# BfR 2 GO

The Science Magazine of the German Federal Institute for Risk Assessment **Issue 1/2018** 

Tattoo Tnks

# A risk that gets under your skin

Cooking shows & germs Kitchen hygiene in the spotlight Magnesium & Co. Food supplements in sports Animal experiments Database shows purpose of research

BfR2GO



It was 5,300 years ago that the "Man from the Ice", better known as Ötzi, died of his injuries. Ever since he was found on 19th September 1991 in the Ötztal Alps, the South Tyrolean ice mummy has opened a window into the Copper Age. One of the most fascinating discoveries was the fact that Ötzi was tattooed. 61 tattoos coloured with carbon "adorned" the body of the man in his midforties. Perhaps they had medical significance and were intended to relieve pain caused by arthritis, parasites or other ailments.

In modern society, people may primarily have other motives for dying their skin, but a touch of magic is still in there somewhere too. The health problems that tattoos can bring are a lot less mysterious and this makes the current trend for tattoos a topic for the German Federal Institute for Risk Assessment (BfR). All the more so, as one in five people in this country has tattoos. Infections, allergies and even carcinogenic effects are being discussed. As the scientific data basis on health assessment is still inadequate, the BfR's commitment to the topic is all the more important. Our Institute has by now become one of the world's leading institutions in the research of the health risks of tattoo inks.

Of course one could ask the question whether the BfR is well advised to dedicate itself to the topic of tattoos. Surely people only have themselves to blame if they expose themselves to this risk, so why is taxpayers' money being used to pay for the research? The answer to this can only be that it is our mandate to identify risks and protect health. This applies all the more when a practice is as widespread as tattooing. Our job is to assess these risks and to inform people about them. Plenty of men and women (tattoos are particularly popular with young women!) will think twice in future before they put themselves "under the needle". Unlike people in Ötzi's day, one thing they can't hope for is a health benefit.

Although the risks of tattooing are the main focus of this new issue of our science magazine BfR2GO, many other exciting topics await you too: kitchen hygiene in TV cooking shows, "botanicals" for athletes, the latest from the European chemicals regulation REACH, an interview with the head of the German Centre for the Protection of Laboratory Animals at the BfR and much more. Nothing has been patched together – or tattooed – in a hurry here!

Wishing you an entertaining and informative read.

**Professor Dr. Reiner Wittkowski** BfR Vice-President



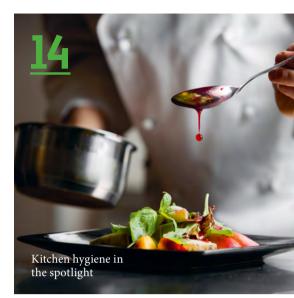
# Incidentally

To remain stable when not fully loaded, freight and passenger ships pump seawater or brackish water into special tanks to act as so-called ballast water. This water is then discharged elsewhere when necessary. In this way, organisms transported in the ballast water can find their way to other habitats and disturb the ecological balance. The Ballast Water Management Convention that came into force in 2017 aims at reducing the spreading of invasive species by regulating the conditions for exchange and treatment of ballast water. However, the treatment of ballast water also involves risks. Chemicals, UV radiation and other methods are used to kill the organisms in tank water prior to discharge. The BfR is responsible for the assessment of possible health risks caused by ballast water treatment. The Institute therefore evaluates chemicals used for ballast water treatment, including disinfection by-products generated through the chemical reaction of strong oxidation agents, such as chlorine, with organic material and dissolved salt in seawater.

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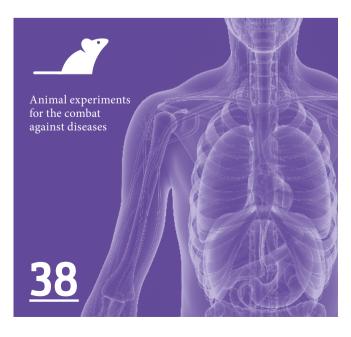








Assess. Research. Communicate.



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**KEY TOPIC: TATTOO INKS** 

# Getting under your skin: Health risks through tattoo inks

Carcinogenic substances, allergies, infections – a wide range of health risks are being discussed in connection with tattoo inks. Up to now it has been mostly unclear which substances have which effects. Various BfR projects are now producing results which are attracting attention all over the world.



They go by the name of Dotwork, Blackout or Double Exposures – the trend techniques of the tattoo scene. Something new hits the market just about every year: watercolour techniques, anatomical motifs, white ink or black light images that illuminate in the dark. What used to be the domain of sailors and criminals turned into a popular mass phenomenon in the 1990s. The trend towards tattooing has remained unbroken ever since, as a current study conducted by the University of Leipzig shows. One in five Germans already has a tattoo and in the age group between 25 and 34, it's even the half of all women. Despite being so widespread, however, tattoos have rarely been discussed up to now in connection with health risks.

# What risks are we talking about?

Tattoos can have various undesired effects on health (see chart). Just like other open wounds, freshly tattooed skin can occasionally become infected through poor hygiene or inks contaminated with bacteria, viruses or fungi. In addition to this, the constituents of tattoo inks can trigger undesired reactions in the body, such as allergies and other complaints. The possibly carcinogenic effect of certain substances is also under discussion, and UV or laser beams can also alter the health effects of the pigments. Sunbathing as well as tattoo removal using laser technology can therefore pose a health risk.

The experts, at least, have been aware of the large number of possible health risks that tattoos can cause for quite some time now, but many questions have remained open up to now when assessing which dyes, ingredients and techniques are of particular concern from a health point of view. The reasons for the uncertainty are of a legal and scientific nature.

## How are tattoo inks regulated?

The tattooing agent regulation contains a negative list of substances which may not be used, while additionally prohibiting other substances on the basis of the cosmetics regulation. The problem is that not all of the dangerous substances that can occur in tattoo inks are regulated here. Moreover, the scientific data required to make the corresponding safety assessment is often missing. A restriction proposal for tattoo inks is currently being prepared within the scope of the European regulation concerning the Registration, Evaluation, Authorisation and Restriction of chemicals (REACH, see page 30) in order to exclude substances whose hazardous properties have already been identified from use in tattoo inks.

# The scientific data basis: uncertainty prevails

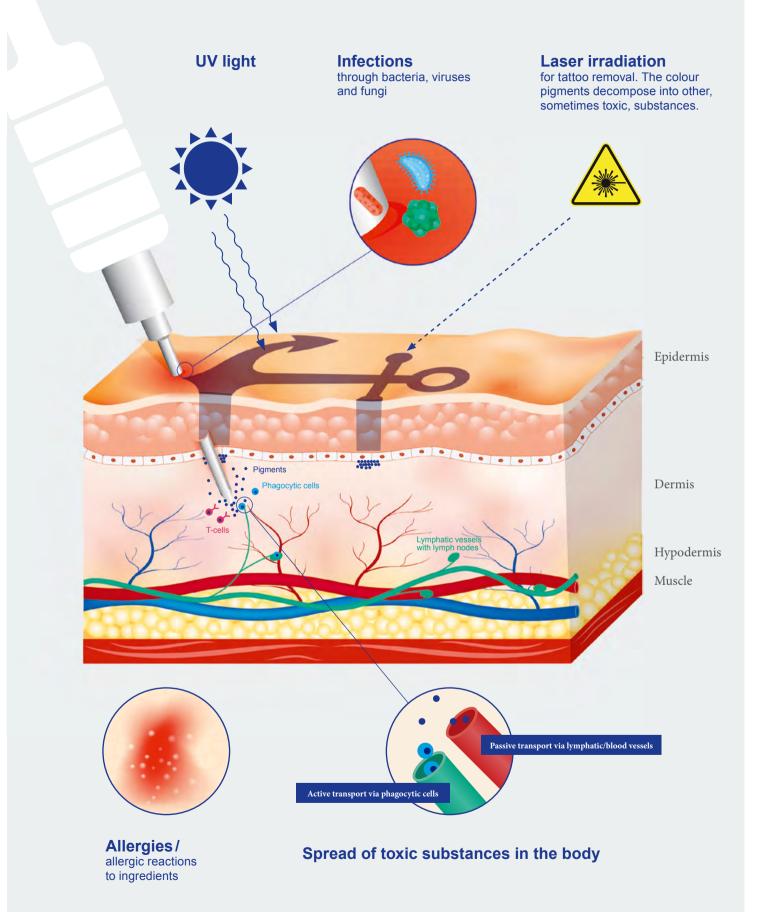
Certain constituents such as dyes and preservatives must be authorised in accordance with cosmetics law. According to European law, however, tattoo inks are not cosmetics as they are applied under and not on top of the top layers of skin, so that the manufacturers are not obliged to prepare toxicological safety reports for their products. Animal experiments of tattoo inks have not been permitted in Germany up to now for ethical reasons, and no epidemiological studies exist.



The health effects of tattoo ink ingredients such as colour pigments are being examined at the BfR.



# **Risks of tattooing**



Colour pigments are filled into test tubes for thermal treatment by mean of pyrolysis.



All of this has had the result that the scientific data basis for the health assessment of tattoo inks is currently insufficient.

A further challenge from a scientific point of view is above all the large number of substances used in tattoo inks. There are organic and inorganic pigments with various chemical structures, additives such as binding agents and preservatives. All of these agents can ultimately be contaminated with elements or other substances, and with each additionally used substance, it becomes more difficult from a scientific point of view to assess the health effects that the agents can bring about.

Where toxicology is concerned, the long-term effects of tattoo inks are of particular importance. "The chronic risks could only be examined by means of the corresponding animal experiments or epidemiological studies conducted on a large number of humans, but animal experiments are not permitted for tattoo inks", explains Professor Dr. Dr. Andreas Luch, head of the Chemical and Product Safety department at the BfR. "And no epidemiological studies have been conducted yet. All we have is an uncoordinated experiment which all people with tattoos conduct on themselves in principle – with open results".

### Tattoo removal per laser – a health risk

Within his department, Professor Luch consolidates various research projects on the subject of tattooing. In 2013, he organised a symposium on the safety of tattoo inks together with Dr. Peter Laux, head of the Product Safety and Nanotechnology unit, after which the BfR started its first experimental examinations. Dr. Ines Schreiver, who today heads the BfR junior research group on tattoo ink research (see interview on page 12), joined the BfR back then as a doctoral candidate. In her work she wanted to identify the substances into which colour pigments decompose when they are lasered during tattoo removal. At that time, this had only been examined for a few pigments using *in vitro* methods and a red pigment on mice. "The animal's skin was tattooed,

then lasered after a few weeks before being extracted and ultimately analysed – a very elaborate process", recalls Schreiver. "We were looking for a simpler method."

And they found one. The idea was based on the observation that the intense heat that develops when lasering leads to the decomposition of the pigments. To simulate this process, the pigment powder was heated to 800 degrees Celsius by means of pyrolysis. The substances produced in this way could then be separated in the usual way by means of gas chromatography and identified per mass spectrometry. A new and successful method for simulating toxicological aspects through laser radiation had been found, because pyrolysis had only been used for pigment identification up to then.

The results showed that when the 36 pigments examined are lasered, some substances that are undesired from a health point of view are produced, such as primary aromatic amines, some of which have a carcinogenic effect. In particular copper phthalocyanine, a blue, particularly lightfast and therefore very popular pigment, was the focus of the study. It decomposed through pyrolysis and after laser irradiation into hydrogen cyanide and benzene among other things – substances with a high toxic potential. Once the study had been published, there were increased reports in the media on the health risks of tattoo removal.

# Where do the pigments migrate to in the body?

The next project at the BfR was dedicated to toxicokinetics. The original question was: What happens to the pigments under the skin? It was known from tests on mice that as much as 30 percent of the pigments disappear from the skin after approx. 42 days. But where are they then? The mouse study and observations in the clinic had shown that the lymph nodes of people with tattoos are often enlarged and coloured. To verify this transport scientifically and characterise the particle structure and composition, the BfR received skin and tissue samples from deceased persons with tattoos from its forensic medicine cooperation partners in Munich for research purposes. Examination of the samples showed that a majority of the pigments accumulate in the adjacent ("regional") lymph nodes. The small, nano-size particles in particular are very mobile. In this way, the BfR analytically proved for the first time what science had long presumed: the toxic elements of tattoos do not remain locally restricted to the skin, they accumulate in the lymph nodes. This news attracted attention all over the world. The study appeared in Scientific Reports journal, a Nature Publishing Group publication, and was one of the most read contributions there in 2017. TV stations and newspapers from Europe, Latin America and the USA, including the BBC several times, reported on the research results.

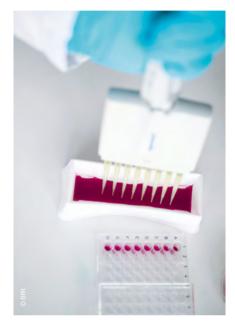
# Risk perception has to change

The BfR is regarded in the meantime as one of the world's most important institutions for the research of the health risks of tattoo inks. A corresponding junior research group was established at the Institute in 2017 (see page 12) to continuously advance experimental research in this field. The BfR is planning a representative population survey in 2018 dedicated solely to the risk perception of tattoo inks. It will be seen in the coming years to what extent scientific findings on tattoo inks contribute towards a change in how risks are perceived by the general public. "The decisive thing is communication. It should be clear to every consumer that it is linked with a health risk which people take voluntarily", says Professor Luch.

More information www.bfr.bund.de/en > A-Z-Index: tattoo

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Colour pigments migrate in the body – nanoparticles in particular are very mobile



Tattoo inks are tested for their toxic effect.



When a tattoo is applied, the natural barrier function of the skin is switched off. Hygienic work in the tattoo studio is therefore particularly important.



# "We want to identify as many allergens as possible"

Dr. Ines Schreiver is head of the new BfR junior research group on tattoo ink research.

# Dr. Schreiver, what is your junior research group working on?

We are currently working on two projects. The first one deals with the allergenic risk of tattoos, starting off with the observation made by dermatologists that many tattoos from the red colour spectrum cause allergies. Medical people lump together pink, purple and orange under the heading "red", even though these substances are completely different from a chemical point of view. We want to find out which pigments have a particularly allergenic effect.

# Why do you believe it is the pigments and not the co-formulants of the tattoo inks that cause the allergy?

Both are possible in theory, but the co-formulants are usually water-soluble, which means that they are excreted after a few days, whereas the insoluble pigments remain in the body. So when we talk about allergies which do not occur in the first few days but only after years, this can only be triggered by the pigments or the substances into which they decompose. As the allergies often only occur after many years, we suspect that the decomposition substances are the actual cause.

### What method do you use in your study?

We have received over 100 tattooed skin samples from allergy patients from our medical cooperation partners. During the analysis of these, we have identified four to six pigments which were used repeatedly and two to three of which are very dominant. We are now conducting tests to find out which substances could have been cleaved off. There are several *in vitro* tests with which this can be analysed. My long-term goal is

### BfR junior research groups

Since 2017, there have been five junior research groups at the BfR which were set up to promote research in selected areas of main emphasis at the BfR, while furthering the scientific career of young scientists at the same time. The groups are dedicated mainly to research and are headed by qualified junior staff shortly after they have obtained their doctorate. They run for a period of three years with the option of extending to a total period of five years. The junior research group on the health risks of tattoo inks started up on 1 September 2017. It currently consists of one leader, two doctoral candidates and a technical assistant.



The junior research group on tattoo ink research headed by Dr. Ines Schreiver started up in 2017.

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# We want to find out which pigments have a particularly allergenic effect.

to identify as many substances as possible which can play a role in the development of allergies.

### What does your second project deal with?

The second project examines how pigments react to UV radiation. We are conducting *in vitro* tests here, too.

# Does that mean that you expose a skin model cultivated in a Petri dish to UV light?

Yes, but we don't use a conventional skin model here. The challenge with this issue is that the tattooing agent is always applied beneath the epidermis, the top skin layer, into the dermis, the thicker skin layer below. This means that the cells of the epidermal cells lie between the dermal cells with the pigments and the UV radiation. It is customary, however, in *in vitro* tests without skin models, to simply place the cells next to one another. By doing so, it cannot be seen how the pigments farther down react to the radiation and how cells in their proximity behave, because the interaction between the cells works differently in space than on a flat surface. That is why we are developing a three-dimensional skin model for our study.

### How are we to envisage this?

To begin with, you mix pigments and cells from the human dermis with collagen to produce a model for the tattooed dermis. Cells from the epidermis are seeded over this which can then form a horny layer. The result is that the skin model is then about 1 to 3 millimetres high and the surface area about the same as a little fingertip. Effects can then be examined on this little cylinder which can only be depicted in three dimensions.

# A personal question to finish off with. Everyone in your group is aged around 30, which makes them the main target group for tattoos. Does this make itself noticed?

As far as can be seen, none of us has a tattoo at the moment but we have had some colleagues with tattoos behind their ears or on their arms. Tattoos are to be found all through society these days. That's also the reason why research in this area is as relevant as never before.

### Many thanks for the discussion, Ms. Schreiver. **a**

### More information:

Schreiver et al. 2017. Synchrotron-based v-XRF mapping and  $\mu$ -FTIR microscopy enable to look into the fate and effects of tattoo pigments in human skin. Sci Rep. 7: 11395, doi:10.1038/s41598-017-11721-z.

Schreiver et al. 2016. Identification and hazard prediction of tattoo pigments by means of pyrolysis-gas chromatography/mass spectrometry. Arch Toxicol. 90: 7, 1639– 1650. doi: 10.1007/s00204-016-1739-2 (Open Access)



# Kitchen hygiene in the spotlight: Exemplary behaviour can prevent illnesses

TV shows about cooking are very popular, but a research project conducted by the BfR shows that kitchen hygiene tends to receive little attention on television and that this can have an impact on the behaviour of viewers copying the recipes. The good news is that TV programmes can use this imitation effect to promote good kitchen hygiene and thus prevent illnesses in private households.

# Kitchen chaos or gala menu?

Professional chefs whisk cream in the spotlight, amateurs cook their favourite recipes at their stoves at home and the recipe is included for free: Different cooking formats on television are evidence of the Germans' love of cooking. The programmes are very diverse and are broadcast on both public and private channels.

However, aside from preparing delicious food, good kitchen hygiene is also part of the art of cooking. Otherwise, ingredients contaminated with pathogens, dirty kitchen sponges or germs on hands could spoil the meal. Each year in Germany, over 100,000 illnesses are reported, many of which are caused by bacteria, viruses or parasites in food. Those who observe the usual measures of kitchen hygiene can protect themselves and others from illnesses of this type.

So how much kitchen hygiene is shown on TV programmes? And what influence does the behaviour demonstrated by TV chefs have on the hygiene behaviour of consumers? To get to the bottom of these questions, the BfR conducted a multi-part research project on the topic of kitchen hygiene in cooperation with other project partners. The first part of the project was an analysis of the hygiene practices shown in cooking shows. The second part comprised an experimental cooking study.

# Kitchen hygiene only has a minor role

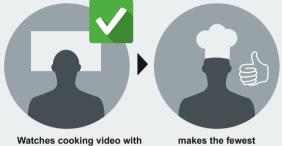
To investigate hygiene practices in cooking shows, the first task was to create an inventory of TV cooking programmes and analyse them. Based on defined criteria, 100 episodes of cooking shows with high viewer numbers, which were intended to represent a broad range of existing formats, were selected. The episodes were then analysed using a list of typical kitchen hygiene lapses to each of which a severity level was assigned.

The results show that important hygiene measures in TV cooking shows are often neglected. "A hygiene lapse can be observed every 50 seconds on average", says

# Cooking shows influence our hygiene behaviour

BfR kitchen hygiene study: methodological procedure and results

# Subsample A\*



Watches cooking video with exemplary kitchen hygiene (exemplary video)

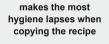
makes the fewest hygiene lapses when copying the recipe

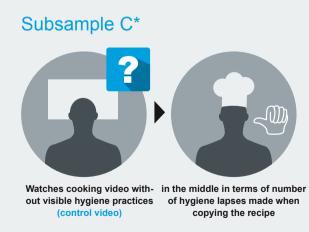
# Subsample B\*





Watches cooking video with poor hygiene practices (poor video)





\*Randomised assignment to three video versions

PD Dr. Gaby-Fleur Böl, head of the Risk communication department at the BfR. "Severe hygiene lapses could be seen approximately every two-and-a-half minutes", continues Böl. Behaviour which could result in the spread of pathogens or cross-contamination was shown most often. Examples were wiping dirty hands on a tea towel instead of washing them or using the chopping board without cleaning it between different work steps (see page 17).

# Copying recipes, copying behaviour

The second part of the BfR project was therefore to investigate the question of what influence hygiene behaviour in the TV programmes has on the viewers' hygiene behaviour when they copy the recipes. For this purpose, three versions of a cooking video were created which differed only with respect to the quantity and severity of the hygiene lapses committed. All three videos show a professional chef with TV experience preparing a chicken salad with homemade mayonnaise and explaining the work steps in a clear and understandable manner. The hygiene behaviour is exemplary in the first video and poor in the second, while the third video completely omits the hygiene sequences. This was the control video.

Participants each watched one of these videos in an individual setting. The selection of who watched which video was determined at random (see figure). Afterwards, the participants prepared the salad with mayonnaise in a test kitchen on their own. While they were cooking, they did not know the actual purpose of the study. They knew that they were being observed but not that their hygiene behaviour was being recorded. The investigators who were keeping the records were not aware either of which video the participants had watched (double-blind study). In this way, no one involved in the study could influence the results.

The analysis of the hygiene behaviour of the participants showed that the hygiene behaviour demonstrated in the video was reflected in the hygiene behaviour of the participants when they copied the recipe. In more precise terms, people who watched the exemplary video made significantly fewer hygiene lapses when copying the recipe than people who watched the video of the chef demonstrating poor kitchen hygiene. On average, people who were assigned to watch the control video without hygiene guidelines fell in between the other two groups in terms of the number of hygiene lapses made. Among the most common hygiene lapses committed by the participants were adding salt to food with their fingers and not washing their hands after contact with potentially hazardous foods such as raw meat. Both of these incorrect practices were also demonstrated by the chef in the video featuring poor hygiene.

# Role models wanted

The results of the BfR research project provide initial indications that the hygiene behaviour shown in TV cooking shows can have an effect on the hygiene behaviour of viewers. The BfR sees a need for further research here. To obtain a valid basis for measures, the BfR is currently conducting a representative survey on the TV and cooking habits of the population. However, even without these representative data measures can already be taken. Promoting risk awareness among professional TV chefs, e.g. through informative material or by making direct contact with prominent representatives of cooking shows, could result in improved hygiene behaviour in TV programmes. It's not all down to the people in front of the camera, though. "Simple changes, such as installing soap dispensers in TV cooking studios, could allow hygiene measures to be integrated in the chefs' normal routines", according to Böl. Increased risk awareness for kitchen hygiene during filming and editing could also ensure that cooking shows set a good example. Camera operators, editors, directors ... many people are involved in creating a programme and make decisions on which sequences are broadcast to viewers.

### More information:

BfR leaflet "Protection against foodborne infections in private households"

BfR-Brochure "Kitchen hygiene in the spotlight: Do TV cooking shows influence our hygiene behaviour?"

Online at: www.bfr.bund.de/en > Publications

# Frequent hygiene lapses in analysed TV cooking shows



Wiping dirty hands on tea towels



Not washing hands after scratching, sneezing, blowing one's nose



Using chopping boards without washing them between different work steps



Not washing hands before preparing food

Adding salt/spices with fingers

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# BfR recommendations on kitchen hygiene

- > Wash your hands thoroughly with soap before beginning
- > Clean work surfaces and hands thoroughly, also between different work steps
- > Use different chopping boards and cooking utensils for cooking and preparing raw foods (particularly poultry and other meat) or wash them thoroughly between different work steps
- > Cook foods, particularly fish and meat, thoroughly to at least 70° C for 2 minutes in the centre of the food
- > Maintain the cold chain and store highly perishable foods in the fridge
- > Keep meat and fish in the coldest compartment
- > Wash lettuce, herbs, vegetables and fruit for raw consumption carefully and peel them if necessary
- > Keep highly perishable foods cool at barbecues or picnics
- > Keep heated foods hot at over 65° C or cool them down to 7° C within a few hours
- > Wash tea towels, dish cloths and sponges at min. 60 °C or change every few days

# Campylo\*...- what? Health risks in the view of the general public



# **Top 5 health risks**

- 1. Smoking
- 2. Climate and environmental pollution
- 3. Alcohol
- 4. Unhealthy/wrong diet
- 5. Unhealthy/contaminated food



# Key word Kitchen Hygiene

Food hygiene in the home continues to be erroneously classed as being of no cause for concern by a majority of respondents. Only 14 % are concerned about it, as opposed to 37 % who are concerned about food hygiene in gastronomy.

### Underlying method:

Each survey was conducted by means of computer-assisted telephone interviews of at least 1,000 German-speaking people aged 14 and over living in private households in Germany. The random sample is generated from land line and mobile telephone numbers. The principle is an omnibus survey.

Underlving sources: Consumer Monitor surveys (issues 2014, June 2015, February 2016, February 2017, August 2017, February 2018)

Can be referenced at: www.bfr.bund.de/en > Publications > Brochures > BfR Consumer Monitor



# Safety of foods

Compared to the first BfR survey (2014: 71 %), food is still regarded as either safe or more safe than unsafe (2018: 81 %). 36 % of the respondents say that the safety of food is on the decline, however. Almost one person in two (45 %) says that its quality is suffering even more.

# Vector/Shutterstock.con

# **Underestimated germs**

\**Campylobacter* are the most common bacterial cause of diarrhoea in Germany. Even though more than 70,000 Campylobacter infections are reported every year in Germany, the pathogen is still widely unknown among the general public. In 2018, only 23 % are aware of these germs and of the people who know these germs not quite the half is concerned about them. By way of comparison, almost everyone is aware of Salmonella (96 %), and every second person (46 %) is worried about them. There were 13,000 reported cases of Salmonella infections in 2016.

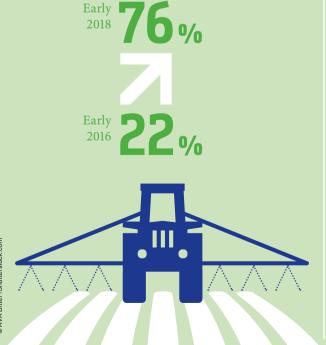
The BfR has been conducting regular representative consumer surveys on the perception of health risks by the general public since 2014. The results are published as the BfR Consumer Monitor. Here are the interim results regarding the consumer topics which the general public know about, fear and underestimate.

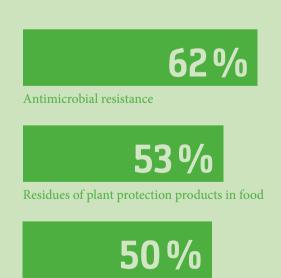


# Glyphosate

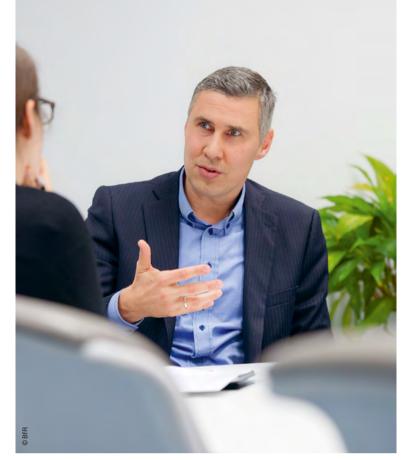
has arrived in the awareness of the general public. At the beginning of 2016, only 22 % of respondents knew about this active substance contained in plant protection products; this figure has risen to 76 % in 2018.







Genetically modified food



**Professor Dr. Stefan Trautmann** holds the chair of Behavioral Finance at the Alfred Weber Institute for Economics at the University of Heidelberg. He has been a member of the BfR Committee for Risk Research and Risk Perception since 2014.

# "Not everyone wants a state that nudges"

Nudging: Professor Dr. Stefan Trautmann is a behavioural economist who deals with people's financial decisions. He contributes to the development of the BfR's risk communication strategies with his specialised knowledge. In this interview, he talks about the method known as nudging and its possible areas of application.

Professor Trautmann, the BfR has the mandate of assessing risks and making them public. But even well-informed citizens take many wellknown health risks, for example by lighting up a cigarette. Does this mean that information alone does not help?

Information about possible consequences always helps when reaching a decision, but people often neglect or ignore risks. So, why is that? In their book "Nudge: Improving decisions about health, wealth and happiness", Richard Thaler and Cass Sunstein describe the psychological obstacles that prevent us from implementing our preferences when making choices. It is clear to them that we often decide in favour of something that we don't actually want. What is known as nudging is supposed to prevent such mistaken decisions.

# So nudging is supposed to help us to implement our own preferences?

Yes, nudging is intended to incite people to clever behaviour without restricting their freedom of choice. Instead of forbidding things, the goal is to neutralise the afore-mentioned psychological obstacles. What we call the choice architecture is altered here. A wellknown example of this is the practice of organ donation. Whereas donors in Germany have to make an active decision in favour of a donor's pass, Austrians can only make a negative decision against it. This difference contributes to the much higher willingness to donate in Austria compared to Germany.

### What forms of nudging are there?

The design depends strongly on the application. First, there is the standard option described above in the case of organ donation. This is known as setting a default and can usually be applied without much effort. Another version would be to make the preferred option more accessible. If fruit is easier to reach in the canteen than a sugary dessert, for example, this could cause a hungry person to choose the fruit. Simple information conveyed in a very accessible manner can also be seen as nudging.

### Do social comparisons also function in nudging?

This is the so-called social peer effect. Consider the example of a family's electricity bill: suppose the bill includes information on the average electricity consumption of a typical household of four in your neighbourhood. If your own family's consumption is considerably higher than this average, this information may encourage a change in behaviour, reducing possibly wasteful consumption. Nudging can therefore take on many different forms.

### How can these applications be evaluated?

To be able to evaluate the applications, two questions have to be addressed. Firstly, will the application have an effect? Although the design of nudges is based on insights from the behavioural sciences, only vague predictions can be made for most interventions. No sound theoretical basis exists for many of the desired effects. To determine the effect, applications typically need to be tested individually. How do consumers react to traffic light labelling which signals the sugar content in foods, for instance? Secondly, it has to be clarified whether the benefits justify the costs. That's the efficiency question. One advantage of nudging is that there are many applications with low implementation costs. For example, a letter is easily adapted with alternative formulations or layouts that simplify and guide the consumer's decisions. A cheap intervention like this can be worthwhile, even if it has only a modest impact on behaviour.

# If applications prove to be effective and efficient, could players in the field of consumer health protection use nudging too?

Yes, consumer health protection applications are conceivable. Suitable areas include the consumption of potentially health-damaging products, or aspects related to a healthy life style. The effective conveyance of relevant information, as well as the structuring of the decision environment, are relevant approaches in this context. Importantly though, the different institutions involved in consumer health protection differ greatly in their mission and mandate. The mandate of the Federal Ministry of Food and Agriculture, for example, is different from that of the BfR. Whereas the ministry can issue bans, or introduce marking and labelling obligations, the role of the BfR lies in the assessment of risks and their transparent communication. There is a clear distinction here between risk assessment and risk management.

## So institutions actively involved in risk management could use nudging?

This is certainly conceivable. In their book, Thaler and Sunstein advocate that the state should use nudges because they presume that people's freedom of choice will not be restricted by it. In many countries, including Germany, there are teams who advise the government on nudging interventions. In Germany there has been much criticism from various sides, however. Not everyone wants a state that nudges.

# BfR Committee for Risk Research and Risk Perception

The Committee for Risk Research and Risk Perception advises the BfR in the choice of its methods for determining the level of information and subjective risk perception in the area of consumer health protection relating to food and feed, consumer products and cosmetics. The committee also supports the conducting and evaluation of target group-specific communication measures and assists the BfR in the context of emerging risks.

### What are the problems with nudging?

First of all, many people fear subtle influencing by the state. Nudging is often seen as state manipulation which lacks the necessary transparency. I do not consider this argument particularly convincing. Through its structure, a supposedly "nudge-free" situation also influences consumers' decisions in a manner that lacks transparency. Another argument against the use of nudging by the state is that it does not only affect people's decisions, but also their preferences. This would contradict the goal of helping people to better implement their own preferences.

### Can you give me an example?

If, for example, we change the inquiry into special dietary needs for a dinner menu from "vegetarian" to "main course containing meat", a norm is changed too. In the first option, the default is a meat dish and in the second a vegetarian dish. In the second instance, people who otherwise eat meat might thus question their preference.

## Are there other points of criticism?

The so-called crowding out of other measures is given as an example. If a product is regarded as damaging to health, discouraging its consumption by taxation or by a potential ban often requires a tedious political process. Nudging could then provide a low-conflict alternative to policy makers. However, if the nudge has a much weaker impact than the more heavy-handed regulation through taxation or prohibition, avoiding political discourse comes at the expense of weakened health protection. In my opinion this is one of the stronger arguments against nudging.

### Many thanks for the interview, Mr. Trautmann. **a**

**Food supplements in sports:** 

# Power packs or false promises?

Magnesium for muscle cramps or caffeine to improve performance? Many sports enthusiasts turn to food supplements to increase their fitness. However, risk assessment of individual supplements shows that negative effects may be possible. A balanced diet remains the best foundation.

E xperts agree that sport is good for you. Physical activity is proven to have a positive effect on combating excess weight, high blood pressure and depression, for example. More and more sports enthusiasts are hoping to reinforce positive effects of physical training through targeted intake of specific substances in food supplements. To protect consumers from being misled or even exposed to possible health risks, the BfR is taking a closer look at such supplements.

Food supplements are classified as food and are therefore subject to the general provisions of food law. Like with other foods, manufacturers and distributors bear responsibility for the safety of their products and for compliance with the provisions of food law. In addition, consumers may not be misled by the information on packaging or in advertisements. The German regulation on food supplements stipulates which vitamins, minerals and specific vitamin or mineral compounds may be added. According to this regulation, additional nutrients and other "substances with nutritional or physiological effects" may be added to the supplements. Which "other substances" these may be is not specified in the regulation.

# Herbal substances and preparations (botanicals)

"Other substances" as referred to above also include certain herbal ingredients. In addition to a wide range of nutrients such as vitamins, trace elements, minerals or amino acids, food supplements often also contain herbal ingredients or preparations referred to as botanicals. Unlike herbal medicines – known as phytopharmaceuticals – food supplements, including herbal food supplements, are not subjected to an official approval procedure before being placed on the market.



# Examples of undesired effects of plant substances

# **Undesired effects of quercetin**

Quercetin is a secondary plant constituent found in many types of fruit and vegetables. It is added to specific food supplements, often with the aim of strengthening the immune system. The BfR would like to point out that interactions between quercetin and specific drugs may occur, depending on the dose. It is possible that quercetin may affect the bioavailability (and thus the efficacy) of certain drugs.

# It's the dose that counts

In risk assessment of food supplement products with isolated plant substances, the dose plays an important role. Adequate levels of secondary plant substances are consumed through fruit, vegetables, herbs and spices as part of a balanced and varied diet. This can have a positive effect on health. Supplements consisting partly of secondary plant substances such as carotenoids, alkaloids or polyphenols often contain high doses in comparison to intake of such substances via a balanced diet. This increased intake could potentially be problematic. Currently, there are no binding maximum levels for herbal ingredients in food supplements, either at the German national or the European level. In addition, the effect of plant substances from traditional foods such as fruit, vegetables, herbs and spices cannot be compared exactly to preparations in food supplements. For example, the bioavailability of plant substances consumed in a concentrated form, e. g. through capsules, could be significantly higher due to a lack of interactions with other substances in foods.

# Difficulties regarding risk assessment

Besides conducting risk assessments on individual plant constituents, the BfR also performs risk assessments on botanicals in food supplements. The scientists involved may encounter a number of difficulties here. Raw material to be examined in the form of herbal extracts or preparations frequently contains a complex mixture of different plant substances, very often in a variable ratio to one another in different batches. Furthermore, there is often insufficient information on the composition of the products. In addition, the safety of many of the herbal ingredients used has not yet been sufficiently investigated, so the basis of data for a health assessment is sparse.

# No substitute for a balanced diet

It is already clear, however, that food supplements are no substitute for a balanced diet. Fitness enthusiasts can cover their nutritional requirements with an individually adapted diet suitable for sportspeople. It is important to understand that there is no superlative comparison of "healthy". The notion of "more is better" does not apply to the intake of food supplements. There are situations in professional and high-performance sports where, depending on sex, training level, time of year, type of sport and competitive stress, targeted intake of certain supplements may make sense. However, such use of supplements should take place under medical supervision after careful consideration of the risks and benefits for health and performance.

More information: www.bfr.bund.de/en > A-Z Index > Food supplements



O Diana Taliun/Shutterstock.com

## **Undesired effects of caffeine**

Caffeine is used in sport with the aim of improving endurance and performance. Depending on the dose and the individual sensitivity to caffeine, undesired effects such as nervousness, insomnia, gastrointestinal problems, increased blood pressure, heart palpitations and even cardiac arrhythmia are possible. From the perspective of the European Food Safety Authority (EFSA), which derived safe intake levels of caffeine in 2015, single doses of caffeine of up to 200 mg or the same amount consumed within a short time (corresponding to up to 3 mg/kg body weight) from all sources have been considered to be safe for healthy adults (not including pregnant women). For habitual caffeine consumption, EFSA derived a safe daily intake of up to 400 mg distributed throughout the day (equivalent to 5.7 mg/kg body weight per day) for healthy adults, not including expectant or nursing mothers.

Ideally, food supplements should only be taken in professional and high-performance sports on medical advice.



## **Undesired effects of synephrine**

Synephrine is a substance found in citrus fruits. Advertised as a weight loss promoting substance, it is added to certain food supplements, often in combination with caffeine and other stimulants. Clinical studies have shown that just a one-off intake of synephrine at doses that are found in some food supplements can cause increased blood pressure and heart rate. There have also been reports of serious effects such as arrhythmia, heart attacks, ventricular fibrillation and high blood pressure. Undesired effects occurred particularly in combination with caffeine during or after physical activity. Consumers ingest an average of 6.7 mg of this substance every day through conventional foods. According to the BfR, this level can be considered safe. Doses of synephrine many times higher than this have frequently been found in food supplements.

# Sick through foodborne germs: Genetic makeup reveals source of infection

The investigation of foodborne disease outbreaks is a race against time for the responsible authorities. The sooner the source is found, the fewer the cases of disease, the fewer fatalities and the lower the costs. The use of whole genome sequencing to characterise the pathogen enables the unequivocal identification of infection sources. The BfR also uses this technology as a test method.

Laboratory diagnostic examination methods to characterise the disease pathogen are used to recognise or exclude a food as the source of an infection. The underlying principle is to isolate the causal germ and compare it with isolates of the same pathogen from foods. Specific properties such as the genetic information of the pathogen – DNA or RNA – are used to make the comparison. The relationship of the isolates is established in this way and a common origin determined accordingly. The more comprehensively a method characterises the pathogen, the more reliable the result of the comparison.



The basis of whole genome sequencing is genetic information such as DNA from isolates of pathogenic germs.

# Whole genome sequencing shines through cells

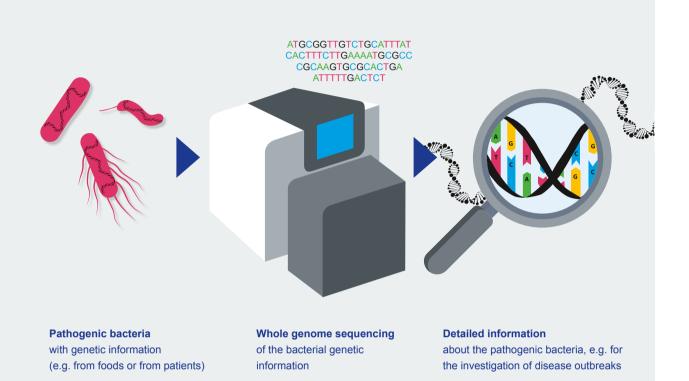
Using conventional methods, such as pulsed-field gel electrophoresis, it has only been possible up to now to determine and compare sections of genetic information. The information acquired in this way was often insufficient to reliably identify a food as the source of infection. In contrast, whole genome sequencing permits the deciphering of the complete genetic information of viruses, bacterial and parasite cells. Whole genome sequencing shines through the genetic information; this is comparable to the notion of switching on a light in a dark room and recognising what is in it. Without the light, the objects could only be recognised vaguely.

# Listeriosis outbreak solved

Whole genome sequencing cast some light into some dark places during the investigation of a protracted listeriosis outbreak in southern Germany. Between 2012 and 2016, 78 people took ill with the same strain of the bacterium *Listeria monocytogenes*, eight of whom died. Patient surveys provided only vague indications as to which foods could be the cause of the outbreak. The reason for this is that with listeriosis, several weeks can often expire between the consumption of the contaminated food and the occurrence of symptoms of disease. For those who have contracted the disease, it is not easy to remember in any detail what foods they had eaten after such a long time. The severity of the disease often means that those affected can often only be questioned long afterwards, if at all.

Thereupon, the National Reference Laboratory for *Listeria monocytogenes* at the BfR typed more than 500 isolates of the listeria pathogen from many different foods. Using methods that were standard up to then, several foods of different origin were identified as the





possible source of infection. The correct allocation was only achieved through the use of whole genome sequencing in March 2016, when an official test laboratory in Bavaria positively tested a pork product for *Listeria monocytogenes*. The sequencing of this isolate at the BfR showed that its genome was identical with the DNA of the *Listeria monocytogenes* isolates found with those who had taken ill. It was also possible to exclude other foods which had previously been suspected as sources of infection due to the methods used.

# New challenges

The new world of whole genome sequencing poses a challenge to science: huge quantities of data are produced which have to be evaluated, administered and made accessible to the laboratories. As with every new method, the goal is to develop international standards and establish databases. To this end, the BfR is involved in national and European third party-funded projects to promote the harmonisation of technology, the buildup of genome databases and the exchange of scientific knowledge. The use of whole genome sequencing makes it possible to follow precisely the spread of microbial pathogens through foods and feeds across national frontiers and stop outbreaks of disease or even prevent them in advance.

### More information:

Lüth et al. 2018: Whole genome sequencing as a typing tool for foodborne pathogens like Listeria monocy-togenes – The way towards global harmonisation and data exchange. Trends Food Sci Technol. 73: 67–75.

Kleta et al. 2017. Molecular tracing to find source of protracted invasive listeriosis outbreak, Southern Germany, 2012–2016. Emerg Infect Dis. 23 (10): 1680–1683.

www.bfr.bund.de/en > Research > Third party projects of the BfR > Exposure estimation and assessment of biological risks

www.engage-europe.eu

# Your questions answered: What is One Health?

There's an old piece of wisdom behind the One Health approach: the health of humans, animals and the environment is closely linked together in many different aspects. Global challenges in the health sector, such as the spread of antimicrobial resistance, add fresh impetus to this realisation. In practice, specialists in human and veterinary medicine work closely with environmental scientists.

The One Health approach plays an important role in the field of food safety. Aside from healthy microflora microorganisms such as bacteria, viruses or parasites can be contaminants in foods of animal and plant origin and can lead to severe health impairments in humans. "To ensure that meat, milk and eggs are safe, the whole food chain has to be observed – from the field to the shed all the way through to processing", says Professor Karsten Nöckler, head of the Biological Safety department at the BfR. "The prerequisite for this is close cooperation between agriculture, veterinary and health authorities, as well as good kitchen hygiene by consumers."

For this reason, the BfR works closely with other institutions on a national level. A good example is the fight against antimicrobial resistance. Whereas the Friedrich-Loeffler-Institute (FLI) conducts research into the spread of animal diseases and zoonotic pathogens in livestock, the BfR assesses the health risk for humans from disease pathogens and resistant bacteria which can be transmitted above all via foods of animal origin. The Federal Office of Consumer Protection and Food Safety examines antimicrobial resistance in pathogens in animals, while the Robert Koch Institute (RKI) researches the disease-causing mechanisms of the pathogens in humans. Together with veterinary and health authorities and universities, a better understanding of occurrence and spread of such diseases in humans and animals is achieved.

Also at an international level, the BfR cooperates closely with players in the health sector. The third party-funded project *European Joint Programme One Health* started in January 2018 with 41 reference laboratories from 19 countries. One organisation responsible for public health and one for food or veterinary medicine usually participate from each country, with the goal of advancing research into pathogens that can be transmitted between humans and animals, setting up a network of specialists and expanding cooperation. The German authorities involved in addition to the BfR are the FLI and RKI.

More information:

www.bfr.bund.de/en > A-Z-Index: One Health www.bfr.bund.de/en > Research > Third party projects of the BfR > Human, animal and environmental health (One Health)

# **SPECTRUM**



# Research alliance for a better understanding of rodent-borne diseases

In the "RoBoPub" research alliance, scientists have been working since 2017 on generating more knowledge about diseases transmitted by rodents, such as leptospirosis and hantavirus infections. RoBoPub stands for rodent-borne pathogens and public health. The BfR's partners in the alliance are the Friedrich-Loeffler-Institute and public health services, as well as university and non-university research institutions. The Federal Ministry of Education and Research is funding this third party project. At the BfR, the consultant laboratory for leptospires is conducting research on pathogen-, rodent- and environment-related aspects of pathogen transmission and diagnosis of the disease in humans. The results are incorporated into the BfR risk assessments, as well as into the development of health recommendations. Leptospirosis is a febrile illness which is transferred to humans by rodents in particular. The symptoms of the disease are non-specific and in general similar to those of flu. However, in some cases infection results into a life-threatening illness.

# Disinfection of slaughtering plants: New methods not very effective

The slaughter of poultry poses a challenge to hygiene. In order to prevent that bacteria from the viscera of the slaughter animals depose on the surface of the equipment and spread to the poultry meat increasing numbers of innovative disinfection methods are being used. They take effect with short application times and low concentrations. A laboratory method was developed at the BfR to test the efficacy of these methods. The method simulates short application times, low ambient temperatures and moist, soiled surfaces. The goal was to find out whether antibiotic resistant *E. coli* can be killed off under these conditions. If this were the case, disinfection after every slaughtered carcass would make good sense without stopping the conveyor. It transpired, however, that the examined bacteria were only killed off to a slight degree under these conditions despite very high concentrations of disinfectant.



### Maximum daily level for magnesium in food supplements

People who consume magnesium as a food supplement should not take more than 250 milligrams per day divided up into at least two portions. After evaluating new nutrition studies, the BfR hereby confirms its recommendations of the year 2004. Accordingly, no negative effects have been observed up to now regarding the uptake of magnesium via conventional foods by healthy persons. If magnesium is ingested additionally, however, as a food supplement, and these products exceed certain daily intake quantities, mild cases of diarrhoea may result. Magnesium is an essential mineral which plays an important role in numerous metabolic processes, as well as in bone mineralisation and in muscle contraction. In general, a balanced and varied diet with plenty of fruits and vegetables supplies a healthy body with all essential nutrients.

More information: BfR Opinion No. 50/2017 of 12 December 2017



Registration, Evaluation, Authorisation and Restriction of CHemicals

# REACH protects against hazardous substances

Chemicals are a part of everyday life. The EU chemicals regulation REACH has been in effect for a decade. The regulation is an important tool for chemicals safety. Currently, it is being used to restrict the use of asthma-triggering diisocyanates throughout Europe on the initiative of Germany.

A t the end of 2017, the European Chemicals Agency (ECHA) came to the conclusion that the use of diisocyanates can pose an unacceptable health risk. This conclusion was based on an assessment report by the BfR and the Federal Institute for Occupational Safety and Health (BAuA). Together, the two institutes had shown that this group of substances can trigger asthma and that no safe limit value can be derived for it. In accordance with the German Chemicals Act (ChemG), the BfR assists in the implementation of the European chemicals legislation as the "assessment body for health and consumer protection". It collaborates with the Federal Institute for Occupational Safety and Health (BAuA), the Federal Environment Agency (UBA) and the coordinating Federal Office for Chemicals (BfC) under professional supervision of the Federal Ministry for the Environment (BMU).

# **Restricted or prohibited substances**

If an unacceptable, insufficiently controlled risk is determined for a substance, the authorities can suggest restricting chemicals according to the European chemicals regulation REACH (EC) 1907/2006. Their use can then be subject to specific conditions or generally prohibited in all EU states. 68 substances or groups of substances have already been restricted throughout Europe. Examples include benzene, asbestos fibres, lead, mercury, arsenic, cadmium, nickel, azo

dyes and certain plasticisers. Many of these substances can cause cancer, damage genes, impair the development of offspring or adversely affect fertility (so-called CMR substances), but substances can also be restricted because of other health risks. In the case of diisocyanates, the main issue is the sensitising effect of these substances on the respiratory tract.

# Reducing new cases of asthma throughout Europe

Diisocyanates are highly reactive chemicals that are used primarily for manufacturing polyurethane plastics with an estimated market volume of 2.5 million tonnes per year in the EU. Cured polyurethane can be found in foams and coatings, for example for insulation boards, mattresses and cushions. Uncured diisocyanates can be contained in special lacquers, seals and in construction foams which are generated and applied on site (so-called in-situ foams). Some of these products are also on the market for DIY use.

# German proposals to restrict chemical substances

**Carcinogenic PAHs:** Certain polycyclic aromatic hydrocarbons (PAHs) are carcinogenic. They can now only be contained up to a defined limit in consumer products and toys. The BfR's health assessment of PAHs and the data on occurrence in consumer products were the basis for the restriction.

**PFOA, -salts and -precursor substances:** Perfluorooctanoic acid (PFOA) accumulates in the human body and is transferred to the foetus through the placenta during pregnancy and to breastfed babies through breast milk. The intake of PFOA and its precursor substances into the human body takes place primarily through food, indoor air and drinking water. In animal experiments, PFOA demonstrates hepatotoxic, carcinogenic and reprotoxic properties. The BfR supported its Norwegian partner authority in the health assessment of PFOA.

More information (in German): www.reach-clp-biozid-helpdesk.de > REACH > Zulassung und Beschränkung > Beschränkungsverfahren > Anhang XVII Beschränkungen In contrast to cured polyurethane, uncured diisocyanates are highly sensitising. Even minimal contact can lead to asthma and skin allergies. Workers who handle products containing diisocyanates are particularly affected. However, uninvolved third parties also need to be protected during construction work with such products so that they do not come into contact with diisocyanates as bystanders. To better protect workers from occupational illness, Germany took the initiative to restrict the use of these chemicals throughout Europe. Together with the BAuA, the BfR performed the toxicological risk assessment and made contributions regarding the safety of bystanders.

# Evidence of an unacceptable risk posed by diisocyanates

The BfR and BAuA proved that the use of diisocyanates can pose an unacceptable health risk. This proof is an important prerequisite for the success of an application for restriction. Proving an unacceptable health risk from diisocyanates was scientifically difficult because measured or estimated exposure of the affected population could not be compared to a limit value for harmful effects, as is otherwise common for restrictions. The BfR and BAuA jointly evaluated several hundred experimental animal studies and epidemiological human data. Even using this comprehensive data base, the reliable determination of a dose without a harmful effect was not possible. At the same time, an extrapolation based on recorded and recognised occupational illnesses and the assumption of a high number of unreported cases, showed that the use of diisocyanates might cause over 5,000 new cases of work-related asthma throughout the EU each year.

The proposal to restrict diisocyanates was submitted to ECHA in October 2016. In December 2017, ECHA's Risk Assessment Committee (RAC) recommended acceptance; the Socio-Economic Analysis Committee (SEAC) of ECHA also agreed with the proposal in March after the end of the official consultation period. Now, the European Commission will present a proposal for the final restriction text. The approval on this will then be made via a comitology procedure involving the member states and the European Parliament.

If the restriction procedure is successful, diisocyanates will only be able to be used above a concentration level of 0.1% if it has been demonstrated that usage poses only a minimal risk, e.g. through the safe design of the product. Alternatively, people who use such products must be informed about the health risk and have received sufficient training on protective measures. In this way, they will be able to better protect themselves and others from substances triggering asthma in the future.

# The REACH Regulation: **Registration – Evaluation – Authorisation – Restriction**

Chemicals produced, imported or procured in the EU in quantities of one tonne and more per year must be registered in accordance with the REACH regulation. For substances which were on the market before REACH came into effect, the registration deadlines have been spread over nine years, depending on the tonnages involved. The last deadline expires on 1 June 2018. To get a substance registered, manufacturers or importers have to submit a registration dossier. The European Chemicals Agency (ECHA) and national authorities of the 28 member states examine these dossiers for data gaps and request any data which might be missing. In order to be able to better assess data quality, the BfR has conducted several research projects (see box), because the quality of the toxicological data plays a key role in health and environmental protection.

ECHA and the national authorities, however, do not only critically examine the risk assessments produced by industry, they also prepare their own risk assessments. Thus, for example, they can identify substances with particularly dangerous properties for humans and the environment as "Substances of Very High Concern" (SVHCs). If necessary, the EU can impose an authorisation obligation on these substances in order to guarantee their safe use and substitute them in the long term with less hazardous substances. All currently known and relevant SVHCs are to be identified and included in a candidate list by the year 2020 (SVHC Roadmap 2020). Subsequently, they can be gradually included in the list of substances requiring authorisation (Annex XIV of the REACH regulation).



If, as in the case of diisocyanates, an unacceptable, inadequately controlled risk is established for a substance, chemicals can also be restricted. Restrictions are listed in Annex XVII of the REACH regulation. Unlike the authorisation requirement, they also apply explicitly to imported substances and/or substances contained in mixtures and articles. "The guiding principle of REACH is that chemicals are only used in such a way that no unacceptable health risk results for humans or the environment," says Dr. Agnes Schulte, head of the Chemicals Safety unit at the BfR. The Institute uses the various REACH instruments – Registration, Evaluation, Authorisation, Restriction – above all else to better protect consumers against CMR and allergenic substances. **■** 

# BfR research project REACH-Compliance I–III: How good is the data provided by industry?

Using a standardised method, 1,814 registration dossiers of chemicals each with an annual market volume of 1,000 tonnes and more were examined to establish whether the necessary data on human toxicity, ecotoxicity, and exposure to the environment is contained in appropriate quality. The result showed that the data basis of many dossiers provided by industry was not in compliance with the requirements. Often data regarding the assessment of the effects of the substance on health and the environment are lacking. As an example: In roughly half of the registration dossiers examined, this was the case regarding the data on developmental toxicity. From the findings made during the project, the BfR has derived recommendations for registrants. The BfR is currently assessing the registration dossiers for substances in quantities of 100 to 1,000 tonnes.

### More information:

www.bfr.bund.de/en > Research > Third party projects of the BfR > Detection of contaminants and for the assessment of chemical risks

www.umweltbundesamt.de/en > Publications



# "We need high-quality exposure data"

Dr. Agnes Schulte, Head of the Chemical Safety unit in the Chemical and Product Safety department at the BfR, about chemical assessment under REACH.

# More than ten years of REACH – do you consider this a success story?

Yes! We work well together with all authorities on the European level. For example, we conducted a substance assessment for bisphenol A, and France took this into consideration in its proposal to restrict the substance for consumer products. There is a great deal of cooperation. Together, we agree on the best options of action in order to improve the safety of chemicals for people and the environment. REACH offers a suitable network for this. Now, non-EU countries also want to introduce a programme like REACH and are using the European chemicals legislation as a good example.

## How has REACH changed the working procedures of authorities in Germany?

Assessment of substances in terms of health, environment and occupational safety is legally divided between three authorities. These authorities can jointly prepare substance dossiers which are then forwarded to the European Chemicals Agency. Many steps in this assessment work rely on the results of other units or require mutual cooperation. This close-knit, interlinked way of working is something special.

# Where is action still required, from the perspective of the BfR?

The registration data often lacks information on the actual exposure of the population, i.e. consumer exposure. When there is a lack of exposure data, risk assessment is difficult. After all, risk assessment means not only assessing the hazard potential of a substance, but also placing this in relation to the dose to which consumers are actually exposed. If we don't know enough about this, our risk assessments are subject to significant uncertainties.

### How can this be changed?

It is important to the BfR that high-quality exposure data is routinely incorporated in the risk assessment under REACH. To improve the basis of data here, the BfR has started several projects on the behaviour of consumers when handling chemicals.





### Toxification of chemicals by microorganisms on the skin

The skin is our largest organ and harbours a microbial population with a density second to the gut. Yet, little is known about the effects of this microbiome metabolism on the human host. Using benzo[a]pyrene (B[a]P) as a model, the BfR has examined for the first time the extent to which skin microbes contribute to the toxification of chemicals. B[a]P is a polycyclic aromatic hydrocarbon (PAH). As combustion products PAHs are contaminants that occur everywhere, in the air as well as in consumer products. Certain PAHs, such as B[*a*]P, are highly carcinogenic. Skin swabs of randomly selected volunteers yielded several B[a]P-degrading microorganisms. The isolated organisms convert B[a]P fully or partially depending on the degradative pathway used. Degradation resulted in the excretion of a mixture of metabolites, several of which unknown. Tests showed these metabolites to have much stronger cytotoxic and genotoxic effects than the corresponding human metabolic products. Studies in microbially competent 3D models confirm this, as well as a possible inhibition of DNA repair in the human host. It now needs to be clarified which effects microbial toxification of B[a]P and other PAHs can have on human health.

### More information:

Sowada et al. 2017. Toxification of polycyclic aromatic hydrocarbons by commensal bacteria from human skin. Arch Toxicol 91 (6): 2331-2341.

### Release of aluminium from uncoated menu travs into foods

In a research project, the BfR showed that aluminium ions are released into acidic foodstuffs from uncoated aluminium menu trays during heating and to a higher extent during warm keeping. Some of the determined concentrations were significantly higher than the specific release limit (SRL) defined by the Council of Europe. The observed aluminium release alone does not result in a harmful intake level, but due to the natural background level of aluminium in drinking water and in untreated foods, exposure is already within the range of the tolerable weekly intake level. Furthermore, consumers may be exposed to aluminium from improper use of other food contact materials consisting of aluminium as well as from cosmetics. In light of this already high intake, the BfR recommends minimising any preventable additional aluminium exposure. This applies in particular to vulnerable consumer groups such as small children and elderly people. Both population groups may - for example in care facilities - consume meals that have been heated and kept warm in aluminium menu trays on a daily or very regular basis.

#### More information: BfR Opinion No. 007/2017 of 29 May 2017 (in German)







### Contamination of feed: Digital tools allow a fast response

Computer tools developed at the BfR help to predict the accumulation and elimination of potentially harmful substances such as per- and polyfluoroalkyl substances (PFAS) in fattening pigs and dairy cows. Should animal feed become contaminated with PFAS, these substances can be passed on to the animals and thus into foods of animal origin. How much ends up on our tables can vary greatly depending on the particular substance and the kind of food. The BfR has developed the digital tools RITOPS and PERCOW. They can calculate the levels of specific PFAS to be expected in foods in the event of animal feed contamination. In this way, the computer tools help the responsible surveillance authorities during cases of feed contamination with PFAS to quickly estimate the associated health risks of consumers. The algorithms used are based on experiments on the transfer of substances from feed conducted at the BfR.

### More information:

Numata et al. 2017. Risk tools for ready-to-use modeling of PFAS transfer from contaminated feed into foods of animal origin. Organohalogen Compd. 79.

Global goods transport Container fumigation – a consumer protection topic?

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### Almost all of the packaged goods conveyed in global trade are transported in containers. A large number of these are fumigated in the exporting country prior to shipment. Within the scope of a research project, the BfR examined the release of fumigants from foods and consumer products.

To prevent the introduction of pests, especially insects from wood, containers are fumigated prior to transport. Fumigation is also carried out to protect the transported goods against infestation with harmful organisms and mould. Regulations to protect humans where fumigation is concerned have been in place in Germany for quite some time.

### International occupational safety has to be improved

Although warning signs are being demanded for the recognition of fumigated containers in the regulations on the sea transport of hazardous goods, only a few of the fumigated sea containers transported to Germany bear markings of this kind. This has resulted in repeated cases of poisoning in Germany in the past when unmarked, fumigated containers were opened. The BfR has been advocating an improvement in the global regulations on the transport of dangerous freight for years. The institute proposed, for instance, that fumigation warning signs in sea transport should be made waterproof. As of this year, this is now internationally mandatory required.

### Fumigants in consumer products

The goods transported in containers may absorb some of the fumigant and – sometimes with a time delay – release it again after fumigation has ended (desorption). In order to enable an estimation of health risks for consumers, a number of open questions still have to be answered with regard to the quantities of fumigant released and the precise chronological order of the release. For this reason, it must be taken into consideration whether, due to ever shorter delivery and storage times, substances are degassing to an increasing extent at home instead of in the warehouse, and whether this poses a health risk. It is also being investigated at the moment whether fumigation alters the transported goods and whether changes of this kind conceal health risks. Where possible fumigant residues in foods are concerned, information is currently available on active substances which are authorised as a plant protection product or biocide for the protection of stored goods, such as phosphine or sulphur fluoride.

### Experimental studies simulate release

In order to better understand the desorption behaviour of fumigants from various consumer products and foods, and to generate sound data for a risk assessment, the BfR initiated research projects with the Central Institute for Occupational Health and Julius Kühn Institute. In these projects, the release of fumigants from foods such as apples, sunflower seeds and wine grapes, as well as various consumer products, such as socks, packing paper and shoes, is determined. It is also examined whether fumigation causes a chemical change in the test objects.

# Released gas quantities within legal limits

First results show that, as expected, the release speed and released quantity of gas depend on the properties of the fumigated freight and the fumigant used. The release of fumigants with a higher boiling point is slower compared to those with a low boiling point. The released gas quantities from the foods examined were found to fall below the maximum residue levels determined by the European Food Safety Authority (EFSA) – before expiry of the legal waiting period, which is the time between the use of the fumigant and the marketability of the produce. Likewise, the reference values for chronic exposure to the examined fumigants from consumer products which were used for the evaluation were no longer exceeded in most cases after a few days.

Although health impairments for consumers seem unlikely after evaluation of the currently available data, there is a need for further research and testing so that possible health risks can be recognised and avoided.

Fumigants protect goods from pests during transport, but residues of these substances remain detectable in the goods.



# Fields of research for more animal welfare

What are the benefits of animal experiments? The BfR "Animal-TestInfo" database provides answers and shows how the protection of laboratory animals can be improved.

The questions are obvious: what is the actual purpose of animal experiments? In which areas it is necessary to do more for animal welfare? Since 4 December 2014, it is possible to answer those questions. On this day, the BfR launched the "animaltestinfo.de" website. The AnimalTestInfo database provides transparent and easily accessible information about experiments with animals. Every authorised project in Germany involving animal experimentation is listed in this database along with easily understandable information in the form of non-technical project summaries (NTS).

# Legislation makes transparency mandatory

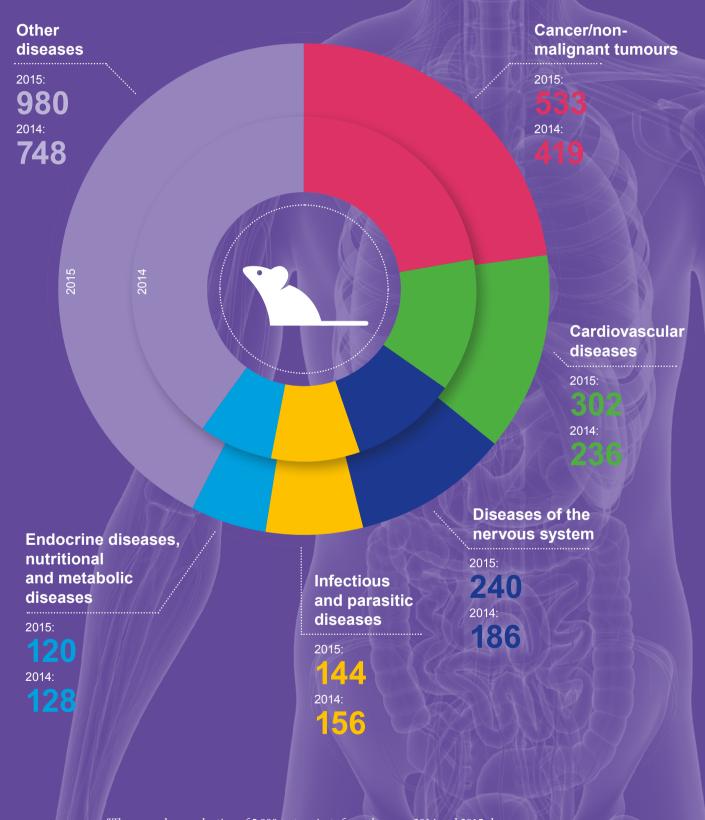
The legal basis for the publication of information on authorised animal experiments is the European Directive 2010/63/EU on the protection of animals used for scientific purposes. This directive stipulates that every EU member state must inform the general public about animal experiments by publishing generally understandable summaries. The directive was implemented into German law in the summer of 2013 through the amendment of the Animal Welfare Act and the enactment of the Regulation on Laboratory Animal Welfare. Ever since, researchers have to submit a non-technical project summary along with their project application. Among other things, this summary must contain details of the expected benefits, number and species of animals to be used, the severity levels they are likely to be exposed to and information on whether or not the requirements of the 3R principle have been applied (see interview on page 42). Once approval has been granted for an animal experiment, the responsible authorities release the corresponding NTS in the AnimalTestInfo database for publication.

### Systematic evaluation of data

Currently, more than 10,000 anonymised non-technical project summaries are listed in AnimalTestInfo and about 2,800 entries are being added every year. Every month, approximately 1,000 users, most of them from Germany but also from other European countries, the USA and Arab and Asian countries, access the database. Although AnimalTestInfo was set up primarily as an information source for the general public, the information contained in the NTS can also be systematically evaluated. And that is exactly what the BfR scientists at the German Centre for the Protection of Laboratory Animals (Bf3R) have done in a study conducted in the third year of the database's existence.

# Animal experiments for the combat against diseases

Number of planned animal experiments per research purpose (evaluation of AnimalTestInfo entries 2014/2015)



"The exemplary evaluation of 5,000 test projects from the years 2014 and 2015 shows that around 80 percent of the animal experiments approved in Germany serve the purpose of investigating the causes, diagnosis and treatment of human illnesses, with main focus on diseases of the cardiovascular and nervous systems, as well as cancer."

### ICD codes as a classification reference

More than 5,000 animal experiments from the years 2014 and 2015 have been systematised with the help of ICD codes. To do so, the experiment purposes described in the NTS were assigned to the corresponding human diseases using the ICD code. The ICD (International Classification of Diseases) code is a classification system used for the precise description of disease diagnoses. For example: an NTS published in 2015 describes an animal experiment examining whether chronic intestinal inflammation increases the risk of colorectal cancer. The plan of the experiment is to switch off some specific enzymes in mice and to transfer certain defence cells. The experiment is intended to help recognise which parts of the immune system and which enzymes contribute to inflammation and cancer. In the Bf3R study, this authorised animal experiment was assigned to the ICD code C15-C26 for malignant tumours of the digestive organs.

### More alternative methods required in cancer research

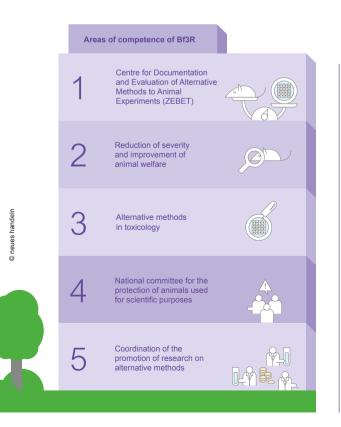
The exemplary evaluation of 5,000 test projects from the years 2014 and 2015 shows that around 80 percent of the animal experiments approved in Germany serve the purpose of investigating the causes, diagnosis and treatment of human illnesses, with main focus on diseases of the cardiovascular and nervous systems, as well as cancer. As the ICD codes deliver a very precise description, the evaluation shows in detail which diseases are researched with animal experiments. Where cancer is concerned, for instance, a great number of the authorised animal experiment projects in this field was dealing with malignant tumours of the digestive system and therapeutic treatment thereof.

In the study, for the first time the concrete fields of research were determined in which a consistently large number of animal experiments were approved over the years, such as the research and treatment of colorectal cancer. The information obtained from the database shows the fields of research in which there is a special need for the development of alternative methods to experiments with animals in line with the 3R principle. The information serves science, research funders and politics as a comprehensive data source opening up areas of action for more animal welfare in the future.

#### More information:

Bert et al. 2017. Rethinking 3R strategies: Digging deeper into AnimalTestInfo promotes transparency in in vivo biomedical research. PLoS Biol. 15 (12): e2003217. (Open Access)

Schönfelder et al. 2015. Laboratory animals: German initiative opens up animal data. Nature. 519: 7541, 33.



### The German Centre for the Protection of Laboratory Animals (Bf3R) at the BfR

For the first time, the Centre combines the various areas of alternative method research on a national level in line with the 3R principle. The Centre coordinates activities all over Germany with the goals of restricting experiments with animals to a level which is absolutely necessary and affording laboratory animals the best possible protection. In addition to this, impetus is to be given to national and international research activities through the work of the Centre while encouraging scientific dialogue at the same time. Bf3R was established in 2015 in the course of the Animal Welfare Initiative of the Federal Ministry of Food and Agriculture. It is an integral component of the BfR which is subdivided into five areas of competence. The AnimalTestInfo database belongs to Area 1, Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET).

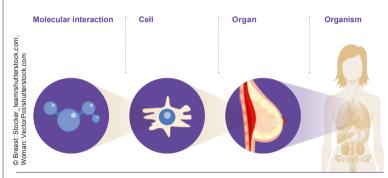
# **SPECTRUM**

### Understanding health hazards without animal experiments

How can hazards and the effects of environmental chemicals be observed without the necessity of animal experiments? Adverse Outcome Pathways (AOPs) were conceived in recent years in order to gain a better understanding of the toxic effects of chemicals on organs and use this knowledge for the development of new, animal-free test methods. AOPs describe on a molecular level how a human or animal body might react to environmental chemicals to cause serious harm to human health. Above all high throughput methods and systems biological approaches are best suited for the implementation of the AOP concept in the risk assessment of chemicals and to target research of alternative methods. The combination of these technologies permits the comprehensive analysis of a large number of chemicals within a short period of time. BfR is now also using these methods to an increasing extent.

#### More information:

Burgdorf et al. 2017. The AOP Concept: How novel technologies can support development of adverse outcome pathways. Appl In Vitro Toxicol. 3: 271–277



Adverse Outcome Pathways depict diseases in detail right down to the level of molecular processes. The AOP of breast cancer looks like this, for instance: bonding to the estrogen receptor activates gene expression, stimulates cell proliferation and cell migration, thus contributing to the occurrence of breast cancer.



### Commitment to the systematisation of animal data

What chances and what limits are there for the use of laboratory animals to research diseases? Neurologist Malcolm Macleod of the University of Edinburgh has dedicated himself to this topic. This spring he was awarded the Maria Sibylla Merian Fellowship by the BfR for his interdisciplinary research achievements and contribution towards improving animal welfare. In the field of stroke research, Malcolm Macleod's systematic studies will enhance the reliability of animal experiments and evidence-based transferability to humans. In this way, unnecessary animal experiments can be avoided in future and new, innovative therapy approaches found. Bf3R will collaborate scientifically with Malcolm Macleod in order to advance research in the field of meta-analysis. With the fellowship programme, awarded for the first time, the BfR honours researchers who have excelled themselves through their extraordinary scientific achievements.

More information: www.bfr.bund.de/en > Research > Fellowship-Programmes

### Bf3R research funding 2017: Sponsored projects have been selected

The BfR included eight external work groups in its current Bf3R research funding programme for the development of innovative alternative methods. Among other projects, the scientists are developing training models for use in animal experimentation courses, as well as improved, cell-based skin models for research on the healing of wounds. The individual projects are scheduled to run for up to three years and are each being sponsored with an average sum of 35,000 euros per annum. The BfR invites bids for the Bf3R research funding every two years. Universities, non-university research institutions and companies with research and development capacity in Germany are all entitled to apply. A high priority is given to methods to substitute or reduce animal experiments in basic biomedical research, as well as research approaches to recognise and alleviate the suffering of laboratory animals. Eight of the 47 applications submitted achieved this priority in 2017. The next invitation for bids for Bf3R research funding will be in spring 2019.

### More information:

www.bfr.bund.de/en > German Centre for the Protection of Laboratory Animals (Bf3R) > Bf3R Research Funding

# "We are creating more transparency in animal experiments"

A focus on animal welfare: Professor Dr. Gilbert Schönfelder heads the German Centre for the Protection of Laboratory Animals at the BfR. In this interview, he reports on the duties of the centre and the search for alternative methods.

### Professor Schönfelder, how did you became interested on the topic "Protection of laboratory animals" and the development of alternative methods to animal experiments?

It is our ethical obligation to avoid unnecessary experiments and spare animals from suffering. On the other hand, we still need animal experiments to achieve medical progress and cure sick people. Working within this field of conflict is a tremendous challenge from both a scientific and a social point of view.

### Critics say that the results of animal experiments cannot be translated to humans.

Reality isn't only black and white. It's true that the results of some animal experiments can only be translated to humans with difficulty, but this can't be generalised. There are some animal experiments which most certainly do permit conclusions with regard to humans. There wouldn't be any medicine without animal experiments!

### One of your main aims is the development of alternative methods – is this a way towards fewer animal experiments?

In the long term, definitely. The guiding principle for us is still the 3R principle put forward by William Russell and Rex Burch in 1959, which states that animal experiments should be replaced, reduced and refined. The latter means that the suffering of the animals should be alleviated. The 3R principle also forms the basis of the European Directive 2010/63/EU on the protection of animals used for scientific purposes. It was implemented into German law in 2013 with the amendment of the Animal Welfare Act.

What does that mean for the Centre for the Protection of Laboratory Animals, which was founded in 2015 and of which you are the head? The range of our tasks has expanded considerably. The Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments, ZEBET, was already established at the BfR. It exists since 1987. What's new is that we now inform the public about every authorised animal experiment in generally understandable form. Four areas of competence have been added to it with the aim of reducing the stress on laboratory animals, identifying alternative methods for toxicological testing and coordinating research funding for alternative methods. The National Committee for the Protection of Animals Used for Scientific Purposes is also located at our Centre. It advises the responsible German authorities and animal welfare bodies at the research institutions.

### Many legal tasks have been transferred to you, but you conduct also research at your Centre. How do you manage this balancing act?

One of the essential strengths of departmental research is that it promotes important areas of science which may have been neglected up to now. We have benefited from this too. We were able to acquire outstanding researchers and build up the necessary infrastructure at the Centre, such as modern technology.

# A centre where alternative methods to animal experiments are developed – that awakens great expectations among the general public.

We have to be honest here – it is not possible to replace all animal experiments within five years. It's simply unrealistic. I hope that in 10 to 20 years the new methods are so good that a measurable decline in animal experiments results.

### Which approaches are particularly promising?

Animal experiments for the development of cosmetics are already prohibited in the cosmetics industry. That's why skin tissue tests have already been introduced to test products for their health safety with regard to skin irritation or corrosion, for instance. Another example are three-dimensional cell culture models which are used more and more in basic research.

### You mean "miniature versions" of organs like the stomach?

For example. Cell cultures are also becoming more important in brain research. To study the development of the nervous system, it can be more beneficial to observe the processes on cells in detail in a Petri dish. You can't simply look inside an animal's skull, on the other hand. There's also a lot of discussions at the moment about



"human" or "organ-on-the-chip" technology. Miniaturised organ systems, such as the liver and brain, are connected on a plastic chip via a kind of blood flow. The interactions between organ systems can be better understood in this way. But the same thing applies here: these methods are not currently capable of completely replacing animal experiments.

### What special impulses can emanate from your Centre?

It is important to increase transparency about research conducted on animals, which is of concern. Our database AnimalTestInfo can provide this important information. As we reported in the journal "Plos Biology", for the first time we were able to provide a more detailed overview about the use of six million animals in experiments. It is important to better understand the purpose why so many animals are used in the research of cancer, disorders of the vascular and immune system? Detailed information can help to make research more efficient. Thereby, we hope to make an important contribution where alternative methods are needed to reduce the suffering of animals. We hope to inspire scientists to dedicate their research efforts more to this subject.

### Professor Dr. Gilbert Schönfelder

is a physician, full-professor at the Institute for Clinical Pharmacology and Toxicology at the Charité Universitätsmedizin Berlin and head of the department Experimental Toxicology and ZEBET, as well as of the German Centre for the Protection of Laboratory Animals (Bf3R) at the BfR. The main focus of Schönfelder's research lies in the field of experimental toxicology, the further development of alternative methods to experiments with animals, and in reproductive and developmental toxicology. He studied Human Medicine at the Freie Universität Berlin, was appointed junior professor at the Charité in 2003, moved to the University of Würzburg in 2007 and returned to the Charité in 2010. Schönfelder has been with the BfR since 2012.

Many thanks for the interview, Mr. Schönfelder. **a** 

### PERSONNEL

### **Commemorative medal**



The Society of German Chemists awarded the Joseph-König commemorative medal to Professor Dr. Reiner Wittkowski, Vice-President of the BfR, at the end of 2017. The prize is in recognition of Wittkowski's work for the promotion and recognition of food chemistry, in particular his studies on the authenticity of foods using modern physico-chemical methods in the course of which he set international standards. Through his research in the field of chemical-analytical detection methods for verifying the geographic origin of foods, he is regarded as one of the founding fathers of authenticity research.

### Award: Overseas Guest Expert in China

Dr. Carsten Fauhl-Hassek, head of the "Product Identity, Supply Chains and Traceability" unit at the BfR, was nominated an "overseas guest expert" by the China National Research Institute of Food and Fermentation Industries (CNRIFFI). CNRIFFI is one of the leading research institutions in the field of the authenticity testing of foods in China and cooperation partner of the BfR since 2016, especially in the area of wine analytics.

### New term for the BfR committees

The new, four-year term of the total of 14 BfRcommittees started in January 2018. The 199 committee members will advise the BfR on an honorary basis until 2021 as independent experts. They consolidate the expert scientific knowledge available in Germany on the highest possible level. The BfR committees "Evidence-based Methods in Risk Assessment" and "Biological Hazards and Hygiene" have been newly established.

### A new beginning and a fond farewell

In August 2017, the Scientific Advisory Board of the BfR was re-appointed for its fourth term of office until 2021. The members advise the BfR on research prioritisation and the staffing of the committees attached to the Institute, and provide support in the expansion of contacts and cooperation projects. The Board will have 16 scientists from various specialised disciplines as members during the coming term of office. Six of the members are new and will be providing fresh expertise in the areas of animal welfare and statistics and strengthening the area of risk communication. In the course of the restaffing, Professor Dr. Monika Schäfer-Korting (Vice-President FU Berlin), who chaired the Board for many years, resigned her office. She has left the committee at her own request after almost 12 years in order to "hand over the tasks to a pair of younger hands". Professor Dr. Tanja Schwerdtle (University of Potsdam), who was deputy until now, was elected the new Chair.



Professor Dr. Tanja Schwerdtle, Chair of the Scientific Advisory Board of the BfR since 2017

### **INTERNATIONAL NEWS**

### Brain circulation in risk assessment

With the scholarship initiative "The European Food Risk Assessment Fellowship Programme", EU-FORA for short, EFSA is promoting the scientific exchange between risk assessment institutions across national frontiers. The goal of the EU-FORA programme is the build-up of a network of "youngster scientists" in risk assessment and their supervisors. By doing so, EFSA is initiating closer cooperation of the next generation of risk assessment specialists in Europe. In addition to their practical work in a European assessment institution, the fellows receive six weeks of accompanying theoretical training in the form of four training modules on the risk assessment of foods and on risk communication. The programme is now moving into the second round. The BfR supports the initiative and has been hosting four of a total of 15 fellows of the programme from Poland, Greece and Norway for a year since autumn 2017.



### Michal Jan Czyz of the Institute of Plant Protection, Poland

is working on the use of Data Science in risk assessment and early warning.

"I applied for the programme because I wanted to expand my skills in the area of modelling. Nowadays, we can go beyond the limits of traditional methods with computer-supported mathematical models. Within a few minutes we can predict analysis results or certain scenarios. In real

life that would take years, cost a lot of money and possibly even damage people or the environment. In Poland I was involved mainly with the risk assessment of pests. Here I am getting to know many different types of risk assessment, especially during the training sessions".



### Ewa Matyjaszczyk of the Institute of Plant Protection, Poland

is preparing a systematic literature analysis of the risk assessment of plants and plant preparations in foods, with focus on willow bark.

"As an expert for food quality in Poland, I tend to work more at the beginning of the food chain, in the area of agriculture. With my project at the BfR I am moving more towards the end of the

food chain, where I am involved with processed foods. It's different from what I've been doing up to now and I've been very fortunate that I've been able to use a part of my formal training that I haven't needed before while learning something new for my work in Poland at the same time".



### Georgios Marakis of the Hellenic Food Authority, Greece

is involved with the risk assessment of substances used in food supplements and enriched foods.

"In Greece I work in an authority that deals with the assessment as well as the management of risks. Although I have personal experience in the field of the management of nutritional risks,

such as salt reduction, I've had less to do with the standardised assessment of risks of this kind. At the BfR, I am now getting acquainted with various methods of risk assessment while working with different scientists and getting to know their way of thinking and working. This 'brain circulation' was my motivation to participate in the EU-FORA programme, because it is the very heart of the European Union: working together for a better future".



### Josef D. Rasinger of the Institute of Marine Research, Norway

is conducting research on the computer-assisted identification and assessment of potentially mutagenic and carcinogenic heat-related contaminants in foods.

"My field of research in Norway is toxicogenomics, so I work a lot with bioinformatics and data mining. In modern toxicology, attempts are being made to avoid experiments with animals and

more and more computer-assisted methods are being tested. I can learn a lot in this area at the BfR, so for me it's a match made in heaven – there are many overlaps between our institutions. The way things are looking at the moment, we will continue our collaboration, even after my year here has expired".

Cover: Cookie Studio/shutterstock.com

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