

INFLUENCE OF ENVIRONMENTAL CHLOROFORM CONCENTRATIONS ON BIOPHYSICS SKIN PARAMETERS

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Abstract

Our studies were focused on chloroform effect on skin properties. Three concentrations have been tested 100, 200 and 300 µg/L. These levels of concentrations can be found in swimming pools, where this compound results after the water chlorination. An original contact method was used, combined with biophysics measurement of several skin parameters: skin hydration (H), transepidermal water loss (TEWL) and sebum secretion (S). Twenty volunteers without clinical signs of dermatological diseases have been monitored, before and 15 minutes after the contact of the product with the skin. The recovery was seen after 50 minutes from the beginning. During the contact, skin hydration increased proportionally with the tested concentrations, while the sebum parameter decreased rapidly. The most important change of TEWL was observed for 100 µg/L, and paradoxically, the return to baseline was higher for 300 µg/L. After 50 minutes, only the sebum parameter didn't return to the initial values. The exposures of such environmental concentrations determine the skin drying by sebum loss, more pronounced if the initial value of the sebum secretion is low.

Rezumat

Studiul nostru a urmărit efectul cloroformului asupra pielii. Au fost testate concentrațiile de 100, 200 și 300 µg/L, care pot fi măsurate în apa bazinelor de înot, unde compusul rezultă prin clorinarea apei. S-a folosit o metodă de contact originală, combinată cu metode biofizice de analiză ce evidențiază: hidratarea pielii (H), pierderea de apă transepidermică (TEWL) și secreția de sebum (S). Au fost monitorizați 20 de voluntari fără semne clinice de afecțiuni dermatologice, înainte și după 15 minute de contact local. Revenirea parametrilor pielii la valorile inițiale a fost analizată după 50 min de la începutul experimentului. Pe durata contactului hidratarea crește proporțional cu concentrațiile testate, în timp ce secreția de sebum scade rapid. Cea mai importantă schimbare pentru TEWL a fost observată la concentrația 100 µg/L și paradoxal, revenirea secreției de sebum la valorile inițiale a fost mai rapidă la 300 µg/L. După 50 min, numai secreția de sebum nu a revenit la valoarea inițială. Expunerea la astfel de concentrații duce la uscarea pielii, cu atât mai accentuată cu cât valoarea inițială a secreției de sebum este mai redusă.

Keywords: dermal contact, environmental chloroform concentrations, hydration, transepidermal transfer, sebum secretion

Introduction

The primary function of epidermis is to produce the protective, semi-permeable *stratum corneum* that allows terrestrial life. Many biomonitoring or case-control studies have been reported in scientific journals based on biophysical methods, with high relevance in evaluation of epidermal integrity, [6, 17, 18]. Impairment of human *stratum corneum* barrier function can lead to increased transepidermal water loss (TEWL) and increased susceptibility to intoxication by penetration of harmful agents [2]. This has been observed in various skin diseases [21]. Chloroform is a volatile organic compound with lipophilic character. Increases of the atopic skin disease by disturbing the epidermal barrier function has been reported in

many studies, as a consequence of volatile organic compound exposure [5, 9-14, 16].

Chloroform concentrations of 100, 200 and 300 µg/L, have been identified in swimming pools. Sandel examined data from 114 residential pools in the USA and reported average concentrations of chloroform of 67.1 µg/L with a maximum value of 313 µg/L [19]. People, who swim in swimming pools, absorb chloroform through their skin. Swimmers (in indoor pools) are exposed to high concentrations of chloroform from both water and air, 78% and 22% of the body burden were due to inhalation and dermal uptake, respectively. Indoor pool areas were associated with illness [1], a large proportion of swimmers being affected. Symptoms were consistent with chloramine exposure and

sometimes they were severe. Although chlorine kills many potential pathogens, it can also react with human wastes such as perspiration, urine, skin particles, and lotion in pool water to form chloramines and trihalomethanes [4].

The skin barrier may be affected by chloroform concentrations found in swimming pools water. Biophysical techniques of skin penetration are routinely used for investigation of human *stratum corneum*, barrier function and in order to study the factors involved in skin pathologies [15, 22]. The methods like tape stripping have been used to study the protection efficiency of barrier creams [20]. These methods are simple, inexpensive, and minimally invasive and can be used in both humans and laboratory animals.

Evaluation of chloroform induced effects is important in understanding skin reaction in different environmental situations. The damage of skin barrier may be evaluated by measuring the transepidermal water loss (TEWL), sebum secretion (S) and water content of *stratum corneum* hydration (H).

Materials and Methods

EXPOSURE GROUP

Twenty healthy volunteers (males and females) between 30-40 years old were selected to participate in the study. The volunteers had no history of allergies, or skin problems. They were informed about the nature of test and about the possible adverse reactions. Furthermore, they did not use any cosmetic products during the study. All participants gave their written consent before starting the study, in accordance with the Declaration of Helsinki.

STANDARD SOLUTION

Three test chloroform solutions with concentrations of 100 µg/L, 200 µg/L and 300 µg/L were prepared. A chloroform (Merck) stock solution, with density of 1.48 g/cm³ at 20 °C has been used, which was instantly diluted with distillate water.

APPARATUS

We used: Corneometer CM 825, Sebumeter SM 815, Tewameter TM 300 (Courage-Khazaka Electronic GmbH, Germany). Three biophysical parameters: Hydration (H), Sebum secretion (S) and Transepidermal water loss (TEWL), were monitored before the experiment and 15, respectively 50 minutes after.

PROCEDURE

A Whatman cellulose filter paper, having quadrate form with margins of 5 mm was suspended in each chloroform dilutions. These quadrate papers were applied in three points: Point 1; Point 2; and Point 3 at the face level, being covered with an impermeable material (aluminium folia) and fixed

during the experiment (Figure 1). In the Point 1 was applied the quadrate paper with the concentration of 100 µg/L; in Point 2 with 200 µg/L and in Point 3 with 300 µg/L. The distance between chosen points was about 3 cm.

The skin response was monitored before and 15 minutes after applying of quadrate paper. After removing the paper with chloroform, the skin parameters were monitored later, at 50 min.

Our procedures correspond with the application in a single point of a very small quadrate filter paper. Chloroform is a toxic compound having CAS#: 67-66-3 and EC#: 200-663-8 numbers, according with EU Classification, Labeling and Packaging (Regulation (EC) No 1272/2008). The compound may cause skin irritation and damage to organs, through prolonged or repeated exposure [11].

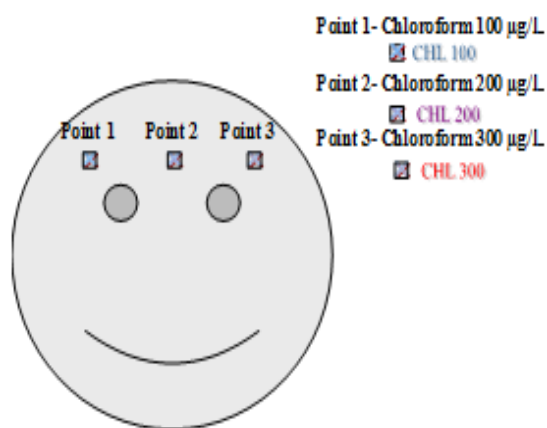


Figure 1.
Experimental design

All measurements were performed in controlled conditions of relative humidity ($45\% \pm 2\%$) and temperature ($22^{\circ} \pm 2^{\circ}\text{C}$). In order to acclimatize at room conditions, volunteers were asked to stay in the test room for at least 1 h prior to the measurements.

Results and Discussion

In the first 15 minutes of chloroform water dilutions exposure, a significant increase of skin hydration could be observed (Figure 2). The increase was proportional with the chloroform applied concentrations that means, the increase of skin impedance and decrease of the corneal layer resistance. After 50 minutes since the topical application, the hydration parameter was nearly to its initial value, in each measured point.

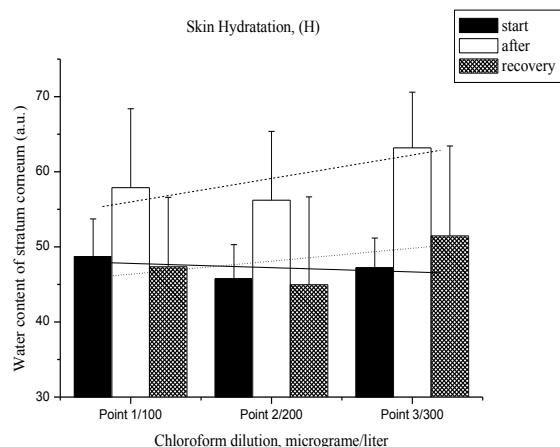


Figure 2.

Influence of chloroform concentrations from swimming pool water on human skin hydration parameter, ($n = 20$, mean $\pm$ SD).

Legend: Point 1/100 = induced effect of 100 µg/L chloroform concentration; Point 2/200 = induced effect of 200 µg/L chloroform concentration; Point 3/300 = induced effect of 300 µg/L chloroform concentration; Start – the value registered at the beginning; After – the values registered after 15 minutes and Recovery – the value registered after 50 minutes.

The sebum quantity has seriously decreased for every point (Figure 3). As a consequence, a local skin drying effect has been installed. The greatest drying effect was observed during the 15 minutes exposure time. At 50 minutes after the topical application, the sebum parameter didn't return at baseline. An increased trend line could be observed in Figure 3, which means the acceleration of sebum secretion after the exposure, especially for the 300 µg/L chloroform dilution.

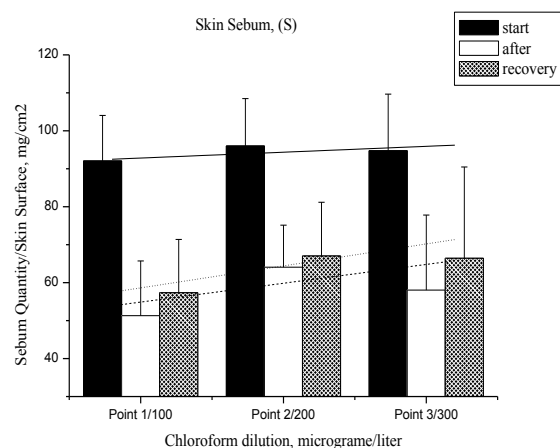


Figure 3.

Influence of chloroform concentrations from swimming pool water on Skin Sebum Parameter ($n = 20$, mean $\pm$ SD).

Legend: Point 1/100 = induced effect of 100 µg/L chloroform concentration; Point 2/200 = induced effect of 200 µg/L chloroform concentration; Point 3/300 = induced effect of 300 µg/L chloroform concentration; Start – the value registered at the beginning; After – the values registered after 15 minutes and Recovery – the value registered after 50 minutes.

Skin barrier function was evaluated also by measuring transepidermal water loss (TEWL). TEWL was measured in the same sites at start, after 15 min, following the barrier disruption and after 50 minutes, following the barrier recovery, Figure 4. After 15 min, was registered the highest barrier disruption for all for all tracked points. The most important alteration was observed for 100 µg/L chloroform concentrations.

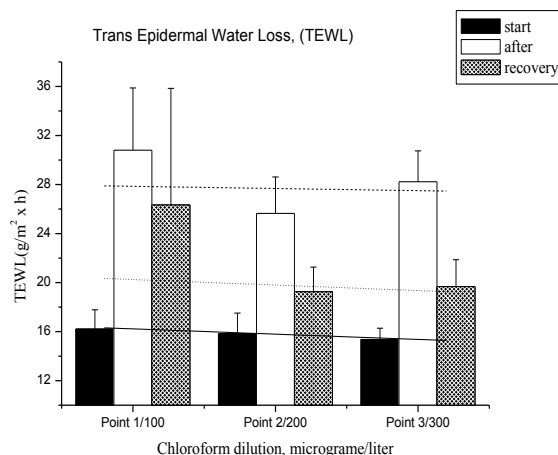


Figure 4.

Influence of chloroform concentrations from swimming pool water on Trans Epidermal Water Loss (TEWL), ($n = 20$, mean $\pm$ SD).

Legend: Point 1/100 = induced effect of 100 µg/L chloroform concentration; Point 2/200 = induced effect of 200 µg/L chloroform concentration; Point 3/300 = induced effect of 300 µg/L chloroform concentration; Start – the value registered at the beginning; After – the values registered after 15 minutes and Recovery – the value registered after 50 minutes.

A high TEWL is indicative of an ineffective or damaged barrier function of the *stratum corneum*. The increase of TEWL can be observed for all volunteers.

Percutaneous absorption of chemicals from a specific vehicle depends on the chemicals partitioning from the vehicle and solubility of a chemical in the vehicle. Due to this lipophilic character, through skin contact, chloroform accumulation will be higher in tissues with high lipid content [3].

The degree of hydration is most frequently determined by measuring electrical properties of skin. Normal skin surface has a negative potential against the inside and the potential decreases immediately after barrier disruption [8]. *In vitro* and *in vivo* studies have shown that low resistance and high impedance correlate with an increase in skin hydration (water content). The measurement is more influenced by changes in the nearby and lower impedance *stratum corneum* layer than is the moist underlying tissue (high impedance).

According with Elias and Menon, 1991, when the corneal *stratum* barrier function is damaged by tape

stripping or treatment with an organic solvent or detergent, a series of homeostatic processes in barrier function is immediately accelerated, and the barrier recovers to its original level. Possibly, the homeostatic process is highly accelerated for the 300 µg/L chloroform concentration.

One of the most important roles of the skin is to generate a barrier against excess transcutaneous water loss. Water is continuously diffusing through the skin to the environment. The barrier to water permeation is not absolute and the normal movement of water through the *stratum corneum* into the atmosphere is known as transepidermal water loss (TEWL) and constitutes part of insensible water loss.

Transcutaneous water flux depends on skin's permeability and the water activity difference of skin relative to the environment. TEWL is often taken as a measure of skin's intrinsic barrier properties. A high TEWL is indicative of an ineffective or damaged barrier function of the *stratum corneum*. The molecular mechanisms, by which the barrier disruption is produced, remain unknown, although changes in water content, ion content and distribution (particularly calcium), may be involved in the signalling pathways [7].

Conclusions

The contact with chloroform may affect the barrier function of *stratum corneum*. All biophysical properties of the skin have been changed: increased hydration and transepidermal water loss and decreased of sebum secretion.

The most affected skin parameter was the sebum, for which the value didn't return to reference standard during our experiment. Dermal exposure to chloroform from pool water can lead to dry skin, more pronounced if the sebum secretion is decreased.

The changes of skin parameters after the local contact with chloroform dilutions similarly with those found in swimming pools are reversible and in the case of a healthy skin they return to normal values in about 90 minutes after washing.

These non-invasive methods offer the opportunities for many other studies in relation with environmental chloroform exposure.

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