Research article

Drug Testing and Analysis

Received: 15 February 2016 Revised: 22 April 2016 Accepted: 5 May 2016 Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.2001

Xenon elimination kinetics following brief exposure

Maximilian S. Schaefer,[a]* Thomas Piper,[b] Hans Geyer,[b] Julia Schneemann,[a] Martin Neukirchen,[a] Mario Thevis[b] and Peter Kienbaum[a]

Xenon is a modern inhalative anaesthetic with a very low solubility in tissues providing rapid elimination and weaning from anaesthesia. Besides its anaesthetic properties, Xenon promotes the endogenous erythropoietin biosynthesis and thus has been enlisted as prohibited substance by the World Anti-Doping Agency (WADA). For effective doping controls, knowledge about the elimination kinetics of Xenon and the duration of traceability are of particular importance. Seventy-seven full blood samples were obtained from 7 normal weight patients undergoing routine Xenon-based general anaesthesia with a targeted inspiratory concentration of 60% Xenon in oxygen. Samples were taken before and during Xenon inhalation as well as one, two, 4, 8, 16, 24, 32, 40, and 48 h after exposure. Xenon concentrations were assessed in full blood by gas chromatography and triple quadrupole tandem mass spectrometry with a detection limit of 0.25 μmol/L. The elimination of Xenon was characterized by linear regression of log-transformed Xenon blood concentrations, as well as non-linear regression. Xenon exposure yielded maximum concentrations in arterial blood of 1.3 [1.1; 1.6] mmol/L. Xenon was traceable for 24 to 48 h. The elimination profile was characterized by a biphasic pattern with a rapid alpha phase, followed by a slower beta phase showing a first order kinetics ($c[Xe] = 69.1e^{-0.26x}$, $R^2 = 0.83$, $t_{1/2} = 2.7$ h). Time in hours after exposure could be estimated by $50*\ln(1.39/c[Xe]^{0.077})$. Xenon's elimination kinetics is biphasic with a delayed beta phase following a first order kinetics. Xenon can reliably be detected for at least 24 h after brief exposure. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: doping in sports; kinetics; performance-enhancing substances; pulmonary elimination; Xenon

Introduction

Xenon was approved as a general anaesthetic in Europe more than 10 years ago. Its very low solubility in tissues and blood is considered to be a major advantage in clinical anaesthesia.[1] Accordingly, when Xenon administration is discontinued in patients under Xenon-based anaesthesia, it is rapidly eliminated via the lungs and associated with almost immediate awakening.[2,3] Besides its anaesthetic properties, Xenon inhibits the metabolism of the sympathetic vasoconstrictor norepinephrine.[4] As a consequence, arterial hypotension is rarely observed during Xenon-based anaesthesia. Xenon was further described to stimulate the release of endogenous erythropoietin,[5-7] which has potentially been recognized by some athletes who were reported to have inhaled Xenon to gain performance advantages.[8] In turn, the World Anti-Doping Agency (WADA) added Xenon to the list of prohibited substances in sports in September 2014.[9] Doping controls are facilitated when the precise elimination characteristics of the substance are available. Accordingly, the time line of Xenon exposure prior to competition can be estimated. Finally, duration of traceability from blood has only been investigated in a single individual.[10] Since ethical concerns were anticipated when planning on exposing athletes to Xenon, Xenon elimination kinetics were determined from patients scheduled for surgery under routine Xenon-based general anaesthesia.

Materials and methods

After IRB approval (Ethikkommission der medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf, Germany, study number 4635), registration at ClinicalTrials.gov (NCT02105077) and written informed consent, patients scheduled for routine Xenon-based general anaesthesia were included into this prospective observational study. Anaesthesia was induced and maintained at the discretion of the attending anaesthesiologist: Inhaled Xenon (Xenon pro Anaesthesia, Air Liquide Deutschland GmbH, Düsseldorf, Germany) was combined with the opioid analgesic remifentanil (0.1-0.5 μg kg^{-1} min^{-1}). Xenon anaesthesia was administered with a closed-circuit anaesthesia machine (Felix Dual, Air Liquide international, Paris, France) at an initial targeted inspiratory concentration of 60%, allowing continuous recording of inspiratory Xenon concentrations. From these data, the total dose of Xenon as inspiratory fraction (in %), multiplied by the duration of Xenon exposure in hours (F_i% h), as well as the median inspiratory concentration was calculated for each patient.

Study subjects

To increase comparability to athletes, we limited our collective to patients with a normal body mass index (20–25) since Xenon elimination may be influenced by body fat content due to a high affinity

* *Correspondence to: Dr Maximilian S. Schaefer, Department of Anaesthesiology University Hospital Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany. E-mail: Maximilian.Schaefer@med.uni-duesseldorf.de*

a *Department of Anaesthesiology, University Hospital Düsseldorf, Germany*

b *Center for Preventive Doping Research – Institute of Biochemistry, German Sports University Cologne, Germany*

to fatty tissue.[11] Also, patients with impaired kidney function (creatinine < 1.2 mg/dL, urea < 56 mg/dL) and abnormal haematocrit values (outside the normal range of 37–45% in female and 42–50% in male patients) were excluded. Further, to reduce influence of significant blood loss during surgery, only patients undergoing minimally invasive vascular surgery were included.

Blood samples

To depict Xenon's elimination kinetics, blood samples were drawn from routinely placed central venous catheters[12] before exposure and at 1, 2, 4, 8, 16, 24, 32, 40, and 48 h after discontinuation of Xenon application. Additionally, to assess maximum blood concentrations, a blood sample was obtained from additional routinely placed arterial catheters shortly before discontinuation of Xenon. Values from this measurement were compared to expected concentrations, as calculated from the respective inhalative Xenon concentration with a blood-gas distribution coefficient of 0.115[1] and the assumption of ideal gas properties at 37 °C[13] with an alveolar pH_2O of 6.3 kPa. Samples were drawn in 3 mL EDTA tubes (Vacutainer®, BD, Heidelberg, Germany), which were immediately sealed airtight (Blenderm™, 3 M Deutschland GmbH, Neuss, Germany). To allow conditions comparable to routine doping testing, samples were stored at +4 °C and a maximum 30 h time to analysis was allowed.

Quantification of xenon concentrations by gas chromatography-mass spectrometry

Xenon concentrations in full blood were analysed using gas chromatography-triple quadrupole tandem mass spectrometry (GC-MS/MS). Sample preparation and the GC-MS/MS protocol were adapted from two earlier studies.[10,14] In brief, 1 mL of each sample was placed in an autosampler vial (Macherey-Nagel, Düren, Germany), fortified with 1.8 μmol of internal standard (d_6-cyclohexanone) and heated at 70 °C for 20 min. Subsequently, 10 μL of the headspace was injected into the GC-MS/MS system with a HP-Ultra 1 column (Agilent, Waldbronn, Germany) in split mode (1:5) with helium used as carrier gas at 1.10 bar. In contrast to the published methodology, the volume injected from the headspace was increased to 10 μL, facilitating a decrease of the detection limit from 0.5 to 0.25 μmol/L. Nitrogen was used as collision gas (collision energy 5 eV). For quantification, blank EDTA blood samples fortified with different Xenon concentrations (0.5, 5, 50, and 500 μmol/L) were prepared by serial dilution, analysed and compared to patient samples to estimate concentrations, providing an accurate measurement of Xenon with a coefficient of variation below 20%.

Sample size estimation and statistical analysis

Xenon concentrations in pig blood during exposure of 67% Xenon have been reported to be 70 ± 9 μl/mL,[12] which corresponds to 63 ± 8.1 μL/mL at 60% inspiratory Xenon concentrations, or 2.4 ± 0.3 mmol/:L at 37 °C.[13] We estimated the characteristic of Xenon elimination to be best described by an exponential decay function. Also, we knew from previous findings that Xenon was traceable for at least 24 h.[10] With these assumptions, we created a model data set by means of a hypothesized decay function, which was described by $c[Xe] = 2.4\ e^{-0.38\ h}$, where *c[Xe]* is the Xenon concentration and *h* the time in hours after discontinuation of inhalation. We found that at least 5 observations per time point were needed to detect a significant decrease of xenon concentrations at a 0.05 alpha level. To compensate for possible loss due to transport, storing or processing of the blood samples, we increased our sample size to a total of 7 patients.

Raw data are expressed as median [interquartile range]. Also, Xenon concentrations were log-transformed and, after visual inspection, linear regression analyses on log-transformed values were performed. Since log transformation of 0 is impossible, values below the limit of detection were set to 0.01 μmol/L. When visual inspection indicated an exponential decay of xenon concentrations, non-linear regression analysis with multiple curve fits was applied to raw values in order to maximize goodness-of-fit, as quantified by the R^2 value. Initially, a single exponential, two-factor function was calculated. Subsequently, multiple-factor and double-exponential functions were calculated and R^2 values were compared. Calculations were made with SigmaPlot 13.0 (Systat Software, Inc., San José, CA, USA) and SPSS 22 (IBM, Armonk, NY, USA).

Results

Seventy-seven full blood samples were obtained and analyzed from seven consecutive patients who underwent Xenon-based anaesthesia. Table 1 depicts patient characteristics as well as anaesthesia and surgery details.

Management of anaesthesia

Duration and concentration of Xenon exposure for all individual patients are expressed in Figure 1. The total dose of Xenon was 137

Table 1. Patient characteristics and anaesthesia details. Values are median [interquartile range] or absolute numbers (%)

Age (years)	74 [66;77]
Female	3 (43)
Weight (kg)	66 [65;77]
Height (cm)	173 [172; 181]
Body mass index	24 [21;24]
Preoperative haematocrit (%)	41 [39; 43]
Duration of anaesthesia (min)	185 [160; 225]
Duration of Xenon inhalation (min)	146 [121;187]
Xenon consumption (L)	22.9 [21; 23]
Type of surgery	
Endovascular aortic repair	6 (86)
Carotid endarterectomy	1 (14)
Estimated blood loss (mL)	80 [50;100]

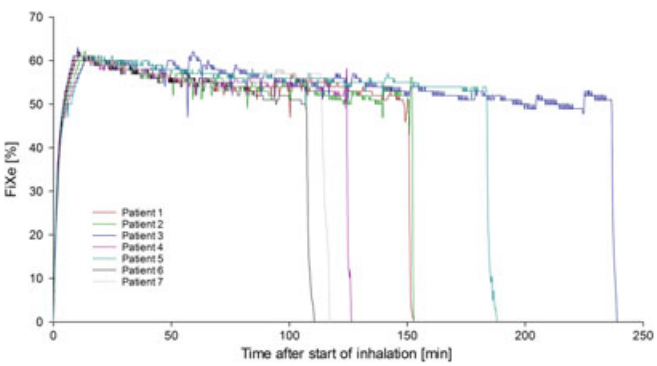

Figure 1. Time course of inspiratory Xenon concentrations (F_iXe) for all 7 patients.

[110; 154] F_i% h with a median Xenon concentration of 54 [54;54] F_i %. At the end of anaesthesia, when arterial samples were obtained, the inspiratory Xenon concentrations were 52 [51; 54] F_i%. To facilitate surgical tolerance, 0.41 [0.40;0.45] μg kg^{-1} min^{-1} intravenous remifentanil was administered additionally.

Xenon concentrations and duration of traceability

Xenon concentrations in full blood were below the detection limit in all samples before exposure. During exposure, the expected calculated concentration was 2062 [2023; 2122] μmol/L. The measured arterial Xenon blood concentration during inhalation in blood was lower, reaching 1341 [1069;1630] μmol/L. Subsequently, Xenon concentrations in central venous blood dropped to 54 [49; 64] μmol/L within the first hour after discontinuation (Figure 2). Xenon was traceable for 48 h in one, 40 h in two, 32 h in two, and 24 h in another two patients.

Elimination profile

Log-transformation of concentrations showed a biphasic pattern with a rapid decrease of concentrations within the first hour (-1.34 log(c[Xe]) per hour, R^2 0.97) and a subsequent slower decrease (-0.07 log(c[Xe])per hour, R^2 0.77, Figure 3). The second elimination phase fitted a single exponential, two-factor function ($c[Xe] = 69.1e^{-0.26h}$, $R^2 = 0.83$). Applying double-exponential and multiple factor functions did not increase goodness-of-fit ($R^2 = 0.83$ for each subsequent curve). The curve revealed a concentration-dependent elimination of first order kinetics (Figure 4). Time in hours after Xenon inhalation could be estimated as $50*\ln(1.39*c[Xe]^{-0.077})$ with a Xenon half-life in the second elimination phase of 2.67 h.

Discussion

The Xenon elimination kinetics presented a rapid initial alpha phase, followed by a much slower beta-elimination that was characterized by a first order kinetics and a Xenon half-life of 2.7 h. Despite its very low solubility in tissues, Xenon exposure was reliably detected for at least 24 h in each patient.

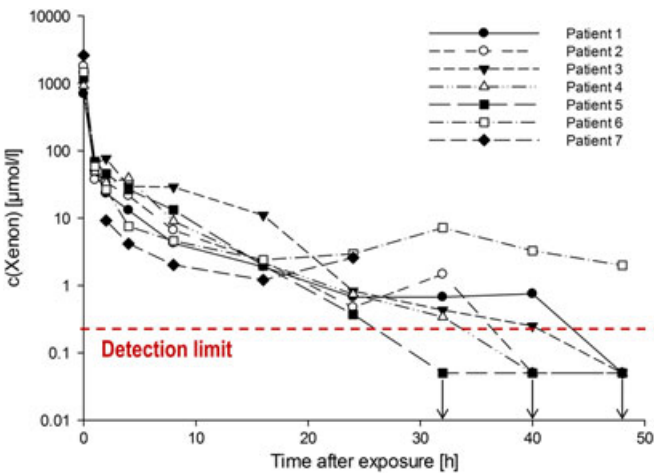

Figure 2. Intra- and postoperative Xenon blood concentrations in all 7 patients.

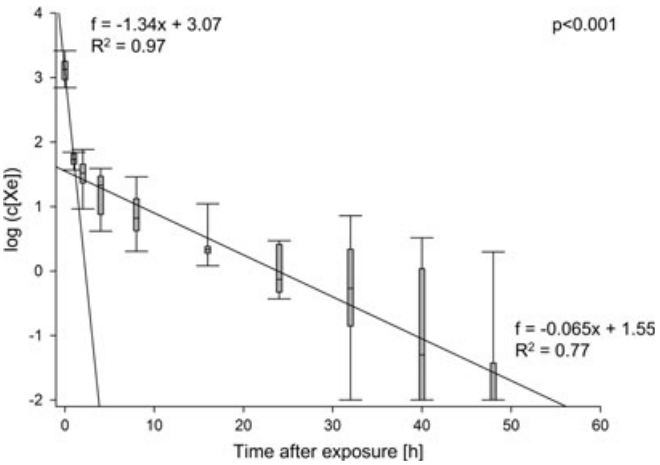

Figure 3. Log-transformation of Xenon concentrations with linear regression analysis showing a biphasic elimination pattern. Mathematical functions for regression lines are indicated next to the respective line with corresponding R^2 values.

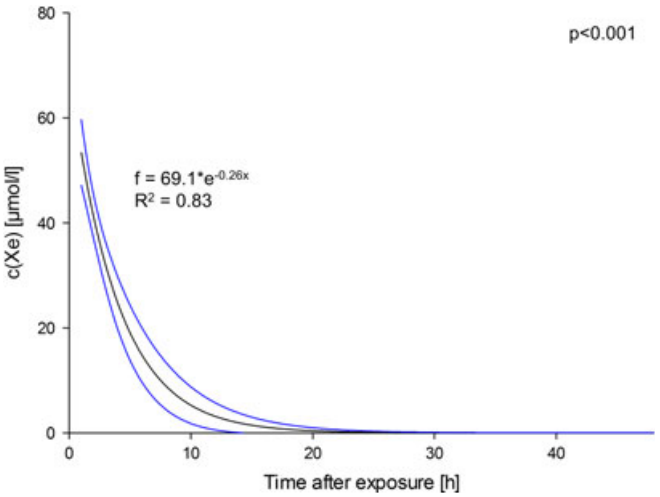

Figure 4. Non-linear regression of the second (beta-) elimination phase showing a first order kinetics. Black: regression line; Blue: 95% confidence interval.

Xenon concentrations and duration of traceability

Our results confirm previously reported data from a single patient, where Xenon could be traced in blood 24 h after exposure.[10] Also, Xenon is consistently detectable in urine from patients at least 24 h after anaesthesia, and is even traceable for more than 40 h after exposure in 40% of patients.[14] In this context, our data confirm the reliable traceability in human blood 24 h after Xenon inhalation and, taken together with previously published data, suggest equal success rates from blood and urine samples. However, testing for Xenon more than one day after exposure will return negative results in many subjects.

During inhalation, measured arterial Xenon concentrations of 1.3 mmol/L differed from calculated expected concentrations of 2.0 mmol/L. This difference can be explained since we allowed a sample storage time for up to 30 h before analysis to facilitate conditions comparable to those observed for detection of doping in athletes. In a previous study, storage times of 20–30 h decreased signal intensity of Xenon isotope 129 by about 35%.[10] With this

assumption, actual arterial concentrations during inhalation can be corrected to 1.3 mmol/L x $(1\text{-}0.35)^{-1}$ = 2.0 mmol/L, and are therefore equal to expected values. For these reasons, storage time before analysis should be reduced whenever possible. Also, superfluous open-close-cycles of doping control sample containers intended for Xenon analyses should be avoided.[10]

Elimination profile

The observed biphasic elimination profile is typical for substances that are stored in multiple compartments, such as inhalational anaesthetics.[15-17] Xenon has the lowest blood-gas-partition coefficient of all anaesthetics,[1] causing rapid elimination from blood when alveolar concentrations decrease. However, after initial alpha-elimination via the lungs, residual Xenon in blood results from its release from deep compartments as indicated by beta-elimination. In this context, tissue affinity might play a role. Xenon has a rather high solubility in oil, with an Ostwald's coefficient of 1.7 to 1.8 at 37 °C,[11] providing affinity to adipose tissue. Also, as with other lipophilic inhalative anaesthetics such as sevoflurane, saturation of deep compartments may take several hours.[17] Therefore, duration of exposure,[18] as well as body fat content may be determinants of the traceability of Xenon after inhalation. Of note, binding to blood proteins may also play a role, since Xenon exerts a high affinity to albumin[19] and is enriched in cellular compartments of the blood.[11]

From our data, the time after single Xenon exposure can be calculated from measured residual concentrations in blood. However, repetitive exposition might increase residual blood concentrations, as opposed to single exposure.[20] Consequently, one next step needs to be the investigation of the influence of repetitive exposition to Xenon, such as described in Russian athletes over several years.[8] Although no published data exist on the efficacy of different patterns of inhalation, a presentation made available to Russian athletes and sport officials recommends repetitive Xenon inhalation 'two to three times over a period of 7 to 10 days'.[8,21] In a recent study, Katz *et al.* established a computer model for the elimination of xenon from the human body after repetitive exposure.[20] Since human data had not been available, the authors validated their calculation on data derived from porcine xenon exposure.[12] It is suggested that residual Xenon may be detected for at least 24 h following a single exposure. Of note, our results are in line with this theoretical model confirming the assumptions made in a clinical situation of Xenon administration. Moreover, repetitive exposition to Xenon may increase residual xenon concentrations,[20] allowing its detection for an even longer period after termination of the last exposure. However, these results after chronic exposure to xenon in a computer model need to be verified prospectively in humans.

Limitations

Due to ethical concerns, we enrolled patients undergoing routine Xenon-based anaesthesia and did not perform Xenon inhalation in young athletes. Naturally, this needs to be considered when transferring our findings to professional sports since patients undergoing vascular surgery differ from athletes in several aspects that might influence Xenon storage in the body, elimination and traceability. Assuming that fatty tissue is a major storage compartment of Xenon, duration of traceability in athletes with minimum body fat content may be reduced. Additionally, haematocrit, age, fluid status and several other factors may diverge between patients and athletes. However, to increase comparability, we excluded patients with an abnormal body mass index (over 25 or under 20). Additionally, any possible influence of surgery was kept to a minimum by restricting to minimally invasive procedures with clinically irrelevant blood loss.

Xenon was administered to our patients with inspiratory concentrations of 54%, depending on the duration of anaesthesia and admixture of nitrogen. Since so far, data on Xenon-induced erythropoietin release are scarce and no dose-response relationship has been established, Xenon concentrations in our study might not necessarily equal concentrations used for doping purposes. Ma *et al.* administered 70% Xenon in oxygen for 2 h to mice and found an increase to 160% in erythropoietin concentrations 24 h later.[6] In patients following cardiac surgery under Xenon-based anaesthesia (median dose 118 $F_{i\%}$ h), erythropoietin plasma concentrations were 48% higher in patients with Xenon anaesthesia than in patients receiving sevoflurane.[7] As a result, and probably based on additional unpublished data, the aforementioned Russian presentation recommends inhalation of a 50:50 Xenon:Oxygen mixture for performance enhancement.[8,21] Therefore, inhaled concentrations in our study reflect recent practice and recommendations of Xenon inhalation for doping purposes.

In conclusion, we were able to show that humans can reliably be tested for Xenon inhalation within 24 h after exposure, and occasional positive test results may even be achieved more than 40 h after inhalation. This prolonged traceability is the result of a biphasic elimination kinetics including delayed beta elimination. Future investigations should focus on the influence of repetitive administration of Xenon on its traceability, and need to further differentiate the influence of body fat content to increase comparability to athletes.

References

[1] T. Goto, K. Suwa, S. Uezono, F. Ichinose, M. Uchiyama, S. Morita. The blood-gas partition coefficient of xenon may be lower than generally accepted. *Br. J. Anaesth.* **1998**, *80*, 255.
[2] T. Goto, H. Saito, Y. Nakata, S. Uezono, F. Ichinose, S. Morita. Emergence times from xenon anaesthesia are independent of the duration of anaesthesia. *Br. J. Anaesth.* **1997**, *79*, 595.
[3] M. Coburn, J.-H. Baumert, D. Roertgen, V. Thiel, M. Fries, M. Hein, O. Kunitz, B. Fimm, R. Rossaint. Emergence and early cognitive function in the elderly after xenon or desflurane anaesthesia: a double-blinded randomized controlled trial. *Br. J. Anaesth.* **2007**, *98*, 756.
[4] M. Neukirchen, J. Hipp, M. S. Schaefer, T. Brandenburger, I. Bauer, M. Winterhalter, P. Kienbaum, R. Werdehausen. Cardiovascular stability and unchanged muscle sympathetic activity during xenon anaesthesia: role of norepinephrine uptake inhibition. *Br. J. Anaesth.* **2012**, *109*, 887.
[5] A. Goetzenich, N. Hatam, S. Preuss, A. Moza, C. Bleilevens, A. B. Roehl, R. Autschbach, J. Bernhagen, C. Stoppe. The role of hypoxia-inducible factor-1α and vascular endothelial growth factor in late-phase preconditioning with xenon, isoflurane and levosimendan in rat cardiomyocytes. *Interact. Cardiovasc. Thorac. Surg.* **2014**, *18*, 321.
[6] D. Ma, T. Lim, J. Xu, H. Tang, Y. Wan, H. Zhao, M. Hossain, P. H. Maxwell, M. Maze. Xenon preconditioning protects against renal ischemic-reperfusion injury via HIF-1alpha activation. *J. Am. Soc. Nephrol.* **2009**, *20*, 713.
[7] C. Stoppe, M. Coburn, A. Fahlenkamp, J. Ney, S. Kraemer, R. Rossaint, A. Goetzenich. Elevated serum concentrations of erythropoietin after xenon anaesthesia in cardiac surgery: secondary analysis of a randomized controlled trial. *Br. J. Anaesth.* **2015**, *114*, 701.
[8] The Economist. Breathe it in. **2014**. Available at: http://www.economist.com/news/science-and-technology/21595890-obscure-gas-improves-athletes-performance-breathe-it [15 February 2016].
[9] WADA. Amendment to the 2014 prohibited list. **2014**. Available at: https://www.wada-ama.org/en/media/news/2014-08/amended-2014-prohibited-list-in-force-september-1 [15 February 2016].

[10] M. Thevis, T. Piper, H. Geyer, A. Thomas, M. S. Schaefer, P. Kienbaum, W. Schänzer. Measuring xenon in human plasma and blood by gas chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* **2014**, *28*, 1501.
[11] R. Y. Chen, F. C. Fan, S. Kim, K. M. Jan, S. Usami, S. Chien. Tissue-blood partition coefficient for xenon: temperature and hematocrit dependence. *J. Appl. Physiol.* **1980**, *49*, 178.
[12] M. Nalos, U. Wachter, A. Pittner, M. Georgieff, P. Radermacher, G. Froeba. Arterial and mixed venous xenon blood concentrations in pigs during wash-in of inhalational anaesthesia. *Br. J. Anaesth.* **2001**, *87*, 497.
[13] É. Clapeyron. Mémoire sur la puissance motrice de la chaleur. *J. Ecole R. Polytech.* **1834**, *14*, 153.
[14] M. Thevis, T. Piper, H. Geyer, M. S. Schaefer, J. Schneemann, P. Kienbaum, W. Schänzer. Urine analysis concerning xenon for doping control purposes. *Rapid Commun. Mass Spectrom.* **2015**, *29*, 61.
[15] C.-C. Lu, L. Tso-Chou, C.-H. Hsu, C.-S. Tsai, M. J. Sheen, O. Y.-P. Hu, S.-T. Ho. Pharmacokinetics of sevoflurane elimination from respiratory gas and blood after coronary artery bypass grafting surgery. *J. Anesth.* **2014**, *28*, 873.
[16] C.-C. Lu, C.-S. Tsai, O. Y.-P. Hu, R.-M. Chen, T.-L. Chen, S.-T. Ho. Pharmacokinetics of isoflurane in human blood. *Pharmacology*. **2008**, *81*, 344.
[17] R. C. Stern, S. C. Towler, P. F. White, A. S. Evers. Elimination kinetics of sevoflurane and halothane from blood, brain, and adipose tissue in the rat. *Anesth. Analg.* **1990**, *71*, 658.
[18] M. S. Schaefer, C. C. Apfel, H.-J. Sachs, R. Stuttmann, B. Bein, P. H. Tonner, M. Hein, M. Neukirchen, M. Reyle-Hahn, P. Kienbaum. Predictors for postoperative nausea and vomiting after xenon-based anaesthesia. *Br. J. Anaesth.* **2015**, *115*, 61.
[19] Ł. Wołoszyn, M. Ilczyszyn, M. M. Ilczyszyn. Experimental evidence on interaction between xenon and bovine serum albumin. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2014**, *125*, 449.
[20] I. Katz, J. Murdock, M. Palgen, J. Pype, G. Caillibotte. Pharmacokinetic analysis of the chronic administration of the inert gases Xe and Ar using a physiological based model. *Med. Gas Res.* **2015**, *5*, 8.
[21] M. de Neef, B. Koh. Xenon gas as a performance-enhancing drug: doping or just hot air? **2014**. Available at: http://cyclingtips.com.au/2014/03/xenon-gas-as-a-performance-enhancing-drug-doping-or-just-hot-air/ [15 February 2016].