Toxicological Evaluation of the Organic Phase Resulted from the COSORB Process

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Abstract
In this work, the toxicological assesment of the organic phase resulted from the COSORB process was performed. For this purpose, an animal model was used to evaluate the effects of the organic phase on skin physiological parameters after topical application to SKH-1 hairless mice. The obtained results revealed that the constituents of the organic phase induce skin toxicity by disturbing the physiological skin parameters status, which represents the first signs of skin pathology.

Introduction
The COSORB process involves a selective elimination of carbon monoxide by means of complexation/decomplexation of CO on a specific catalyst, in an appropriate aromatic solvent. [1] By this process, it is possible to complexate and recover more then 99% of the CO content [2].

The calalyst is a bimetalic complex of $Me_IMe_{II}X_n$ type, where Me_I is often Cu(I), and M_{II} is Al(III), X being a halogen, such as chlorine. During the process, the catalyst is involved in several complexation/decomplexation processes, resulting finally in a partialy poisoned catalyst due to the accumulation of some sulfures and/or other secondary alkylation or polymerisation products. The disposal of the used catalyst involves several environmental risks, due to its high metal content, and the recovery and subsequent use of these metals presents a great deal of interest. Thus, several processes of Cu and toluene have been developped [3-8]. All of these technologies have advantages and disadvantages, and more or less specific shortcomings, their efficiency being strongly dependent by the type of compound. However, none of the technologies can remove all of the compounds from wastewaters.

Based on these considerations, it seems that the developpement of an unitary technology able to recover all of the usefull materials from the used catalyst should be of great interest. Moreover, the toxicological evaluation of all stages involved in such a technology will bring more added value to this technology.

Experimental
The analysis of the organic phase was carried-out by means of gas chromatography/ mass spectroscopy (GC/MS), using a Hewlett Packard Gaz Chromatograph HP 6890 associated with a Mass Spectrometer HP 5973. The GC column was of ZB-5MS type, and had a 30m×0.25mm inner diameter and a film thickness of 0,25 μm. The stationary phase was a mixture of 95% dimethyl siloxane and 5% phenyl-aryilene. The column temperature program was of 6 ^{o}/ min, in the temperature range of 50-300°C.

The animals used in the present study were SKH1 hairless male mice (12-14 weeks-old) purchased from Charles River Laboratories, Budapest, Hungary. All experimental procedures were conducted in accordance with the Directive 2010/63/EU on the protection of animals used for scientific purposes. The experimental protocol was approved by the Committee for Ethics Research of the University for Medicine and Pharmacy of Timisoara, Romania. Animals were fed ad libitum and kept under standard conditions: constant temperature of 22.5 ± 2° C, humidity 55 ± 5% and a 12-h light/dark cycle.

In order to accomplish the present study, the mice were divided in 2 groups (n=5/mice group): group 1 - control group – no interventions were applied; group 2 – the mice were treated with the organic phase solution (100µl) which was applied on the dorsal area twice a week for 5 weeks.

For the evaluation of skin response to organic phase effect, we measured several physiological skin parameters (erythema and TEWL – transepidermal water loss) by the means of a non-invasive technique (mexametry and tewametry) using MPA5 System from Courage-Khazaka.

Results and discussion

In this work, the toxicological evaluation of the organic phase resulted from the COSORB process was carried-on, using an animal model.

The content of the organic phase was evaluated by GC/MS and the results are illustrated in Table 1. The percentage composition of the organic phase reveal the presence of 84,46 % toluene (RT = 1,9), 10,1% oxydation products of toluene (RT=21-23 derivatives of bis-(methyl-phenyl) ketone, RT= 28-32 derivatives of trimethyl tritil alcohol), 5,3% xylenes (RT = 3-4) and 0,1% benzene (RT = 1.3).

Table 1. The content of the organic phase resulted from the COSORB process

Nr.	Retention time (RT)	Peak area	Area %
1	1,323	59577	0,081
2	1,946	62040506	84,466
3	2,632	196840	0,268
4	2,832	256827	0,35
5	3,021	1320320	1,798
6	3,346	359668	0,49
7	4,318	156802	0,213
8	4,455	165834	0,226
9	21,659	704812	0,96
10	22,407	190586	0,259
11	22,882	543778	0,74
12	23,316	1284443	1,749
13	23,665	124753	0,17
14	28,146	146443	0,199
15	28,734	392714	0,535
16	29,317	393223	0,535
17	29,872	141317	0,192
18	30,083	354484	0,483
19	30,729	1807869	2,461
20	31,369	2215855	3,017
21	31,992	593709	0,808

As it can be seen from the results depicted in Table 1, toluene is the major constituent of the organic phase resulted from the COSORB process, and it is known that this volatile organic compound, affects the central nervous system, as well as the heart, but its noxious effects at skin level are not fully elucidated [9].

Further, the effects of this organic phase were investigated at skin level, after topical application on SKH1 mice, as described in Experimental part. The evaluation of these efects were carried out by means of non-invasive methods, e.g. tewametry and mexametry. The main reason for our choice was the fact that hairless mice represent a great tool for the evaluation of the changes that occur at cutaneous level, changes that can be associated with the application of a toxic or a new drug in the testing phase or after absorption of some substances that possess the capacity to modify skin parameters [10,11].

Moreover, Tewametry and mexametry are established non-invasive methods, used very frequently in the diagnostic of skin pathologies both in humans and in *in vivo* experiments [12, 13].

One of the skin parameters measured in this experiment using a Mexameter probe was the erythema value.

The topical application of the organic phase to the SKH1 mice led to relevant changes regarding the skin parameters evaluated. Our results showed that the application of the test solution induced a significant degree of erythema as compared with the control group (see Figure 1).

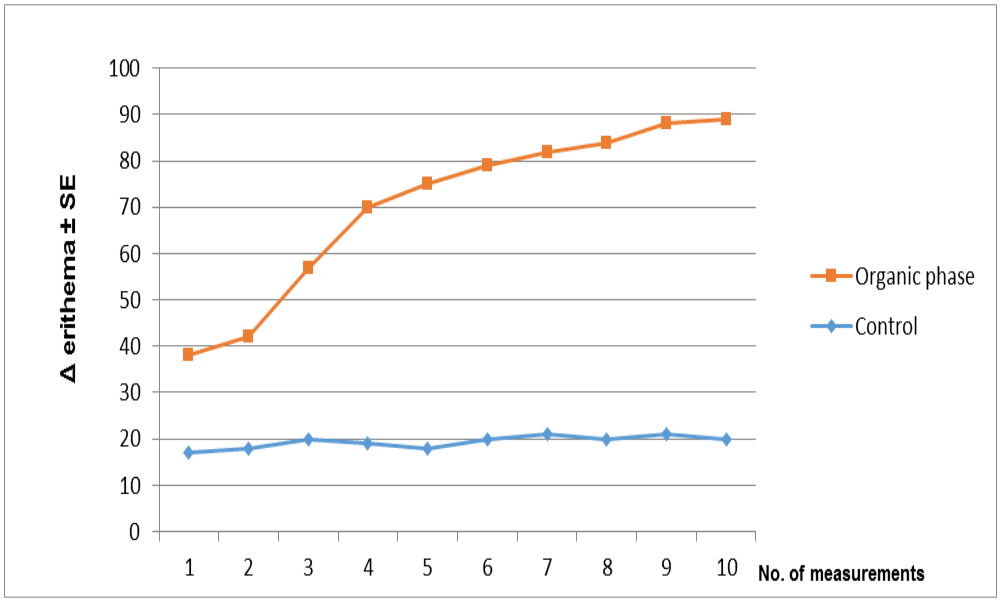

Figure 1. Control group and organic phase group - topically applied (data are expressed as differences ± SE). The units are arbitrary.

An increase was also observed in the values measured for transepidermal water loss (TEWL) in the group treated with the test solution in comparison with the control group, as illustrated in Figure 2.

TEWL value is considered an index for the estimation of the degree of injury of skin barrier and it measures the rate of skin water loss (it is expressed in g/hm^2) [14]. An increased value of TEWL indicates an injury or a skin pathology [15].

Our results showed that the TEWL values were higher in the group that was topically treated with the organic phase what indicates a noxious effect of this solution at cutaneous level.

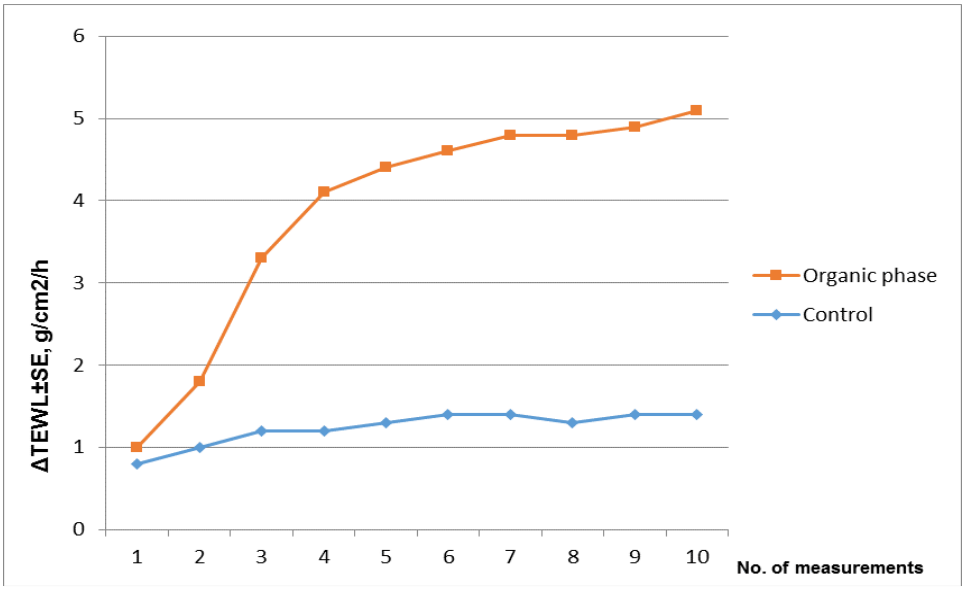

Figure 2. Transepidermal water loss (TEWL) evaluation: Control group and Organic phase group - topically applied (data are expressed as differences ± SE). The units are arbitrary.

Conclusion

Our preliminary results indicate that the constituents of the organic phase induce skin toxicity by disturbing the physiological skin parameters status, which represents the first signs of skin pathology. Further studies are required in order to elucidate the mechanism involved.

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References

[1] A. Keller, R. Schendel, AICHE Summer Meeting, August 23, 1988.
[2] F. Rahmani, M. Bayar, M. Haghighi, M. R. Rahimpour, Iranian J. Chem. Eng. 7(3) (2010) 29.
[3] R. B. Long, H. H. Horowitz, D. W. Savage, US 3754047, 1973.
[4] D. G. Walker, B. Haase, D. J. Haase, US 3845188,1974.
[5] J.R. Sudduth, D. A. Keyworth, US 3960910,1976.
[6] D. A. Keyworth, J. R. Sudduth, US 4153669, 1979.
[7] C. P. Christenson, G. M. McNamee, R. D. Delaune, Method of disposing if spent organic complexing solutions containing couprous halides, US 4249939, 1981.
[8] I. B. Plecas, S. Dimovic, Bull. Mater. Sci., 27(2) (2004) 175.
[9] A. Saito, H. Tanaka, H. Usuda, T. Shibata, S. Higashi, H. Yamashita, N. Inagaki, H. Nagai, Environ Toxicol. 26(3) (2011) 224.
[10] C. Danciu, D. E. Coricovac, C. Soica, V. Dumitrascu, G. Simu, D. Antal, K. Lajos, C. A. Dehelean, F. Borcan, Rev. Chim. (Bucharest) 65(10) (2014) 1195.
[11] D. Minda, D. Coricovac, I. Pinzaru, C. Dehelean, F. Borcan, D. Muntean, Physiology 25.3 (87) (2015) 25.
[13] B. Nedelec, N. J. Forget, T. Hurtubise, S. Cimino, F. de Muszka, A. Legault, W. L. Liu, A. de Oliveira, V. Calva, J. A. Correa, Skin Res. Technol. 2015. doi: 10.1111/srt.12256.
[14] Y. Cheng, Y. Y. Dong, M. X. Dong, C. Wang, N. Su, Y. T. Sun, J. Liu, H. Y. Zheng, A. Schrader, M. Rohr, W. Liu, Skin Res Technol. 14(1) (2008) 45.
[15] C. Rosado, P. Pinto, L. M. Rodrigues, Int. J. Cosmet. Sci. 27(4) (2015) 237.