

Halothane Exposure and Serum Bromide Measurements for Healthcare Professionals Working in Surgical Theatres in Jordanian Hospitals

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Abstract

Background Aims: To evaluate and measure serum bromide levels for health care professionals working in operation rooms upon exposure to Halothane anesthetic in attempt to support worldwide calls to eradicate halothane from the use as a general anesthetic before surgical operations.

Materials and Methods: This is a quantitative descriptive study which used the Inductively-coupled plasma-mass spectroscopy (ICP-MASS) to investigate serum bromide levels in blood samples of fifty two healthcare professionals working under Halothane atmosphere inside operation rooms.

Results: This study revealed 3-10 times higher levels of serum bromide serum than the allowed non toxic levels (3-4.4 mg/l). The measured concentration ranges were 10-39.9 mg/l.

Conclusions: The observed high level of serum bromide strongly suggested an urgent call to exclude Halothane from the use as a general anesthetic in surgical operation. Meanwhile, serum bromide levels for all workers exposed to Halothane must be frequently monitored to predict the level of toxicity.

Keywords: Halothane, ICP-MASS, Serum Bromide, Surgical Theater, nurse, anesthesia, Jordan.

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Introduction

Inhalational halogenated anaesthetic agents were first introduced early in the twentieth century as the main general anaesthetics used before major surgical operations; they have replaced diethyl ether because of high flammability especially in presence of rich oxygen atmosphere. Halothane, enflurane,

isoflourane, desoflurane and sevoflourane among the mostly used halogenated hydrocarbons used (Figure 1).

The main advantages for these agents over diethyl ether are the ease of use and low flammability as well as the rapid onset of action that made these agents the mainstay of general anesthesia especially the halogenated ether; Sevoflurane and isoflourane.

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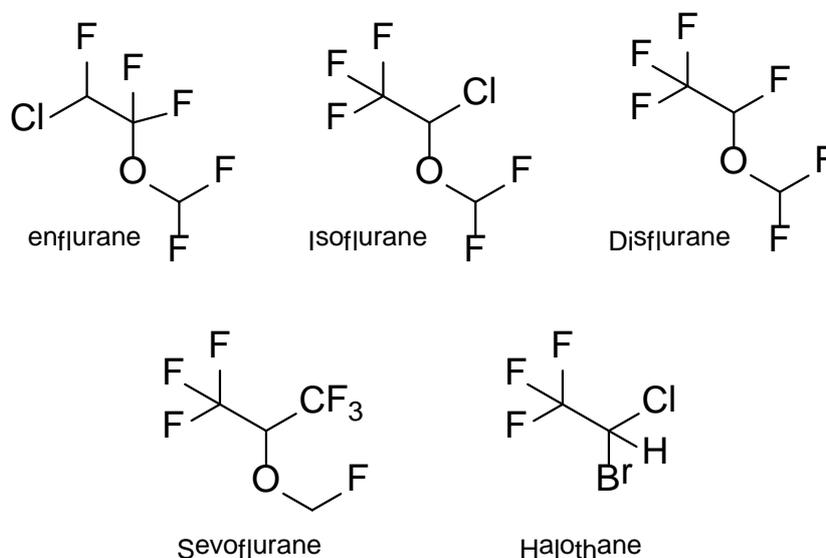


Figure 1: The chemical structure for commonly used halogenated anesthetics

Halothane, which was the first clinically used one from this family, is the only inhalational anesthetic containing bromine. It is normally provided in dark bottles because it is unstable upon exposure to light. Halothane is on the World Health Organization (WHO) model list of essential Medicines. Although of halothane superiority in terms of activity and safety to diethyl ether, not too many years were needed to recognize the first clinical presentation of a side effect, later termed halothane-induced hepatitis, in a 39-year-old woman in 1958.^(1, 2) This occurrence became a major trigger for the production of less harmful anesthetic gases⁽³⁾.

The mechanisms of Halothane toxicity in particular have been well described first by De Goot and Noll,⁽⁴⁾ the most proposed mechanism is by oxidation/reduction metabolism (**Scheme-1**); the oxidative pathway is catalysed by CYP 2E1 and 2A6 and gives trifluoroacetyl chloride intermediate which can acylate liver proteins.⁽⁵⁾ Moreover, this reactive intermediate could covalently

bind to cellular macromolecule such as proteins, lipids and render them immunogenic complexes which later could be recognized by immune system as antigenic and lead to serious tissue injuries especially liver necrosis.⁽⁶⁾

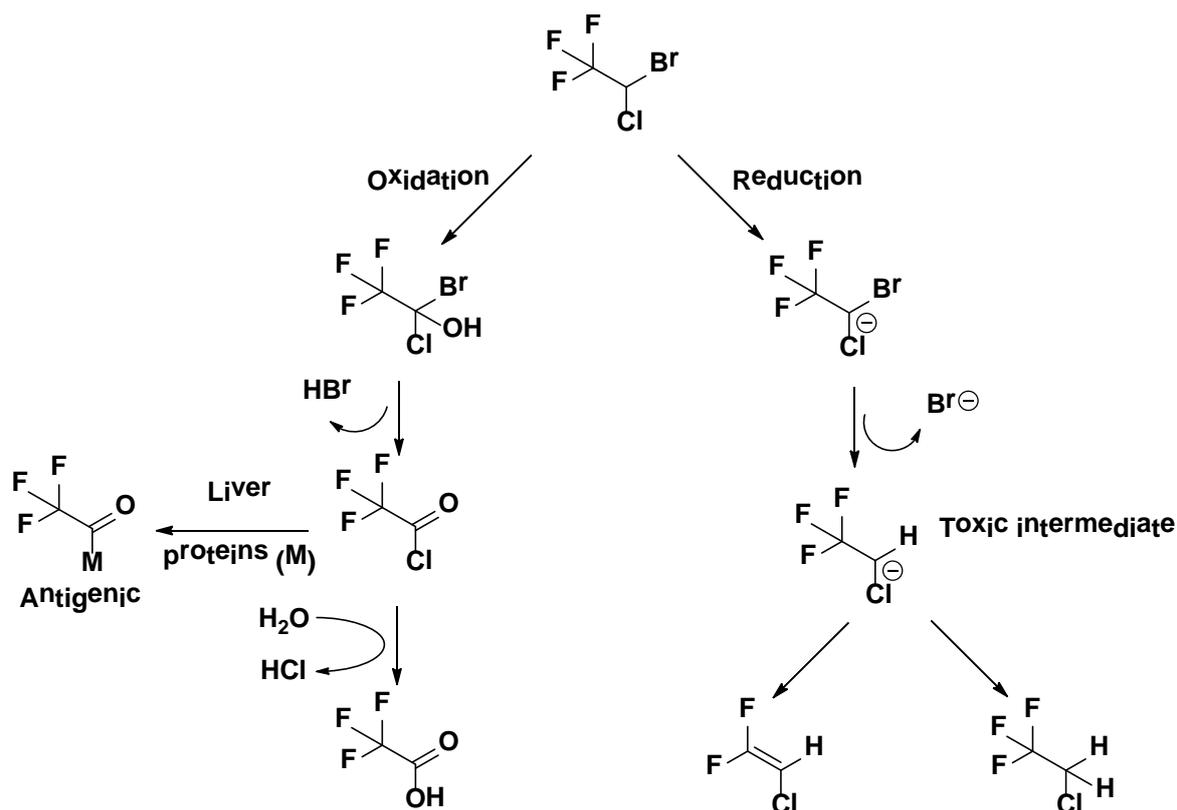
The reductive pathway, on the other hand, is catalysed by CYP 3A4 and 2A6 and resulted in the formation of chlorotrifluoroethane and chlorodifluoroethylene in exhaled breath in addition to fluoride and bromide ions; the latter is known for its neurotoxicity including alteration in consciousness, ataxia, upper and lower motor neuron tetra paresis and par paretic especially in animal models.^(7,8)

In addition to Halothane toxicity on patients, occupational exposure to halothane has resulted in liver damage for surgeons, anaesthetists and operating theatre personnel.⁽⁹⁾

Attempts to inhibit or minimize the possibility of halothane metabolism have been conducted; one of them is to prepare *d*-

halothane in which the oxygen insertion reaction at the C-H bond will be impaired. Results from this study revealed that the increased stability of the C-D bond explains the reduction in oxidative metabolites observed after exposure to d-halothane. In other studies, although the co-administration

of CYP2E1 and CYP 2A6 inhibitors such as Methoxalen and Troleandomycin has decreased TFA and bromide urine levels, the use of such inhibitors to counteract the elevated levels of such metabolites is not recommended because of many possible drug-drug interactions and complications.⁽⁸⁾



Scheme 1: Metabolic biotransformation of Halothane into toxic metabolites (4)

There is a lower risk of liver toxicity after consumption of newer halogenated anesthetics, such as enflurane, isoflurane, sevoflurane, and desflurane, since they have lower metabolism rates in the liver. But, compared to halothane, these agents are much more expensive⁽¹⁰⁾ The liver related complications of these agents, although they have decreased, have not disappeared,

Bromide ion, which is the other toxic metabolite for Halothane, is rapidly and almost

completely absorbed from gastrointestinal tract then distributed to almost all tissues and into extracellular fluid except central nervous system, this distribution is notably affected by chloride ion concentration which means that possible pharmacokinetics interactions are expected between bromide and chloride ion.⁽¹¹⁾ It has been reported in literature that the half life ($t_{1/2}$) in human is about 12 days that might be increased by salt-deficient diet and the main route of excretion is by renal in a rate of 5% the administered dose per hour.⁽¹²⁾ It has been

reported in literature that the maximum allowed serum bromide level is 3.2-4.8 mg/l and as this level increased, bromide toxicity increased.⁽¹³⁾

Researchers assume that healthcare workers in operation rooms (Ors), because of their long exposure to anesthesia, are the most susceptible to Halothane toxicity and high levels of bromide and Trifluoroacetate are expected in their blood and strongly support the use of proper scavenging system in the anesthetic circuit to control the level of gases in Ors. Moreover, compared to other countries such as USA and Europe, in which Joint Commission on Accreditation of Healthcare Organizations (JCAHO) set a group of regulations on the allowed concentrations (in ppm) for detectable gas levels in Ors⁽¹⁴⁾ most of Jordanian Hospitals do not have such monitoring procedures. Therefore the aim of this study is to investigate the serum bromide levels of healthcare professionals working in halothane atmosphere to highlight the health risk upon using Halothane as a general anesthetic and to support the calls to stop using this anesthetic in Ors.

A number of quantitative laboratory methods have been used for measuring both negatively and positively charged ions in blood, such as colorimetry which is based on the use of Gold chloride method, this was previously described by Dunlop and Underwood.⁽¹⁵⁾ Neutron activation was another method more specific for Iodine and bromide detection.^(16,17) Other methods used for the same purpose are Polarography,⁽¹⁸⁾ Gas chromatography, High Performance liquid chromatography (HPLC),⁽¹⁹⁾ and energy dispersive X-ray fluorescence.⁽²⁰⁾

Practically, all above mentioned methods were tedious and serum pretreatment procedure is recommended to exclude the interference of other elements and ions in the accuracy of measurement. Moreover, the detection limits for such methods were very high; meaning low levels of serum bromide will not be accurately detected and measured. Inductively-coupled plasma-mass spectroscopy (ICP-MASS) was the most accurate method with the ability to have an internal standard as well as a blank to subtract the interference of other anionic and cationic species such as chloride, sodium, magnesium and potassium that will be discussed in the methodology section.^(21,22) Therefore, ICP-MASS was utilized as a method of choice for serum bromide levels in our work.

The aim of this study is to examine the serum Bromide levels in health care professionals working in ORs (i.e. surgeons, anaesthesiologists and nursing staff), because they are frequently exposed to unlimited amounts of halothane daily.

Materials and Methods

Settings

Jordan has three main sectors provide healthcare; governmental, military and private. As mentioned earlier no regulations in any of the sectors on the allowed ppm in ORs for detectable gas levels. In any of healthcare settings in Jordan, excluding the King Hussein Cancer Center (KHCC), there is no active system that allows the compulsory leave for healthcare professionals from the ORs for a time to allow serum bromide to clear. The theatres in the setting of this study do not have scavenging system in the anesthetic circuit, but theatre rooms are supplied with vacuum. In cardiac ORs for example, the bypass circuit

has a vaporizer as part of it, but not connected to scavenging. Orthopedic and Pediatric ORs have high turnover rate compared to others.

Technique:

Serum bromide concentration was measured for medical staff working inside surgical theaters and rooms including surgeons, anesthetic specialists, nursing and welfare persons. The routine work procedure for those individuals makes them passively susceptible to heavy exposure to halothane; this means that staffs working in surgical theaters are more susceptible to Halothane toxicity than those in other hospital departments. Therefore, an urgent call firstly to measure halothane metabolites levels; both trifluoroacetic acid and bromide in medical staff blood, then to withdraw its use as general anesthetic to be replaced with safer alternatives.

Al-Bashir hospital, where the participants are working, was purposively selected for many reasons; firstly it is one of the biggest hospitals in Amman. Secondly, surgical operations are undergone almost daily in all hospital sections and in high frequency.

Thirdly and most importantly, Halothane is the main gaseous anesthetic used in all surgical operations.

Fifty two participants were asked to voluntarily participate in this study using convenient sampling technique. Researchers asked all staffs working in ORs (surgeons, anesthesiologists and nursing staff), and comply with inclusion criteria, to participate in this study. The total number of staff members was eighty one (Table 1). Eighteen members were excluded because they did not meet the inclusion criteria; to be full-time Jordanian workers who worked in ORs for at least one year. Eleven staffs refused to take part in the study (seven females and 4 males).

A third party which was a lab technician has been recruited to withdraw blood samples from participants at a schedule set by researchers. Both participants and this technician were blind to each other. Additional blood sample was withdrawn from this technician, who did not ever exposed to Halothane, also be examined as a negative control.

Table 1. Characteristics of staffs refused participation

Total number: 11	
Gender	7 females, 4 males
Qualifications:	
Surgeons	3 (mean of expertise = 9 yrs)
Anaesthetists	2 (mean of expertise = 13.4 yrs)
Nurses	6 (mean of expertise = 6.7 yrs)

Blood samples (5ml) were collected from 12:00 afternoon to 3:00 pm because it was that time of the day with highest level of halothane exposure. Blood samples were placed then in plain plastic blood tubes, tubes were left for 10 minutes on bench to facilitate blood

coagulation, after that clotted blood was centrifuged for 15 minutes at a speed of 2500 RPM in order to separate serum from blood clot. The haemolyzed blood specimens were discarded since they might give false results. Clear serum samples were transferred into new

plastic plain tubes and labeled with sampling time, name of participant and the medical section that participant belongs to. All above mentioned information was confidential and was available for the use of researchers only. Serum specimens were kept in fridge at a temperature of 6°C prior to shipping to our collaborators; the Royal Scientific Society/ Amman, for ICP-MASS analysis to measure serum bromide levels.

The ICP-MASS instrument used was from Shimadzu/Japan, model number 8500. The mass spectrometer was quadruple and ions obtained inside the instrument was detected by a secondary electron multiplier while the plasma source was crystal oscillator with high frequency power supply reaching 27.12 MHz \pm 0.05%. Other essential components and parameters used in this instrument was the mini plasma torch, coaxial nebulizer, spray chamber and peristaltic pump. The important parameters were set as follow: the flow rate for Argon was controlled by a flow meter to be around 7.9 L/ min and 0.2-1.4 L/min for the carrier gas. The detection unit used Channeltron detector which was set to a right angle against the quadruple.

Quantitative analysis method was used for the data processing software unit after drawing a calibration curve by using internal standard correction blank. Each analysis including rinse time lasted for 80 seconds although the response for the selected ions were integrated for only 40 seconds.

On the bases of 10 fold dilution, serum samples were pretreated prior to ICP-MASS analysis by diluting 200 μ l with 1800 μ l of diluent which was previously prepared as 1% nitric acid aqueous solution. A standard

solutions containing 3.3 g/l Na ion, 0.15 g/l K ion, 0.1 g/l Ca ion and 0.02 g/l Mg ion were prepared with and without bromide in order to eliminate the interference of such elements with the final bromide results. Moreover a series of bromide solution with different concentrations (2-20 mg/l) were prepared using nitric acid diluent in order to draw the calibration curve that will be used for measuring serum bromide levels in the unknown samples. The calibration curve obtained automatically by the data processor integrated in ICP-MASS using the data points of prepared known bromide concentrations and found to be linear enough over a range of 0-70 mg/l which was reasonably satisfactory to include almost all expected clinical values without farther dilution steps. On the other hand, the standard addition plot constructed by the data processor was nearly parallel for artificial and serum bromide concentrations and the recovery of bromide being added to serum from 10 subjects were close to 100% with a coefficient of variation of less than 2%. Moreover, the within-day and between-days reproducibility (n = 10) determination under routine conditions using the lowest serum permitted bromide concentration was about 3.5 mg/l which is considered sufficient for clinical applications.

Results and Discussion

The detection limit for bromide levels calculated as being equivalent to twice the standard deviation of the background signal and found to be 0.5 mg/l. The calibration curve previously obtained by the machine for a range of 0-70 mg/L bromide solutions was used for the measurement of serum bromide samples without the need of farther dilution steps. Moreover as the standard addition plots constructed by the data processor for the

artificial bromide solutions are almost parallel to that of serum samples, the signal was

approximately the same in both serum and standard solutions.

Table 2. Serum bromide concentration of medical staff working in surgical theaters

Sample ID	Gender	Age	Exposure period (Years)	Average Serum bromide level (mg/L), n =2
NE1	F	32	0.5	6.25
NE2	F	44	15	6.6
NE3	M	39	15	14.2
NE4	M	33	10	7.8
NE5	M	41	15	9.1
NO1	F	48	17	10.0
NO2	F	52	30	9.5
NO3	F	50	28	10.2
NO4	M	44	18	13.1
NO5	M	48	22	13.2
NO6	F	36	7	12.4
NO7	M	40	17	14.2
NO9	M	30	0.5	6.8
NO13	F	32	4	18.5
NO14	M	40	0.5	15.5
NO16	M	35	8	20.1
NO17	M	31	0.5	17.1
NN06	F	30	1	3.7
NN07	M	32	2	19.4
NN10	M	31	1	19.4
NN12	F	43	21	5.6
NN14	M	35	0.67	19.8
NN15	F	27	0.4	5.0
NN20	M	38	16	6.0
NG3	F	25	1	4.8
NG6	F	43	19	21.8
NG7	F	40	12	18.7
NG10	M	27	0.4	14.4
AE7	F	27	0.3	8
AE6	M	30	2	12.7
AO8	M	34	2	16.7
AO10	M	26	5	15.2
AO11	M	21	1	21.6
AO12	M	40	8	22.9
AO15	M	22	0.4	21.2
AO18	M	30	0.2	23.7
AO19	M	53	25	20.5

Table 2....continued

Sample ID	Gender	Age	Exposure period (Years)	Average Serum bromide level (mg/L), n =2
AG2	M	53	18	17.7
AG4	M	30	2	20.2
AG5	F	38	19	24.7
AG8	F	25	5	24.1
AG9	F	21	2	39.9
AN1	M	60	32	23.4
AN2	F	35	10	12.8
AN3	F	23	1	16.7
AN4	F	27	5	9.99
AN5	M	37	5	16.7
AN8	M	30	2	21.9
AN9	M	30	1	7.4
AN11	M	34	14	14.7
AN13	F	25	0.4	13.8
SG1	M	38	14	13.5
-ve Control	F	42	-	3.7

NE: Nurse in ENT surgery, NO: Nurse in orthopedic surgery, NN: Nurse in neurological surgery, NG: Nurse in general surgery, AE: Anesthesiologist in ENT surgery, AO: Anesthesiologist in orthopedic surgery, AG: Anesthesiologist in general surgery, AN: Anesthesiologist in neurological surgery, SG: surgeon in general surgery, -ve Control: Lab technician working in the central labs.

Statistically, the recovery of bromide added to the serum from ten ordinary subjects was close to 100% with a coefficient of variation of less than 2%. Also the within-day and between days reproducibility (n = 10) under routine conditions after using the lowest serum bromide concentration was about 3% which is considered satisfactory for clinical applications.

Regarding the interference of other elements and impurities, it has been observed that no interference was detected for bromide (m/z = 79) instead of isotope (m/z =81), this is expected and reported before by Janghorbani.⁽²³⁾

The fifty two Participants belonged to four different surgical sections; General surgery

(G), Ear-Nose-Throat (E), Orthopedic (O) and Neurological surgery (N). Participants were nurses, anesthesiologist and surgeons with age range of 21-60 years and work experience in surgical theaters of up to 32 years.

As shown in Table 2, serum bromide levels measured were much higher than the allowed levels, which is 4 mg/l, and almost all samples having levels more than 10 mg/l. when comparing serum bromide levels between different hospital sections, it can be concluded that no significant difference between participants from different sections although staff from orthopedic and general surgery theaters had the highest levels among other areas in the hospital. The maximum bromide concentration found was for an

anesthesiologist with a level reaching 39.9 mg/l. Interestingly, this level is unexplainable since this participant was among the least exposed person to halothane with only 2 years period which strongly suggests that other factors might affect bromide level such as physiological and pathological factors, kidney and liver functions. Other participants, on the other hand, were in direct contact with halothane for up to 32 years, have average bromide levels of 14.6 mg/l.

As mentioned before, staff working in both general surgery and orthopedic sections had the highest serum bromide levels ranging from 15.2 to 39.9. This was expected since those two sections were the busiest sections according to the number of surgical operations. Moreover, operations in such sections lasted for a minimum of 6 hours which means high level of halothane in atmosphere could be inhaled by the medical staff working around the operation table. In addition to that, upon inspecting the operation theaters especially for general surgery of pediatric operations; those theaters were poorly ventilated rooms that will increase the buildup concentration of Halothane in air.

No clear effect of gender or age on serum bromide concentration although many studies have shown that the action of CYP 450 enzymes might be affected by those factors especially patient age.^(24,25) Therefore, additional work is needed to figure out the factors affecting the extent of halothane metabolism as well as its pharmacokinetic properties especially that concerns about factors affecting halothane $t_{1/2}$, fat storage, protein binding and kidney excretion.

The results of this study strongly recommend that the use of halothane as a

general anesthetic agent must be replaced with other safer alternatives since it will be metabolized into toxic bromide anion. In the meantime, the build-up blood concentration of bromide over a long period of time must be monitored for all medical staff working under halothane environment and their vital physiological functions must be checked periodically for possible signs and symptoms of bromide toxicity. Moreover, the findings of this study strongly recommend that all surgical theaters must be well-ventilated and supplied by enough sterile suction filtration units to reduce the concentration of halothane in environment.

Conclusion and Future Remarks

Monitoring serum bromide level in medical staff working under Halothane environment in surgical operation theaters is strongly recommended. Serum bromide levels for 52 medical staff; nurses, surgeons and anesthesiologists, from Al-Bashir Hospital/ Jordan were measured using ICP-MASS instrument. Results have shown high bromide concentration ranging from 10.1-39.9 mg/l in most of cases which are at least twice the allowed serum bromide. Staffs working in orthopedic and general surgery sections were the most susceptible individuals exposed to Halothane due to the long operational time. Moreover, operational theaters with inadequate ventilation such as pediatric surgery resulted in high levels of bromide.

No clear effect of gender or age on the extent of Halothane metabolism into bromide which needs more investigation. Future studies that emphasize the effect of operation duration, ventilation conditions and all factors affecting pharmacokinetic properties of halothane are needed since this study has not

approached such limitations where the main aim of it was to examine the level of bromide descriptively not in a correlation manner.

This study suggests that Halothane is among non safe gaseous anesthetic agents due to its biotransformation into toxic bromide anion, this recommends the withdrawal of halothane from being used in surgical operations to be replaced with much safer alternatives or at least a continuous serum bromide monitoring for all staff working under

contaminated environments.

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قياس مستويات البروميد في مص الدم للكادر الطبي والتمريضي العامل في غرف العمليات في المستشفيات الأردنية التي تستخدم مادة الهالوثين

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الملخص

خلفية البحث: تشير الدراسات إلى أن معظم المشتقات في العالم قد توقفت عن استخدام مادة الهالوثين كمادة مخدرة في العمليات الجراحية، إلا أن هذه المادة ما زالت تستخدم في كثير من المستشفيات الأردنية. ناتج أيض هذه المادة داخل الجسم يعطي أيون البروميد بالإضافة إلى ثلاثي فلورات حامض الأستيك، وكلتا المادتين معروفة بسميتها لجسم الإنسان. تهدف هذه الدراسة إلى قياس مستويات البروميد في مصل اثنين وخمسين من الكادر الطبي والتمريضي العامل داخل غرف العمليات الجراحية بعد التعرض لمادة الهالوثين في مستشفى البشير، الأردن.

طريقة البحث: البحث هو عبارة عن بحث كمي وصفي لقياس مستويات البروميد في مصل بشري (25 عينة) من الكادر الطبي والتمريضي (أطباء تخدير، أطباء جراحين، ممرضين عمليات، فنيين تخدير) العامل في غرف العمليات باستخدام جهاز ال ICP-MASS. **النتائج:** كشف تحليل عينات المصل البشري عن وجود مستويات مرتفعة أكثر (بثلاثة إلى عشرة أضعاف) من المعدل المسموح عالمياً حيث وصلت في بعض العينات إلى 39.9 مغ/لتر بينما المستوى المسموح به يجب ألا يتجاوز 3-4.4 مغ/ لتر.

الاستنتاجات: إن المستويات العالية المقاسة للبروميد في هذه الدراسة تسلط الضوء على ضرورة التوقف عن استخدام الهالوثين في غرف العمليات بسبب سمية هذه المادة خصوصاً بعد تحولها إلى أيون البروميد السام، كما و تؤكد على ضرورة قياس هذه المستويات بشكل دوري للعاملين في غرف العمليات في حال الاستمرار باستخدام هذه المادة.

الكلمات الدالة: الهالوثين، ICP-MASS، بروميد المصل، غرف العمليات، ممرضين، تخدير، الأردن.