

# ANTI-SMOKING EDUKIT 3 - *SMOKE OF E-CIGARETTES*

An **ANTI-VAPING EDUCATIVE KIT** for a simple experiment  
to determine the effects of the **SMOKE OF E-CIGARETTES**  
On health



## USER MANUAL

# CONTENTS

	Page
- SMOKING...AND THE CONSEQUENCES FOR HUMANS	2
- E-CIGARETTE SMOKE: HARMFUL OR NOT?	3
- DETERMINATION OF THE EFFECTS OF THE SMOKE OF E-CIGARETTES	5
- CONCEPT OF THE ANTI-SMOKING EDUKIT 3 – <i>SMOKE OF E-CIGARETTES</i>	8
- PREPARATION OF THE EXPERIMENT	9
- PERFORMANCE OF THE EXPERIMENT	10
- OBSERVATIONS	11
- CONCLUSIONS	16

# SMOKING... AND THE CONSEQUENCES FOR HUMANS

Cigarette smoke contains thousands of chemical compounds many of which are very toxic and some even carcinogenic.

The experiment with the ANTI-SMOKING EDUKIT 1 – *CIGARETTE SMOKE* shows the harmful effects of smoking on humans, and the tests of the ANTI-SMOKING EDUKIT 2 – *CIGARETTE BUTTS* demonstrate the impact of the millions of cigarette butts which are thrown away daily, on organisms in water and soil.

For several years there has been an increasing pressure to convince adults to stop smoking, as well as to convince youngsters not to start smoking.

In 2003 the Chinese pharmacist Hon Lik thought up an alternative for smoking after his father had died from lung cancer. This was based on the patent of a small device, already invented in 1963 by a scientist in the USA, in which a solution comprised of scent and taste compounds is “evaporated” in an “aerosol vapor”, which can then be inhaled.

That was the start of the “**e-cigarette**” which has since rapidly grown commercially as an alternative for smoking of tobacco cigarettes.

According to the producers of e-cigarettes there are indeed (and contrary to the smoking of tobacco) “no harmful chemical compounds” in the inhaled (and blown out smoke), and “**vaping**” is hence “not unhealthy”.

The industry of e-cigarettes has made vaping even more popular by adding attractive fruity aromas to the liquid which is vaporized.

**The consequence is that more and more youngsters start to vape, and even as early as 12 years of age!**

The effect which vaping produces is “a pleasant stimulus” in the lungs or in the throat (called “throat hit”) when the e-cigarette mist is inhaled.

This gives a “soothing sensation” comparable to the sensation felt by smokers in their daily use of cigarettes, but which is unfortunately at the basis of their addiction to this “silent killer” ...

NB : A publicity from an “E-Cigarette Shop” even describes 6 kinds of “throat hits” which vaping fans can “try out” with different types of electronic cigarettes, to determine which one(s) give them “the most pleasant sensation”!

One of the consequences of vaping by youngsters is that it even triggers some of them “to shift” after a while from e-cigarette smoking to smoking tobacco cigarettes!!

## **E-CIGARETTE SMOKE: HARMFUL OF NOT ?**

The statement by the e-cigarette industry that vaping smoke is “harmless” for humans can be contested for several reasons.

First of all, the liquid of e-cigarettes which is evaporated also contains “**nicotine**”.

The amount of nicotine in an e-cigarette differs from product to product and must be “explicitly” indicated on all commercial packages.

This information is important since it is well-known that nicotine is at the basis of “the addiction” to smoking, and the question can hence

be asked if this will not also be the case in the long term for those who are vaping from day to day ...

The need to decrease a possible (eventual) addiction to nicotine by vapers is even treated specifically in a handout of the e-cigarette industry, by a “step-by-step progression”. The vaper is advised to gradually select electronic cigarettes containing a lower and lower amount of nicotine, to eventually brands with “no nicotine”.

Besides nicotine, the smoke of e-cigarettes contains several other compounds such as propylene glycol, glycerols and aldehydes which can irritate or damage the respiratory tract.

Although all European countries accept vaping, each country has its own regulations for e-cigarettes. The European Union has laid down rules for electronic cigarettes sold as consumer products. The EU Directive sets a maximum nicotine concentration and health warnings must advise consumers that e-cigarettes contain nicotine and should not be used by non-smokers.

In Belgium, the Health Council issued in June 2022 an updated advice on electronic cigarettes that vaping is not without risk and is even potentially harmful. This advice is actually at the basis of a Belgian Royal Decree that states that as of January 1, 2025, the sale of “disposable” e-cigarettes will be forbidden. Belgium is in this regard the first country in Europe promulgating such a drastic legislation!

In the UK and the USA the sale of vaping products is prohibited to individuals respectively under 18 years and 21 years old, and in the USA pods with flavors other than tobacco and menthol are banned.

# DETERMINATION OF THE EFFECTS OF THE SMOKE OF E-CIGARETTES

As shown by scientific research, the “direct” determination of the effects of inhaled smoke on humans is very difficult and complex, and this is also the case for the determination of the possible effects of vaping.

In 1975, the research team of Professor Gräf in Germany developed a simple technique which shows that cigarette smoke perturbs the functioning of the cilia of protozoan ciliates, similarly to what also happens with the cilia of the respiratory tract of smokers.

This allowed the development of a simple experiment with a species of ciliated protozoans exposed to the smoke of cigarettes. This test is actually at the basis of the ANTI-SMOKING EDUKIT 1 – *CIGARETTE SMOKE* which is presently used successfully in many schools.

It thus makes sense to develop a test on “a living organism” exposed to e-cigarette smoke, to determine if the smoke generated by vaping has harmful effects.

Since 1990, the research group of Professor Drewes at Iowa State University in the USA performs extensive investigations on the effects of a variety of chemical compounds on a species of small worms, namely the blackworm *Lumbriculus variegatus*.

These small transparent worms have a size of 5-6 cm and a width of 1-1.5 mm. They are very common in freshwater sediments and they are well-known by aquariologists because they are an excellent live food for small tropical fishes.

The body of blackworms is composed of 150 to 200 segments and they have a closed blood circulation system which propels the blood from the tail to the top by “rhythmic pulses” (as a kind of “heart beating”).

The interest of the American research group for blackworms was vitalized by the finding that these organisms have a remarkable behavior pattern and reaction “when they are touched”!

When the head or the frontal segments are touched, the worm contracts and turns around. When the tail or the posterior segments are touched, the blackworm escapes “in a flash” in a “zigzag” movement.



During the last decade of the 20th century, extensive research was performed in the laboratory of Prof. Drewes on these blackworms, where exposure to a variety of chemical compounds determined their specific behavior during stimulus of the head and the tail. Measurements were also made on the speed of blood pulsation in the segments.

Investigations have in this regard also been specifically made on “nicotine” (which is the well-known cause of the addiction of smokers for tobacco cigarettes)

It was found that at an exposure to a certain concentration of nicotine (pure nicotine dissolved in water) there is not only a decrease of the

blood pulsation speed through the segments (even up to virtually standstill), but that the blackworms only react slowly or not at all to the stimulation of the head or the tail.

The interesting findings of Professor Drewes and his team have led to the establishment a few years ago of the “Worm Integrative Research Laboratory (SWIRL)” at Swansea University in the United Kingdom.

Extensive research has since been performed on blackworms in this Laboratory, under the supervision of Professor Seeley.

**Nicotine** is not only an important component of the smoke of tobacco cigarettes, **but is actually also the “major component” of the smoke of electronic cigarettes !**

Similarly to the laboratory of Prof. Drewes, experiments have in the SWIRL also been performed **on nicotine**.

However, all investigations have been performed with solutions of “pure nicotine”, but until now no research has been performed (neither by these scientists nor by others), **on possible effects of e-cigarette smoke on blackworms!**

Nevertheless, the findings of the American and British scientists that stimulation of these small worms exposed to nicotine has significant effects on their reaction speed, are certainly an interesting departure point to find out possible effects of e-cigarette smoke on these test organisms.

Blackworms can be easily cultured, and cultures have therefore been established to analyse their “behavior” after exposure to the smoke of electronic cigarettes.

These preliminary investigations gave interesting observations and it was therefore decided to work out a concept for an **ANTI-SMOKING**

EDUKIT 3 – *SMOKE OF E-CIGARETTES*, with selection of the materials for performance of simple experiments in school classes.

## CONCEPT OF THE ANTI-SMOKING EDUKIT 3- *SMOKE OF E-CIGARETTES*

The concept of the ANTI-SMOKING EDUKIT 3 – *SMOKE OF E-CIGARETTES* is similar to that of the ANTI-SMOKING EDUKIT 1 – *CIGARETTE SMOKE*, namely that the smoke (the vape) of the e-cigarette is also blown in a tube filled with tap water.

The chemical compounds in the vape dissolve in the water and with a pipette, a small volume of “vape water” is transferred to a small transparent container in which one has first introduced one blackworm.

A control test is performed in parallel, in an identical small container also containing one worm, and filled with a same volume of tap water.

In the container with “vape water”, the blackworm is in continuous contact with all the compounds which have dissolved from the smoke. After a short time of exposure (about 5 minutes) the “behavior” of the test organism is analysed in both containers.

Similarly to the experiments of the American and the British researchers, the behavior of the worms is analysed after stimulation at their head and tail with the top of a probing needle.

Blackworms have a length of several centimetres and a width of 1-1.5 mm, so they are quite visible with the naked eye and one can observe their behavior “directly” after stimulation, without the need for enlargement with a magnifier or a microscope.

Observations can be repeated after a longer time of exposure (e.g. 15 minutes, 30 minutes).

## PREPARATION OF THE EXPERIMENT

The ANTI-SMOKING EDUKIT 3 – *SMOKE OF E-CIGARETTES* contains all the materials with which one can perform in school classes a total of 6 individual experiments.

In practice, 2 separate sets of 3 tests each are performed, either simultaneously, or at different times. For each experiment the analyses can be made by one or by several students.

*For each of the 2 sets of tests (and similarly to the test of the ANTI-SMOKING EDUKIT 1 – CIGARETTE SMOKE), an e-cigarette has to be “vaped” in advance and “outside of the classroom (!!)* by a volunteer (or a smoker of electronic cigarettes).

*After each puff, the smoker must blow the vape through a straw in the large tube filled in part with tap water. The blowing of the smoke dissolves the chemicals in the water.*

*In the classroom, the “vape water” is then transferred to a small transparent container in which one has first introduced one worm from a tube containing a number of blackworms.*

*A control test is set up, in an identical container with one worm and an identical volume of tap water.*

1. Take one of the 2 large (30 ml) tubes + its stopper, and one straw from the kit.
2. Fill the tube “exactly” to the mark (about 15 ml) with tap water and close the tube with the stopper.
3. Give the tube and the straw to the vaper.

## Smoking of an e-cigarette and dissolving of the vape in the water

*The vaper is asked to blow after each puff the vape through the straw in the water in the tube.*

*The blowing generates “smoke air bubbles” in the water, and the tube becomes totally filled with a thick white smoke.*

*The blowing out of the smoke must be performed carefully to avoid water spilling from the tube.*

*To make sure that enough chemical compounds dissolve in the tap water, one must ask the vaper to blow out about “10 puffs” through the straw in the tube water.*

## PERFORMANCE OF THE EXPERIMENT

### CONTROL TEST

1. Take one container, one pipette and one tube containing several worms (= worm tube) from the kit.
2. Draw up with the pipette one worm from the worm tube and transfer the water and the blackworm to the container.  
*N.B. It will be observed that in the worm tube the blackworms are aggregated in a “blob”, from which they can, however, easily be dissociated by gently shaking the tube.*  
*The worms are very active and when one tries to draw one up, they resist very strongly, and several attempts will likely have to be carried out before succeeding to get a worm in the pipette!*
3. Check to see whether the organism reacts quickly to head or tail stimulation. If the reaction is slow (or absent) the (weak) worm must be replaced by a more active one.

4. After the transfer of the blackworm in the container, one must “suck out” with the pipette most of the water from the container, such that the organism is in a “minimal volume” of water.
5. Fill the pipette subsequently twice with tap water to the 2.5 ml mark and squirt its contents into the container.

## TEST WITH VAPE WATER

1. Take a second container from the kit, and transfer (as for the control test) one blackworm from the worm tube in this container.
2. Eliminate also by pipetting most of the water from the container.
3. Fill the pipette twice to the 2.5 ml mark with “vape water” from the tube and squirt its contents in the container.

*N.B : Since there are 15 ml of “vape water” in the large tube, 3 separate tests can be performed in each of the 2 series of tests, (each with one “control container” and one “test container”, and with 5 ml liquid in each container).*

For each series of tests, 6 containers will be used, as well as one pipette, one straw and one worm tube.

## OBSERVATIONS

Observations can already be made after 5 minutes of exposure.

Take the control container and shake it (carefully) a couple of times.

The blackworm will move and swim actively.

Take one of the 2 probing needles from the kit, and gently touch the head of the worm with the tip of the needle.

This stimulation will rapidly make the blackworm contract and turn around.

Then touch the tail with the needle tip.

The worm will immediately “swim away” in a “zigzag corkscrew” movement.

*N.B : This reaction to stimulation also occurs when the blackworm is touched in the middle of the body.*

Afterwards, take the test container with the worm exposed to vape water and perform the same actions (stimulation of the head and the tail with the needle tip).

You will see that - contrary to the behavior of the blackworm in the control container – the organism is no longer active and reacts very little or not at all to stimulations.

Observations after longer exposure (e.g. 15 minutes or 30 minutes) will show that in the control container the worm still reacts immediately when stimulated, whereas it will not in the test container with vape water.

### **Important additional information and a number of suggestions**

1. Blackworms live and grow in a temperature range between 5°C and 25°C, but their best “survival time” is at low temperature.  
It is therefore advised on reception of a kit to put the 2 worm tubes in the refrigerator (but not in the freezer)

because the organisms will then die!) until the time of performance of the tests.

2. For a sound observation of the worm, the container is best placed on a white substrate (e.g. a sheet of white paper). One can even see the organisms better by “illuminating” the container (e.g. by the light of a smartphone).
3. The blackworms “don’t die” by exposure to the vape water, but they are “inactivated” (= paralysed) and react very little or not to stimulation.
4. The “amount” of nicotine in an e-cigarette (which is indicated on each package) varies from “0 mg/ml nicotine” up to 36 mg/ml!!
5. A very important finding is that when a test is performed with an electronic cigarette with “0 mg/ml nicotine” (indicating absence of nicotine in the smoke) **there is “no inactivation” of the worm in the container with vape water**, and the organism is as active when stimulated as in the control container.

The most important conclusion of this test with “no nicotine” - in comparison with tests on e-cigarettes containing nicotine - is thus that **the “inactivation (paralysis)” of the blackworms is totally due to “nicotine”**.

6. Tests with e-cigarettes containing nicotine have shown that “the degree of inactivation” is proportional with “the amount” of nicotine in the smoke (i.e., the more nicotine, the stronger the paralysis).

*N.B. It is therefore important to know and to note “how much” nicotine there is in the e-cigarette used for the experiment!*

7. The explanation for the paralysis of blackworms exposed to nicotine is that this compound has an explicit effect on their nervous system. The nervous system of blackworms consists of a long nerve chain along their whole body. The

nerve ends are in contact with the muscles in the segments which ensure worm movement. The nerves are “inactivated” when the worm is exposed to nicotine, which paralyzes the muscles and makes the organism insensitive to the behavioral stimuli.

The muscles also “propel” the blood through the body, and their “inactivation” (in part or totally) also has an impact on the pulsation speed of the blood.

8. The paralysis from exposure to nicotine is only “temporary”, and the investigations from the American and English scientists revealed that worms which have been exposed to nicotine become again totally active (and react to stimulation) when they are reintroduced in “clean water”.
9. One can therefore continue the experiment, and after a certain time of exposure to vape water, draw out the water from the container and replace it by tap water.

It will then be observed that stimulation will rapidly give the same behavioral activity as for the worm in the control container.

*N.B. A very good way to see “reactivation” after the transfer from vape water back to tap water is to try to draw up the worm with the pipette. It will be noted that the organism will escape “as a flash” (to avoid being aspirated), similarly to what happens when one tries to draw up the worm from the control container.*

### Additional observations under the microscope

Besides direct observations “with the naked eye”, one can also make interesting observations on the “blood pulsation” of the worms.

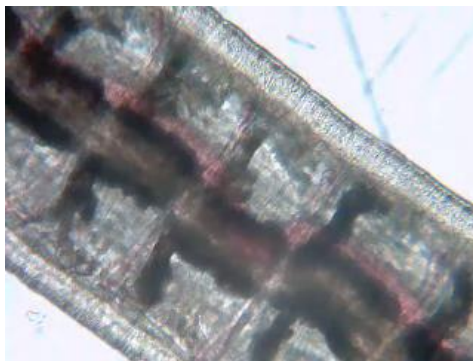
Due to the small size of the blackworms (a width of only 1-1.5 mm), these observations can only be made “under the microscope”.

With the pipette, the worms from the control container and the test container are each transferred to a glass slide, in a minimal volume of water, and then covered with a cover slide.

*NB : In order to keep the (actively moving) worm from the control container “as quiet as possible” for the observation, one has to remove as much water as possible from under the cover slide (e.g. absorption with the tip of a piece of filter paper) without, however, aspirating all the water.*

The worm must subsequently be centered under the microscope “at an appropriate magnification” to see the individual segments, and the pulsation (“heart beating”).

The photo hereunder shows 4 segments of the blackworm at high magnification.



The blood pulsation is rhythmic, with several “pulses” per minute, and the pulses move from segment to segment, from the posterior part of the worm to the head. The pulsation speed will normally be higher in the “control” worm than in the worm exposed to vape water.

*N.B. The interpretation of the pulsation speed has, however, to be made “with care”, because this speed is different in the anterior, middle, and posterior parts of the worm body.*

Analyzing the impact of compounds in the vape smoke on the blood pulsation in the worms is therefore also of interest!

## CONCLUSIONS

As mentioned above, it is presently agreed worldwide that smoking of electronic cigarettes is not “totally harmless”, and that vaping should be discouraged because of the possible addiction to nicotine as shown to occur with smoking of tobacco cigarettes.

The extensive research on the effects of nicotine on the blackworm *Lumbriculus variegatus*, and the test of the ANTI-SMOKING EDUKIT 3 – *SMOKE OF E-CIGARETTES* with the vape of e-cigarettes are an important “additional proof” that “vaping” has negative effects on “a living organism”, and by extension also to humans.

The experiment with the vape of an e-cigarette “with no nicotine” (which shows that there is no negative effect on the test organism) provides a clear proof that **nicotine** is “the culprit” which causes the effects.

The findings on blackworms as test organisms are a signal that smoking of electronic cigarettes is **not healthy either “for humans”** and that, similarly to smoking of tobacco cigarettes, vaping will lead to addiction.



The key message of the  
ANTI-SMOKING-EDUKIT 3 –  
*SMOKE OF E-CIGARETTES*  
is hence:

**PREVENTION IS BETTER  
THAN CURE**

Because vaping is definitely  
harmful for health



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