# Dual-nuclei $^{19}$F-$^{1}$H MRI for studying administration and clearance of perfluorooctane in rat lungs

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## PURPOSE

Total Liquid Ventilation (TLV) is a liquid breathing procedure with high capacities of transport and diffusion for respiratory gases. One application is fast hypothermia using liquid perfluorocarbons (PFC) that has been shown to be cardioprotective [1]. TLV procedure includes a filling phase, a ventilation per se, and a final clearance phase during which PFCs are eliminated from the lungs by evaporation or endocytosis. For potential clinical transfer of such protocol, tolerance is crucial. Fluorine imaging of the lung has been proposed for gas imaging [2], or in the context of partial liquid ventilation [3]. In a previous work, using dual-nuclei MRI on a clinical system, it was shown *post-mortem* in pigs that perfluorooctane (PFOC) is better tolerated than other PFCs [4]. Here, we present the first *in vivo* evaluation in rats of the fast PFOC clearance from the body after lung administration.

## METHODS

***Administration procedure:*** 6 Wistar rats (male, 6/7 weeks old, 180-200g) were anesthetized (2,5% isoflurane, 1L/min $O_2$). They were held still in the MRI in the prone position using a dedicated crib and body temperature was maintained with hot water circulation. Respiration was monitored (SA Instruments Inc., NY, USA). 5mL of PFOC ($C_8F_{18}$; Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) were aerosolized using an ultrasonic nebulizer (Aerogen, Galway, Ireland) located close to the rat nose in the anesthetic input line. Nebulization lasted 5 to 10 min. Rats were euthanized in the end of the imaging session.

***MRI experiments:*** Experiments were carried out at 1.5T (Philips, Best, the Netherlands) with a multi-nuclei imaging system. A transmit-receive 47-mm diameter circular dual-resonant $^{19}$F/$^{1}$H coil was built in-house, allowing imaging the entire rat chest region. The $^{1}$H mode was used for scout imaging and anatomical localization. A 3D FFE sequence was performed with parameters: TR=6.1ms, TE=2.7ms, 20º flip angle, FOV=128x128mm², resolution=1x1x5 mm$^3$, 14 slices. The $^{19}$F acquisition protocol consisted of the following: 1) 5mL of PFOC were nebulized, dynamic MR-spectra were acquired during the administration phase, the nebulization was stopped, dynamic spectra were acquired during the clearance phase. 2) 5mL of PFOC were nebulized again, dynamic MR-spectra were acquired during the administration phase, nebulization was stopped followed by a 3D FFE sequence during clearance. MRS parameters were as follows: free induction decay acquisition after a non spatially-selective excitation, TE=0.09ms, 90º flip angle, 16 signal averages, 32 dynamic scans. 3D FFE sequence parameters the same as in the $^{1}$H mode with 128 signal averages. The selective RF pulse had a bandwidth of 1828Hz allowing to image PFOC main peak only within ±15 ppm span.

***Data processing:*** The acquired spectrum contained 2 regions (Fig.1a), resonance at 0 ppm corresponding to isoflurane and PFOC ($CF_3$) and resonance at -40 ppm (PFOC-specific peak corresponding to $CF_2$ resonances). The area under the curve was computed for each region for all dynamic scans and plotted as a function of time. The temporal evolution was analyzed by estimating 4 parameters (Fig.1b): delay between administration start and PFOC arrival $t_{delay}$, time-to-peak $t_{to\_peak}$, peak value $S_{peak}$ and $t_{1/2}$ corresponding to a signal reduction by half to characterize clearance. $^{19}$F images were superimposed to $^{1}$H images considered as anatomical reference, which allowed locating $^{19}$F signal.

## RESULTS

The rats survived the whole experimental protocol without noticeable change in respiratory rate during PFOC nebulization indicating a good tolerance of the procedure. $^{1}$H and $^{19}$F image superposition is shown in Fig. 2. Before PFOC administration, there is a small $^{19}$F signal coming from isoflurane present in fat-regions. After PFOC nebulization, at the beginning of the clearance phase, the PFOC uptake appears scarcely in the lungs but in large amounts in fat, especially in the ventral area (Fig. 2). Typical MRS data are shown in Fig.1, displaying a delay before PFOC signal is measured ($t_{delay}$= 2.32±1.29 min), an increase of PFOC reaching a peak ($t_{to\_peak}$= 4.78±1.49min), followed by a clearance phase ($t_{1/2}$=11.86±1.43min). The time from peak to $t_{1/2}$ was thus 4.76min indicating a fast clearance from the body.

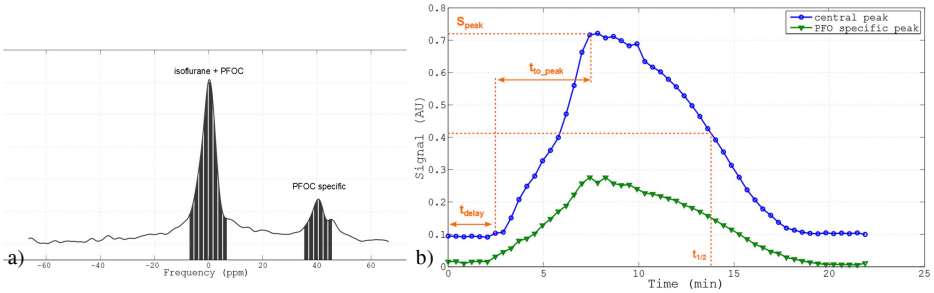


**Figure 1**: MRS for PFOC wash-in and wash-out (a) Spectrum after PFOC administration. Striped surfaces are selected for computing the area under the curve. (b) Spectrum trough time showing the rise of the signal during PFOC administration and the decrease of the signal during the clearance phase. 4 values were estimated on the curve to characterize the PFOC kinetics.

## DISCUSSION AND CONCLUSION

It was shown that nebulized PFOC accumulate in tissue, in particular fat regions, similarly as isoflurane [5]. After nebulization start, a short delay of ~2.3min is needed to be able to measure PFOC signal in the chest region. Signal then increases until the end of nebulization, and is rapidly cleared from the body in ~5min. The nebulizer produces small particles with diameters in a scale of 1µm. It is possible that PFOC particles reach the alveolar region as small particles, or already vaporized. PFOC particles or gas in the lung were not detectable, either because of a low concentration or due to unfavorable relaxation parameters of PFOC within the lungs. A transfer to circulation and accumulation in tissue around the chest were then observed. This accumulation is probably systemic, and in the form of dissolved PFOC gas. Once administration is stopped, fast clearance is observed, compatible with lung elimination due to a progressive reduction of PFOC alveolar partial pressure. These preliminary *in vivo* experiments provide elements on PFOC pharmacokinetics showing fast clearance from the body, predominantly through respiration. This observation is in favor of its use in total liquid ventilation, confirming previous *post-mortem* tolerance studies [4].

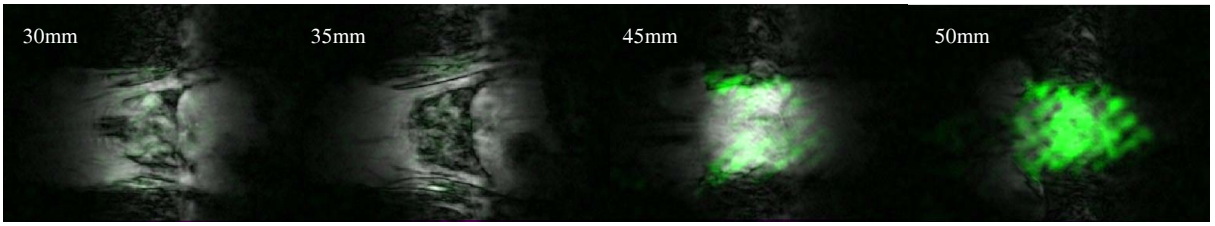


**Figure 2:** Superimposed $^{19}$F (in green) and $^{1}$H images at different slice locations acquired during the clearance phase. It shows how the PFOC is scarcely spread inside the lungs whereas it is found abundantly in fat tissue.

## REFERENCES

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