ORIGINAL ARTICLE

EVALUATION OF THE IMPACT INDUCED BY THE ENVIRONMENTAL EXPOSURE TO A MIXTURE OF ORGANIC SOLVENTS ON SKH1 MICE

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Abstract

The present study aimed to evaluate the local and systemic acute effects associated with the environmental exposure to a mixture of aromatic organic compounds resulting from the COSORB process. The experiment was performed on SKH1 hairless male mice according to the following experimental design: exposure for 30 minutes/day/5 days to the organic mixture. To assess the potential toxicity, were verified several parameters: mice weight, skin physiological parameters using a non-invasive method and the muscular strength by the means of the inverted screen test. The test group of mice (the mice exposed to organic mixture) exhibited weight loss, changes in the skin parameters values and muscular weakness. Altogether, our results showed that the environmental exposure to the organic mixture was associated with noxious effects both at skin and systemic levels.

Rezumat

Acest studiu a avut ca și scop evaluarea efectelor acute locale și sistemice induse de un amestec de solvenți organici aromatici rezultați din procesul COSORB. Experimentul s-a realizat pe șoareci fără păr SKH1 masculi conform următorului protocol de lucru: animalele au fost expuse la amestecul de solvenți organici zilnic, timp de 30 minute/zi, pe o perioada de 5 zile. Pentru a evalua potențialul toxic, au fost evaluați următorii parametrii: greutatea șoarecilor, parametri fiziologici ai pielii cu ajutorul unei metode neinvazive și performanțele musculare, cu ajutorul testului *inverted screen*. Grupul șoarecilor test (cei expuși la amestecul de solvenți organici) au prezentat scădere în greutate, modificări ale valorilor parametrilor fiziologici ai pielii și slăbiciune musculară. În ansamblu, rezultatele obținute arată că expunerea la componenții prezenți în amestecul de solvenți organici a determinat efecte nocive, atât la nivel cutanat, cât și la nivel sistemic.

Keywords: COSORB process, toluene, SKH1 mice, inverted screen test

Introduction

The COSORB process is a selective and low cost solvent extraction process, which provides an almost complete recovery of carbon monoxide of high purity [10, 19]. During the process, carbon monoxide (CO) is adsorbed and desorbed under mild conditions in a toluene solution onto cuprous aluminium chloride ($CuAlCl_4$). By this process, it is possible to obtain a recovery of carbon monoxide higher than 96%. These characteristics make this process as one of the most valuable sources of carbon monoxide in the case of downstream manufacture of chemicals and pharmaceuticals. Among the downsides of this process it could be mentioned the following: the partially poisoning of the copper catalyst, as well as some environmental risks which are associated to the disposal of the used catalyst, and the resulting organic and inorganic phases [10, 19].

It is well known that acute or chronic exposure to aromatic organic solvents such as toluene and its oxidation derivatives, xylenes and benzene can harm humans and animals, as well as the environment. By instance, the primary target organ for toluene and its oxidation derivatives toxicity is the central nervous system, whose dysfunction and narcosis were observed for both acute (short-term) and chronic (long-term) exposures. Among the symptoms associated to theses intoxications, there are: sleepiness, headaches, fatigue, dizziness, and nausea [15]. Several studies denoted effects as cardiotoxicity, nephrotoxicity, hepatotoxicity, irritation of eyes and the upper respiratory tract, sore throat, skeletal muscle damage associated with the exposure to aromatic organic solvents [3, 4, 9, 30].

Mice models represent valuable tools for experimental investigations in the biomedical and toxicological research fields. Among the main reasons for the use of

mice as animal models, is the fact that mice share substantial similarities with the human physiology and pathology, so their use allows the investigation of numerous diseases development, as well as their progression and further, the testing of some novel therapies [8, 17, 22, 23, 25, 28, 33]. There are several mice strains which present interest in the research field as experimental models. SKH1 is a corresponding hairless strain that is indicated for multiple applications, including the acute toxicological evaluations after external administration of toluene based formulations [12, 27]. The age of mice plays a significant role regarding the pre-carcinogenic potential of different chemical agents after external administration [14].

The main aim of the present work was to assess the impact induced by the environmental exposure to a mixture of aromatic organic compounds resulting from the COSORB process on murine organism by using SKH1 male mice. According to these considerations, toxicological studies of the organic phase mixture could offer valuable information regarding its impact on human and environmental safety. It was evaluated the possible neurotoxic effect associated with the external exposure by skeletal muscle weakness observation and, also, the physiological skin parameters by the means of an established non-invasive method.

Materials and Methods

The organic phase composition obtained during the COSORB process and used in the present study was described in our previous work [27]. In brief, the components of the organic phase mixture were: 84.46% toluene, 10.1% oxidation products of toluene, 5.3% xylenes and 0.1% benzene. The test animals, SKH1 adult (26 - 28 weeks old) male mice were purchased from Charles River, Budapest, Hungary and were allowed to accommodate to our laboratory conditions.

Design of the experimental procedure: SKH1 male mice were divided in 2 groups (n = 6 mice/group), as follows: control group - no intervention was applied; test group - mice exposed to organic phase - 30 min/day - daily for 5 days (under sub-chronic interval). There were respected all conditions imposed by NIHA and the Directive 2010/63/EU regarding the protection of animals used for scientific purposes, as well as the UMFVBT rules: humidity 55 ± 5%, 12 h/12 h light/dark cycle, a constant and normal temperature 22.5 ± 2°C and food and water *ad libitum*. Animal's exposure to the organic phase mixture was realized in a special assembly (Figure 1).

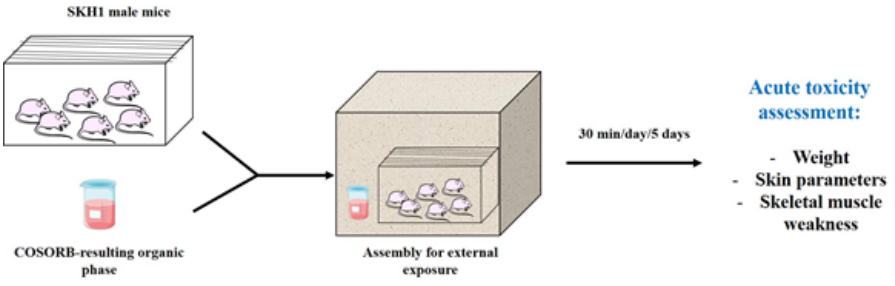

Figure 1.
Design of the experimental procedure

The experimental procedures were approved by the Committee for Ethics Research of the University for Medicine and Pharmacy of Timişoara, Romania.

The main observations evaluated consisted of: daily measurements of mice weights during the experiment, external modifications at skin level: skin colour (melanin, erythema or redness) and water content and, also, the muscles strength. The skin physiological parameters verified were: melanin, erythema and skin hydration and were measured at different time points (0, 1, 24, 48, 72, 96 and 120 h).

Non-invasive skin parameters measurements: The skin parameters were measured in a minimally invasive way using the devices from Courage-Khazaka, Germany: an electronic Skin Colorimeter CL 400 and the Corneometer® CM 825. The Skin Colorimeter CL 400 principle relies on the tri-stimulus colorimetry and uses the *Commission Internationale de l'Eclairage* (CIE) L*a*b* colour system to determine skin colour modifications. The colour is expressed using the 3-digit output L*a*b*, where: L* measures skin reflectance or lightness (a grey scale with values ranging from 0 to 100 where 0 is black and 100 is white); a* measures the colour saturation from red to green (scale from +60 to -60, where positive values indicate varying intensities of red); b* measures the colour saturation from yellow to blue (scale +60 to - 60, where positive values indicate varying intensities of yellow) [1]. The L*a*b* are expressed as arbitrary units. Another index value determined by the colorimeter is the °ITA score (individual typology angle). °ITA score can be described where the higher the °ITA value

the lighter the skin colour and the other way around [32]. The hydration of the *stratum corneum* was measured using the Corneometer® CM 825 probe.

Kondziela's inverted screen test: This test was used to verify if the exposure to the organic phase mixture affected the mice muscle strength. It was applied the same protocol as the one described by Deacon [13]. In brief, the mice were placed in the centre of the screen, it was started a stopwatch followed by the rotation of the screen in an inverted position, the mice head declining first. It was noted the time when the mice fell off and based on the data obtained, it was applied the following scoring: score 1 = falling between 1 - 10 s; score 2 = falling between 11 - 25 s; score 3 = falling between 26 - 60 s; score 4 = falling between 61 - 90 s; score 5 = falling after 90 s [13].

Statistical analysis: The data are expressed as mean ± SD and were recorded in triplicate for each determination. Data were analysed using Student t-test and One-way Anova followed by Tukey's Multiple Comparison post-test by applying Graph Pad Prism 5 software. Levels of significance were indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001.

Results and Discussion

The main objective of this article was to characterize from a toxicological point of view the effects associated with the environmental exposure (in a special assembly) of hairless SKH1 male mice to an organic mixture based on toluene obtained during the COSORB process. In order to gather toxicological data to fulfil our objective, the mice were divided in 2 groups (n = 6 mice/group): control group - no intervention was applied and test group – the mice were exposed to the organic phase mixture for 30 minutes/daily/for 5 consecutive days. The time of exposure (30 minutes) was chosen according to the literature [7, 11]. This study was aimed to obtain data regarding the possible toxicity induced by the organic volatile compounds after repeated exposure for a short period of time (daily for 5 days, which can be considered equivalent with a working week), the acute period.

Effects of the organic mixture exposure on body weight

One of the parameters that we investigated was the influence of the organic mixture on mice body weight. The body weights were recorded daily and, as it can be seen in Figure 2, the control group presented not significant changes within the group having a constant pattern, whereas the test group weights presented decreasing values starting with the second day of exposure, the greatest decrease being recorded at 120 h (weight 0 h *vs.* weight 120 h: 36.20 ± 0.55 *vs.* 34.80 ± 0.13 g body weight). The differences between the two groups were not statistically significant (Figure 2).

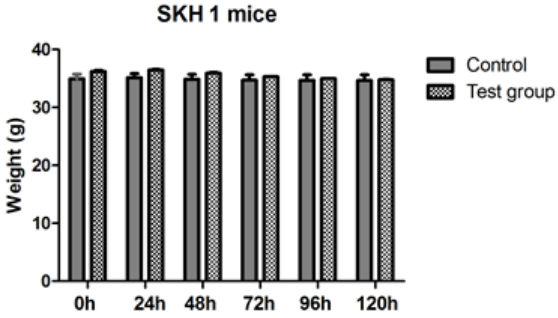

Figure 2.
The evolution of weight/group during the 5 days of exposure. The values were expressed as mean ± SD and were recorded in triplicate for each mouse of each group (n = 6 mice/group)

These data indicate that exposure to toluene and the other organic components of the organic phase mixture was associated with a tendency to weight loss.

Several studies mentioned that the exposure of female and male mice to toluene for 6.5 hours/day for 14 weeks (concentrations ≥ 100 ppm and 2,500 ppm) led to a decrease of body weight [24]. Similar data were recorded for rats exposed to toluene (4 hours/day for 7 or 20 days) [18, 20]. Furthermore, other studies performed on mice and rats exposed to toluene (6 hours/day, 5 days/week for 20, 28, 42 or 90 days) mentioned no related weight loss effects [5, 6].

Effects of the organic mixture exposure at skin level: Another aspect that was tested in the present work, was the impact of the organic volatile mixture on skin physiological parameters after the inhalation exposure. The parameters were measured at different time points, as follows: 1 h, 24 h, 48 h, 72 h, 96 and 120 h (the values were recorded before each exposure). The skin parameters evaluated were: skin hydration by using the corneometry technique, melanin and erythema by the means of tristimulus colorimetry using skin colorimeter CL 400 (Courage–Khazaka, Germany). Skin hydration, also known as hydration of *stratum corneum* is a very important marker in the evaluation of skin integrity and in the diagnosis of different cutaneous disorders. Our results showed that the exposure to the organic mixture led to a decrease of skin hydration in the first hour post-exposure (Figure 3) as compared to control group. In addition, the measurements recorded after 24 h indicated that the skin hydration value in the test group was close to the control group, what could express the capacity of skin recovery after the toxic agent was removed from the environment. It was interesting to note that after the second exposure until the end of experiment,

the values of skin hydration in the test group suffered a statistically significant decrease as compared to control group, the highest decrease being measured at 120 h post-exposure (Figure 3).

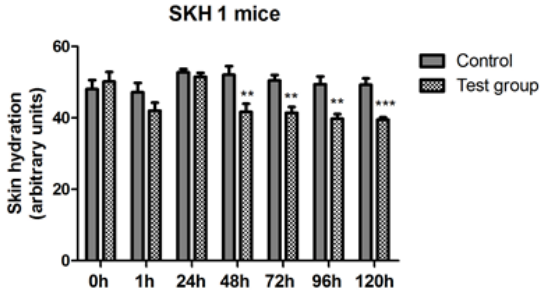

Figure 3.
Skin hydration evolution. The values were expressed as mean ± SD and were recorded in triplicate for each mouse of each group (n = 6 mice/group)

The use of the skin colorimeter CL 400 offered information regarding the changes in skin colour as a consequence of the organic mixture exposure. In this study, the skin colour modifications were characterized by analysing the numerical coordinates L*, a* and b* (arbitrary units) recorded. L* can be defined as the value of skin reflectance or lightness (only positive values in the range of 0 - 100) and b* (yellowness) - is the measure of colour saturation from yellow to blue (range between +60 to - 60). Furthermore, it was shown that both L* and b* are related to the measurement of melanin content and tanning [1]. Based on these considerations and on our results, there were calculated the melanin related-measurements (Figure 4).

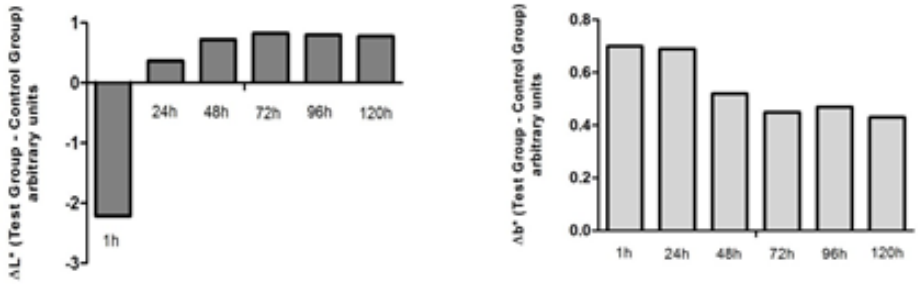

Figure 4.
Melanin-related measurements. The values were expressed as ΔL* and Δb*, Δ – is the difference between the mean value of the test group obtained for L* and b*, respectively (for each time point) and the mean values for these coordinates in the control group

According to our results, the measurements at 1 h post-exposure indicate a decrease of L* value in the test group as compared to control group, that can be considered as a lower brightness (Figure 4). The values of L* at the other time points indicated an increase of this index as compared to the control group, but not statistically significant (Figure 4). In the case of b* values recorded for the test group it can be observed an increase at 1 h and 24 h post-exposure and a decrease at the other time points as compared to the control group (Figure 4). A decrease of L* value and an increased b* value are usually associated with a darker skin [2]. Taking these facts in consideration, it could be said that the exposure to organic mixtures led to a pigmentation only in the first 24 h post-exposure, the following exposures having a lower effect on melanin values.
Another numerical coordinate recorded by the skin colorimeter was a*. The red-green chromaticity coordinate (a* - values with the range between +60 and -60) [1] is often utilized for the quantification of the erythema degree induced by physical or chemical agents [29]. The higher the a* value is, the presence of redness and erythema is observed [1, 2]. Our results showed increased values of the a* index (redness) in the test group as compared to the control group starting from the first hour post-exposure becoming higher after 24 h and keeping at an almost constant value until the end of experiment (Figure 5).

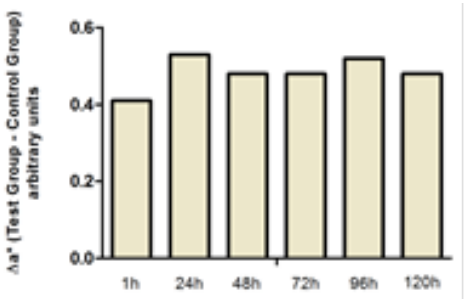

Figure 5.
Erythema-related measurements. The values were expressed as Δa*, where Δ – is the difference between the mean value of the test group obtained for a* (for each time point) and the mean value for this coordinate in the control group

According to these results, it could be stated that the exposure to organic mixture based on toluene induced a small degree of erythema.

To the best of our knowledge, there are very few data regarding the noxious effects of inhaled toluene at dermal level, what lead us to the appreciation that there are required further studies in order to explain thoroughly the results that we obtained in the present study. In contrast, it is well-known that toluene undiluted applied topically induces irritation and swelling [26]. Moreover, in our previous studies, we demonstrated that topical application of the organic phase induced toxicity at skin level [27].

Effects of the organic mixture exposure on muscle strength: It is well-known that inhalation of toluene induces reversible neurological symptoms in case of acute exposure that varies from fatigue and decreased manual dexterity to narcosis, and that the chronic exposure was associated with changes of cognitive function and neuromuscular performance, of hearing and colour discrimination [31].

The central nervous system toxicity induced by different agents is usually associated with a deficit of skeletal muscle strength (muscle weakness). The inverted screen test is used to measure the changes in mice muscles strength as a consequence of some motor disorders that might have a central nervous system origin [13, 16]. As the central nervous system is considered the primary target of toluene-induced toxicity, we decided to perform the inverted screen test for the evaluation of the muscular strength. The latency to fall was recorded up to 120 seconds, after this time the mice were removed from the assembly and returned to their cages. Three independent measurements were taken with a period of pause of 2 - 3 min between the tests. The possible systemic toxicity was appreciated as muscular strength decrease of mice and presented as mean/group by scores characteristic for Kondziela's inverted screen test in Table I.

The scores mentioned in Table I were applied as described by Deacon [13]. The inverted screen test was performed for both groups before mice exposure to organic mixture (0 h), at 1 h post-exposure, at 24 h before the second exposure and daily before the exposure to the organic phase (48, 72, 96 and 120 hours).

Table I

Inverted screen test results after 5 days of exposure to organic phase mixture

Time, hours after exposure	**Groups**	
	Control	Test
	Falling seconds/Score	Falling seconds/Score
0	63.33/4	36.33/ 3
1	71.50/4	13/2
24	71.67/4	10.33/1
48	72.33/4	9.67/1
72	> 90/5	7.75/1
96	> 90/5	6.67/1
120	> 90/5	4.67/1

Our data indicate that the mice from the control group presented a higher locomotor response (muscular strength) with every test they performed, the latency to fall becoming longer (Table I). In contrast, the mice exposed to organic phase mixture showed a lower latency to fall, what could indicate that the neuromuscular performance was affected and the mice presented muscular weakness of neurological origin. The decrease of time until falling in the test group increased with the number of exposures, so we could assume that the effect of the aromatic solvents was a cumulative one, whereas further studies are required to prove this hypothesis.

In a previous study, it was demonstrated that repeated prenatal exposure of pregnant mice to a high concentration of toluene led to developmental toxicity to the progeny characterized by weight reduction and some deficits concerning the neuromuscular behaviour, such as: delay in the righting reflex and a reduced grip strength [21]. These data are in compliance with the results that were obtained in the present study.

Conclusions

The evaluation of the impact of an organic mixture resulted from the COSORB process on animal organism led to the following results: exposure to toluene and related compounds determined weight loss in the exposed group; the skin barrier was mildly affected (decreased skin hydration values and a slight pigmentation and erythema) and the muscular performance was perturbed. These data offer valuable information regarding the safety of the personal involved in the COSORB process. In order to explain the underlying mechanism of toxicity there are required further studies.

Acknowledgement

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