# Laboratory Investigations

# Reconstituted thiopentone retains its alkalinity without bacterial contamination for up to four weeks

Clinton L. Wong MD,* C. Brian Warriner FRCPC,*
James P. McCormack PharmD,† Alison M. Clarke FRCPC‡

*The manufacturers of thiopentone recommend that after reconstitution, it should be kept only for 24 hr to reduce the risk of contamination. However, there are no studies to support this practice and compliance with this recommendation has economic implications. The reasons for discarding a reconstituted bottle of thiopentone are related to concerns about chemical and physical (pH) stability, contamination with infectious agents, and contamination with cellular material. We studied the incidence of bacterial contamination and pH stability of thiopentone in clinical use, as well as the pH stability of thiopentone not in clinical use, and surveyed the eight hospitals affiliated with the University of British Columbia to determine their protocols for thiopental preparation and storage. Cost comparisons were made between our current practice of discarding thiopentone when depleted and the practice of routinely discarding it 24 hr after reconstitution. Samples of thiopentone in clinical use were cultured daily and the pH was measured. The bottles had been in clinical use from 1 to 25 days (mean 4.23 ± 4.32 SD). Of 106 samples there were no positive bacteriological cultures and there were only minor changes in pH. The telephone survey of the eight hospitals revealed that only one had a policy to discard thiopentone after 24 hr. Cost comparisons indicate that discarding thiopentone 24 hr after reconstitution would result in increased cost. In conclusion, reconstituted thiopentone retains its alkalinity for up to four weeks, and has an acceptably low risk of bacterial contamination for periods beyond 24 hr, therefore thiopentone need not be discarded after 24 hr.*

*Afin de diminuer le risque de contamination du thiopental, le fabricant recommande de jeter le produit 24 h après dilution pour utilization clinique. Cependant, une telle pratique est coûteuse et sans fondement objectif. Les raisons en faveur de la recommandation sont l'instabilité chimique et physique (pH) du médicament, ainsi que sa contamination avec des agents infectieux ou d'autres particules organiques. Nous avons déterminé l'incidence de contamination bactérienne et la stabilité du pH de solution de thiopental utilisée en clinique, ainsi que la stabilité du pH de thiopental non utilisé en clinique. De plus, nous avons évalué les protocoles de préparation et de conservation de thiopental dans huit centres hospitaliers affiliés à l'Université de Columbie Britannique. Les coûts associés à la pratique recommandée par le fabricant ont été comparés à ceux de notre pratique actuelle qui consiste à utiliser les flacons de thiopental jusqu'à ce qu'ils soient vides. Des échantillons de thiopental disposible en clinique étaient mis en culture à chaque jour et leur pH était mesuré. Les solutions utilisées dataient de 1 à 25 jours (moyenne de 4,23 ± 4,32 jours). Sur 106 échantillons, il n'y a eu aucune croissance bactérienne et seuls des changements mineurs de pH ont été observés. L'evaluation des huit centres hospitaliers a rélévé qu'un seul centre suivait la recommandation du fabricant. L'étude de coût a indiqué que la pratique de jeter le thiopental 24 h après sa dilution entraînait de coûts supplémentaires. En conclusion, le thiopental dilué maintient son alcalinité pendant au moins quatre semaines, avec des risques minimes de contamination bactérienne après 24 h. Il*

**Key words**
ANAESTHETICS, INTRAVENOUS: thiopentone;
COMPLICATIONS: infections.

From the departments of Anaesthesia,* Microbiology‡ and Pharmacy† at St. Paul's Hospital, University of British Columbia, Vancouver, B.C., Canada V6Z 1Y6.

*Address correspondence to:* Dr. C.B. Warriner, Department of Anaesthesia, St. Paul's Hospital, 1081 Burrard St., Vancouver, B.C. Canada V6Z 1Y6.

*Accepted for publication 1st January, 1992.*

*n'est donc pas nécessaire de jeter le thiopental 24 h après dilution.*

Reconstituted solutions of thiopentone "... should be freshly prepared and used promptly. Reconstituted solutions ... may be kept, tightly stoppered, under refrigeration up to 24 hours – unused portions should be discarded after 24 hours," according to the product monograph supplied by Abbott Laboratories, Montreal, Canada. However, at many institutions, including this hospital, these guidelines are not followed. A study was undertaken to evaluate the effect of storage conditions for thiopentone on pH and bacteriological contamination. A small survey was made of the institutions affiliated with the University of British Columbia with regard to these guidelines. The cost of discarding thiopentone after 24 hr was calculated.

## Methods

*Part A: pH stability of thiopentone*

Four solutions of thiopentone (Pentothal Kit, Abbott Laboratories, five grams in 200 ml of sterile water for a 2.5% solution) were prepared from the same lot number and with the same date of expiration. Bottle A was kept at room temperature with its dispensing Luer fitment tightly capped. Bottle B was also kept at room temperature, but both the cap and the air-inlet filter were removed. Bottles C and D were kept in the refrigerator at all times, but the top of bottle C was tightly capped and bottle D was uncapped and the air-inlet filter was removed. All four solutions were kept away from patient care areas and were not in clinical use.

Ten mL samples were obtained immediately after reconstitution and then daily for three days and then three times weekly for a total of four weeks. These were analysed by our hospital chemistry laboratory on a Radiometer PHM 71 pH meter, with a scale from pH 1.0 to 14.0 calibrated with standard buffer solutions from 7.0 to 10.0 before and after each batch of measurements, and accurate to ±0.1 pH units.

*Part B: Bacterial contamination of thiopentone*

Ten mL samples were taken daily from reconstituted thiopentone bottles which were in clinical use. The anaesthetists were not informed of the collection. All reconstituted thiopentone bottles were stored at room temperature on top of the anaesthetic cart. There is no formal policy as to how long thiopentone is kept before discarding; usually the bottle is discarded when empty. Samples were obtained in the morning before surgery began. Note was made of the date the thiopentone bottle was reconstituted (or if undated, an inconspicuous mark was made on the label to help to identify the bottle in subsequent samplings), the date sampled, whether a cap was present, and whether precipitate was grossly visible.

Samples were sent to the microbiology lab on the morning of collection and were filtered through a 0.45 μ filter. The filter was then cultured on a blood agar plate culture in ambient air for five days. A 1 mL portion of the sample was also assayed for pH as in part A.

*Part C: Survey*

The seven other teaching hospitals affiliated with the University of British Columbia (UBC) were surveyed to determine how long reconstituted thiopentone solutions were stored and whether they were refrigerated.

*Part D: Cost comparisons*

The cost of discarding unused portions of thiopentone was calculated. Cost comparisons were made between the five methods for dispensing thiopentone.

For data from part A, a repeated measures analysis of variance (ANOVA) was performed to test for differences among the four bottles and differences over time. When statistically significant differences were found, Newman-Keul's tests were used to determine more precisely where the differences existed. For part B statistical analysis involved obtaining the 95% confidence interval for a binomial distribution. Values expressed are mean ± standard deviation.

## Results

*Part A: pH stability of thiopentone*

Thiopentone in bottle A had an initial pH of 11.1 and after 29 days, its pH was 10.9. Thiopentone in bottle B had an initial pH of 11.1 and this declined to 10.8. Thiopentone in bottles C and D both started at a pH of 11.2 and declined to 11.0. The lowest pH recorded was in bottle B on day 22 when it measured 10.7. With the times combined, the mean pH of bottle B (10.97 ± 0.17 s.d.) was less than that of bottle C (11.05 ± 0.15 s.d.) and of bottle D (11.03 ± 0.14 s.d.) ($P < 0.01$). However, these differences are small and are within the laboratory error of 0.1 pH units. With the bottles combined, the pH decreased slowly over time, and was different from control values from day 13 and beyond ($P = 0.0001$). Although it reached statistical significance, the magnitude of the difference was small; the mean pH on day 0 was 11.15 ± 0.06 while the lowest mean pH, which occurred on day 22, was 10.85 ± 0.10.

*Part B: Bacterial contamination of thiopentone*

A total of 106 samples was obtained. No positive bacteriological cultures were found (95% confidence interval 0% to 3%). The bottles had been in clinical use from 1 to 25

TABLE I Survey of anaesthesia departments

| *Hospital* | *Storage temperature* | *Routine discard* |
|---|---|---|
| A | Room temp. | No routine |
| B | Room temp. | One week |
| C | Room temp. | No routine |
| D | Refrigerated | Six days |
| E | Refrigerated | 24 hr |
| F | Room temp. | Depleted in 10 days or less |
| G | Room temp. | One week |
| H | Refrigerated | Until depleted or expiry date |

days (mean 4.23 ± 4.32). The majority of bottles (92%) were depleted within eight days of reconstitution. Fourteen percent of the bottles were undated and 16% were uncapped. None of the bottles showed any sign of visual cloudiness or precipitation. The pH ranged from 10.7 to 11.1.

*Part C: Survey*

The telephone survey revealed that of the eight hospitals, three stored their reconstituted solutions of thiopentone in the refrigerator, and only one had a policy to discard unused portions after 24 hr (Table I).

*Part D: Cost comparisons*

(Table II).

TABLE II Cost of thiopentone

| *Option* | *Unit cost*[a] | *Annual cost* |
|---|---|---|
| 1 5 g bottle multiple-dose used beyond 24 hr until empty (Abbott Laboratories) | $24.00 | $18,000.00[b] |
| 2 5 g bottle multiple-dose discarded after 24 hr. (Abbott Laboratories) | $24.00 | $64,896.00[c] |
| 3 500 mg. vial single-dose[d] (Abbott Laboratories) | $7.25 | $87,000.00[e] |
| 4 1000 mg. vial double-dose[d] (Abbott Laboratories) | $10.35 | $62,100.00[e] |
| 5 500 mg syringe single-dose[d] (IMS)[f] | $7.70 | $92,400.00[e] |

Notes: (a) All costs effective June 1991, in Canadian dollars; (b) Based on current use of 750 bottles annually; (c) Assuming ten operating rooms on weekdays and one on weekends; (d) Assuming drawing up 500 mg for an adult patient; (e) Based on 12,000 cases per year; (f) International Medication Systems.

## Discussion

This study demonstrated that none of the 106 samples of reconstituted thiopentone in clinical use developed bacteriological contamination over the study period. Thiopentone became slightly less alkaline over time, which became statistically significant after 13 days, and storage without a cap at room temperature resulted in lower pH than refrigerated storage with a cap. However, the magnitude of these differences was small and is not clinically important.

The reasons for discarding a reconstituted bottle of thiopentone can be classified according to concerns about (1) chemical and physical stability (i.e., potency and pH), (2) contamination with infectious agents including viruses, bacteria and their endotoxins (pyrogens), and (3) contamination with cellular material (red and white blood cells, epithelial cells, etc.).

The powder form of thiopentone is stable at room temperature indefinitely.[1] According to Stoelting's review,[2] reconstituted solutions of barbiturates remain stable at room temperature for up to two weeks. The solution is buffered in $NaCO_3$ 6% to maintain a pH of 10.5–11.0,[3] since thiopentone is insoluble unless in an alkaline solution with a pH >9.90.[4] The lowest pH observed in our study was 10.7. The stability depends on the storage conditions. Jones *et al.*[3] found that after reconstitution, thiopentone 2.5% showed visible precipitate due to loss of alkalinity when stored at 25° C for 60 days. At 5° C this loss of alkalinity occurred after 117 days. Our data on the pH stability is consistent with Jones's data. Exposure to atmospheric carbon dioxide with formation of carbonic acid could make the solution less alkaline, which might be more apparent in an uncapped bottle.[5] Our study showed that solutions in capped and uncapped bottles retained their alkalinity, whether or not they were refrigerated.

Chemical stability, as measured by potency, has been reported in several papers from the 1940's. Most found no loss of potency up to 16 days, although one author found that ten-day-old solutions produced slightly longer anaesthetic induction times.[5–8] More recently, Jones *et al.*[3] found that thiopentone retained 95% of its potency for four to seven days when stored at 25° C., and refrigerated solutions maintained their potency at more than 95% for at least 100 days.

The concerns about microbial contamination have resulted in Abbott Laboratories stating that since there is no added bacteriostatic agent, unused portions of thiopentone should be discarded after 24 hr. The source of this recommendation is unclear, but may be related to the Centres for Disease Control's suggestion that injectable medications in multiple-dose vials (MDVs) be discarded within 24 hr of opening, although there were no formal

guidelines.[9] Also, the US Pharmacopoeia gives a 24 hr limit after opening multiple-dose vials.[10]

Surprisingly, the literature on bacteriological contamination of thiopentone in clinical use is sparse. The studies, dating from the 1940's and 1950's, were based on small sample sizes ranging from one to five samples only.[5–7,11] None showed any bacterial contamination, but definitive conclusions should not be based on such small sample sizes. Our study is the first to utilize a larger sample size of thiopentone in clinical use.

Data exist on purposely innoculated solutions of thiopentone and assessment of subsequent cultures over time.[5,7] Young *et al.*[11] in 1958 innoculated 100,000 organisms of *Staphylococcus aureus* into a solution of thiopentone. Cultures showed 51% survival at 30 min, 10.7% survival at two hours, and 0% at 24 hr. In 1982, Highsmith *et al.*[12] innoculated various MDVs, including thiopentone, with 13 different pathogens that are common causes of nosocomial infections. After innoculation, samples were obtained at 24, 48, 72, 96 and 168 hr. Only *Enterococcus* and *Candida* were able to survive briefly in thiopentone and did not survive beyond 24 hr and 48 hr respectively. The other 11 species died within 24 hr. In contrast, nine of these 13 species were still present after 168 hr in lidocaine, consistent with other reports.[13]

The rate of contamination that justifies discarding a MDV after a fixed time is a matter of judgement.[20] An incidence of contamination ranging from 0 to 27% has been reported for other drugs in MDVs.[9] Since our Pharmacy Department uses 3–4% as their upper acceptable limit of contamination for intravenous admixtures,[14,15] we adopted this figure for our study. We found none of the 105 samples were contaminated, giving a 95% confidence interval of 0 to 3%. This occurred despite long storage times (up to 25 days), uncapped bottles (16.2%), undated bottles (14.3%), and lack of refrigeration.

Most pathogenic bacteria prefer a narrow pH range of 6.0 to 8.0, and strong alkalis exert marked bactericidal effects.[16–19] If lack of contamination can be correlated to the highly alkaline pH of thiopentone,[2] time should not be a concern with respect to risk of infection since the pH is stable for 60 days even at room temperature, and our study showed that even uncapped bottles maintained their pH for at least four weeks. The innoculation studies, which showed a declining bacterial count over time, suggested that a policy of discarding after 24 hr does not make microbiological sense. Thiopentone has been in clinical use for five decades. There are no reports of clinical infection arising from contaminated bottles of thiopentone.

The pH stability was assessed in thiopentone from one manufacturer, and may not neccessarily apply to thiopentone from others, although we have no reason to believe there would be any differences. Anaerobic bacteria were not tested for in this study since thiopentone is kept in room air. We did not assess endotoxins or pyrogens, which could persist in the absence of viable bacteria. There has been one study that addressed this issue in some MDVs, but did not look at thiopentone MDVs.[12] We also did not address the issue of viral contamination. This issue is highlighted by the finding that haemoglobin has been found in the intravenous injection port closest to the patient,[21] and that reusing syringes and refilling of used syringes from MDVs is common practice amongst anaesthesia personnel.[22] Also, viral infection of patients has occurred with other anaesthetic MDVs.[23] There is a potential risk for contamination of thiopentone solutions. However, viruses are usually stable between pH 5.0 to 9.0, and all viruses are destroyed by alkaline conditions.[17] Thus it is unlikely that solutions of thiopentone could become contaminated by viruses.

Innoculation studies indicate that the risk of thiopentone contamination is low, while that of lidocaine is higher. Future studies should focus on other high-risk MDVs, followed by bacteriological sampling of such MDVs in clinical settings. These studies would help to establish objective guidelines for storage and handling of multiple-dose vials used in anaesthesia.

In conclusion, reconstituted thiopentone maintains its alkaline pH under various clinical settings for at least four weeks. This maintains its physical stability in solution, and may play a role in its ability to remain free of contamination from infectious agents. There is an acceptably low risk of bacterial contamination with our current practice for handling thiopentone, including storage at room temperature for periods up to 25 days.

Abbott Laboratories' recommendation that thiopentone be discarded within 24 hr of reconstitution is not supported by our study based on bacteriological data; it is costly, and was disregarded by seven of the eight affiliated institutions.

**Acknowledgements**

Special thanks to Dr. C.K. Harris, Ph.D., Division of Clinical Chemistry for performing the pH assays, to Ms. Pamela Merrick, B.S.N., Department of Anaesthesia, University of British Columbia, for the statistical analysis, and to Ms. Colleen McGimpsey, R.T., Division of Medical Microbiology for processing the microbiology samples.

**References**

1 *Parker EA.* Compatibility digest. Am J Hosp Pharm 1969; 26: 653–5.

2 *Stoelting RK.* Pharmacology and Physiology in Anesthetic Practice. Philadelphia: JB Lippincott, 1987: 102.

3 *Jones RW, Stanko GL, Gross HM.* Pharmaceutical compatibilities of pentothal and nembutal. Am J Hosp Pharm 1961; 18: 700–4.
4 *Morton WD, Lerman J.* The effect of pancuronium on the solubility of aqueous thiopentone. Can J Anaesth 1987; 34: 87–9.
5 *Mallinson FB.* Apparatus for preparing pentothal in bulk. Lancet 1944; 2: 473–4.
6 *Stevens EJ.* Pentothal sodium: its use in continuous intravenous anesthesia and a method of preserving it in solution. Anesthesiology 1945; 6: 378–80.
7 *Anderson BM.* Letter. Anesthesiology 1944; 5: 87–8.
8 *Robinson MH.* Deterioration of solutions of pentothal sodium. Anesthesiology 1947; 8: 166–75.
9 *Longfield R, Longfield J, Smith LP, Hyams KC, Strohmer, ME.* Multidose medication vial, sterility: an in-use study and a review of the literature. Infection Control 1984; 5: 165–9.
10 *Heller WM.* Time limits on the use of opened multiple-dose vials (letter). Am J Hosp Pharm 1980; 37: 1610.
11 *Young J, Collete TS, Brehm WF.* Sterility of multiple-dose vials after repeated use. American Surgeon 1958; 24: 811–4.
12 *Highsmith AK, Greenhood GP, Alien JR.* Growth of nosocomial pathogens in multiple-dose parenteral medication vials. J Clin Microbiol 1982; 15: 1024–8.
13 *Langille DB.* Contamination of multiple-dose vials due to repeat use of syringes. Can Med Assoc J 1989; 140: 529–31.
14 *Ravin R, Bahr J, Luscomb F, Gooch J, Mutter S, Spittell SD.* Program for bacterial surveillance of intravenous admixtures. Am J Hosp Pharm 1974; 31: 340–7.
15 *Hanson AL, Shelley RM.* Monitoring contamination levels of in-use intravenous solutions using "total sample" techniques. Am J Hosp Pharm 1974; 31: 733–5.
16 *Volk WA, Benjamin DC, Kadner RJ, Parsons JT.* Essentials of Medical Microbiology. 3rd ed., Philadelphia: JB Lippincott, 1986: 267.
17 *Jawetz E, Melnick JL, Adelberg EA, Brooks GF, Butel JS, Ornston LN.* Medical Microbiology. 18th ed., Norwalk Conneticut/San Mateo California: Appleton & Lange, 1989: 51, 363.
18 *Willett HP.* Physiology of bacterial growth. *In*: Joklik WK, Willett HP, Amos DB (Eds.). Zinsser Microbiology, 18th ed., Norwalk Conneticut: Appleton-Century Crofts, 1984: 69.
19 *Wolin MJ, Miller TL.* Bacterial anatomy, physiology and genetics. *In*: Freeman BA (Ed.). Burrows Textbook of Microbiology, 22nd ed., Philadelphia: WB Saunders, 1985: 134.
20 *Bawden JC, Jacobson JA, Jackson JC, Anderson RK, Burke JP.* Sterility and use patterns of multiple-dose vials. Am J Hosp Pharm 1982; 39: 294–7.
21 *Trepanier CA, Lessard MR, Brochu JG, Denault PH.* Risk of cross-infection related to the multiple use of disposable syringes. Can J Anaesth 1990; 37: 156–9.
22 *Kempen PM, Learned DW.* Anesthesia practice – a vector of infection? Anesthesiology 1989; 71: A948.
23 *Alter MJ, Ahtone J, Maynard JE.* Hepatitis B virus transmission associated with a multiple-dose vial in a hemodialysis unit. Ann Intern Med 1983; 99: 330–3.