Resolving medicolegal issues using pharmacokinetic/pharmacodynamic simulations

Johan Coetzee

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There are more things in heaven and earth, Horatio,
Than are dreamt of in our philosophy.
- Hamlet (1.5.167-8)

Introduction:

The first report in the medical literature in which pharmacokinetic-pharmacodynamic (PKPD) simulations were used as an aid to resolve a medicolegal matter was by Lotsch and coworkers regarding an inquest [1]. The inquiry concerned a young adult female who following knee surgery, received repeated intravenous (iv) doses of morphine totaling 35 mg, during 1¾ hours in an attempt to control her pain in a post anesthetic recovery unit (PACU). Cardiorespiratory arrest occurred 40 minutes after the last dose dose. The delay in severe respiratory depression was shown to be mainly due to the slow transfer of morphine between plasma and the effect site. Morphine's hydrophyllic molecules are transferred slowly into the effect site and likewise are slow to exit. PKPD simulations demonstrated that when morphine plasma concentrations had already decreased to low values, the effect-site concentrations were approaching peak values. This confirms the statement by Rigg 22 years previously, that *"Plasma concentrations of morphine are not an objective indicator of pharmacological activity"* [2]. Lotsch et al. compared their simulated results with those from an earlier volunteer study of morphine respiratory depression [3] and demonstrated that the deceased patient's estimated effect-site concentrations at the time of her arrest were similar to those of the volunteers at the stage when they were observed to suffer severe respiratory depression. This concentration was estimated to be about 40 ng/ml (144 nM).

The most publicised case was Steven Shafer's testimony in the The trial of Conrad Murray concerning the death of Michael Jackson (People of the State of California v. Conrad Robert Murray). Prof Shafer demonstrated that neither the lorazepam or the propofol postmortem blood concentrations could have resulted from ingestion by the deceased; furthermore the propofol blood concentrations could only have been achieved by means of administering a large dose by intravenous infusion.

Case No. 1: Death post-tonsillectomy

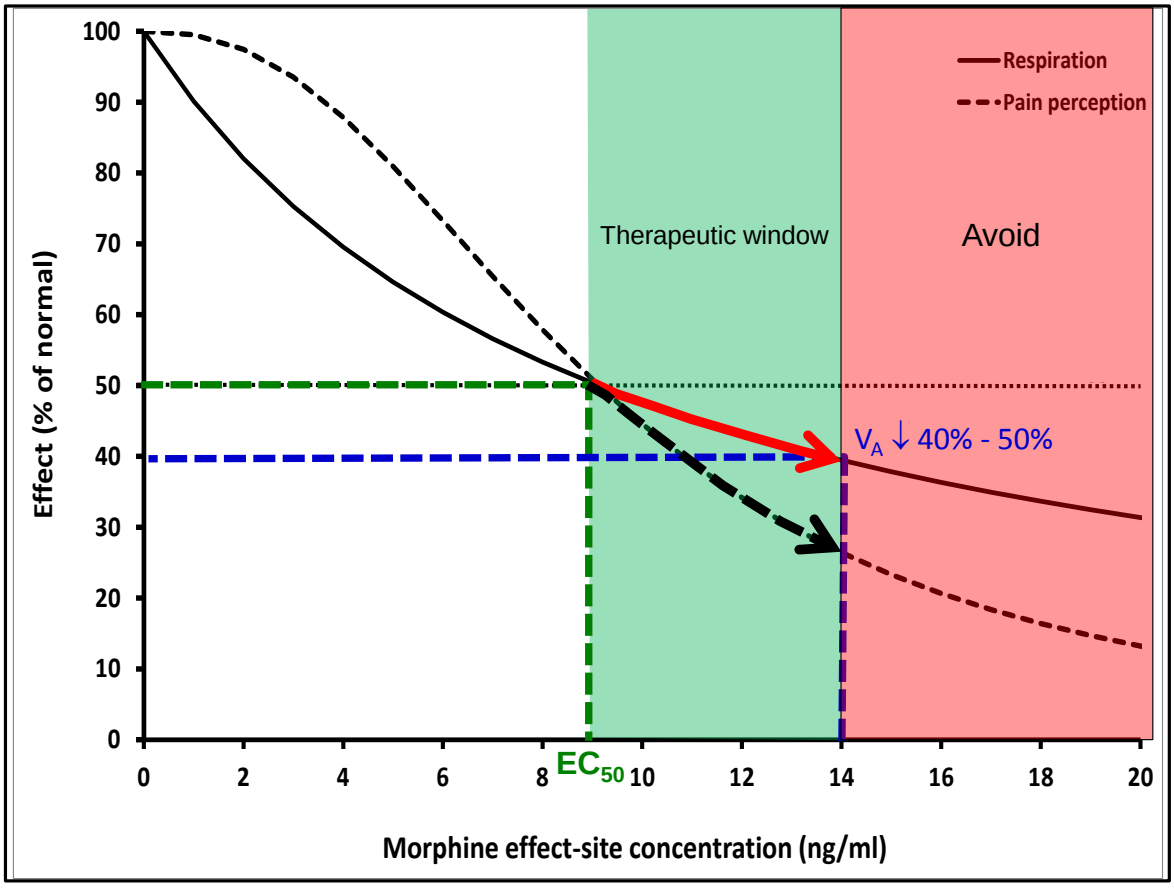
A healthy 26 year old, 59 kg female, underwent a tonsillectomy for a peritonsillar abcess. Anesthesia was unremarkable (drugs administered sufentanil 10mg, propofol 150 mg, desflurane in 50% N_2O, no muscle relaxant). Morphine 10 mg was administered iv shortly after induction of anesthesia. In the PACU one hour later she was awake, stable with a good airway and complained of pain. She received morphine 5 mg iv and was admitted to the ward 20 minutes later. Three hours later she was seen by the surgeon who noted "vitals stable, pain free". Two hours later (i.e. six hours after the first morphine dose), a fellow patient reported "She was breathing funny and now she is quiet". Postmortem examination revealed hypoxic cerebral damage. No morphine was detected in femoral blood.

A PKPD model for computing the respiratory and analgesic effects of morphine:

Dahan and coworkers [4] studied the PKPD of 16 healthy, young adult, opioid-naïve volunteers. They measured morphine's analgesic effects as well as respiratory responses to hypercapneic and hypoxic challenges. They demonstrated that measureable respiratory depression occurred even at morphine effect-site concentrations that produced little analgesia. Importantly they noted that depression of respiratory responses to 40% of control (i.e. by 60%) constituted a dangerous level of depression equivalent to an increase of end-tidal PCO_2 by 10-15 mmHg (1.5 – 2 kPa), and a reduction of minute volume by 40-50% during spontaneous breathing. At these levels of hypercapnia, sedation progressing to unconsciousness begins to supervene [5]. Furthermore they pointed out that sedation/sleep potentiates the respiratory depressant effects of morphine [6-8] and that a patient who falls asleep in the presence such a degree of respiratory depression, may stop breathing completely. Dose-response curves resulting from their pharmacodynamic model are insightful. In Figure 1 the solid line represents the relationship between morphine effect-site concentrations and their effect on the measured respiratory responses as a percentage of control values; the dotted line depicts the relationship between morphine and pain perception. The EC50 of both curves is the same (9 ng/ml). Depression of respiration to 40% of control occurs at about 14 ng/ml and this value can be regarded as a threshold above which the effects on respiration of a typical opioid-naïve young adult are severe. A "therapeutic window" of 9-14 ng/ml can therefore be defined: Effect-site concentrations of less than 9 ng/ml would result in less than 50% reduction of pain perception and concentrations greater than 14 ng/ml would produce unacceptable respiratory depression. It is important to note that the slope of the curve representing respiratory depression is steeper than the analgesic curve at "subtherapeutic" concentrations, but as

morphine concentrations increase to values greater than the EC_{50}, the steepness changes; i.e. within the "therapeutic window" the effects of morphine on pain are greater than the effects on respiration. Considering the findings of Lotsch et al. [3], apnoea is likely to ensue at effect-site concentrations about 40 ng/ml when respiratory responses are less than 20% of baseline. However patients may stop breathing at lower or higher concentrations, depending on (1) potentiating factors such as interactions with other drugs, sleep and airway obstruction (2) antagonising influences such as the presence of pain and opioid tolerance.

Figure 1: Morphine dose-response curve.



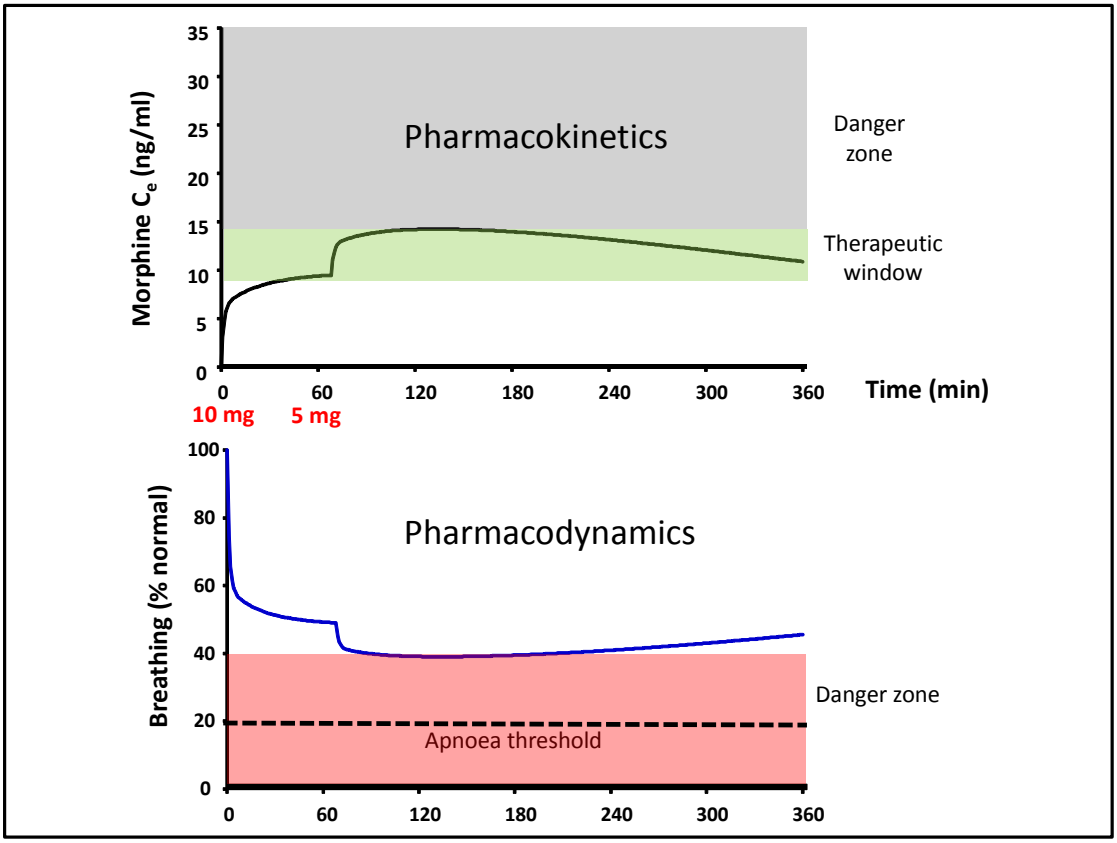
Dotted line plot: Effect of morphine on pain perception

Solid line plot: Effect of morphine on respiratory responses to hypercapneic and hypoxic challenges

V_A: alveolar ventilation

Figure 2, displays a PKPD simulation of the dosage regimen that the patient received, using the PKPD model of Dahan et al. [4] The effect-site concentrations reach the threshold for severe respiratory respiratory depression (40% of normal respiratory responses to the hypercapneic and hypoxic challenges).

Figure 2: Results of a PKPD simulation of the morphine dosage to a 59 kg person.



10mg 5mg: morphine doses administered 1 hour apart

Upper graph: Morphine effect-site concentrations (ng/ml) versus time.

Therapeutic window: Theoretical range of safe, effective concentrations for an opioid-naïve young adult.

Danger zone: Theoretical concentrations at which there may be dangerous degrees of respiratory depression.

Lower graph: Effect of morphine on breathing responses (expressed as a percentage of normal). The dotted line represents depression of respiratory responses from normal by 80%.

The question arises whether this degree of respiratory depression would have been sufficient to cause the patient's demise. Central depression of respiratory drive is not the sole mechanism by which opioid induced ventilatory impairment (OIVI) occurs [9]. OIVI results from three interrelated factors,:

1. Central depression of respiratory drive.
2. Sleep, sedation and the presence/absence of pain; i.e. alteration of level consciousness.
3. Atonia of pharyngeal and tongue musculature, predisposing to airway obstruction.

1. *Central depression of respiratory drive*

   It is a basic PKPD tenet that there is considerable between-subject variation in PKPD. Factors include:

   - Weight: this slightly-built patient received a large dose (0.25 mg/kg)
   - Gender: Women have been shown to be more sensitive to the analgesic effects [10] as well as to the respiratory effects [11, 12] of morphine.

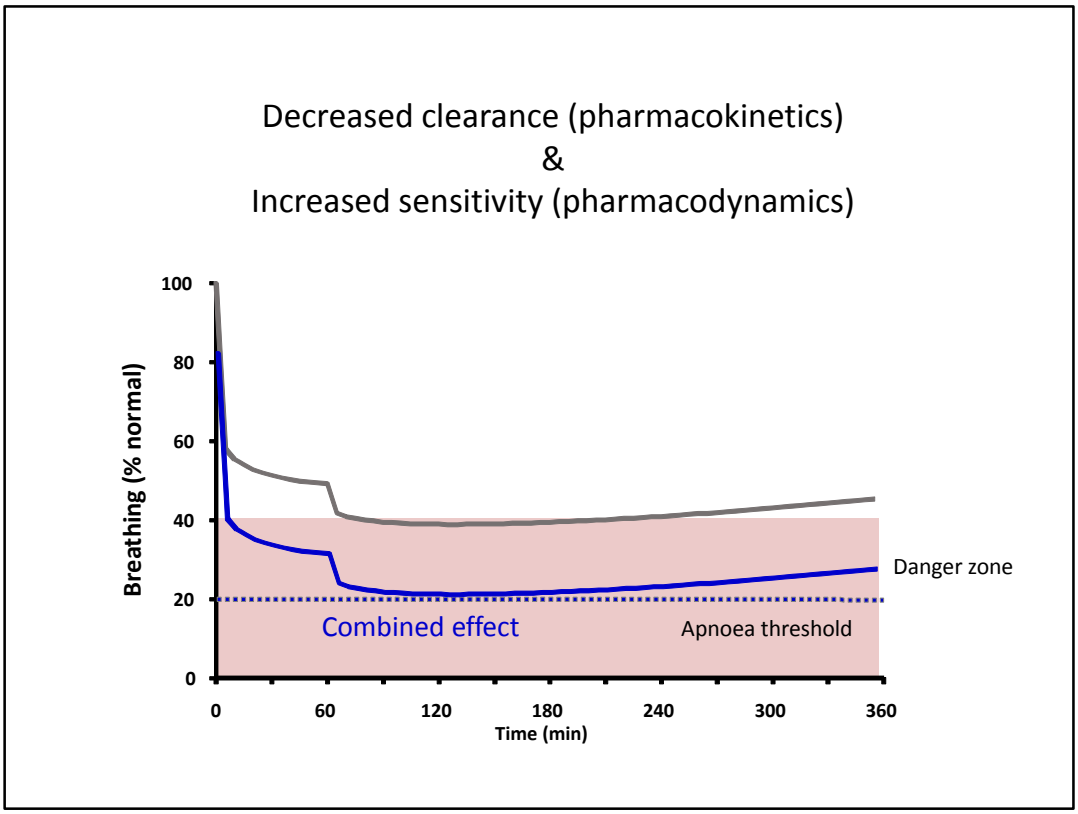
   Unknown factors with regard to the patient include:

   - Pharmacogenomics of metabolism: Allelic variants of the enzyme UGT2B7 are thought to be responsible for reduced rates of glucuronidation of morphine, resulting in accumulation after repeated doses. The evidence is conflicting however [13-16].
   - μ-Receptor polymorphisms: The genetic variant A118G of the μ1-receptor (OPRM1) results in decreased receptor binding and increases morphine requirements [17].

   Age was not a factor, nor were there any comorbidities or possible drug interactions.

   Figure 3 depicts the expected respiratory effect of the same dosage to a person with reduced clearance (lower 95% confidence limit in reference [4]) and increased sensitivity to morphine (lower 95% confidence limit of the EC_{50}). The result is markedly increased central depression.

Figure 3: The result of decreased clearance (pharmacokinetics) and increased sensititvity to morphine (pharmacodynamics) on respiratory responses



Upper plot (grey): Expected effects on respiration after morphine 10 mg iv followed by 5 mg one hour later, to a 59 kg young adult.

Lower plot (blue): The combined effects on respiratory responses due to a decrease of morphine clearance to the lower 95% confidence limit and increased sensitivity due to a shift of the EC50 to the upper 95% confidence limit.

2. *Sleep, sedation and the presence/absence of pain; i.e. depression of consciousness.*

   Falling asleep increases opioid-induced central respiratory depression [6, 7], as do interactions with sedative drugs (benzodiazepines, phenothiazines). It is well known that pain antagonises opioid-induced respiratory depression, but less well-known that pain relief unmasks latent depression [18-20].

3. *Airway obstruction:*

   Morphine has been shown to exert a direct influence on the hypoglossal motor nucleus, thereby reducing the tone of the genioglossus muscle, an important dilator of the upper airway [9, 21]. A recent human study [22] demonstrated that morphine 0.1 mg/kg resulted pharyngeal dysfunction and discoordinated breathing and swallowing.

It is significant that a fellow patient testified that the deceased "*was asleep and snoring"* and that "*she lay flat on her back*". Thus it is likely that several factors that contribute to OIVI played a role in the deceased's cardiorespiratory arrest, namely central depression from a large dose to a possibly sensitive, opioid-naïve, female patient, who on experiencing pain relief from her peritonsillar abcess, fell asleep in the supine position, tolerating subsequent airway obstruction. The resulting hypercapnea, hypoventilation and hypoxemia aggravated her depression of consciousness and a vicious circle ensued. Inquest judgement was that death was the result of a morphine overdose and that no person was culpable.

Case No. 2: Brain damage after shoulder surgery

A 32-year old male, weighing 87 kg (BMI 31), underwent shoulder surgery lasting 1½ hours in a semi-sitting position. Anesthetic drugs administered were propofol, rocuronium, remifentanil and desflurane. A brachial plexus block was performed using ropivicaine 300 mg. In addition, parecoxib 40 mg, paracetamol (acetaminophen) 1g and Cyclimorph® 15 mg (a mixture comprising morphine 15 mg & cyclizine 50 mg) were administered intra-operatively. Patient-controlled analgesia (PCA) was started in the PACU consisting of a 50 ml solution containing Cyclimorph® (morphine 1 mg/ml & cyclizine 3.3 mg/ml), to be administered using a background infusion of morphine 1 mg/hour plus demand bolusses of morphine 2 mg with a lockout interval of 6 minutes. The following occurrences were noted in the ward between 15h30 and 06h00 (Table 1).

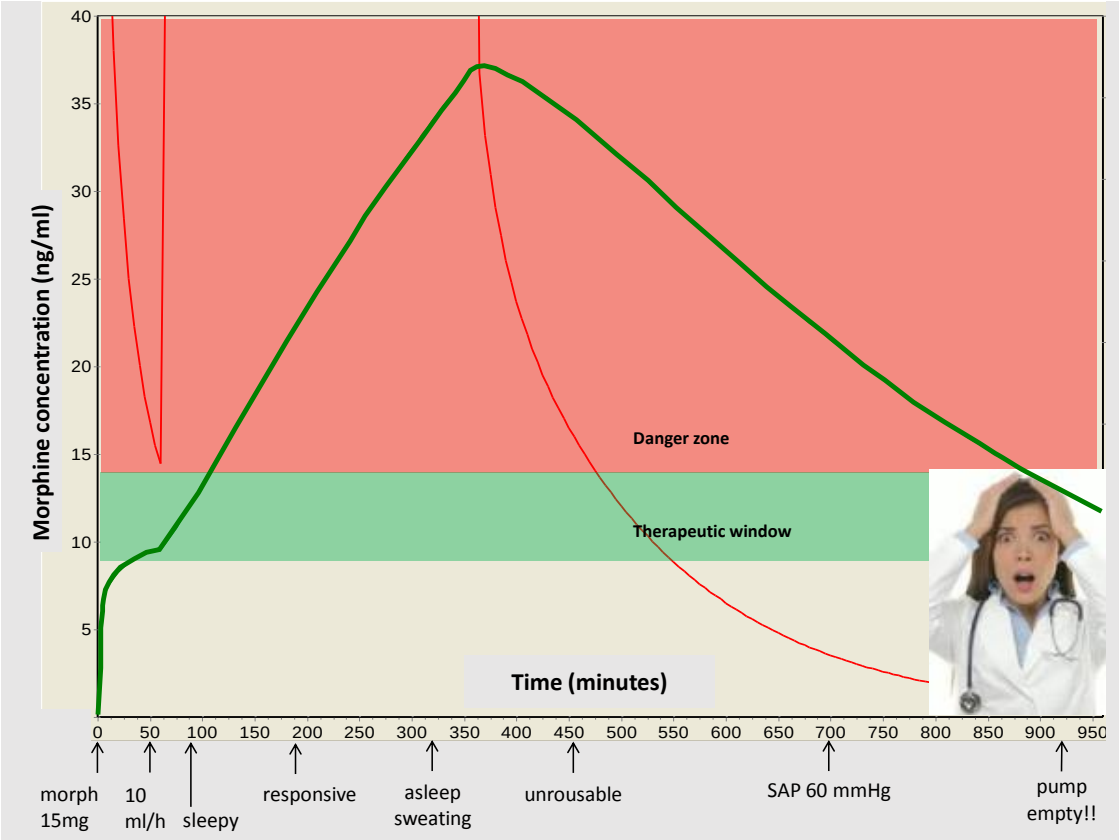
Table 1: Case No. 2; postoperative events.

Time	Patient condition
15h30	Coughed blood (±30ml)
16h00	Patient "sleepy"
17h30	Patient awake & responsive; Coughed blood again
20h00	Paitent asleep, perspiring, normal temperature
22h00	Patient unrousable
02h00	Patient asleep, systolic BP 60 mmHg
06H00	Patient unrousable, GCS 3/15, cyanotic, BP 69/48, pinpoint pupils

The patient was transferred to the intensive care unit (ICU) where it was discovered that the PCA syringe was empty. In spite of successful resuscitation, he suffered permanent brain damage to the extent that he needs care and supervision. Legal action was instituted.

Differential diagnoses for the cause of the brain damage included morphine overdose, cerebral hypoperfusion (possible hypotension in the sitting position) and collapse from a pulmonary thrombo-embolism (history of hemoptysis). Considering the rapid, unobserved emptying of the PCA syringe and the fact that a high concentration of opioids was detected in the urine, a diagnosis of drug overdose was considered to be the most likely. One of the commonest causes of adverse events during PCA are programming errors [23]. Figure 4 depicts the result of a typical programming error the result of which is an infusion rate of 10 mg/hour instead of the intended 1.0 mg/hour. This could occur if either the wrong background infusion rate was entered into the pump software (10 mg/h instead of 1.0 mg/hour) or the wrong concentration (0.1 mg/ml instead of 1.0 mg/hour).

Figure 4: Pharmacokinetic simulation of a PCA pump programming error.

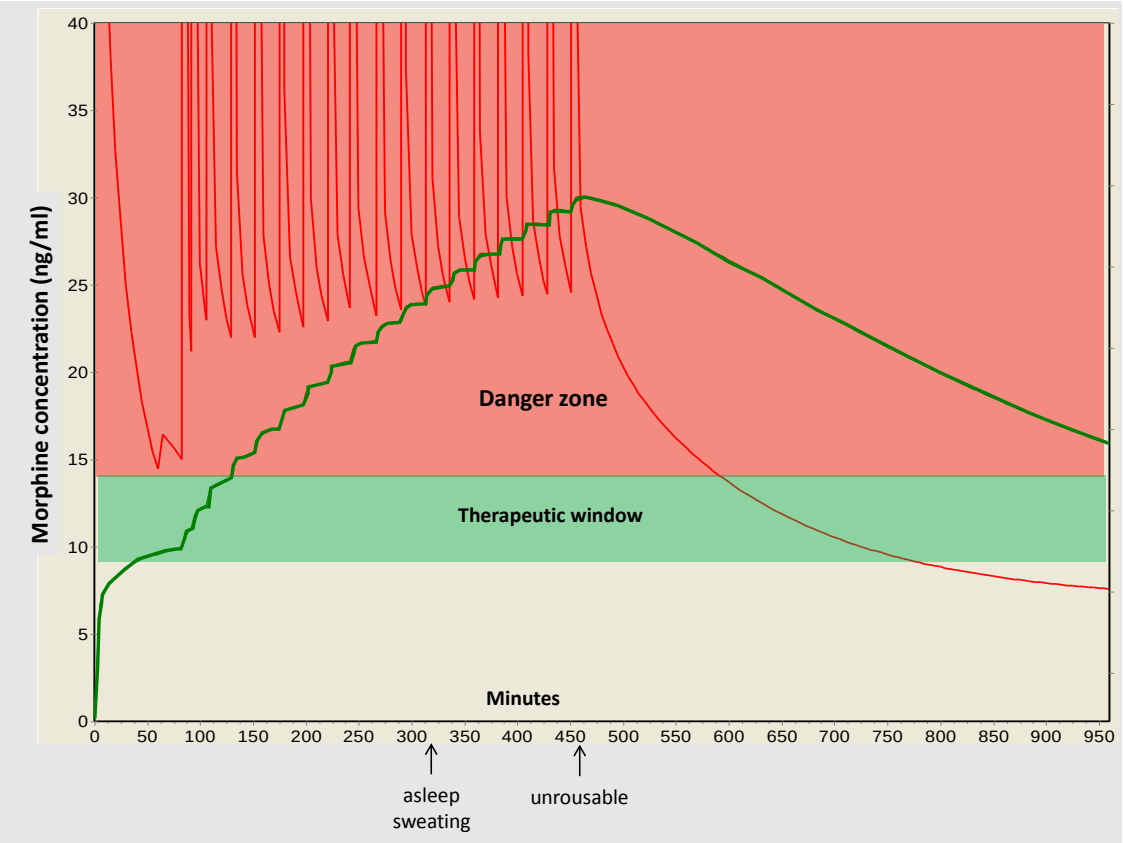


Green plot: morphine effect-site concentrations; Red plot: morphine plasma concentrations.

The graph illustrates how the morphine effect-site concentrations exceed the therapeutic window after two hours and progressively increase well into the "danger zone", reaching a peak value of 37 ng/ml after pump emptying. The sweating episode probably resulted from hypercapnia. The large dose of cyclizine (166 mg) undoubtedly contributed to the severe hypotension.

Another possibility is that the patient could have self-administered some of the 50 ml of Cyclimorph®. He could only have managed that while conscious, and this would have required 17 x 2mg doses during a period of 6½ hours in addition to the purpoted background infusion of 1 mg/hour. It is unkown if and when the patient self administered, however it is reasonable to perform a conservative estimate by evenly spacing the 2 mg bolusses at 23-minute intervals. The pharmcokinetic simulation in Figure 5, illustrates that similar to the previous scenario, morphine effect-site concentrations increase to a peak value of about 30 ng/ml, which coincides with the time at which the patient became unrousable.

Figure 5: Pharmacokinetic simulation of morphine 2 mg doses spaced at 23 minutes, superimposed upon a morphine background infusion of 1 mg/hour



Thus the most likely causes of the adverse event were a combination of excessive analgesia, unwisely chosen (Cyclimorph® 15 mg, brachial plexus block, PCA, parecoxib, paracetamol); compounded by a programming error and poor nursing during which multiple opportunities for rescuing the patient were missed. The claim was settled for a large sum.

Case No. 3: Brain damage after Cyclimorph® (again)!

A 50-year old patient, weighing 48 kg (BMI 20.2), underwent an hysterectomy under general anaesthesia. She received Cyclimorph at the following times (Table 2):

Table 2: The Cyclimorph® dosing schedule:

After induction of anaesthesia	Cyclimorph (morphine 15 mg) intravenously
2¼ hours later	Cyclimorph (morphine 5 mg) intravenously
2¼ hours later	Cyclimorph (morphine 10 mg) intramuscularly
4 hours later	Cyclimorph (morphine 15 mg) intramuscularly

Note: Each ampule of Cyclimorph® contains morphine 15 mg & cyclizine 50 mg.

An hour after the last dose she was noted to be "unconscious and breathing noisily" and 30 minutes later she suffered respiratory arrest, from which she was successfully resuscitated. However she subsquently developed partial deafness as well as cognitive and emotional impairment.

A simple solution to the problem of simulating extravascular absorption (intramuscular injection) was devised by calculating morphine's absorption rate constant, given the absorption half-time. If drug absorption into the bloodstream from an extravascular site occurs according to a first order process, the amount absorbed at any time after injection is given by the expression:

$$A_t = F.D.(1 - e^{-kt}) \qquad \textit{Equation (1)}$$

Where F = bio-availability; D = dose administered; e = base of the natural logarithm; k = the rate constant;

The relationship between the absorption halflife ($T_{½}$) and the rate constant (k) is given by the expression: $k = \frac{ln(2)}{T_{½}}$ *Equation (2)*

So that if the halflife is known, the rate constant can easily be calculated.

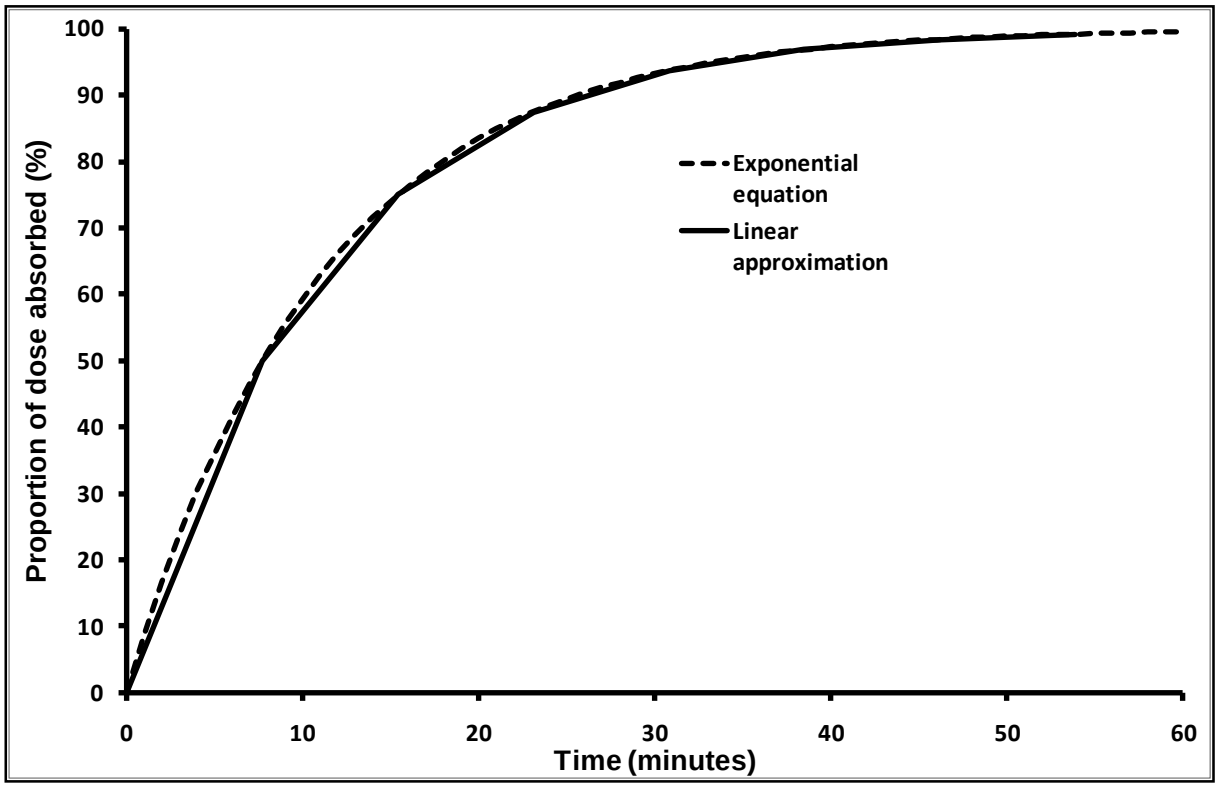
Stanski [24] in a study of the pharmacokinetics of intramuscularly administered morphine, demonstrated that the drug is fully absorbed from the injection site (F=1), that it occurs according to a first order process and that the mean absorption halflife is 7.7 minutes (standard deviation 1.6 min). The mean rate constant (k) is therefore 0.090019 min^{-1}. Inserting this value into equation (1), it is possible to approximate the diminishing rate of absorption by a series of stepped-down, zero-order intravenous infusions that are based on absorption halftimes: Table 3 depicts the approximations for an intramuscular dose of morphine 15 mg:

Table 3: A dosage scheme to approximate first-order absorption of morphine 15 mg by means of a series of zero-order intravenous infusions:

Time (min)	Halflives	Amount absorbed (%)	Infusion Rate (mg/h)
0	0	0.0	58.44
7.7	1	50.0	29.22
15.4	2	75.0	14.61
23.1	3	87.5	7.31
30.8	4	93.8	3.65
38.5	5	96.9	1.83
46.2	6	98.4	0.91
53.9	7	99.2	0.00

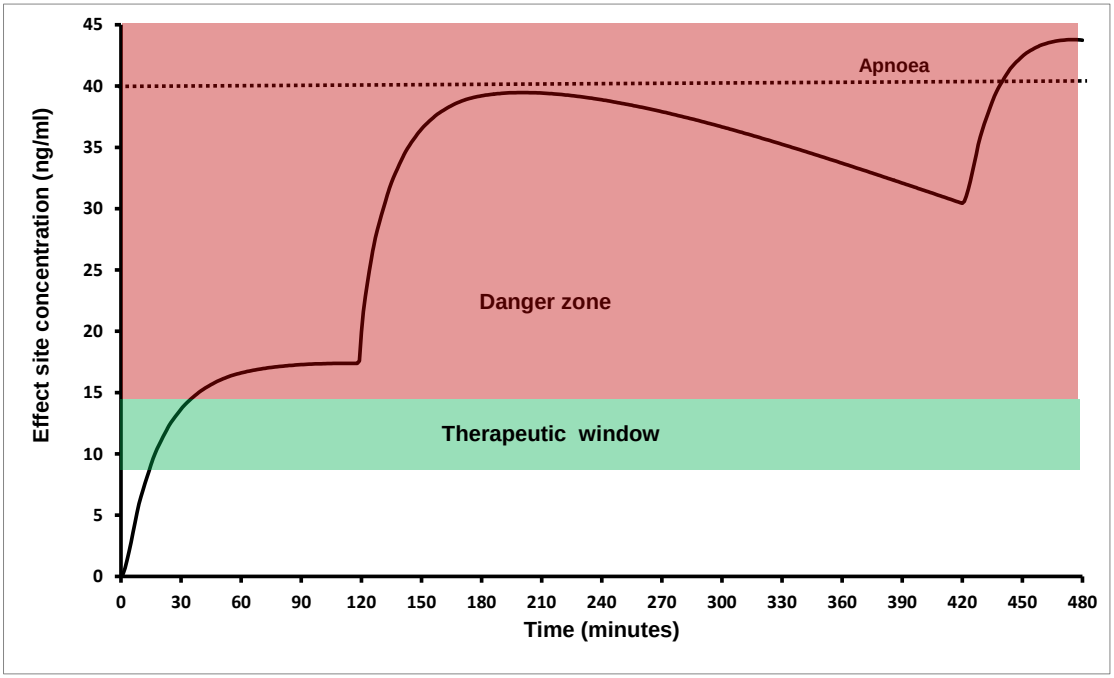
Figure 6 depicts the linear approximation. The error with regard to the total dose absorbed after 54 minutes is 0.7% (14.9 mg vs. 15.0 mg).

Figure 6: Approximation of an exponentially decreasing absorption rate by a series of stepped-down constant-rate intravenous infusions.



The result of a pharmacokinetic simulation of the dosage schedule to the 48 kg patient is portrayed in Figure 7. The graph shows how the expected effect-site concentrations enter the danger zone 15 minutes after the first intravenously administered Cyclimorph® injection and remain within the danger zone until the time of the second administration of Cyclimorph® in the recovery room 2 hours and 15 minutes later. The result of the second large dose was to drive the effect-site concentrations even higher into the zone where apnoea can be expected to ensue in the typical, opioid-naïve patient. The third intramuscular dose of Cyclimorph® 15 mg was administered while the morphine effect-site concentrations were still dangerously high, resulting in even greater morphine effect-site concentrations than before. Once again, it is likely that the accompanying cyclizine would have contributed to somnolnence and OIVI.

Figure 7: Simulation of morphine concentrations in the effect-site that resulted from the repeated injections of Cyclimorph® to a 48 kg patient.



The first morphine dose at time zero was 15 mg i.v. This was followed by a second morphine administration at 135 min consisting of 5 mg i.v. and 10 mg i.m. The third morphine dose of 15 mg was administered i.m. four hours after the second dose. The theoretic therapeutic window (green area) and danger zone for severe respiratory depression (red area) are indicated. Apnoea can be expected at about 40 ng/ml in the typical young, opioid-naïve adult.

Case No. 4: Death by methadone and diazepam?

This case concerns an inquest into the death of a 26 year old multi-drug female addict, weighing 89 kg who was admitted to a rehabilitation centre for initiatiion of methadone maintenance therapy. Drugs of addiction were heroin, diazepam (30 mg/day), cannabis, methamphetamine and cocaine. She was also taking the antidepressant, paroxetine 20 mg/day. On examination she appeared to be agitated and in withdrawal. Urine tested positively for cocaine, opiates and tetrahydrocannabiol. Oral methadone and diazepam therapy was begun on day-2. Table 4 presents the schedule of medications administered. An extract of the Aloe vera plant was prescribed twice daily for its laxative action.

The patient retired to bed at 21h00. At 04h30 a fellow patient observed that she was snoring heavily. At 07h30 a nurse found her to be unrousable and this was followed shortly afterwards

by cardiorespiratory arrest. Resuscitative attempts were unsuccessful and death was pronounced at 09h30.

Table 4: Schedule of medication

Day	Time	Medication
Day 2	08:00	Paroxetine 20 mg
	17:30	Methadone 20 mg, Diazepam 30 mg
Day 3	08:00	Methadone 20 mg, Diazepam 20 mg, Paroxetine 20 mg, Aloe vera
	12:00	Methadone 20 mg, Diazepam 20 mg
	20:00	Methadone 20 mg, Diazepam 20 mg; Aloe vera

In a blood sample obtained during CPR there were raised liver enzymes and albumin concentration 27 g/L. Postmortem examination revealed pulmonary aspiration of gastric contents and bronchopneumonia. There was fatty degeneration of the liver. Brain pathology was absent. Toxicology revealed the following drug concentrations (Table 5).

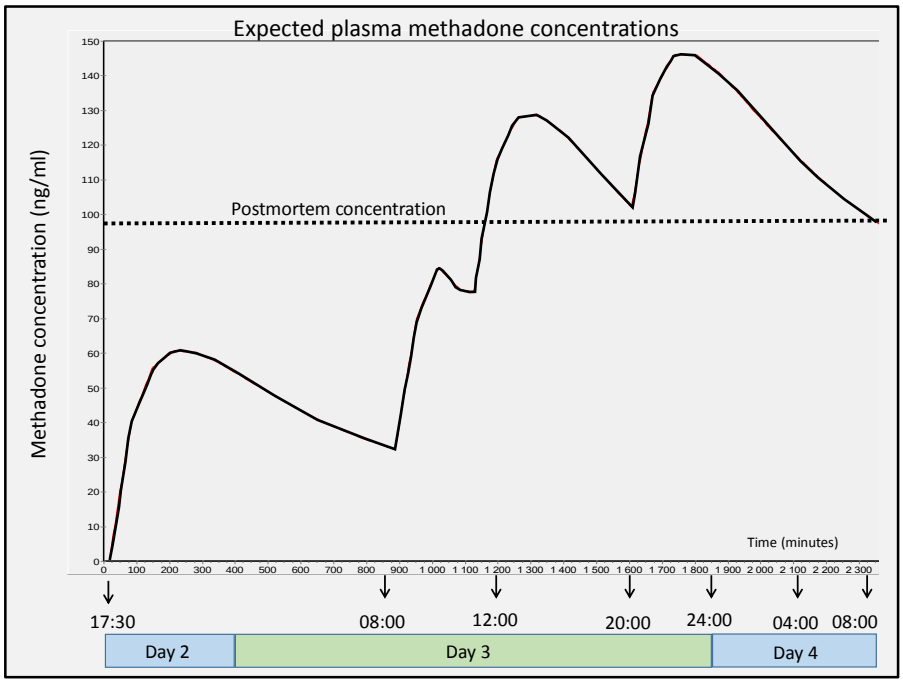
Table 5: Drug concentrations determined in postmortem samples.

Drug	Stomach	Femoral blood
Methadone (ng/ml)	278	97
Diazepam (ng/ml)	42	< 40

First impressions lead one to perhaps conclude that this is a clear-cut case of death due to methadone and midazolam overdose, especially when considering the following regarding methadone maintenance therapy [25-29]: Most methadone deaths occur during initiation of therapy, at night and are often associated with bronchopneumonia. Postmortem blood concentrations are generally high, but deaths have been recorded with concentrations as low as 60 ng/ml. Official guidelines by various bodies recommend *"Start low and go slow"*, i.e. initial methadone dosage should not exceed 30 mg/24 hours (she received 60 mg) and concomitant benzodiazepines should be avoided (she received diazepam 60 mg/24 hours).

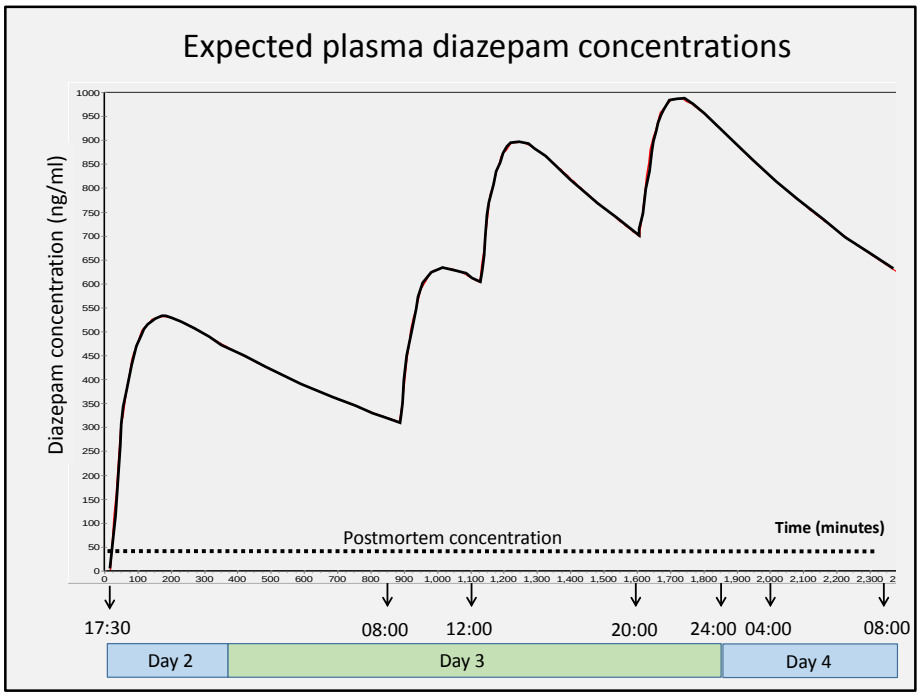
Pharmacokinetic simulations of extrahepatic absorption employing the pharmacokinetic parameter set of Wolff et al. for methadone in opiate users [30] and of Eatman et al. [31] for diazepam, revealed the following results (Figures 8 & 9).

Figure 8: Expected plasma methadone concentrations versus time resulting from the dosage regimen depicted in Table 5 to an 89 kg adult opiate user.



Methadone is slowly excreted, and accordingly the expected plasma concentrations increase progressively after each ingestion. The expected concentration at the time of cardiorespiratory arrest coincides with the value obtainied at postmortem.

Figure 9: Expected plasma diazepam concentrations versus time resulting from the dosage regimen depicted in Table 5 to an 89 kg adult.



Contemplating these results, reveals certain incongruencies. The postmortem concentrations of diazepam in both the stomach contents and in blood were at the lower limit of quantifiability (approximately 40 ngml), whereas the expected concentration at the time of death is greater than 600 ng/ml. Thus it appears that the patient did not ingest the 8 pm dose. With regard to the postmortem methadone concentrations, whereas the stomach content concentration was considerably greater than that in the blood, according to the simulation, the reverse should have been true. Thus it appears that the patient's circulation had become compromised before the 8 pm dose had been completely absorbed. That the simulated blood methadone concentration at the time of death coincided with the postmortem concentration is probably perchance.

Greater doubt regarding methadone-diazepam overdose as the cause of death, arises on perusal of the clinical notes. It was noted that during the previous day, the patient walked into the pharmacy to collect her medications. On each of the three occasions she was alert and orientated with normal gait, speech and pupils. These are pehaps indicators that she was opioid tolerant. Closer examination of the histology report revealed the following statement, *"No microscopic pathological or structural abnormality identified; specifically no ischaemic-hypoxic change, no oedema or inflammatory changes are observed".* These are not findings consistent with death resulting from OIVI.

During the attempted resuscitation a femoral blood sample revealed a blood glucose concentration of 1.6 mmol/L (28.8 mg/dL), a level low enough to induce coma per se. Repeated administrations of dextrose were required to bring the glucose concentration to normal levels. It is important to note that methadone and other opioids have been documented to cause hypoglycemia [32-36]. Furthermore Aloe vera extract is used in certain regions to treat diabetes [37, 38], apparently by means of stimulating insulin secretion. Thus a more likely explanation for the cause of death is that firstly there were low glycogen stores due an underlying chronic hepatic condition as indicated by the findings of fatty degeneration, raised liver enzymes and a low blood albumin concentration. Large doses of methadone resulted in hypoglycemia, which may have been aggravated by the actions of Aloe vera. Hypoglycemic coma was complicated by aspiration of stomach contents, bronchopneumonia and finally death.

Learning points:

1. PKPD simulations can help to explain adverse events. However it is imperative to keep an open mind and to interpret the results in the light of the clinical events.
2. It is possible to simulate extravascular drug absorption by means of a series of zero order intravenous infusions, given the absorption halftime.
3. There is a poor relationship between changing blood morphine concentrations and effect-site concentrations.
4. Opioids predispose to airway obstruction due to direct effects on genioglossus and pharyngeal muscle function.
5. Sleep, sedation and pain relief individually and combined, potentiate opioid induced ventilatory impairment.
6. These cases highlight nursing deficiencies in certain areas in South Africa that need to be addressed urgently.

References

1. Lotsch J, Dudziak R, Freynhagen R, Marschner J, Geisslinger G. Fatal respiratory depression after multiple intravenous morphine injections. ClinPharmacokinet. 2006; 45:1051-60.

2. Rigg JR. Ventilatory effects and plasma concentration of morphine in man. BrJ Anaesth. 1978; 50:759-65.

3. Lotsch J, Skarke C, Schneider A, Hummel T, Geisslinger G. The 5-hydroxytryptamine 4 receptor agonist mosapride does not antagonize morphine-induced respiratory depression. ClinPharmacolTher. 2005; 78:278-87.

4. Dahan A, Romberg R, Teppema L, Sarton E, Bijl H, Olofsen E. Simultaneous measurement and integrated analysis of analgesia and respiration after an intravenous morphine infusion. Anesthesiology. 2004; 101:1201-9.

5. Sieker HO, Hickam JB. Carbon dioxide intoxication: the clinical syndrome, its etiology and management with particular reference to the use of mechanical respirators. Medicine (Baltimore). 1956 Dec;35(4):389-423.

6. Catley DM, Thornton C, Jordan C, Lehane JR, Royston D, Jones JG. Pronounced, episodic oxygen desaturation in the postoperative period: its association with ventilatory pattern and analgesic regimen. Anesthesiology. 1985 Jul;63(1):20-8.

7. Forrest WH, Jr., Bellville JW. The effect of sleep plus morphine on the respiratory response to carbon dioxide. Anesthesiology. 1964 Mar-Apr;25:137-41.

8. Nieuwenhuijs DJ, Olofsen E, Romberg RR, Sarton E, Ward D, Engbers F, et al. Response surface modeling of remifentanil-propofol interaction on cardiorespiratory control and bispectral index. Anesthesiology. 2003 Feb;98(2):312-22.

9. Macintyre PE, Loadsman JA, Scott DA. Opioids, ventilation and acute pain management. Anaesth Intensive Care. 2011 Jul;39(4):545-58.

10. Sarton E, Olofsen E, Romberg R, den Hartigh J, Kest B, Nieuwenhuijs D, et al. Sex differences in morphine analgesia: an experimental study in healthy volunteers. Anesthesiology. 2000 Nov;93(5):1245-54.

11. Dahan A, Sarton E, Teppema L, Olievier C. Sex-related differences in the influence of morphine on ventilatory control in humans. Anesthesiology. 1998; 88:903-13.

12. Sarton E, Teppema L, Dahan A. Sex differences in morphine-induced ventilatory depression reside within the peripheral chemoreflex loop. Anesthesiology. 1999 May;90(5):1329-38.

13. Fujita K, Ando Y, Yamamoto W, Miya T, Endo H, Sunakawa Y, et al. Association of UGT2B7 and ABCB1 genotypes with morphine-induced adverse drug reactions in Japanese patients with cancer. Cancer Chemother Pharmacol. 2010 Jan;65(2):251-8.

14. Holthe M, Rakvag TN, Klepstad P, Idle JR, Kaasa S, Krokan HE, et al. Sequence variations in the UDP-glucuronosyltransferase 2B7 (UGT2B7) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients. Pharmacogenomics J. 2003;3(1):17-26.

15. Sadhasivam S, Krekels EH, Chidambaran V, Esslinger HR, Ngamprasertwong P, Zhang K, et al. Morphine clearance in children: does race or genetics matter? J Opioid Manag. 2012 Jul-Aug;8(4):217-26.

16. Sawyer MB, Innocenti F, Das S, Cheng C, Ramirez J, Pantle-Fisher FH, et al. A pharmacogenetic study of uridine diphosphate-glucuronosyltransferase 2B7 in patients receiving morphine. Clin Pharmacol Ther. 2003 Jun;73(6):566-74.

17. Chidambaran V, Mavi J, Esslinger H, Pilipenko V, Martin LJ, Zhang K, et al. Association of OPRM1 A118G variant with risk of morphine-induced respiratory depression following spine fusion in adolescents. Pharmacogenomics J. 2015 Jun;15(3):255-62.

18. Borgbjerg FM, Nielsen K, Franks J. Experimental pain stimulates respiration and attenuates morphine-induced respiratory depression: a controlled study in human volunteers. Pain. 1996; 64:123-8.

19. Hanks GW, Twycross RG, Lloyd JW. Unexpected complication of successful nerve block. Morphine induced respiratory depression precipitated by removal of severe pain. Anaesthesia. 1981 Jan;36(1):37-9.

20. Wells CJ, Lipton S, Lahuerta J. Respiratory depression after percutaneous cervical anterolateral cordotomy in patients on slow-release oral morphine. Lancet. 1984; 1:739-.

21. Hajiha M, DuBord MA, Liu H, Horner RL. Opioid receptor mechanisms at the hypoglossal motor pool and effects on tongue muscle activity in vivo. J Physiol. 2009 Jun 1;587(Pt 11):2677-92.

22. Hårdemark CA, Sundman E, Bodén K, Hedström H, Kuylenstierna R, Ekberg O, et al. Effects of morphine and midazolam on pharyngeal function, airway protection, and coordination of breathing and swallowing in healthy adults. Anesthesiology. 2015;122(6):1253-67.

23. Paul JE, Buckley N, McLean RF, Antoni K, Musson D, Kampf M, et al. Hamilton acute pain service safety study: using root cause analysis to reduce the incidence of adverse events. Anesthesiology. 2014 Jan;120(1):97-109.

24. Stanski DR, Greenblatt DJ, Lowenstein E. Kinetics of intravenous and intramuscular morphine. Clin Pharmacol Ther. 1978 Jul;24(1):52-9.

25. Chou R, Cruciani RA, Fiellin DA, Compton P, Farrar JT, Haigney MC, et al. Methadone safety: a clinical practice guideline from the American Pain Society and College on Problems of Drug Dependence, in collaboration with the Heart Rhythm Society. J Pain. 2014 Apr;15(4):321-37.

26. Leavitt SB. Methadone dosing & safety in the treatment of opioid addiction . Vol. 12. No. 2. 2003. Addiction Treatment Forum. 2003;12(2):1-8.

27. Weich L. South African Guidelines for the Management of Opioid Dependence. The South African Addiction Medicine Society; 2013.

28. Wolff K. Characterization of methadone overdose: clinical considerations and the scientific evidence. Ther Drug Monit. 2002 Aug;24(4):457-70.

29. WHO. Guidelines for the psychosocially assisted pharmacological treatment of opioid dependence. Geneva: World Health Organization; 2009.

30. Wolff K, Rostami-Hodjegan A, Shires S, Hay AW, Feely M, Calvert R, et al. The pharmacokinetics of methadone in healthy subjects and opiate users. Br J Clin Pharmacol. 1997 Oct;44(4):325-34.

31. Eatman FB, Colburn WA, Boxenbaum HG, Posmanter HN, Weinfeld RE, Ronfeld R, et al. Pharmacokinetics of diazepam following multiple-dose oral administration to healthy human subjects. J Pharmacokinet Biopharm. 1977 Oct;5(5):481-94.

32. Faskowitz AJ, Kramskiy VN, Pasternak GW. Methadone-induced hypoglycemia. Cell Mol Neurobiol. 2013 May;33(4):537-42.

33. Fung H, Cheung K, Lam S, Wong O, Leung K, Leung S. A case of unintentional methadone overdose followed by hypoglycaemia. Hong Kong Journal of Emergency Medicine. 2011;18(4):239.

34. Gjedsted J, Dall R. Severe hypoglycemia during methadone escalation in an 8-year-old child. Acta Anaesthesiol Scand. 2015 Jun 19(Published ahead of print June 2015).

35. Maingi S, Moryl N, Andrew F. Symptomatic hypoglycemia due to escalating doses of intravenous methadone. The Journal of Pain. 2008;9(4):37.

36. Moryl N, Pope J, Obbens E. Hypoglycemia during rapid methadone dose escalation. J Opioid Manag. 2013 Jan-Feb;9(1):29-34.

37. Ghannam N, Kingston M, Al-Meshaal IA, Tariq M, Parman NS, Woodhouse N. The antidiabetic activity of aloes: preliminary clinical and experimental observations. Horm Res. 1986;24(4):288-94.

38. Sahu PK, al. e. Therapeutic and Medicinal Uses of Aloe vera: A Review. Pharmacology and Pharmacy. 2013;4:599-610.