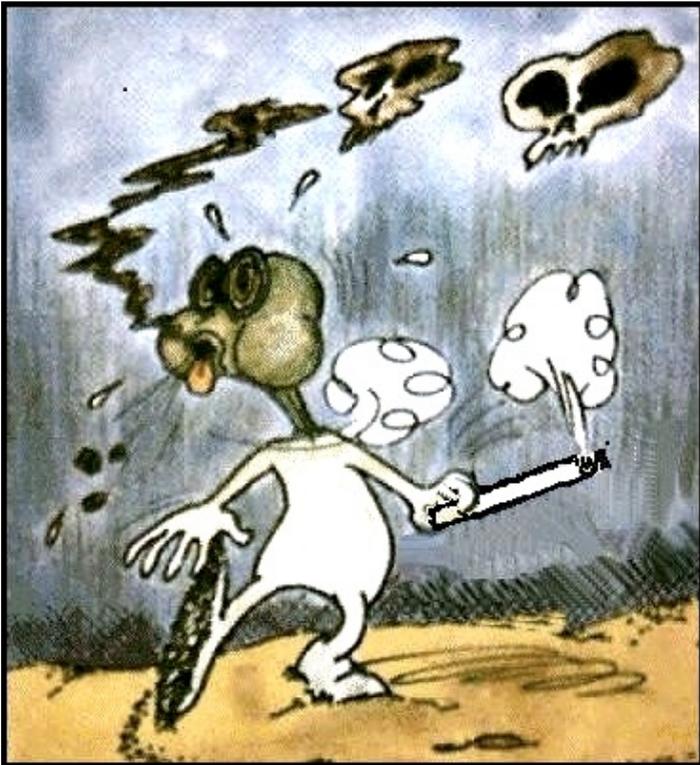


ANTI-SMOKING EDUKIT 1

CIGARETTE SMOKE

An **EDUCATIVE KIT** with a simple experiment to determine the effects of **CIGARETTE SMOKE** on human health



USER MANUAL

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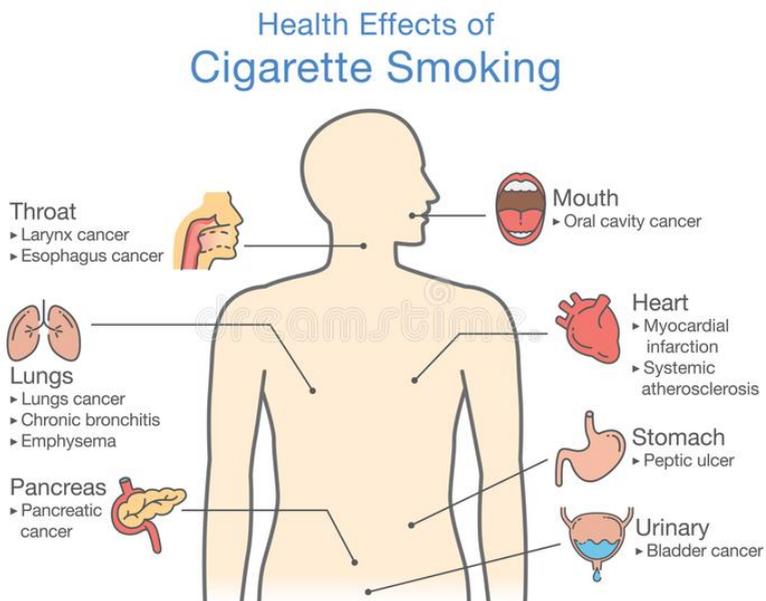
SMOKING... AND THE CONSEQUENCES FOR HUMANS

Worldwide more than **10 billion (10.000.000.000)** cigarettes are smoked every day, and millions of people are “voluntary slaves” of this “silent killer”.

The harmful effects of smoking on humans are well-known and are shown on cigarette packages by explicit photos.

In all countries, authorities warn for the health risks of smoking (active and passive), with data and illustrations of the direct and indirect consequences of smoking.

Scientific research has shown that cigarette smoke contains thousands of chemicals of which many are **very toxic and some even **carcinogenic**.**



According to data of the World Health Organisation (WHO) more than 5 million people die yearly from the effects of smoking. The number of deaths from “smoking-related” diseases is higher than that of tuberculosis, HIV and malaria jointly. Clearly the worst illness caused by smoking is **lung cancer**.

CIGARETTE SMOKE AND THE RESPIRATORY SYSTEM

As indicated above, the combustion of tobacco in cigarettes generates thousands of chemicals of which many are **very toxic** and some even **carcinogenic**.

Most types of cigarettes are now provided with a filter which – at least according to the producers – adsorbs the “dangerous” compounds present in cigarette smoke.

In reality, a substantial part of the combustion chemicals pass through the filter and are hence “inhaled” by the smoker. **All these chemicals thus reach the respiratory tract after passing through the mouth !**

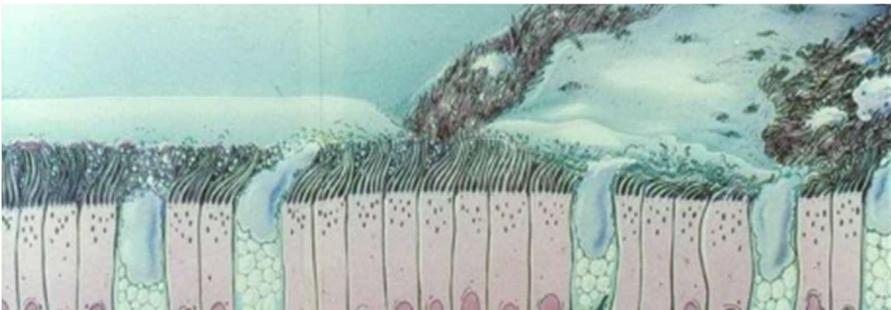
Cigarette smoke is an “aerosol” which besides a large number of chemicals in “gaseous form” also contains many (very small) “solid particles”.

The top layer of cells (the epithelium) of the respiratory system consists of “mucus cells” and “ciliated cells”.

The mucus cells produce a layer of slime (“mucus”) which is spread on top of the ciliated cells. As indicated by their name, ciliated cells have “cilia” which are fine hairlike “protrusions” on top of the cells. The cilia are in continuous movement, with a frequency of about 15 beats per second.

The respiratory epithelium plays an important role in the elimination of chemicals which get into the respiratory tract (by respiration and by smoking). The mucus slime layer “literally catches” these chemicals, and the ciliary beating transports them upwards, as a kind of “flying carpet”, back to the nose and the mouth.

The drawing hereunder provides a nice illustration of the structure of the respiratory epithelium and shows the mucus cells, the ciliated cells, the mucus layer on top of the cilia, and the patches of “inhaled” chemicals on top of the slime layer.



Besides the transportation and the uptake of oxygen in the lungs, the respiratory system also ensures the elimination of chemicals which are inhaled through the production of mucus and the ciliary beating activity.

EFFECT OF CIGARETTE SMOKE ON THE CILIA OF THE RESPIRATORY SYSTEM

Scientific studies performed more than 70 years ago already showed that cigarette smoke has an influence on the motility (the beating) of the cilia in the respiratory tract.

The inhaled smoke decreases the frequency of the ciliary beating leading to a slower draining of the chemicals and the particles of the smoke.

It was also found that smoking ultimately decreases the length of the cilia, and that the number of ciliated cells is reduced.

The disturbance of the ciliary beating activity has at first glance no immediate negative consequences for the health of smokers. Yet, the decrease of the draining of harmful chemicals is a first sign of the “domino effect” which will impact the health of long-term smokers.

Research on the effects of chemicals and cigarette smoke on the ciliary activity in the human respiratory tract was in the 1950's performed in a few highly specialized laboratories.

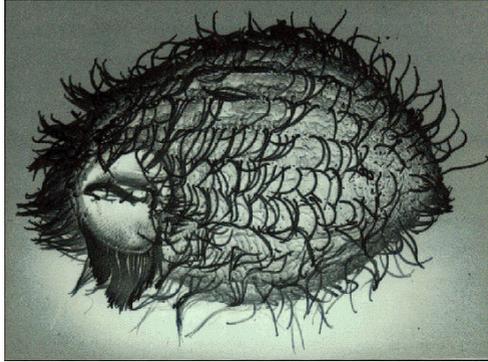
In 1975, Prof.Dr.Gräf and his research team at the University of Erlangen-Nuremburg in Germany tried to develop a simple technique to measure quantitatively the disturbance of ciliary beating by chemicals.

Since it appeared that using “human” ciliary cells would not enable a simpler technique, alternatives were explored with “not human” cilia.

The extensive research eventually revealed that some free living “unicellular” organisms, and more specifically those of the group of “ciliated organisms” (Ciliates) have cilia which have the same structure and composition as those of the human respiratory tract, and also have the same “ciliary beat activity”.

A choice was eventually made for the ciliate *Tetrahymena*, a very small unicellular organism of a size of only 0.05 mm, which is a very common inhabitant in natural waters. The body is completely covered by cilia which, like human respiratory cilia, are in continuous movement and with which these small animals constantly swim.

The microscopic photo hereunder shows the multiple rows of cilia on the body of *Tetrahymena*.



Tetrahymena ciliates can be cultured very easily in the laboratory, and the research team of Prof.Gräf developed with *Tetrahymena* cultures a “Ciliate Mobility Test” which measures the swimming speed of the ciliates after exposure to various chemicals, also including cigarette smoke.

The results of the Ciliate Mobility Test, similarly to tests performed on cilia of the human respiratory tract, indicated that cigarette smoke also brings about a significant decrease of the ciliary beating activity in *Tetrahymena*, as shown in the latter by a decrease of their mobility (i.e. their swimming velocity).

It was even observed that after a short exposure time to cigarette smoke the ciliates become totally “inactive”.

DEVELOPMENT OF AN ANTI-SMOKING EDUKIT 1

CIGARETTE SMOKE

As indicated above, the disturbance of the good functioning of the respiratory cilia by chemicals and also by cigarette smoke is a “first signal” of the negative effects of long-term smoking.

It would certainly be good if this “first signal” could also be shown in class to youngsters, with the aid of a simple experiment.

Based on the same principle as the Ciliate Mobility Test, an ANTI-SMOKING EDUKIT has therefore been developed to demonstrate effects of inhaled cigarette smoke on the ciliate *Tetrahymena*.

The idea of the ANTI-SMOKING EDUKIT TEST 1 – *CIGARETTE SMOKE* is that “inhaled smoke”(= the smoke which gets in the mouth during the smoking of a cigarette), is “blown out” in a test tube containing tap water.

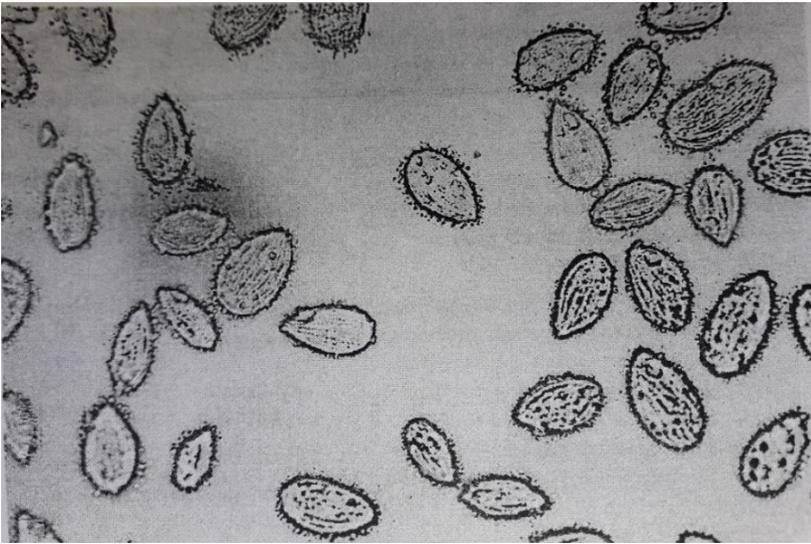
The chemicals in the cigarette smoke dissolve in the water and this water is then transferred into a “cuvette” to which a number of *Tetrahymena* cells from a stock culture are added.

The organisms in the water in the cuvette are hence exposed directly (and continuously) to the cigarette smoke chemicals.

A control test is performed in parallel, in a second cuvette with tap water and ciliates, but without cigarette smoke.

The motility of the *Tetrahymena* ciliates in the two transparent cuvettes are observed under a dissecting microscope, or alternatively with a magnifying glass with a high magnification (minimum 60 X).

The photo hereunder shows a number of *Tetrahymena* cells in the observation field of the microscope at high magnification.



PREPARATION OF THE EXPERIMENT

The ANTI-SMOKING EDUKIT 1 – *CIGARETTE SMOKE* contains all the materials for class performance of up to 6 experiments.

The experiments can be performed concurrently (by 6 groups of scholars) or at different times.

For the experiments a cigarette has to be smoked in advance and “outside of class (!!) by a volunteer (or a smoker).

“With each puff” the smoker has “to blow” the smoke from his/her mouth via a straw in a large tube of which the bottom part is filled with tap water. The chemicals in the smoke dissolve in the tap water and this water is subsequently (in class) poured in a cuvette. Tetrahymena ciliates from a stock culture are then added to the cuvette in order to bring the ciliates in contact with the dissolved chemicals of the cigarette smoke, for subsequent observation.

1. Take the large (30 ml) tube and its screw cap, and the straw from the kit.
2. Fill the tube “exactly to the mark” with tap water (about 8 ml).
3. Close the tube with the screw cap.
4. Give the tube and the straw to the smoker.

Smoking of the cigarette and dissolving of the smoke in the water

The smoker is asked to blow “after each puff” the smoke via the straw in the water of the tube.

This will generate “smoke bubbles” in the water which will fill the tube with a thick white smoke.

N.B. The blowing out of the smoke in the water via the straw must be performed gently to avoid that water is spilling from the tube !!

After each puff and blowing out of the smoke, the straw has to be quickly removed from the tube and the tube immediately closed with the screw cap.

The tube is then shaken thoroughly to mix the smoke with the water and to dissolve the chemicals in the water.

This procedure is repeated till the cigarette is completely smoked.

One will note that the cigarette smoke compounds which have dissolved in the water gradually color the tap water yellow.

PERFORMANCE OF THE TEST

CONTROL EXPERIMENT

1. Open zipper bag 1 and take out one transparent cuvette and one small pipette..
2. Remove the lid from the cuvette.
3. Fill the pipette with water and dispense it in the cuvette.
4. Repeat this operation till the bottom part of the cuvette (the narrow part) is totally filled.

5. Shake the glass bottle containing the stock culture of *Tetrahymena* cells, to distribute the ciliates evenly in the liquid.
6. Open the bottle and draw up a small volume of stock culture in the small pipette.
7. Hold the pipette vertically and dispense **exactly 6 drops** of stock culture in the cuvette.
8. Squirt the rest of the stock culture in the pipette back into the glass bottle and close the bottle.
9. Close the cuvette with its lid and shake it several times to thoroughly mix the suspension of ciliates in the tap water.

EXPERIMENT WITH THE CIGARETTE SMOKE

1. Open zipper bag 2 and take out one transparent cuvette, one small pipette and the pipette with long stem.
2. Remove the lid from the cuvette.
3. Open the tube with the tap water containing the dissolved cigarette smoke.
4. Put the end of the pipette with the long stem in the water of the tube, and draw up part of the water in the pipette.
5. Squirt the water from the pipette in the cuvette till the **bottom part** of the cuvette is totally filled with water with dissolved cigarette smoke.
6. Squirt the rest of the water from the pipette back in the tube and close the tube with the screw cap.
7. Shake the glass bottle containing the stock culture of *Tetrahymena* to distribute the ciliates evenly in the liquid.
8. Open the bottle and draw up a small volume of stock culture in the small pipette.
9. Hold the pipette vertically and dispense **exactly 6 drops** of stock culture in the cuvette.

10. Squirt the rest of the stock culture in the pipette back into the glass bottle and close the bottle.
11. Close the cuvette with its lid and shake it several times to thoroughly mix the suspension of *Tetrahymena* ciliates in the tap water.

OBSERVATIONS

Place the control cuvette on the stage of the dissecting microscope and observe the *Tetrahymena* ciliates at high magnification.

One will see that the ciliates actively swim in all directions.

The observations can also be made with a magnifying glass at high magnification (60 X).

N.B. A LED 60x magnifying glass, provided with built-in illumination, is included in each kit.

Perform the same observations with the cuvette containing water with dissolved cigarette smoke.

One will see that even after a short time (a few minutes) the ciliates are much less active and swim more slowly than in the control.

The observations shall be repeated at different time intervals (15 minutes, 30 minutes), each time after shaking the cuvettes so as to distribute the ciliates evenly in the water.

Observations will show that in the control cuvette the ciliates still move actively whereas in the cuvette with the water with dissolved cigarette smoke they gradually become “totally inactive” (absence of motility).

Additional observations under the microscope

Besides the observations under the dissecting microscope and/or with the magnifying glass, one can also take a look at the activity of the ciliates under a microscope at high magnification.

Microscope slides can therefore easily and rapidly be prepared as follows :

After closing the control cuvette with the lid and shaking to distribute the *Tetrahymena* evenly in the water, a small volume of water is drawn up from the cuvette in the small pipette.

Three drops from the pipette are then dispensed “on top of each other” on a glass slide, which is then covered with a cover slide.

The slide is then placed under the microscope for the observations.

A slide is prepared the same way with water and *Tetrahymena*'s from the cuvette with dissolved cigarette smoke.

*N.B. : The volume of liquid in the 2 cuvettes allows to make **up to 12 slides** with the control cuvette, and **up to 12 slides** with the cuvette with dissolved cigarette smoke, for observations by a large number of students.*

CONCLUSIONS

The experiment clearly shows that similarly to what scientific research had already revealed for ciliated cells of the human respiratory tract, “inhaled cigarette smoke” has the same harmful effects on cilia of *Tetrahymena* ciliates.

The many chemicals which are present in inhaled cigarette smoke – and which ultimately end up in the lungs of the smoker – have the same negative effect on the “ciliary beat frequency” of *Tetrahymena* ciliates as in human ciliated cells.

The experiment shows “visually” that the motility (swimming) of the *Tetrahymena* ciliates gradually decreases, and finally completely stops.

**The negative effect of
cigarette smoke on
the ciliary activity
of the ciliates is,
like in humans
a first signal for
“the domino effect”
of the harmful consequences
of smoking on human health.**

The key message conveyed by the ANTI-SMOKING-EDUKIT 1 – *CIGARETTE SMOKE* experiment is unambiguously that :

**PREVENTION IS BETTER
THAN CURE**

and smoking is
definitely harmful
for human health



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