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Biological monitoring of exposure to sevoflurane in operating room personnel by the measurement of hexafluoroisopropanol and fluoride in urine

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The objectives of this study were to evaluate the value of urinary hexafluoroisopropanol (HFIP) and fluoride (F^-) measurement for the biological monitoring of operating room personnel exposed to sevoflurane. Fifty members of operating room staffs from eight different hospitals took part in the study. To assess external exposure to sevoflurane, air samples were collected during the whole anaesthesia period by a passive sampling device (3M 3500 organic vapour monitor) attached close to the breathing zone of each subject. Urine was collected before (BA) and at the end of anaesthesia (EA) for the determination of HFIP, fluoride and creatinine. Average airborne concentration of sevoflurane was 19.0 ppm (range: ND–139.9 ppm) with a mean duration of anaesthesia of 221 min (range: 60–435 min). There was a better correlation between external and internal exposure as estimated by EA urinary HFIP ($r = 0.78$; $p<0.0001$) compared with EA urinary F^- ($r = 0.41$; $p = 0.0031$). Furthermore determination of urinary HFIP seemed more suited than that of F^- for the assessment of sevoflurane exposure because of lower background in BA samples (86 % of BA HFIP values were under the limit of detection). Based on these results, values of 9.6 and 4.3 mg HFIP g^{-1} creatinine correspond to airborne concentrations of 50 and 20 ppm of sevoflurane, respectively. Among the confounding parameters investigated (body mass index (BMI), sex, cytochrome P450 polymorphism) only BMI showed statistically significant influence on sevoflurane metabolism at these low levels of exposure. The measurement of HFIP in urine at the end of the surgical procedure constitutes a good index to assess occupational exposure to sevoflurane. Further studies will be necessary to propose an health-based limit value which remains to be determined from the relationship between effects and internal dose as can be assessed by HFIP measurement in urine.

Keywords: sevoflurane, biological monitoring, occupational exposure, cytochrome P4502E1.

Introduction

Sevoflurane (fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl)ethyl ether, CAS no. 28523-86-6 mol. wt 200.07) is a relatively new volatile inhalational anaesthetic with some advantages such as a low blood/gas partition coefficient that makes induction of anaesthesia and awakening more easy to control. Depending on the

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ventilator type and the fresh gas flow added, it can be used in closed or in (semi-) open circuit. Although most of the modern anaesthesia ventilators are used in semi-open circuit with an efficient scavenging system, some circumstances such as induction of anaesthesia and paediatric anaesthesia can contaminate the operating room atmosphere with the halogenated agent. Occupational exposure to significant concentrations of inhaled anaesthetics may result in various forms of adverse health effects such as headaches and neurobehavioural changes (Brodsky and Cohen 1985). This raises the need for appropriate methods for the monitoring of exposure to this gas.

Sevoflurane undergoes biotransformation to the primary metabolites fluoride (F^-) and hexafluoroisopropanol (HFIP) (figure 1). HFIP circulates in blood primarily as a glucuronide conjugate, with unconjugated (or free) HFIP representing <15 % of the total metabolites. HFIP is excreted in urine as a glucuronide conjugate. A recent clinical study in anaesthetized patients showed that primary metabolites (F^- and HFIP) are rapidly formed from sevoflurane and eliminated within minutes in urine. The overall extent of sevoflurane metabolism was estimated to be approximately 5 % from kinetic data of F^- and HFIP (Kharasch *et al.* 1995a).

Moreover since sevoflurane absorption occurs exclusively by inhalation, this compound provides a good opportunity to analyse the relationship between external and internal parameters. Using disulfiram, an effective inhibitor of CYP2E1, Kharasch *et al.* (1995b) have shown a substantially decreased production of HFIP and fluoride during and after sevoflurane anaesthesia, suggesting that CYP2E1 is the predominant P450 isoform responsible for the biotransformation of sevoflurane in humans. Some physiological conditions already known to enhance CYP2E1 activity, like chronic alcohol consumption and obesity (Lieber 1997) should therefore interfere with sevoflurane metabolism. Furthermore, as *CYP2E1* has been shown to be genetically polymorphic (reviewed by Rannug *et al.* (1995)), determination of *CYP2E1* genotypes may offer useful information to refine the interpretation of the relationship between ambient sevoflurane and urinary metabolites concentration.

The aim of this study was therefore to assess the value of urinary HFIP and fluoride measurements for the monitoring of the medical staff exposed to low concentrations of sevoflurane.

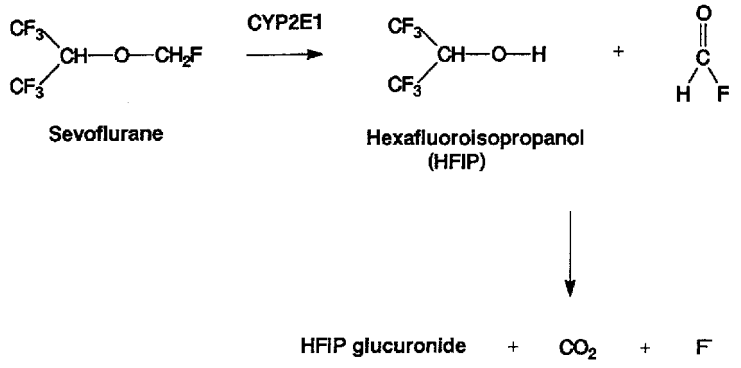

Figure 1. Metabolic pathway of sevoflurane.

Table 1. Characteristics of the study population.

Subgroup	*n*	Mean age in years (SD)	Percent smokers
Anaesthetists	15	40.7 (10.0)	47
Surgeons	14	39.1 (7.9)	7
Auxilliary nurses	21	39.5 (8.1)	29

SD: standard deviation.

Materials and methods

After approval of the protocol by the local Ethical Committee, 50 members of operating room staffs from eight different hospitals (seven in Belgium and one in France) accepted to take part in the study. The main characteristics of the study population are summarized in table 1.

The survey was carried out on selected days when no fluorinated anaesthetic other than sevoflurane was used. To assess external exposure of operating room personnel to sevoflurane, air samples were collected during the whole duration of anaesthesia by a passive sampling device (3M 3500 organic vapour monitor; St Paul, Minn., USA) attached to clothing within the breathing zone of each member of the medical staff. The sampler was eluted with carbon disulphide and the eluate analysed by gas chromatography with flame ionization detection (FID) on a capillary column CP SIL 5CB (length 25 m, internal diameter 0.32 mm, film thickness 1.2 μm) with an oven temperature maintained at 60 °C for 5 min. Under these conditions the detection limit was 0.1 ppm.

Urine was collected before (BA) and at the end of the anaesthesia period (EA) for HFIP, fluoride and creatinine determination. On the day of the sampling, all urinary samples were transported at +4 °C to the laboratory within 6 h. Aliquots of urine were kept at +4 °C for a maximum of 1 week for the determination of fluoride while others aliquots were frozen at –20 °C until analysis for HFIP and creatinine measurements.

Total HFIP in urine was determined after slight modifications of the method described by Morgan *et al*. (1994). Briefly 2 ml of 0.1 M sodium acetate buffer (pH 5), 10 μl of ß-glucuronidase (Sigma G-0876) and 50 μl of 2,2,3,3,3-pentafluoro-1-propanol solution (20 μl per 100 ml H_2O) (internal standard, Aldrich 25,747-8) were added to 1 ml urine sample in a 5 ml glass vial sealed with a teflon stopper. The samples were incubated at +37 °C overnight. One ml of the solution was then placed in a 20 ml head-space vial containing 1 g of sodium sulphate. Samples were thermostated 25 min at 85 °C before injection. For separation, a capillary column CP WAX 52 (length 25 m, internal diameter 0.32 mm, film thickness 1.2 μm) was used with FID detection. The column temperature was programmed from 70 to 160 °C at a rate of 10 °C min^{-1}, increased to 230 °C at a rate of 35 °C min^{-1}, and post-run oven temperature was kept at 230 °C for 3 min. Under these experimental conditions, retention times for internal standard and HFIP were 5.2 and 8.0 min, respectively (limit of detection, LOD: 0.5 mg l^{-1}).

Fluoride in urine was determined with an Orion® model 94-09 fluoride electrode. The laboratory regularly participates in the interlaboratory comparison programme organized by the Centre de toxicologie du Quebec (CHUQ, Quebec G1V 4G2, Canada). During the study period, the accuracy was between 89.3 % and 106.9 % (mean: 99.8 %) as compared with target values (LOD: 0.05 mg l^{-1}).

Creatinine concentration in urine was measured by the method of Jaffe (Narayanan and Appleton 1980).

Three *CYP2E1* polymorphisms, respectively polymorphisms c1→ c2 (Watanabe *et al*. 1990), D→ C (Uematsu *et al*. 1991) and A1→ A2 (McBride *et al*. 1987) were determined by restriction fragment-length polymorphism (RFLP) analysis on a 'first urine of the morning' sample as described elsewhere (Haufroid *et al*. 1998).

All statistical analyses were performed with the SAS package version 6.12. When the concentration of a parameter was <LOD it was assigned a value equal to half the LOD. The strength of the relationships between parameters was evaluated by Pearson's correlation. The *t* test used was one sided and the level of significance was chosen as 5 %.

Results

Ambient sevoflurane measurements

Concentrations measured in the breathing zone were very low when sevoflurane was used in closed circuit (table 2). Under these conditions only one result was above the LOD. This value (0.3 ppm) was observed with an anaesthetist nurse who

Table 2. External and internal exposure parameters.

	Closed circuit (n=6)	Open (semi-open) circuit (n=44)	Total (n=50)
Sevoflurane in air ppm	0.1 (ND–0.3)	21.7 (2.2–139.9)	19.0 (ND–139.9)
HFIP in urine[a]			
mg l^{-1}	ND (ND-ND)	7.4 (ND–58.8)	6.4 (ND–58.8)
mg g^{-1} creatinine	—	4.8 (0.2–24.8)	4.2 (0.2–24.8)
Fluoride in urine[a]			
mg l^{-1}	0.48 (0.23–1.17)	1.00 (0.17–3.00)	0.94 (0.17–3.00)
mg g^{-1} creatinine	0.31 (0.15–0.80)	0.74 (0.11–3.29)	0.68 (0.11–3.29)

Values are means (range), ND not detected (at or below the limit of detection).
Sevoflurane was detected in the breathing zone of 45 members of the staff.
[a] End of anaesthesia samples (EA).

was in the vicinity of the sevoflurane evaporator system during the whole anaesthesia period. In open or semi-open circuit, significant exposure was measured up to 139.9 ppm.

Urinary metabolite measurements

The quantification of low levels total HFIP in the urine of operating room personnel was possible using head-space GC/FID analysis. Figure 2(a) illustrates a typical chromatogram obtained from a pooled urine sample originating from individual samples collected directly after exposure to sevoflurane during anaesthesia. This pool was used as internal control during the study (8.6 mg l^{-1}). Figure 2(b and c) also shows a chromatogram obtained from a control urine (non-exposed individual) before and after spiking with 5.0 mg l^{-1} HFIP, respectively. The calibration curve was linear in the tested range of 0 to 162 mg l^{-1}.

As shown in table 2, total urinary HFIP concentrations ranged from ND (LOD 0.5 mg l^{-1}) to 58.8 mg l^{-1} for the whole study population. All results (n = 6) were below the LOD when sevoflurane was used in closed circuit. In contrast, in open (or semi-open) circuit more than 95% of the individuals (42 out of 44) had detectable HFIP levels in urine with a mean value of 7.4 mg l^{-1}.

Regarding urinary fluoride, in closed or open circuit, all individuals showed substantial concentrations (table 2) which may, in part, be explained by the fact that even in non-exposed personnel there is a background concentration of fluoride. Consistent with this observation all BA urinary samples showed fluoride concentration >LOD with a mean value of 0.60 mg l^{-1} (n = 50). In contrast only 14 % out of the latter samples had HFIP levels slightly >0.5 mg l^{-1}. For the rest of the study only EA samples were considered.

When HFIP and fluoride metabolites were plotted on the same graph and both expressed as μM g^{-1} creatinine, a positive correlation was observed with a slope of the regression line close to unity (figure 3) as would be expected from the equimolar formation of these two metabolites. The x-axis intercept value corresponds to a fluoride concentration of 5.7 μM g^{-1} creatinine (0.11 mg g^{-1} creatinine), a concentration which represents a normal background in the absence of exposure.

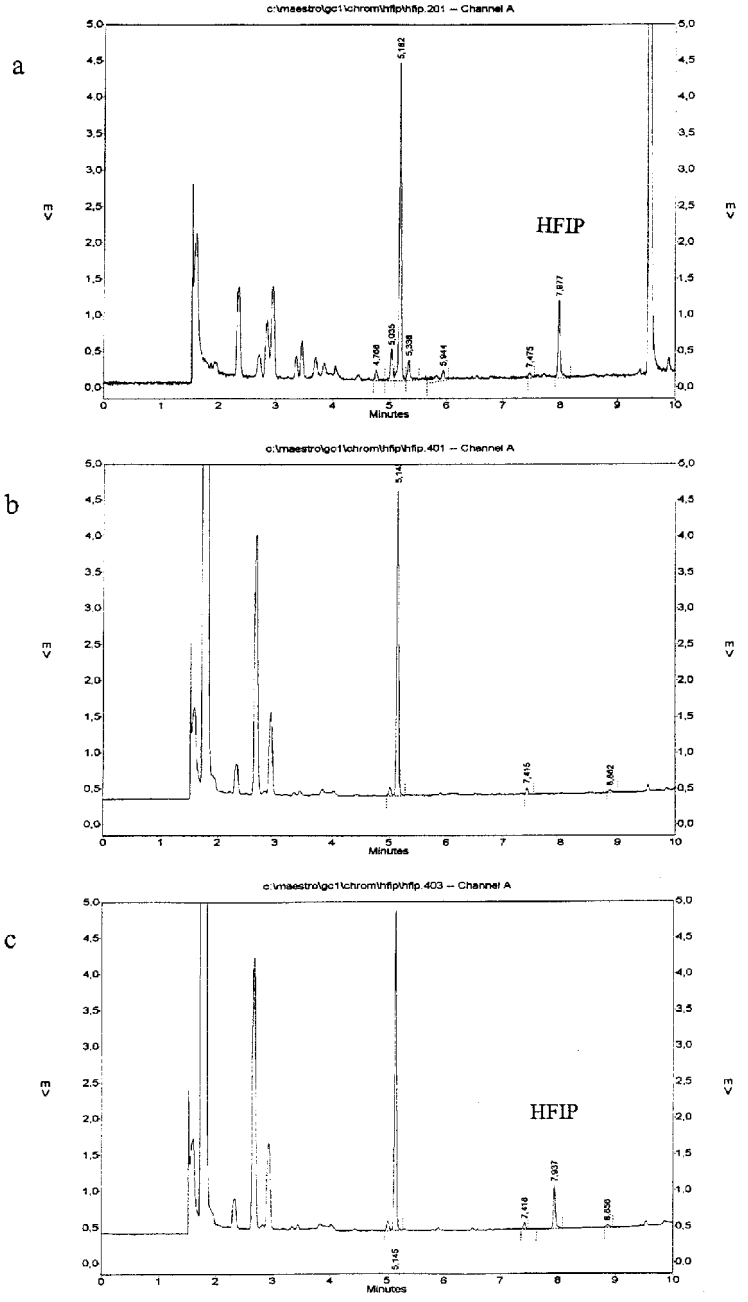

Figure 2. Chromatograms representing (a) a pool urine sample from occupationally exposed personnel (b) a control urine sample (non-exposed individual) and (c) urine b spiked with 5.0 mg l^{-1} HFIP.

Relationship between external and internal parameters

The urinary level of HFIP was closely correlated ($r = 0.81$; $p<0.0001$, not shown) to the sevoflurane concentration in the operating room. The HFIP axis intercept was at 0.11 mg l^{-1} which is below the detection level for the metabolite (LOD : 0.5 mg l^{-1}). A similar relationship was observed with fluoride although less

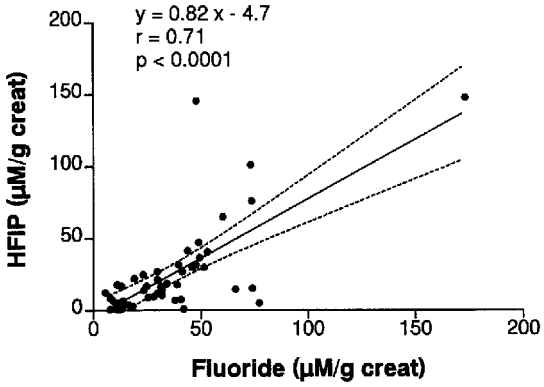

Figure 3. Relationship between the two metabolites (HFIP and fluoride) expressed as µM g^{-1} creatinine.

robust ($r = 0.56$; $p<0.0001$, not shown) and characterized by a background level (y-axis intercept: 0.64 mg l^{-1}) of the same order of magnitude as the majority of the experimental results.

When normalized according to urinary creatinine concentration correlations were not improved, neither for HFIP ($r = 0.78$; $p<0.0001$, not shown) nor for fluoride ($r = 0.41$; $p = 0.0031$, not shown). Another correction accounting for the duration of exposure (sevoflurane expressed as ppm x min) did not yield significant improvement of the relationship.

Because of the large distribution observed both in HFIP (expressed as mg l^{-1} or mg g^{-1} creatinine) and sevoflurane (expressed as ppm) values, a logarithmic transformation of the data was applied. As shown in figure 4 the logarithmic transformation improved the relationship between HFIP and sevoflurane ($r = 0.89$; $p<0.0001$).

Influence of sex and body mass index (BMI) on sevoflurane metabolism in vivo

To test the possible influence of these parameters on sevoflurane metabolism at low exposure level, a 'metabolization index' (defined as the ratio between urinary

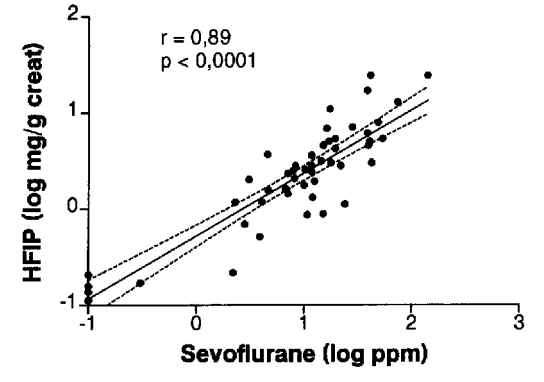

Figure 4. Relationship between airborne sevoflurane and HFIP.

HFIP expressed as mg g^{-1} creatinine and ambient sevoflurane expressed as ppm) was calculated for the 45 individuals who had experienced substantial exposure to sevoflurane (ambient level >LOD).

The results are presented in table 3. While no statistically significant difference was observed between both sexes, individuals with high BMI (>25) had a significantly higher 'metabolization index'than those with low BMI.

Influence of CYP2E1 genetic polymorphisms on sevoflurane metabolism

The distribution of polymorphic alleles in the study population is presented in table 4. Allele frequencies observed were similar to those observed by other authors for a Caucasian population (Kato *et al*. 1992, Persson *et al*. 1993, Stephens *et al*. 1994, Kim *et al*. 1995).

Based on the above described 'metabolization index' no statistically significant differences were observed between individuals homozygous for the wild alleles (c1c1, DD or A2A2) and the heterozygotes (c1c2, DC or A1A2). A slight but non-significant reduction of the metabolization indices was noticed for individuals with DC and A2A2 alleles compared with DD and A1A2 subjects, respectively.

Table 3. Influence of sex and BMI.

Variable	Individual status	Number (frequency)	Metabolization index (SD) *p* value
Sex	Male	*n* = 25 (56 %)	0.276 (0.194)
	Female	*n* = 20 (44 %)	0.254 (0.147)
			p = 0.332
BMI	<25	*n* = 35 (78 %)	0.227 (0.138)
	>25	*n* = 10 (22 %)	0.405 (0.219)
			p = 0.016*

SD: standard deviation.
BMI: body mass index.

Table 4. Cytochrome P4502E1 genotyping.

Polymorphism (location of polymorphic site)	Genotypic status	Number (frequency)	Metabolization index (SD) *p* value
c1/c2	c1c1	*n* = 43 (96 %)	0.267 (0.177)
(5′-flanking region)	c1c2	*n* = 2 (4 %)	0.265 (0.088)
	c2c2	*n* = 0 (0 %)	*p* = 0.492
D/C	DD	*n* = 38 (84 %)	0.275 (0.185)
(intron 6)	DC	*n* = 7 (16 %)	0.219 (0.071)
	CC	*n* = 0 (0 %)	*p* = 0.086
A1/A2	A2A2	*n* = 36 (80 %)	0.260 (0.150)
(intron 7)	A1A2	*n* = 8 (18 %)	0.313 (0.267)
	A1A1	*n* = 1 (2 %)	*p* = 0.302

SD : standard deviation.
Metabolization index = HFIP (mg g^{-1} creatinine) / sevoflurane (ppm).
Allele frequencies (*n* = 50): c1(0.98), c2(0.02), D(0.93), C(0.07), A2(0.88), A1(0.12).

Discussion

Some authors have assumed that scavenging of waste anaesthetic gases was now universally adopted and therefore that operating room staff members were no more exposed to the levels of waste gases that may affect other health professionals such as dental personnel (Mazze and Lecky 1985). In a study on occupational exposure to sevoflurane and nitrous oxide in operating room personnel, Hoerauf *et al.* (1997) concluded that when all recommendations for a modern working environment were followed, the trace concentrations measured were under the recommended NIOSH values (see below) in most, but not in all, cases. It must however be stressed that some particular utilization modes of halogenated anaesthetics, like in paediatric anaesthesia when a modified 'T piece' is used, lead to important contamination of the workplace atmosphere because scavenging of expired gases is more difficult to achieve. The assessment of occupational exposure seems therefore to be indicated in such circumstances. Up to now there is no TLV (ACGIH) or MAK (DFG) value for airborne sevoflurane. Only a very strict value of 2 ppm has been recommended by the NIOSH for all halogenated anaesthetics (NIOSH 1977) and a target level of 20 ppm as an 8-h time weighted average (TWA) is under consideration at the European level (Hall *et al.*1997). In this study, the NIOSH limit value was respected in all cases where sevoflurane was used in closed circuit. However the use of sevoflurane in open or semi-open circuit (i.e. patient exhaled air is released totally or partially in operating room) led to airborne concentrations largely exceeding the limit proposed by NIOSH. The detection of HFIP in 95 % of EA urine from the operating room personnel exposed when sevoflurane is used in open or semi-open circuit clearly indicates that these conditions allow significant exposure, raising the general problem of the efficiency of the scavenging systems used in operating rooms.

Following sevoflurane oxidation by cytochrome P450 monooxygenases, HFIP and fluoride are produced in equimolar quantities, which is confirmed by our results showing an equimolar concentration of both metabolites in urine. Furthermore our results are in line with those of Kharasch *et al.* (1995a) who had previously investigated the relationship between 24 h urinary excretion of HFIP and fluoride in a cohort of anaesthetized patients ($y = 1.18\ (x) + 2$; $r = 0.89$; $p < 0.001$). Although their values were one order of magnitude higher than ours, the slope of regression line close to the unity in both studies indicates that an extrapolation is possible from medical situations (anaesthetized patients) to occupational settings (operating room personnel).

In the light of the results of the present study the determination of HFIP in a spot urine sample obtained at the end of the anaesthesia period represents a reliable biomarker of occupational exposure to sevoflurane. Urinary fluoride which was previously proposed to assess exposure to sevoflurane (Hoerauf *et al.* 1997) is less useful. First, HFIP is more specific of sevoflurane exposure as illustrated by a lower background level near to the analytical detection limit and second a better sensitivity of this biomarker is expected in view of its rapid increase in urine when ambient concentrations of sevoflurane raise.

Based on the existence of a close relationship between ambient sevoflurane and urinary HFIP at the end of the anaethesia period, HFIP biological indices corresponding to different airborne sevoflurane concentrations have been determined. The values shown in table 5 are proposed as biological exposure indices to evaluate the contamination at the workplace. Compared with ambient

determination of sevoflurane, this approach is clearly more easy to implement in the operating room personnel and integrates several biological variability factors such as metabolism, absorption and excretion.

CYP2E1 has been identified as the main CYP isoform responsible for the metabolism of sevoflurane (Kharasch *et al* 1995b). Since obesity is a physiological condition well known to enhance CYP2E1 activity (Lieber 1997), we have tested the influence of this variable on sevoflurane metabolization rate in the study population. It was found that individuals with high BMI and therefore with conceivably higher CYP2E1 activity had higher 'metabolization indices' than individuals with lower BMI. Another variable of interest in this context would be chronic alcohol consumption. There were, however, no differences in reported alcohol consumption between participants and no objective measurements could be performed that would have allowed us to examine the influence of this potential confounder.

Moreover since several polymorphic sites have been reported in the human *CYP2E1* gene (Rannug *et al*. 1995), with a particular one located in the regulatory 5´-flanking region (c1/c2) of the gene that may affect its transcriptional regulation (Hayashi *et al*. 1991), it was therefore interesting to test the possible influence of these polymorphisms on sevoflurane metabolism. None of the subjects were homozygous for the rare allele (c2c2), and only two heterozygous individuals c1c2 were identified (table 3). This low frequency of the c2 allele (0.02) is in accordance with previous studies in Caucasian populations (Kato *et al*. 1992, Persson *et al*. 1993, Stephens *et al*. 1994, Kim *et al*.1995). No difference was observed in the 'metabolization index' between c1c1 and c1c2 individuals (table 3) but the low incidence of rare alleles in Caucasian populations would require a larger study population to confirm this result. As already observed by Kim *et al*. (1995), the presence of the c2 allele was always associated with the rare mutant DraI C allele (c2C haplotype) leading to individuals c1c2 *and* DC. However the latter mutation associated with the DraI RFLP is much more frequent than these in 5´-flanking region so that a particular haplotype c1C is also possible leading to individuals DC without any c2 mutation. Consistent with the observations of Kim *et al*. (1995) who found a lower chlorzoxazone clearance in DC heterozygous subjects compared with the homozygous wild-type genotype, DC heterozygous subjects in our study showed a lower 'metabolization index' than their DD counterparts but the difference was not statistically significant, probably because of the small size of the population studied. Interestingly, although more frequent, the presence of the mutant A1 allele was never associated with rare alleles c2 or C; individuals with the rare mutant A1 allele had always an associated c1D haplotype. Consistent with this 'reverse linkage', heterozygous A1A2 expressed a slightly higher 'metabolization index' than homozygous A2A2 but the difference was not statistically significant.

In summary, while this study clearly identifies an influence of BMI on

Table 5. Proposed biological exposure indices for sevoflurane.

Ambient Sevoflurane	2 ppm	20 ppm	50 ppm
HFIP			
mg l^{-1}	0.8	6.8	16.7
mg g^{-1} creatinine	1.2	4.3	9.6

sevoflurane metabolism, the possible impact of *CYP2E1* genetic polymorphisms, if any, appears relatively limited. Further studies testing phenotypic traits (such as the determination of chlorzoxazone clearance) will be necessary to assess the *in vivo* influence of CYP2E1 variations on the HFIP excretion rate following occupational exposure to sevoflurane in order to integrate genotypic characteristics and additional environmental influences.

Conclusion

The measurement of HFIP in urine at the end of the surgical procedure represents a good index to assess occupational exposure to sevoflurane. So far HFIP determination constitutes only a biomarker of *exposure* which can be implemented on a routine basis to monitor exposure of the medical staff to this gas. It could also be used intermittently to verify the efficacy of new scavenging devices and ventilation systems in operating rooms. Future studies aiming to investigate a health-based limit value may use HFIP determinations in urine as a specific marker of exposure.

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