JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, March 2016, Vol. 61, No. 2
doi: 10.1111/1556-4029.12972
Available online at: onlinelibrary.wiley.com

CASE REPORT

TOXICOLOGY

Parvaz Madadi,[1] Ph.D.; Lauren E. Kelly,[2] Ph.D.; Colin J. Ross,[3,4,5] Ph.D.; Charis Kepron,[6] M.D.; James N. Edwards,[7] M.D.; and Gideon Koren,[2,8] M.D.

Forensic Investigation of Methadone Concentrations in Deceased Breastfed Infants

ABSTRACT: There is a paucity of data to aid in assessing whether postmortem methadone findings in breastfed infants are clinically and/or toxicologically significant. Two cases are reported in which methadone was detected in deceased neonates whose mothers were enrolled in methadone maintenance programs and were breastfeeding. In addition to a complete autopsy and toxicological testing for alcohol, prescription medications, and drugs of abuse, pharmacogenetic analysis was performed for variants in genes related to methadone metabolism and response. In both cases, the postmortem methadone concentration measured in neonatal heart blood was higher than the maximum serum methadone concentration reported in living breastfed infants whose mothers were receiving methadone. However, additional analysis of antemortem blood indicated postmortem redistribution of methadone. Pharmacogenetic results were suggestive of a potential predisposition to methadone toxicity based on studies in adults; the significance of these findings in breastfed neonates requires further research. The medical cause of death was unascertained in both cases.

KEYWORDS: forensic science, toxicology, methadone, infant, breastmilk, opioids, CYP2B6, ABCB1

For the medical forensic team, death investigations in children under the age of one are challenging, especially when autopsy and clinical findings do not clearly demonstrate an anatomical or pathological cause of death (1). In such context, toxicological test results may be of particular importance to the case investigation. However, for many drugs, the dose–toxicity relationship in neonates and infants has not been established, and evidence from the literature is sparse.

In Ontario, like many other North America jurisdictions, the growing prevalence of opioid dependency has led to an increase in the number of individuals receiving methadone maintenance treatment. In pregnancy, methadone maintenance treatment has been the standard of care for opioid dependence as it is associated with improved outcomes for both the mother and child when compared to untreated maternal opiate dependency (2). In the postpartum period, maternal methadone use is considered compatible with breastfeeding based on undetectable to low concentrations of methadone in breastmilk (3–10) and low serum levels of methadone in breastfed infants (ranging from undetectable to 8.1 mcg/L) (4,8,9). Breastfeeding may decrease neonatal withdrawal symptoms in infants who were exposed to methadone *in utero* (3,11,12).

Past reports of young children whose causes of death were attributed to methadone overdose have been related to accidental or intentional methadone administration. In breastfed neonates, there is a paucity of data to aid in assessing whether postmortem methadone findings are clinically and/or toxicologically significant. Herein, we report two cases of elevated methadone findings in deceased neonates who were exclusively breastfed by mothers enrolled in methadone maintenance programs and the potential role for pharmacogenetics in these findings.

Case One

An exclusively breastfed 3-week-old male infant was born at 36 weeks' gestation to a mother who was maintained on 65 mg/day of methadone during pregnancy and into the postpartum period. The neonate was found in his bassinet without vital signs 3 h after nursing. No somnolence or grogginess of the infant was reported by the mother in the hours prior to this event. In hospital, his resuscitation was initially successful, but he succumbed to hypoxic brain injury several hours later. Blood collected upon arrival to the emergency room indicated a methadone concentration between the lower detection limit of 10 mcg/L and the lower quantitation limit of 42 mcg/L, as analyzed by liquid chromatography tandem–mass spectrometry (LC-MS/MS). Forensic analysis of admission and postmortem blood using gas chromatography (GC)-MS, GC flame ionization detection, and GC nitrogen phosphorus detection ruled out the presence of alcohol, common medications, and drugs of abuse

[1]Toxicology Section, Centre of Forensic Sciences, Toronto, ON, Canada.
[2]Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, and Ivey Chair in Molecular Toxicology, Western University, London, ON, Canada.
[3]Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, BC, Canada.
[4]Child and Family Research Institute, University of British Columbia, Vancouver, BC, Canada.
[5]Division of Translational Therapeutics, Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada.
[6]Ontario Forensic Pathology Service, Ottawa, ON, Canada.
[7]Office of the Chief Coroner of Ontario, Toronto, ON, Canada.
[8]Division of Clinical Pharmacology and Toxicology, Department of Pediatrics, Hospital for Sick Children, Toronto, ON, Canada.

Received 24 Oct. 2014; and in revised form 16 Feb. 2015; accepted 11 April 2015.

except for lorazepam and phenobarbital which were used during the resuscitation. The postmortem heart blood methadone concentration, sampled 17 h after death, was 79 mcg/L.

Pharmacogenetic analysis for variants associated with methadone metabolism and response was performed. DNA was purified from a postmortem blood sample using the QIAmp DNA purification system (Qiagen, Toronto, ON, Canada) and targeted genotyping performed using a custom Illumina GoldenGate single nucleotide polymorphism (SNP) genotyping assay (Illumina, San Diego, CA) and TaqMan® genotyping assays (Applied Biosystems, Foster City, CA), as previously described (13). Key genotype results for this infant are summarized in Table 1.

The infant was found to be homozygous for the *CYP2B6*6* haplotype, which has been associated with methadone-related mortality in adults (14,15) due to an impaired ability to metabolize methadone (16,17). The infant was also heterozygous for SNPs in the *ABCB1* gene (encoding for p-glycoprotein), which is associated with partially impaired efflux activity (18), and may potentially render an individual more sensitive to the opioid-mediated effects of methadone.

Postmortem examination, including a complete autopsy, did not reveal any pathological etiologies to explain the death. Growth parameters and organ weights were all within normal limits. Metabolic screens, biochemical analysis of vitreous fluid, karyotype, and microbiological assessment of blood, lung, tissue, and cerebrospinal fluid were all noncontributory. The neonate was also not noted to have neonatal abstinence syndrome after birth. Police investigation ruled out foul play. The medical cause of death was unascertained.

Case Two

An 18-day-old male infant was born at 35 weeks' gestation (birthweight 2.34 kg) to a mother who was prescribed methadone (85–115 mg), and was also using cocaine and smoking cigarettes during pregnancy. The neonate remained in hospital for 17 days for management of respiratory distress syndrome, and subsequently neonatal abstinence syndrome on a tapering dose of morphine, with his last 0.1 mg dose of morphine given 2 days prior to his discharge home. On his first night at home, the neonate was reportedly fussy in his crib and was breastfed and bottle-fed prior to being bundled and placed supine with a pillow to prop him up on a double bed with the parents. Two hours later, he was noted to be unresponsive and resuscitation attempts were unsuccessful.

Both methadone and benzoylecgonine (a major metabolite of cocaine) were qualitatively detected in a baby bottle filled with breastmilk; the concentration of drug in breastmilk was not quantitated. The neonate's postmortem heart blood contained methadone at a concentration of 26 mcg/L, and mixed blood collected from autopsy contained a methadone concentration of 33 mcg/L. Naloxone was also detected, as well as traces of benzoylecgonine in heart blood.

Pharmacogenetic analysis was performed for the determination of potential polymorphisms in variants known to be involved in methadone metabolism and response as described in case one. Results are summarized in Table 1.

This infant was also heterozygous for the three SNPs in ABCB1 associated with decreased P-glycoprotein activity. This efflux transporter is expressed in the luminal membrane of the blood–brain barrier (BBB), and functional impairment in its activity has been shown to significantly increase the amount of methadone that reaches the brain (19).

A thorough police investigation and scene assessment was performed. The postmortem exam, including a complete autopsy, did not reveal any signs of traumatic injury. There was evidence of early bronchopneumonia with focal extension to complete lobules, which was not felt to be sufficiently significant to have solely caused death. Case conferences involving police, child protection services, the coroner, and the forensic pathologist were held. The case concluded with the medical cause of death being unascertained with the identified contribut-

TABLE 1—*Genotype results associated with reduced metabolism and increased sensitivity to methadone in two deceased neonates whose mothers were taking methadone.*

Gene SNP	Function	Impact	Wild-type Allele	Polymorphism	Case 1 Result	Case 2 Result
CYP2B6 rs3745274	Coding NONSYN Q172H (NP_000758.1) *CYP2B6*6*	Decreased protein expression, decreased methadone clearance	C	A	**A_A**	*n/a
CYP2B6 rs2279343	Coding NONSYN K262R (NP_000758.1) *CYP2B6*6*	Decreased protein expression, decreased methadone clearance	A	G	**G_G**	*n/a
ABCB1 rs2032582	Coding NONSYNON (G2677T) (Ala893Thr)	Impaired p-glycoprotein efflux activity; methadone dose adjustments in adults	C	A	**A_C**	**A_C**
ABCB1 rs1045642	Coding SYNON (C3435T) (Ile1145Ile)	Impaired p-glycoprotein efflux activity; methadone dose adjustments in adults	G	A	**A_G**	**A_G**
ABCB1 rs1128503	Coding SYNON G412G (NP_000918.2)	Impaired p-glycoprotein efflux activity; methadone dose adjustments in adults	A	G	**G_A**	**G_A**
ABCB1 rs2229109	Coding NONSYN S400N (NP_000918.2)	Partially impaired p-glycoprotein efflux activity	G	A	G_G	G_G
ABCB1 rs9282564	Coding NONSYN N21D (NP_000918.2)	Partially impaired p-glycoprotein efflux activity	A	G	A_A	A_A
OPRM1 rs1799971	Coding NONSYN Asn40Asp	Associations with opioid response and dose requirements	A	G	A_A	A_A

SNP, single nucleotide polymorphism; SYNON, synonymous (genetic polymorphism is not thought to have a functional effect on protein expression); NONSYNON, nonsynonymous (genetic polymorphism is thought to have a functional effect on protein expression). *Other variants tested (nonsignificant findings): CYP2B6* (rs8192709, rs8192709, rs2279341), *UGT2B7* (rs12233719, rs7439366), *ABCB4* (rs7785206, rs1202283), *CYP3A5* (rs41303343, rs55965422), *CYP3A7* (rs55798860), *CYP3A4* (rs55785340, rs17342647), *CYP3A* (rs800667), *COMT* (rs740602, rs4633, rs4818, rs165815).

*n/a: Due to limited sample available at autopsy and poor-quality DNA that contained a high amount of impurity, CYP2B6 results were unobtainable by two different laboratories, even after multiple extractions, for this case.

ing factors of bronchopneumonia, unsafe sleep environment, (bed-sharing with two adults while propped with a pillow), and potential methadone toxicity. The manner of death was undetermined.

Discussion

Toxicology Findings

From a toxicological perspective, methadone is a challenging drug, given the phenomenon of opioid tolerance and the fact that metabolism and clearance are highly variable between individuals. Liver metabolism by cytochrome P450 (CYP) isoenzymes CYP3A4 and CYP2B6 is the main routes of elimination (20), although various other CYP enzymes are also involved. Interindividual variability in CYP3A4 and CYP2B6 activity can arise from age-related differences in the ontogeny of drug metabolizing enzymes, drug interactions resulting in induction or inhibition of these enzymes, and from genetic polymorphisms which may have various consequences on enzymatic activity and function. Moreover, the R- and S-methadone enantiomers possess different pharmacological properties; while S-methadone has limited pharmacological activity at the mu opioid receptor as compared to the R-methadone enantiomer, it has been associated with QT interval prolongation and may increase the risk of cardiac arrhythmias and sudden death (21).

In infants, several additional factors need to be considered when interpreting methadone concentrations. For one, little is known about the inherent ability of a neonate to metabolize and eliminate methadone, and the relationship between blood concentration and toxicity has not been established. Second, as most mothers are maintained on methadone throughout pregnancy and into the postpartum period, the contribution of fetal methadone exposure *in utero* to blood concentrations measured in the early postpartum period is unknown. Moreover, overall infant opioid tolerance and sensitivity to methadone, especially with respect to neonatal abstinence syndrome (NAS), needs to be considered. Third, in the context of breastfeeding, maternal pharmacokinetic parameters affecting the amount of drug excreted into breastmilk may exacerbate or protect neonates based on their intrinsic clearance capacity for methadone.

In the literature, serum methadone concentrations in breastfed infants have not exceeded 8.1 mcg/L (4,8,9); this level is equivalent to a blood methadone concentration of 6.1 mcg/L using a blood-to-plasma ratio of 0.75 (22). In case two, the presence of methadone in breastmilk supports the likelihood that methadone was transferred to the baby through breastfeeding. In case one, the availability of admission blood for analysis offers the first evidence in the literature of the extent by which methadone concentrations may be subject to postmortem redistribution (PMR) in infants. The postmortem methadone concentration in heart blood was anywhere from 2 to 10 times higher than the admission blood concentration range measured in this infant. The extent of PMR depends on a number of factors including postmortem interval and drug properties (e.g., lipophilicity). Heart blood drug concentrations may be elevated due to drug distribution from proximal organs such as the liver. For methadone, differences between heart and peripheral blood concentrations are variable (23). In addition, the postmortem methadone concentrations for both cases are substantially lower than the one published case report of methadone intoxication in a breastfed infant (24). In this case from the year 1977, the breastfed infant had a methadone blood concentration of 400 mcg/L and was also deemed to be "malnourished" at autopsy (24).

Pharmacogenetic Findings

A candidate-gene pharmacogenetic analysis was performed as part of the death investigation in order to identify genetic variants that may have rendered these infants as susceptible to methadone accumulation or sensitivity. In adults, there has been much recent attention given to the contribution of pharmacogenetics as it pertains to methadone clearance and response. In addition to CYP3A4, CYP2B6 has recently emerged as an important enzyme predicting methadone clearance (25,26). The *CYP2B6*6* variant, in particular, has been associated with lower protein expression and a "poor metabolizer" phenotype (16,27), and studies by one group have shown a high prevalence of *CYP2B6*6* in methadone-related adults deaths (14,15). Subsequently, another team showed that mean methadone doses required by subjects homozygous for the **6* variant of *CYP2B6* were significantly lower than heterozygotes and noncarriers (17), and a recent meta-analysis has concluded that methadone metabolism is significantly slower in *6 homozygous carriers (28). An estimated 6% of individuals with European ancestry carry the **6* variant as do over 40% of West Africans (27), while data from the International HapMap project indicate that the incidence of homozygous *6 carriers ranges from 3% to 20% depending on ethnicity. Importantly, CYP2B6 appears to plays a stereoselective role in the metabolism of S-methadone; the *CYP2B6*6* genotype has been associated with specific increases in S-methadone plasma concentrations (16).

In infants, CYP2B6 enzymatic levels have been reported to increase by approximately twofold after the neonatal period (birth to 30 days of postnatal); however, between individuals, the overall level of CYP2B6 protein expression can vary over 25-fold (29). Studies to date suggest that this variation may be pharmacogenetically mediated. In case one, symptoms of accumulating opioid toxicity (i.e., infant somnolence) were not noted based on parental report. It follows that theoretically, enzymatic deficiency in CYP2B6 may have potentially rendered the infant susceptible to QT prolongation or another cardiac event due to specific increases in the S-methadone enantiomer plasma concentration. On the other hand, there is evidence to show that the R-methadone enantiomer is found in higher concentrations in breastmilk than the S-enantiomer (10), but this study did not account for maternal *CYP2B6* genotype status, which may affect the relative amount of the S-enantiomer circulating in the plasma (30). In case one, the mother carried at least one *CYP2B6*6* variant based on her infant's homozygous genotype.

Both infants were heterozygous carriers of the lower activity form of the P-glycoprotein transporter, which plays an important role in mediating the transport of opioids across the BBB. In neonates, the BBB is structurally and functionally immature, and current evidence suggests that at birth, the expression of P-glycoprotein is low (31). How polymorphisms in *ABCB1* when combined with the ontogeny of P-glycoprotein expression and the structural and functional immaturity of BBB may predispose infants to opioid-related toxicity is an area of current investigation (31). It has been shown that polymorphisms in the *ABCB1* gene have been associated with increased risk of central nervous system depression from codeine in neonates (13), although there are inconsistent findings in regard to the role of *ABCB1* polymorphisms and methadone toxicity in adults (28).

Other Clinical Considerations

Case two illustrates the potential interplay of sleep-associated risk factors and social risk factors (1) which should be considered as part of a neonatal death investigation. Specifically, bed-sharing, sleeping on a surface not intended for infant sleep, and/or sleeping in a cluttered environment are potentially unsafe practices which have been deemed as contributing factors in many cases of infant deaths in Ontario (1). Moreover, there is evidence that a subpopulation of methadone-maintained mothers may continue to utilize other drugs throughout pregnancy, placing infants at risk of multidrug exposures both *in utero* and into the postpartum period (32). In addition to general systemic issues resulting in a lack of addiction support services for these individuals, it is also possible that methadone doses during pregnancy are not appropriately adjusted to account for changes in pharmacokinetic parameters, particularly in the second and third trimesters. This may result in subtherapeutic dosing creating withdrawal symptoms leading to additional substance use or abuse of other drugs (32).

To aid in the interpretation of elevated postmortem methadone concentrations in infants, one needs to consider exposure to methadone *in utero*, pharmacological treatment of neonatal withdrawal symptoms in the postpartum period, breastfeeding, neonate sleeping and care conditions, and the capacity of the neonate to metabolize and clear methadone from his or her system as part of the death investigation. Moreover, as exemplified in case one, the contribution of PMR of methadone concentrations after death needs to be accounted for. The results of pharmacogenetic testing in these two cases may have identified a possible explanation for elevated postmortem methadone levels. There have been case reports of infants and young children dying as a result of accidental or intentional administration of methadone, and caregivers have faced criminal conviction as a result (33). Therefore, in pediatric fatalities where the infant is found to have higher than expected methadone levels in the postmortem samples, the death investigation team should also consider a possible contribution from inborn variations in enzyme activity.

This study aims to describe the complexity of interpreting elevated postmortem methadone concentrations in breastfed infants for the forensic science community. It would be inappropriate to assume that the methadone concentrations presented in this study are indicators of cause of death in these infants. It is possible that the interplay of clinical and polygenetic factors may render some infants more sensitive to the effects of methadone (i.e., bronchopneumonia, reduced methadone efflux at the BBB, accumulation of methadone blood concentrations due to potentially reduced neonatal metabolism, bed-sharing, exposure to other drugs or medications). Larger systemic investigations are needed to characterize the clinical significance of such factors and to understand how the culmination of potentially life-threatening risks may be prospectively averted.

Acknowledgments

The authors would like to acknowledge the Death Under Five Committee and Dr. Michael B. Wilson of the Office of the Chief Coroner of Ontario, Dr. Liza Boucher formerly of the Ontario Forensic Pathology Service, Drs. Yvona Buczek, Amy Peaire, and Ms. Patricia Solbeck from the Centre of Forensic Sciences, Dr. Bruce C. Carleton from the University of British Columbia, and Dr. Tara Paton from the Hospital for Sick Children for their contributions to the analysis, interpretation, and discussion of these cases.

References

1. Office of the Chief Coroner of Ontario. Paediatric Death Review Committee and Deaths Under Five Committee annual report 2013; http://www.mcscs.jus.gov.on.ca/stellent/groups/public/@mcscs/@www/@com/documents/webasset/ec163306.pdf (accessed September 5, 2014).
2. Wong S, Ordean A, Kahan M, Maternal Fetal Medicine Committee, Family Physicians Advisory Committee, Medico-Legal Committee, Society of Obstetricians and Gynaecologists of Canada. Substance use in pregnancy. J Obstet Gynaecol Can 2011;33(4):367–84.
3. Geraghty B, Graham EA, Logan B, Weiss EL. Methadone levels in breast milk. J Hum Lact 1997;13(3):227–30.
4. Wojnar-Horton RE, Kristensen JH, Yapp P, Ilett KF, Dusci LJ, Hackett LP. Methadone distribution and excretion into breast milk of clients in a methadone maintenance programme. Br J Clin Pharmacol 1997;44(6):543–7.
5. McCarthy JJ, Posey BL. Methadone levels in human milk. J Hum Lact 2000;16(2):115–20.
6. Begg EJ, Malpas TJ, Hackett LP, Ilett KF. Distribution of R- and S-methadone into human milk during multiple, medium to high oral dosing. Br J Clin Pharmacol 2001;52(6):681–5.
7. Jansson LM, Choo RE, Harrow C, Velez M, Schroeder JR, Lowe R, et al. Concentrations of methadone in breast milk and plasma in the immediate perinatal period. J Hum Lact 2007;23(2):184–90.
8. Jansson LM, Choo R, Velez ML, Harrow C, Schroeder JR, Shakleya DM, et al. Methadone maintenance and breastfeeding in the neonatal period. Pediatrics 2008;121(1):106–14.
9. Jansson LM, Choo R, Velez ML, Lowe R, Huestis MA. Methadone maintenance and long-term lactation. Breastfeed Med 2008;3(1):34–7.
10. Bogen DL, Perel JM, Helsel JC, Hanusa BH, Thompson M, Wisner KL. Estimated infant exposure to enantiomer-specific methadone levels in breastmilk. Breastfeed Med 2011;6(6):377–84.
11. Abdel-Latif ME, Pinner J, Clews S, Cooke F, Lui K, Oei J. Effects of breast milk on the severity and outcome of neonatal abstinence syndrome among infants of drug-dependent mothers. Pediatrics 2006;117(6):e1163–9.
12. Isemann B, Meinzen-Derr J, Akinbi H. Maternal and neonatal factors impacting response to methadone therapy in infants treated for neonatal abstinence syndrome. J Perinatol 2011;31(1):25–9.
13. Sistonen J, Madadi P, Ross CJ, Yazdanpanah M, Lee JW, Landsmeer ML, et al. Prediction of codeine in infants and their mothers using a novel combination of maternal genetic markers. Clin Pharmacol Ther 2012;91(4):692–9.
14. Bunten H, Liang WJ, Pounder DJ, Seneviratne C, Osselton D. OPRM1 and CYP2B6 gene variants as risk factors in methadone-related deaths. Clin Pharmacol Ther 2010;88(3):383–9.
15. Bunten H, Liang WJ, Pounder DJ, Seneviratne C, Osselton D. Interindividual variability in the prevalence of OPRM1 and CYP2B6 gene variations may identify drug-susceptible populations. J Anal Toxicol 2011;35(7):431–7.
16. Dobrinas M, Crettol S, Oneda B, Lahyani R, Rotger M, Choong E, et al. Contribution of CYP2B6 alleles in explaining extreme (S)-methadone plasma levels: a CYP2B6 gene resequencing study. Pharmacogenet Genomics 2013;23(2):84–93.
17. Levran O, Peles E, Hamon S, Randesi M, Adelson M, Kreek MJ. CYP2B6 SNPs are associated with methadone dose required for effective treatment of opioid addiction. Addict Biol 2013;18(4):709–16.
18. Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, et al. Identification of functionally variant MDR1 alleles among European American and African Americans. Clin Pharmacol Ther 2001;70:189–99.
19. Wang JS, Ruan Y, Taylor RM, Donovan JL, Markowitz JS, DeVane CL. Brain penetration of methadone (R)- and (S)-enantiomers is greatly increased by P-glycoprotein deficiency in the blood-brain barrier of Abcb1a gene knockout mice. Psychopharmacology 2004;173(1–2):132–8.
20. Wang JS, DeVane CL. Involvement of CYP3A4, CYP2C8, and CYP2D6 in the metabolism of (R)- and (S)-methadone *in vitro*. Drug Metab Dispos 2003;31(6):742–7.
21. Eap CB, Crettol S, Rougier JS, Schläpfer J, Sintra Grilo L, Déglon JJ, et al. Stereoselective block of hERG channel by (S)-methadone and QT interval prolongation in CYP2B6 slow metabolizers. Clin Pharmacol Ther 2007;81(5):719–28.
22. Inturrisi CE, Colburn WA, Kaiko RF, Houde RW, Foley KM. Pharmacokinetics and pharmacodynamics of methadone in patients with chronic pain. Clin Pharmacol Ther 1987;41(4):392–4.
23. Levine B, Wu SC, Dixon A, Smialek JE. Site dependence of postmortem blood methadone concentrations. Am J Forensic Med Pathol 1995;16(2):97–100.
24. Smialek JE, Monforte JR, Aronow R, Spitz WU. Methadone deaths in children. A continuing problem. JAMA 1977;238(23):2516–7.

25. Gadel S, Crafford A, Regina K, Kharasch ED. Methadone N-demethylation by the common CYP2B6 allelic variant CYP2B6.6. Drug Metab Dispos 2013;41(4):709–13.
26. Kharasch ED, Stubbert K. Role of cytochrome P4502B6 in methadone metabolism and clearance. J Clin Pharmacol 2013;53(3):305–13.
27. Zanger UM, Klein K, Saussele T, Blievernicht J, Hofmann MH, Schwab M. Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. Pharmacogenomics 2007;8:743–59.
28. Dennis BB, Bawor M, Thabane L, Sohani Z, Samaan Z. Impact of ABCB1 and CYP2B6 genetic polymorphisms on methadone metabolism, dose and treatment response in patients with opioid addiction: a systematic review and meta-analysis. PLoS ONE 2014;9(1):e86114.
29. Croom EL, Stevens JC, Hines RN, Wallace AD, Hodgson E. Human hepatic CYP2B6 developmental expression: the impact of age and genotype. Biochem Pharmacol 2009;78(2):184–90.
30. Gerber JG, Rhodes RJ, Gal J. Stereoselective metabolism of methadone N-demethylation by cytochrome P4502B6 and 2C19. Chirality 2004;16 (1):36–44.
31. Lam J, Koren G. P-glycoprotein in the developing human brain: a review of the effects of ontogeny on the safety of opioids in neonates. Ther Drug Monit 2014 Dec;36(6):699–705.
32. Delano K, Gareri J, Koren G. Rates of fetal polydrug exposures in methadone-maintained pregnancies from a high-risk population. PLoS ONE 2013;8(12):e82647.
33. Glatstein M, Finkelstein Y, Scolnik D. Accidental methadone ingestion in an infant: case report and review of the literature. Pediatr Emerg Care 2009;25(2):109–11.

Additional information and reprint requests:
Parvaz Madadi, Ph.D.
Centre of Forensic Sciences
Toxicology Section
25 Morton Shulman Avenue, 3rd Floor
Toronto, ON, Canada
M3M0B1
E-mail: parvaz.madadi@gmail.com