744	96.189	4.585	0.267	5.829	91.700
	10.000	9.866	0.196	1.991	98.662	9.621	0.507	5.272	96.213

Table 6. Interday and intraday accuracy and precision (n = 5) for glyphosate in human urine and blood

sample	concentration(μg/ml)	Interday				Intraday			
		Mean (μg/ml)	SD	C.V.(%)	Accuracy (%)	Mean (μg/ml)	SD	C.V.(%)	Accuracy (%)
Urine	2.500	2.381	0.108	4.542	95.237	2.376	0.047	1.962	95.054
	5.000	4.768	0.122	2.561	95.366	4.861	0.049	1.012	97.224
	10.000	9.990	0.316	3.167	99.904	9.988	0.205	2.053	99.880
Blood	2.500	2.590	0.160	6.193	103.580	2.529	0.286	11.303	101.161
	5.000	4.671	0.403	8.619	93.426	4.489	0.515	11.478	89.772
	10.000	9.734	0.717	7.362	97.340	9.573	0.614	6.419	95.733

Table 7. Interday and intraday accuracy and precision (n = 5) for glufosinate in human urine and blood

sample	concentration (μg/ml)	Interday				Intraday			
		Mean (μg/ml)	SD	C.V.(%)	Accuracy (%)	Mean (μg/ml)	SD	C.V.(%)	Accuracy (%)
Urine	2.500	2.466	0.030	1.231	98.634	2.453	0.089	3.612	98.128
	5.000	4.875	0.078	1.593	97.507	4.901	0.082	1.675	98.020
	10.000	9.840	0.195	1.981	98.404	9.977	0.131	1.314	99.765
Blood	2.500	2.419	0.054	2.238	96.760	2.410	0.051	2.102	96.391
	5.000	4.816	0.032	0.661	96.316	4.867	0.148	3.043	97.350
	10.000	9.930	0.228	2.295	99.299	9.819	0.180	1.838	98.190

iv. Postmortem Samples

Table 8. Results of the analysis of paraquat and glyphosate in post-mortem samples using LC-MS/MS

Case number	Sample type	Concentration (μg/ mL)	
		Paraquat	Glyphosate
1	Blood	18.52	3.39
2	Blood	2.95	-
3	Blood		269.12
4	Blood	-	293.79

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Zolpidem-Related Deaths In Taiwan 2004-2012: A Report On 117 Fatalities

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Aims: Zolpidem, a short-acting nonbenzodiazepine hypnotics commonly used for treating insomnia, was investigated in this retrospective study of 881 forensic autopsy cases from Taiwan during 2004-2012 at our institute. Zolpidem concentrations found in these cases were studied, in particular, with respect to the manner and cause of death. **Methods:** Postmortem blood specimens were routinely screened for zolpidem using liquid-liquid extraction (by a Toxi-tubes® A protocol), followed by GC/MS and/or LC/ion-trap/MS methods of analysis. Positive specimens were confirmed for zolpidem and quantified by LC/MS/MS using zolpidem-d_6 as the internal standard. This method has been validated earlier for the quantification of 26 benzodiazepines and Z-drugs (zolpidem, zopiclone and zaleplon), with 0.005 μg/mL as the limit of quantitation for zolpidem. **Results:** Complete autopsy records for 117 cases were obtained, reviewed, and analyzed. Yearly distributions of these zolpidem-positive cases were: 2012, 11; 2011, 10; 2010, 22; 2009, 24; 2008, 12; 2007, 7; 2006, 14; 2005, 13; 2004, 4. Among these 117 cases, the mean age was 42.3, ranging from 10 to 81; with 79 (67.5%) of these deaths were women. Various drugs including ethanol were detected in 94 cases. The manners of death for these cases were suicide (57.3%), accident (15.4%), uncertain (15.4%) , homicide 6.8%), and disease (5.1%). The causes of death were intoxication - including single and multiple drugs (43.6%), drowning (21.4%), charcoal-burning suicides (15.4%), other asphyxia (7.7%), fire disaster (5.1%), hanging (3.4%), and falling (3.4%). Blood zolpidem concentrations ranged from 0.50 to 95.3 μg/mL; mean concentrations, with respect to manner of death, were 5.05, 2.46, 1.28, 3.95 and 1.08 μg/mL for suicide, accident, homicide, uncertain, and disease, respectively. Mean concentrations, according to cause of death, were 2.80, 3.93, 9.71, 1.45, 2.35, 2.41 and 1.25 μg/mL for intoxication, drowning, charcoal-burning suicides, other asphyxia, fire disaster, hanging, and falling, respectively. **Conclusions:** Zolpidem-positive specimens were frequently detected in forensic autopsy cases at our institute .Yearly distribution data indicated zolpidem-related fatalities increased dramatically, especially in the most recent 4 years included in this study. Among the zolpidem-positive cases, the most common manner of death and cause of death were suicide (57.3%) and intoxication (43.6%), respectively. The concentrations of zolpidem in charcoal-burning cases were substantially higher, indicating zolpidem was also commonly used in non-pharmaceutical suicides in Taiwan.

Zolpidem-related Deaths In Taiwan 2004-2012: A Report On 117 Fatalities

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Introduction

Zolpidem is an imidazopyridine derivative and used since 1986 in Europe and 1993 in United States. It is sold under the brand names Ambien, Stilnox, Intermezzo, Sublinox, and Edluar. In case of acute zolpidem overdose, adverse effects inculde dissocative behaviors, dizziness, anterograde amnesia, headache, nausea, sleep driving and even death. A short-acting nonbenzodiazepine hypnotics, Zolpidem commonly used for treating insomnia, was investigated in this retrospective study of 881 forensic autopsy cases from Taiwan during 2004-2012 at our institute. Zolpidem concentrations found in these cases were studied, in particular, with respect to the manner and cause of death.

Methods and Materials

Postmortem blood specimens were routinely screened for zolpidem using liquid-liquid extraction (by a Toxi-tubes® A protocol), followed by GC/MS and LC/ion-trap/MS methods of analysis. Positive specimens were confirmed for zolpidem and quantified by LC/MS/MS using zolpidem-d6 as the internal standard. This method has been validated for the quantification of 26 benzodiazepines and Z-drugs (zolpidem, zopiclone and zaleplon), with 0.005 μg/mL as the limit of quantitation for zolpidem.

Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100 mm × 2.1 mm i.d., dp = 1.8μm) analytical column operated at 50°C. The mobile phase consists of 0.1% formic acid (v/v) in water (A) and methanol (B), operated at a flow rate of 0.32 ml/min. Mass spectrometric analysis was performed in positive-ion mode, applying multiple reaction monitoring (MRM) using appropriate collision energy for each precursor ion. Then, all information and data were recorded and analyzed with Excel software.

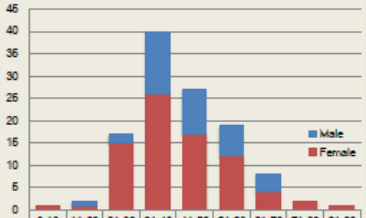
Results

117 cases were obtained, reviewed, and analyzed. Yearly distributions of these zolpidem-positive cases were: 2012, 11; 2011, 10; 2010, 22; 2009, 24; 2008, 12; 2007, 7; 2006, 14; 2005, 13; 2004, 4. (Figure1) Among these 117 cases, the mean age was 42.3, ranging from 10 to 81; with 79 (67.5%) of these deaths were women. (Table1,figure2) Various drugs including ethanol were detected in 94 cases. The manners of death for these cases were suicide (57.3%), accident (15.4%), uncertain (15.4%) , homicide (6.8%), and disease (5.1%). (Figure3)

Results

The causes of death were intoxication - including single and multiple drugs (43.6%), drowning (21.4%), charcoal-burning suicides (15.4%), other asphyxia (7.7%), fire disaster (5.1%), hanging (3.4%), and falling (3.4%). (Figure4) Blood zolpidem concentrations ranged from 0.50 to 95.3 μg/mL; mean concentrations, with respect to manner of death, were 5.05, 2.46, 1.28, 3.95 and 1.08 μg/mL for suicide, accident, homicide, uncertain, and disease, respectively. (Table2) Mean concentrations, according to cause of death, were 2.80, 3.93, 9.71, 1.45, 2.35, 2.41 and 1.25 μg/mL for intoxication, drowning, charcoal-burning suicides, other asphyxia, fire disaster, hanging, and falling, respectively. (Table3)

Figure 2. Age and gender distributions of the 117 zolpidem fatalities during 2004-2012 in Taiwan.



	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90
Male	0	1	2	14	10	7	4	0	0
Female	1	1	16	26	17	12	4	2	1

Figure 3. The percentage of Manner of death of zolpidem related 117 fatalities.

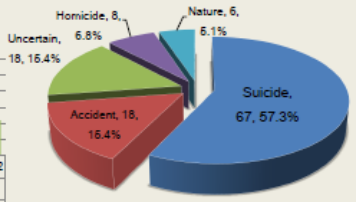
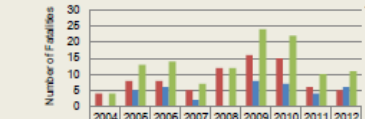


Figure1. Yearly distribution of Zolpidem related 117 fatalities during 2004-2012 in Taiwan.



	2004	2006	2006	2007	2008	2009	2010	2011	2012
Female	4	8	8	5	12	16	15	6	5
Male	0	5	6	2	0	8	7	4	6
Total Fatalities	4	13	14	7	12	24	22	10	11

Figure 4. The percentage of Cause of death of zolpidem related 117 fatalities .

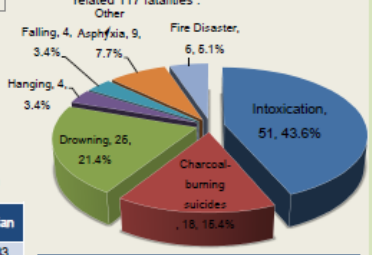


Table1. Age and Sex Distributions of 117 Fatalities for zolpidem during 2004-2012 in Taiwan.

Sex	n	Age	Mean±S.D.	Median
Male	38	13-68	43.26±12.25	41
Female	79	10-81	41.76±12.80	39
Total	117	10-81	42.25±12.59	40

Table 2. Manner of death of blood zolpidem concentration related 117 fatalities.

Manner of death	n	Range	Mean±S.D.	Median
Suicide	67	0.511-95.307	5.05±12.71	1.533
Accident	18	0.519-11.953	2.46±2.86	1.037
Homicide	8	0.501-3.783	1.28±1.07	0.948
Nature	6	0.560-1.516	1.08±0.32	0.966
Uncertain	18	0.518-23.315	3.95±6.28	1.248

Table 3. Cause of death of blood zolpidem concentration related 117 fatalities.

Manner of death	n	Range	Mean±S.D.	Median
Intoxication	51	0.527-23.315	2.80±3.79	1.727
Charcoal-burning suicides	18	0.517-95.307	9.71±22.9	1.333
Drowning	25	0.518-27.469	3.93±6.65	1.301
Hanging	4	0.511-6.402	2.41±2.71	1.357
Falling	4	0.526-2.555	1.25±0.93	0.967
Other Asphyxia	9	0.501-4.022	1.45±1.14	1.048
Fire Disaster	6	0.514-6.991	2.35±2.48	0.834

Conclusions

In summary, zolpidem-positive specimens were frequently detected in forensic autopsy cases at our institute. Yearly distribution data indicated zolpidem-related fatalities increased dramatically, especially in the most recent 4 years included in this study. Among the zolpidem-positive cases, the most common manner of death and cause of death were suicide (57.3%) and intoxication (43.6%), respectively. The concentrations of zolpidem in charcoal-burning cases were substantially higher, indicating zolpidem was also commonly used in non-pharmaceutical suicides in Taiwan.

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