Comments on “A Simple, Rapid, Comparative Evaluation of Multiple Products for Decontamination of Actinide-contaminated Rat Skin Ex Vivo “

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Title: Comments on "A Simple, Rapid, Comparative Evaluation of Multiple Products for Decontamination of Actinide-contaminated Rat Skin *Ex Vivo* "

Dear Editors,

This letter is regarding the paper written by Griffiths et al. (Griffiths et al., 2022). We read with interest this article describing a simple and rapid test to compare the efficiency of various products to remove cutaneous contamination by actinides with a rat skin *ex vivo* model.
We would like to draw your attention to some experimental biases in the work of Dr Griffiths and her co-authors that may mislead the interpretations of the published results.
According to our analysis of this paper, we have 3 main remarks.
Our first and main remark relates on the difference in the modalities of treatments application on the skin after the contamination step:
Number of treatment applications: According to the materials and methods section and Table 1, liquid products (water, DTPA 5 mM, or prediluted Trait Rouge) were applied three times on the contaminated area (3 × 0.5 mL) whilst other products (calixarene nanoemulsion, osmogel, hyaluronic acid or wound dressings) were applied once and rinsed three times by water wash (3 × 0.5 mL H_2O). Thus, the performances of these liquid products could be overestimated and attributed to an undue greater number of applications compared to other products (gels and wound dressings).
Volume/amount of treatment: various volumes of treatments have been used in this study without explanation about these differences (0.5 mL for liquid treatments, 0.1 g for calixarene nanoemulsion, 0.3 g for gel products (osmogel, hyaluronic acid and algoplaque paste), 1 g for French green clay).
Treatment application on dry or wetted skin: It was not clearly stated in the Figures 2 and 3 whether the different products were applied on wetted skin or dry skin. Skin wetting seems to be crucial condition for gel or low fluidity products (such as calixarene nanoemulsion, osmogel, hyaluronic acid or dressings) as mentioned by the authors themselves, and probably less so for aqueous products (water, DTPA, prediluted Trait Rouge). According to the results mentioned in the discussion section and focused on the comparison of the efficacy between Trait Rouge and calixarene nanoemulsion treatments and cited hereafter:
"Similarly, for Pu 17.3 ± 3.1 % remained in skin after Trait Rouge treatment and 15.6 ± 2.1 % (dry skin) or 2.8 ± 1.2 % (wetted skin) after calixarene nanoemulsion",
it seems that the results of Figure 2 refer to application of calixarene nanoemulsion on dry skin, despite the protocol described by the authors in Material and Methods section: "the manufacturer's instructions (Cevidra) were followed: the contaminated skin area was wetted with 100 µL water and the calixarene nanoemulsion (100 mg) was added".
Consequently, we believe that the differences in application conditions (different volumes and amounts of each product, number of applications, wetted or dry skin) makes the comparison of the published results dubious. This clearly limits the interpretation of these results. Since the applications

differ from fluid products (water, DTPA, Trait Rouge) to less fluid products (Osmogel, Calixarene, wound dressings), the statistical analyses and comparison presented in Table 2 are irrelevant. The authors should have used other tests such as non-parametric or ANOVA tests, or paired Student t-test for products of the same category (fluid or less fluid).

Secondly, the proposed ex vivo model of the Griffiths' study may be simple and rapid but does not comply with the Association Française de Normalisation (AFNOR), which recommends the use of Franz diffusion cells and porcine skin which is a better surrogate for human skin in order to assess the performance of "nuclear, radiological, biological, chemical" decontamination products on intact skin (AFNOR 2016). Yet, the authors have already used such protocols in their previous studies (Tazrart et al. 2018) but this was not mentioned in the discussion. Franz diffusion cells are relevant for the assessment of the potential diffusion of contaminants through the skin when the actinides are deposited for a long time such as 2h.

Our last remark concerns the citation of the reference Grives et al 2017 (Grivès et al. 2017) at page 9. The authors have referenced the wrong paper and have misinterpreted the results. In the referenced study, contaminations were performed in vivo on wounds model in the rats and not on intact skin as in the present study. The authors probably confused it with the paper Grives et al 2015 (Grives et al. 2015).

To conclude, the authors should reinterpret the results of the experiments in which the products were applied in the same conditions and clearly state that the application conditions are not completely comparable. Otherwise, the authors could perform supplementary tests, ideally on Franz diffusion cells for fair comparison purpose (for example Trait Rouge and calixarene nanoemulsion applied both not diluted and in the same amount/volume on dry skin or wetted skin, or dilute 1:1 both products Trait Rouge and calixarene nanoemulsion before their application).

We thank you for taking into consideration our comments and suggestions.

Sincerely,

Dr Céline Bouvier-Capely and Dr Guillaume Phan,

Fontenay-aux-Roses, January 24th 2022

The authors declare to have developed the calixarene nanoemulsion with financial support of Direction Générale de l'Armement (DGA), Centre National de la Recherche Scientifique (CNRS) and Institut de Radioprotection et Sûreté Nucléaire (IRSN). This product has been patented and is now commercialized by Cevidra Laboratory.

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