

Chemical Considerations Regarding the H-Value Methodology and Its Relation With Toxicity Determination

We are writing about the recently published paper “How to ward off retinal toxicity of perfluorooctane and other perfluorocarbon liquids?” by Menz et al.¹ The article is of great interest to us and other vitreoretinal surgeons because of the huge number of cases in which blindness has occurred after vitreoretinal surgery during which perfluorocarbon endotamponades were used. The perfluorocarbon (PFO) liquids were manufactured by different companies, one German, one Indian, and one Turkish. The existence of these toxic compounds and the insufficient control by European Union authorities were also part of a report made by the International Consortium of Journalists that was released in many mass media outlets in Europe in December 2018, causing alarm among patients and ophthalmologists.

In their article, Menz et al.¹ reported an analytical method to determine the concentration of incompletely fluorinated compounds assuming that its presence is the cause of cytotoxicity, increasing in a dose-dependent manner. We found several aspects that need clarification in the referenced paper. The basis of any scientific publication is that the analytical procedures should be easily reproduced in any laboratory in the world. Thus, an essential feature of any published report is the absolute clarity and accuracy of the methodology. In this sense, we have some issues about the methods, as detailed below.

When a digestion process to cleavage C-F bonds from a complex molecule is performed, it is important to calculate the yield of the reaction to ensure that all of the fluoride ions are extracted during the digestion. In the reported experiments, the yield was not calculated, nor was it reported in the literature cited in the references.^{2,3} The reaction was not evaluated to ensure that the three fluoride ions were always released. Further, it is doubtful that the amine used in the protocol could easily break the C-F bonds. This is an important issue because the stoichiometric factor 1/3 is used in the equation to calculate the concentration of incompletely fluorinated contaminants.

Moreover, as the methodology is described, it seems to be specific to those compounds with only one or two C-H bonds in the perfluorocarbon molecule. Other contaminants, such as the -COOH (acid group), with high toxicity have been found in the toxic PFO samples, producing cases of severe and acute toxicity in patients.⁴ If these specific toxic contaminants cannot be identified with the current protocol, it is a major limitation of the procedure and should be reported and detailed.

The method also does not detail the molecular weight and density that the authors used in the equation. It is important to know if the authors measured them from the tested sample. If so, then they should have reported how both parameters (weight and density) were measured. Alternatively, the authors could have used the values for the fully fluorinated perfluorooctane (438.06 g/mol and 1.77 kg/L).

Moreover, according to the article, 10 ppm was the toxicity detection limit, which is the most important parameter described. Therefore, to validate this parameter,

all details of how the detection limit was calculated should be disclosed.

Finally, the authors tested the dose dependency of the H-value and cell growth inhibition (Table 5) by adding different amounts of an ultra-purified sample (with H-value <10 ppm) into the toxic batch 061014 sample (with an H-value of 2800 ppm, according to the authors). However, in doing so, it is clear that the so-named H-value (related to the incompletely fluorinated compounds) will be reduced. In the same way, the concentration of other toxic compounds not related to the incompletely fluorinated ones and present in batch 061014 also will be reduced to the level of nontoxicity (Fig.). To verify that toxicity is really caused by incompletely fluorinated contaminants, the dose-dependency experiment must be done by adding one-by-one or a mixture of incompletely fluorinated compound standards into the sample. As the experiment was described in the article, other toxic compounds not related with the incompletely fluorinated compounds could influence the results. Examples of such compounds include hydroxyl groups (-OH), perfluorooctanoic acid, and/or dodecafluoro-1-heptanol, which have been identified in this same AlaOcta batch 061014.⁵ Furthermore, the real relationship between the so-named H-value and cytotoxicity is an issue still not proven. In our opinion, this is a crucial point and one of the weaknesses of the paper.

The analytical method to determine the concentration of incompletely fluorinated compounds was developed earlier than 1990, as referenced by Groß et al.² However, none of the references provided by the authors detail the H-value as part of the chemical analysis itself, or how the assays were performed and verified. Importantly, with this technique, only fluoride ions are measured, not hydrogen, which is the important element. Currently, there are other well-known analytical techniques, such as nuclear magnetic resonance,^{6,7} to determine and quantify hydrogen in the samples. This technique is easier to perform and more precise due to the absence of any pre-analytical preparation of the sample, small amount of sample required, sample recovery, rapidity, and, finally, all hydrogen atoms in the samples can be analyzed and quantified for.

In summary, we believe that the analytical method described by the authors does not provide enough scientific evidence to be proposed as a reference for safety of the perfluorocarbon liquids use in intraocular surgery.

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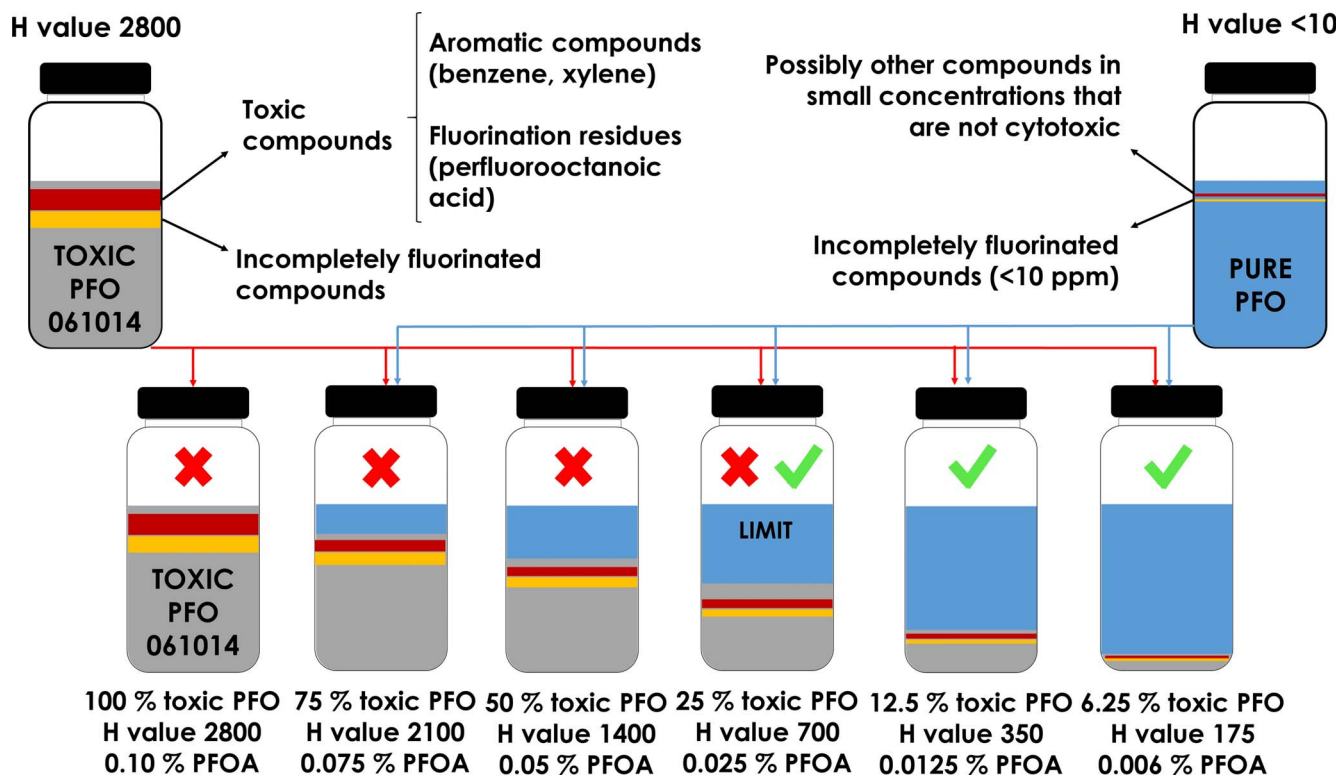


FIGURE. Graphic interpretation of the procedure followed by Menz et al.¹ to establish the dose dependency between H value and cytotoxicity. Different amounts of “pure” PFO with a H-index of 10 ppm were added to the original AlaOcta toxic sample lot 061014. By doing that, H-index was reduced, but also the concentration of the contaminants not related to incompletely fluorinated compounds present in the above-mentioned lot.

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