The Krefelder Ciliatetest: A New Experimental Method to Study the Toxicity of Chemicals

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During the last years, skin compatibility of textiles has gained importance for consumers. Therefore, the development of suitable test methods for the evaluation of skin compatibility is a vital challenge. The fundamental requirements for these test methods are low experimental complexity combined with minor costs. The Cilitatetest has been developed for the cosmetic and pharmaceutics industry. Due to the optical measurement of the number of living microorganisms, this method is not suitable for colored or heterogeneous samples. However, using a calorimetric detection, these problems are resolved. This method allows very sensitive detection of the metabolism of the microorganism. The presence of any toxic substance results in a reduction of the heat of metabolism.

Key words: *Ciliatetest, microorganism, calorimetry, toxicity*

Introduction

For the determination of the toxicity of chemical substances in water, sewage and sludge, standard methods are mainly used. However, these methods require duration between 7 and 72 hours. The metabolism of the organisms used in the standard methods differs from the metabolism of the human organism. Therefore, it is difficult to predict the toxicity of chemical substances with regard to the human organism. To test the irritating effect of chemical substances on human skin, the Draize test is normally used.[1] This test applies a chemical substance to the skin of a rabbit under standardized conditions. To reduce the number of animal experiments, a search was run for alternative methods. Thus, different cell lines are used. For all these tests, special laboratory equipment and well-trained employees are essential. This results in high costs for each testing.

For the detection of cytotoxic and cytostatic properties of substances used in hygienic, cosmetic, toxicological and pharmaceutical applications, the microorganism *Tetrahymena pyriformis* (*Ciliates*) is used.[2,3] They show similar sensitivity as human cell cultures.[4] This microorganism has an axial filament, the rotation of which causes the microorganism to move through the aqueous medium. This organism also possesses an organelle that enables *Tetrahymena pyriformis* to incorporate substances as particles or dispersions. The experimental methods for the study of the cytotoxic and cytostatic properties of chemical substances are based on optical measurements of the population for a period of 24 h up to 96 h.[4–8] Unfortunately, these detection methods can not be used if the test solutions are colored, turbid or even non-homogeneous. A new detection method used in the DTNW is based on the measurement of the heat effects caused by movement and metabolism.[9] In the presence of toxic substances the heat produced by *Tetrahymena pyriformis* is reduced. These heat effects can be measured with a high sensitivity. Thus, even the detection of very small amounts of toxic substances in aqueous systems is possible. Using this method, textile auxiliaries and dyes have been examined.[10,11] The calorimetric measurements are even possible using solid probes like textile materials.

Material and methods

The organism *Tetrahymena pyriformis* is easily cultivated in a liquid fluid (γ = 5 g l^{-1} proteose peptone (Merck 1.07229), γ = 5 g l^{-1} peptone from casein (Merck 1.07213) and γ = 0.2 g l^{-1} dinatrium hydrogenphosphate dihydrate Fluka 71644) for stabilization). The culture medium is distributed in 60 ml portions among Erlenmeyer flasks of 100 ml. They are then closed and autoclaved. 1 ml of the overgrown culture is added to each fresh culture medium and incubated at ϑ = 28 °C.

Calorimetry

For the calorimetric measurements, 40 ml of at least 48–hour-old cultures were used. The measurements were performed using an isoperibolic calorimeter (Tronac Model 450, Tronac Inc., Orem, USA). Fig. 1 shows a schematic draft of an isoperibolic calorimeter. The chemical substances were added directly and at once to the solutions containing *Tetrahymena pyriformis* before starting the measurements. Textile probes were pulverized and then 1 g of this powder was mixed with the solution containing the microorganism. Afterwards the heat production was measured over a period from 2 to 20 h. For comparison, one measurement was performed without the addition of a chemical substance and a second with the pure culture medium.

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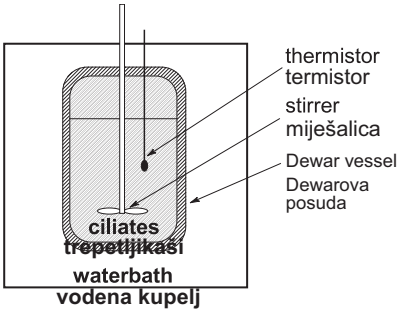


F i g. 1 – *Schematic draft of an isoperibolic calorimeter*

S l i k a 1 – *Shematski prikaz izoperiboličnog kalorimetra*

Results and discussions

The measured heat Q is the sum of the heat of metabolism and movement of *Tetrahymena pyriformis* Q_{met}, the heat of stirring Q_{stirr} and the heat exchange with the surroundings Q_{exch}:

$$Q = Q_{met} + Q_{stirr} + Q_{exch} \quad (1)$$

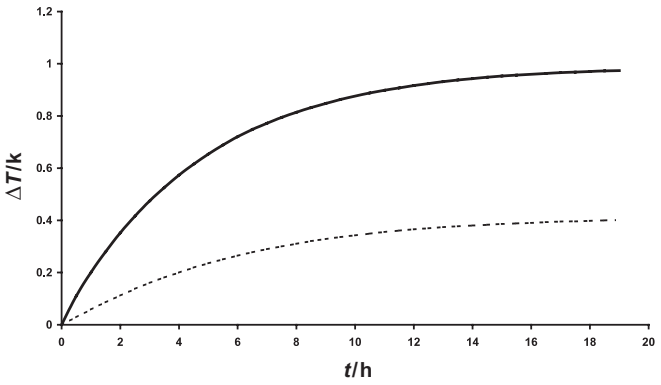
With a pure culture medium, the experimentally measured heat Q_{cm} is given by eq (2):

$$Q_{cm} = Q_{stirr} + Q_{exch} \quad (2)$$

These curves are shown in Fig. 2. The solutions for the measurements are within a Dewar vessel. As a result, the heat flow with the surrounding is extensively reduced. The Dewar vessel is located within a heat bath the temperature of which is kept constant with an accuracy of 1/1000 degree. To achieve a temperature homogeneity the solution is stirred at a constant rate. This mechanical heat input results in an increase of the temperature within the vessel. All the time a small temperature exchange with the surrounding water bath is observable. In the case of a pure culture medium in the reaction vessel, an equilibrium state is reached when both heat effects for stirring and for the exchange are identical. Under these conditions, the temperature in the vessel is constant. Identical effects also take place in the presence of microorganism in the vessel. Now the observed heat effect is higher due to the heat of metabolism.

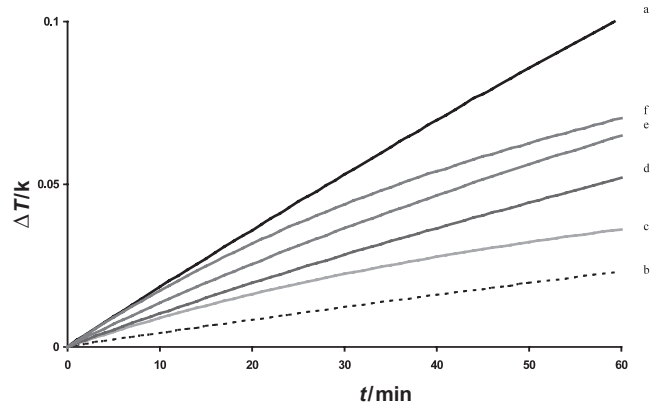
In the presence of toxic substances, the heat of metabolism is reduced. For the demonstration of the influence of the concentration of the toxic substance tributyltin chloride is chosen. The calorimetric measurements are shown in Fig. 3. Although tributyltin chloride is not soluble in water the addition as pure substance to the cultures of *Tetrahymena pyriformis* has a distinct influence upon the metabolism. Even at the lowest concentration used the metabolism is reduced. Using the measured values of ΔT after a constant time (60 min) a dose-response relationship is obtained, see Fig. 4. From this curve the concentration of the toxic substance can be calculated at which the heat of metabolism is reduced to 50 % (LC_{50}). In the case of tributyltin chloride the following result is obtained: $LC_{50} = 18.2\ \mu l\ l^{-1}$.

Even solid samples, as textile material, can be added directly to the cultures of *Tetrahymena pyriformis*. If any toxic substance is present on the textiles, the metabolism of the microorganism is influenced. This is shown in Fig. 5. No



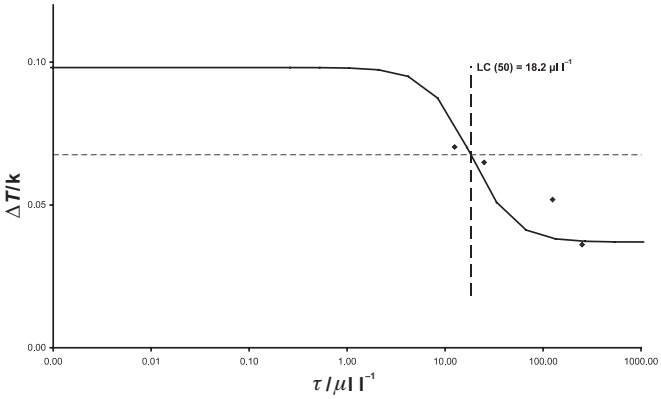
F i g. 2 – *Temperature changes in the reaction vessel with a solution containing Tetrahymena pyriformis (a) and with the pure culture medium (b)*

S l i k a 2 – *Temperaturne promjene u reakcijskoj posudi s otopinom koja sadrži Tetrahymena pyriformis (a) i s medijem čiste kulture (b)*



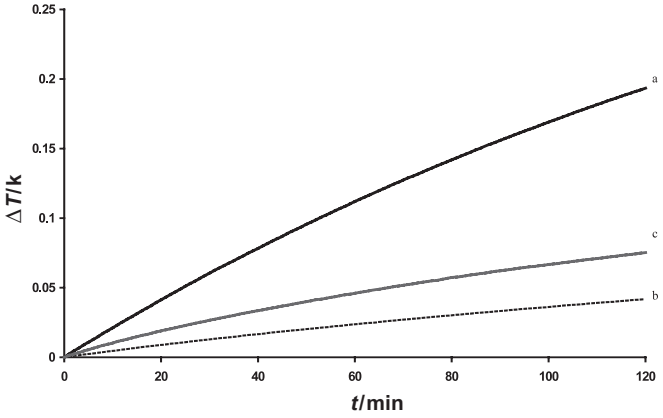
F i g. 3 – *Temperature changes in cultures of Tetrahymena pyriformis in the presence of different amounts of tributyltin chloride (a: pure culture of Tetrahymena pyriformis, b: pure culture medium, c: after the addition of 250 μl l⁻¹, d: 125 μl l⁻¹, e: 25 μl l⁻¹, f: 12.5 μl l⁻¹)*

S l i k a 3 – *Temperaturne promjene u kulturama Tetrahymena pyiriformis u prisutnosti različitih količina tributilkositrova klorida (a: čista kultura Tetrahymena pyiriformis, b: medij čiste kulture, c: nakon dodavanja 250 μl l⁻¹, d: 125 μl l⁻¹, e: 25 μl l⁻¹, f: 12,5 μl l⁻¹)*



F i g. 4 – *Dose-response relationship for tributyltin chloride upon Tetrahymena pyriformis*

S l i k a 4 – *Odnos reakcije doze tributilkositrova klorida na Tetrahymena pyriformis*



F i g. 5 – *Temperature changes in the reaction vessel with a solution containing Tetrahymena pyriformis (a), with an added textile sample (b) and with the pure culture medium (c)*

S l i k a 5 – *Temperaturne promjene u reakcijskoj posudi s otopinom koja sadrži Tetrahymena pyriformis (a), s dodanim tekstilnim uzorkom (b) i s medijem čiste kulture (c)*

pretreatment of the textile samples is necessary. The chemical substances migrate from the surface of the fabrics into the aqueous solution and are able to influence the metabolism of *Tetrahymena pyriformis*. Obviously, the textile sample examined is contaminated with a toxic substance. From these measurements, no information is obtained about the chemical nature of the toxic substance and about their concentration on the textile sample.

Conclusions

All these results show the suitability of the "Krefelder Ciliatetest" to act as a test method for the detection of toxic substances on textiles or other probes. Due to the calorimetric measurements of the metabolism of *Tetrahymena pyriformis* even solid samples or turbid solutions can be examined. This is an important advantage over the spectrophotometric measurements already used.

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List of symbols
Popis simbola

Q – heat, J
– toplina, J

t – time, h
– vrijeme, h

T – thermodynamic temperature, K
– termodinamička temperatura, K

γ – mass concentration, g L^{-1}
– masna koncentracija, g L^{-1}

τ – volume concentration, µl l^{-1}
– obujmna koncentracija, µl l^{-1}

ϑ – temperature, °C
– temperatura, °C

SAŽETAK

Krefelderski test – novi eksperimentalni postupak za proučavanje toksičnosti kemikalija

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Kompatibilnost tekstila prema koži postajala je tijekom posljednjih godina sve važnija potrošačima. Zato je razvoj prikladnih postupaka ispitivanja za prosudbu kompatibilnosti s kožom primjeran izazov. Osnovni zahtjev postavljen za ove postupke ispitivanja jest eksperimentalna jednostavnost povezana s nižim troškovima. Ispitivanje trepetljikašima razvijeno je za kozmetičku i farmaceutsku industriju. Zbog optičkog mjerenja broja živih mikroorganizama taj postupak nije prikladan za obojene ili heterogene uzorke. Međutim, ti problemi su riješeni primjenom kalorimetijskog otkrivanja. Taj postupak otkrivanja omogućuje vrlo osjetljivo prepoznavanje metabolizma mikroorganizama. Postojanje bilo koje otrovne tvari znači smanjenje topline metabolizma.

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