Prof. Dr. Dr. W. Gräf

Specialist in Hygiene and Environmental Medicine em. o. Prof. in Hygiene and Med. Microbiology of the University of Erlangen-Nuremberg



91054 Erlangen, 10-12-2001 Saranstrasse 11 Telephone 09131/55163

Expert Opinion concerning the lung tolerance to the air disinfectant WESSO[®] AIR

The agent WESSO[®] Air, used as an aerosol and brought to the air by means of a cold nebulizer, effectuates a considerable reduction of air germs.

Apart from the reduction of germs in the air reached by using the agent, it is of greatest importance for the practical application that the WESSO[®] Air aerosol has no harmful impacts on human and animal health. From the toxicological point of view it is essential that, by using the aerosol, no damage to or impairment of the human ciliated epithelia or of the ciliary apparatus in the bronchial system is to be expected by the WESSO[®] Air agent.

As is known it is, by experiment, very difficult to record quantitatively the activity and, where relevant, the impairment of the ciliated epithelium in the lung. For this reason, already a long time ago people went over to using microorganisms as a reference test system for these purposes. Several years ago we developed the so-called <u>Ciliate Mobility Test (CMT)</u>, which has a wide variety of uses since then (1, 2). The principle consists in using animal, ciliated protozoa (<u>Tetrahymena pyriformis</u>). By means of a special experimental arrangement the ciliate activity (= mobility of the cilia) is registered on the basis of their locomotion speed and used as a reference for the activity of the bronchial ciliated epithelium. Thorough comparative tests have produced evidence that the ciliary apparatus of human ciliated epithelia is almost identical to that of the ciliate locomotor apparatus and that, consequently, both systems are definitely comparable (2).

In the CM test, the locomotion speed of the ciliate cells is determined in a simple way and the influence by air pollutants is registered via the so-called <u>mobility quotient</u> (= $Q_{(1h)}$). Details are given in the relevant publication (2).

The mobility quotient Q (1h) is to be assessed as follows:

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$Q_{(1h)} < 1.0$	reduced mobility
$Q_{(1h)} = 0.0$	immobility (cilia paralysis)
$Q_{(1h)} = 1.0$	no impairment of mobility
$0^{1} \times 10^{1}$	·

 $Q_{(1h)} > 1.0$ <u>increase</u> of mobility

Description of the experiment

The agent WESSO[®] Air, in a 10 % stock solution was atomised as aerosol into the room air (room volume 50 m³) using cold nebulizer technology. The substance was atomised <u>continuously</u> for 12 hours each time. This <u>aerosolising was aimed at creating the extremest possible conditions of exposure for the toxicological evaluation of the effect on cilia.</u>

The aerosol sampling was made with the aid of a standardised air sampler (volume 150 ml). The suckedoff air was, as described in 2, conducted into special reaction vessels and <u>the mobility quotient</u> (=Q_(1h)) was determined after an 1-hour exposure, as described.

In order to create reproducibility conditions required for the evaluation, in each test series the aerosol was taken in the <u>test room</u> as well as directly in front of the atomising opening of the device, i.e. at the point of its <u>highest concentration</u>.

Results of the test series

3 different test series were carried out. In each of them one sample taken from the <u>room air</u> and one sample taken directly from the <u>device opening</u> (= extreme conditions) was measured. The results of these <u>altogether 6 determinations</u> of the mobility quotient are shown in the following tables 1.1, 1.2; 2.1, 2.2; 3.1, 3.2.

<u>1.1 Ciliate Mobility Test (CMT)</u>

BLANK REAGENT			MEASUREMENT REAGENT		
$t = 0 \min$	$t = 10 \min$	Difference	$t = 0 \min$	$t = 10 \min$	Difference
= 100 %	= 46.7 %	= 53.3 %	= 100 %	= 50.0 %	= 50.0 %
= 100 %	= 52.6 %	= 47.4 %	= 100 %	= 52.4 %	= 47.6 %
Mean difference = 50.4 %		Mean difference =		48.8 %	

Test material: WESSO[®] Air (directly at the device)

 $Q_{(1h)}$ = mobility quotient = mean difference of measurement reagent : mean difference of blank reagent = $1.0 = \frac{48.8}{50.4}$

<u>1.2 Ciliate Mobility Test (CMT)</u>

Test material: WESSO[®] Air (room air)

BLANK REAGENT			MEASUREMENT REAGENT		
$t = 0 \min$	$t = 10 \min$	Difference	$t = 0 \min$	t = 10 min	Difference
= 100 %	= 50.0 %	= 50.0 %	= 100 %	= 47.6 %	= 52.4 %
= 100 %	= 50.0 %	= 50.0 %	= 100 %	= 50.0 %	= 50.0 %
Mean difference = 50.0 %		Mean difference	=	51.2 %	

Q_(1h) = mobility quotient = mean difference of measurement reagent : mean difference of blank reagent = $\frac{51.2}{1.0 = 50.0}$

2.1 Ciliate Mobility Test (CMT)

Test material: WESSO[®] Air (directly at the device)

BLANK REAGENT		MEASUREMENT REAGENT			
$t = 0 \min$	$t = 10 \min$	Difference	$t = 0 \min$	$t = 10 \min$	Difference
= 100 %	= 48.8 %	= 51.2 %	= 100 %	= 41.2 %	= 58.8 %
= 100 %	= 46.7 %	= 53.3 %	= 100 %	= 49.0 %	= 51.0 %
Mean difference = 52.3 %		Mean difference	=	54.9 %	

Q (1h) = mobility quotient = mean difference of measurement reagent : mean difference of blank reagent = $\frac{54.9}{1.0 = 52.3}$

2.2 Ciliate Mobility Test (CMT)

Test material: WESSO[®] Air (room air)

BLANK REAGENT		MEASUREMENT REAGENT			
$t = 0 \min$	$t = 10 \min$	Difference	$t = 0 \min$	$t = 10 \min$	Difference
= 100 %	= 46.8 %	= 53.2 %	= 100 %	= 48.1 %	= 51.9 %
= 100 %	= 47.8 %	= 52.2 %	= 100 %	= 48.1 %	= 51.9 %
Mean difference = 52.7 %		Mean difference	=	51.9 %	

 $Q_{(1h)}$ = mobility quotient = mean difference of measurement reagent : mean difference of blank reagent = $\frac{51.9}{1.0 = 52.7}$

3.1 Ciliate Mobility Test (CMT)

Test material: WESSO[®] Air (directly at the device)

BLANK REAGENT		MEASUREMENT REAGENT			
$t = 0 \min$	$t = 10 \min$	Difference	$t = 0 \min$	$t = 10 \min$	Difference
= 100 %	= 33.3 %	= 66.7 %	= 100 %	= 44.4 %	= 55.6 %
= 100 %	= 36.4 %	= 63.6 %	= 100 %	= 44.4 %	= 55.6 %
Mean difference = 65.2 %		Mean difference	=	55.6 %	

Q_(1h) = mobility quotient = mean difference of measurement reagent : mean difference of blank reagent = $0.9 = \frac{55.6}{65.2}$

3.2 Ciliate Mobility Test (CMT)

Test material: WESSO® Air (room air)

BLANK REAGENT		MEASUREMENT REAGENT			
$t = 0 \min$	$t = 10 \min$	Difference	$t = 0 \min$	$t = 10 \min$	Difference
= 100 %	= 44.0 %	= 56.0 %	= 100 %	= 40.8 %	= 59.2 %
= 100 %	= 44.0 %	= 56.0 %	= 100 %	= 43.4 %	= 56.6 %
Mean difference = 56.0 %		Mean difference =		57.9 %	

 $Q_{(1h)}$ = mobility quotient = mean difference of measurement reagent : mean difference of blank reagent = $1.0 = \frac{57.9}{56.0}$

$$1.0 = 56$$

Evaluation of the test results

The obtained results, indicated in the above six tables show that the so-called mobility quotients in all test series are 1.0.

That means that in the Ciliate Mobility Test the WESSO[®] Air aerosols have not caused any impairment of the ciliate activity, neither in the room air tested, nor directly at the output opening of the device.

It can therefore be assessed that the preparation in the above applied aerosol concentration does not impair the bronchial ciliated epithelia.

Overall evaluation

In the event of relatively high aerosol concentrations in the room air and extremely high concentrations at the output opening of the device, damaging effects on the lung or ciliated epithelium cannot be detected.

Literature

- (1) = Biologischer Nachweis von Luftschadstoffwirkungen auf das menschliche Respirationssystem mit dem Ciliaten-Mobilitäts-Test (CMT). [Biological detection of the effects of air pollutants on the human respiratory system with the Ciliate Mobility Test (CMT).] Forum Städtehygiene 1 (1993) Jan./Feb., pages 17-21.
- (2) = Tetrahymena pyriformis im Ciliaten-Mobilitäts-Test. Validierung und Beschreibung eines Prüfverfahrens zur Erfassung von Luftschadstoff- sowie Zigarettenrauchwirkung (Passivrauch) auf das menschliche Respirationsepithel. [Tetrahymena pyriformis in the Ciliate Mobility Test. Validation and description of a testing procedure for the registration of the effects of air pollutants and cigarette smoke (passive smoke) on the human respiratory epithelium.] Zentl. bl. Hy. Umweltmed. 201 (1998/99), pages 451-472.

[Signed]

(Prof. Dr. Dr. W. Gräf)