

**BfR**  
**2**  
**GO**

The Science Magazine of the  
German Federal Institute for Risk Assessment  
**Issue 1/2017**



Food authenticity

## **Tracking down the food fraudsters**

BfR Consumer Monitor  
**Keeping an eye on  
perceived risks**

Pyrrolizidine Alkaloids  
**Undesirable  
in food**

Multiple residues  
**The mixture  
is all important**

# Dear Readers,

In times of global networking, foods, products and materials change rapidly. New scientific findings are made every day and the dynamics of public perception are also subject to constant change. We aim to respond to these processes with a suitable communication format – a format that is up to date while still providing sound information on the research and assessment of possible health risks. As of now, twice a year you will receive the BfR to takeaway in a compact form but still crammed full of knowledge: BfR 2 GO.

In every issue, the science magazine BfR 2 GO illuminates one of the BfR's current topics and provides information on the latest developments in assessment and research in the field of consumer health protection. You can get acquainted with the topic of authenticity in this issue, gain an insight into how the fingerprints of foods are determined in the laboratory and also find out which research projects the BfR is currently accompanying. You can read about which foods tend to get adulterated and an interview about where the risks of the fraud lie. In addition to the main topic, you will find reports, interviews and concise messages split up into the work areas of the BfR: risk communication, food safety, product and chemicals safety as well as the protection of laboratory animals. In this issue, you will also find out among other things that vegetarianism favours the decision towards adopting a vegan diet. On top of all of this, you can use the neatly arranged infographics to learn how antimicrobial resistance spreads. You may also care to read about how animal experiments can be reduced through the choice of a suitable model and the risks that are connected with multiple residues of plant protection products in foods.

Opinions, press releases, videos, scientific reports, infographics and annual reports – many communication tools have been developed in the course of the 15-year history of the BfR, with the goal of informing the general public and policy makers about possible health risks. All of them are based on the three principles of the BfR: transparency, reliability and the greatest possible openness. In this way, the foundation is laid for an objective and social discourse involving all interest groups and the strengthening of trust in the process of research, assessment and risk communication. In addition to the communication of possible health risks, the BfR makes an impartial contribution towards the safety of foods and feeds, products and chemicals through its independent scientific assessments and research. This tradition is continued by the science magazine BfR 2 GO.

I wish you lots of fun exploring the BfR.

**Professor Dr. Dr. Andreas Hensel**  
BfR President



### BfR-MEAL-Study

#### Meals for the exposure estimation and analysis of foods

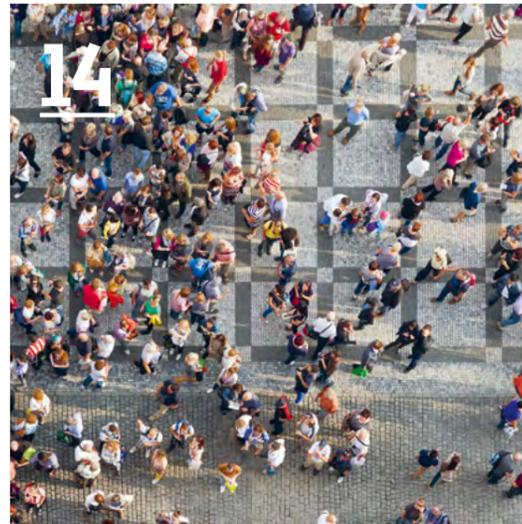
For the first time in Germany, the BfR MEAL Study analyses foods in a ready-to-eat condition systematically and representatively and follows a so-called Total Diet Study (TDS) with regard to the methodology. TDS are recommended by the United Nations Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) to determine the mean levels of substances in the average human diet. The first results of the BfR MEAL Study will probably be available at the beginning of 2019 and used to estimate the intake of substances via food.



**BfR  
MEAL Studie**  
Was im Essen steckt



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Assess.  
Research.  
Communicate.

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## Imprint

BfR 2 GO – The BfR Science Magazine  
Published twice a year

## Publisher:

German Federal Institute for Risk Assessment  
Institution under public law

Represented by the President of the BfR,  
Professor Dr. Dr. Andreas Hensel  
Responsible under the German Press Act:  
Dr. Suzan Fiack

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## Edited by:

BfR Press and Public Relations Unit

## Layout, graphics & photo editing:

Studio GOOD, Berlin

## Printed by:

Ruksaldruck, Berlin

## Copies printed:

4.000 (german), 1.500 (english)

## ISSN

Print-ISSN 2567-8949  
Online-ISSN 2567-8957

DOI 10.17590/20171024-095010

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please contact the editorial department at  
[publikationen@bfr.bund.de](mailto:publikationen@bfr.bund.de)

Printed on PEFC-certified paper



Made in Parma?



Was it cold pressed?



KEY TOPIC: AUTHENTICITY

# Tracking down the food fraudsters

Laced, cut, adulterated – food fraud is a global problem. The BfR is examining how methods for establishing the authenticity of foods can be harmonised. To do so, the researchers at the BfR slip into the role of the food fraudsters too.

Is it pure wine?



Does it come from the Allgäu region?



How much sheep's milk is in it?



A monitor, a PC, an infrared spectroscopy and lots of little sample vessels filled with fat extracted from hard cheese – Allgäuer Emmentaler. A lab assistant draws a sample of fat the size of a pin head and takes it to an analysis device. Seconds later, a wavy line made up of 1,800 data points appears on the screen: the spectrum of the cheese fat sample. A second laboratory, another analysis: test tube by test tube, a gripper arm picks up Allgäuer Emmentaler samples and inserts them into the strong magnetic field of a nuclear magnetic resonance spectroscope. The measurement produces up to 130,000 data points that are characteristic for each cheese sample. A third laboratory, a third test method, in which isotope ratio mass spectrometry is used to determine the origin of the hard cheese samples. A total of 150 to 200 samples are to be procured and analysed. “All of the data are processed and fed into evaluation software which calculates a characteristic data spectrum for each method – the authentic fingerprint of Allgäuer Emmentaler,” explains Dr. Susanne Esslinger who coordinates the tests to establish the authenticity of Allgäuer Emmentaler.

### The search for the fingerprint

In the BfR laboratories, scientists are working hard to find the typical, unmistakable fingerprints of various foods and feeds,

including wines, spirits, herbs and spices, hard cheeses, vegetable oils and corn. Their goal is to be able to verify the authenticity of these products or deviations which indicate falsification. If this is successful, it will make faking foods more difficult. The awarding of the EU quality mark “Protected Designation of Origin” guarantees that for the specific product designation “Allgäuer Emmentaler”, the cheese must actually have been produced, processed and manufactured in the Allgäu region. What is the use of the quality mark though if there is no method to distinguish the original from the fake. For this reason, the BfR has set up the trainee group “Authenticity along the supply chain” to strengthen research and interdisciplinary networking for the standardisation of data collection to verify authenticity.

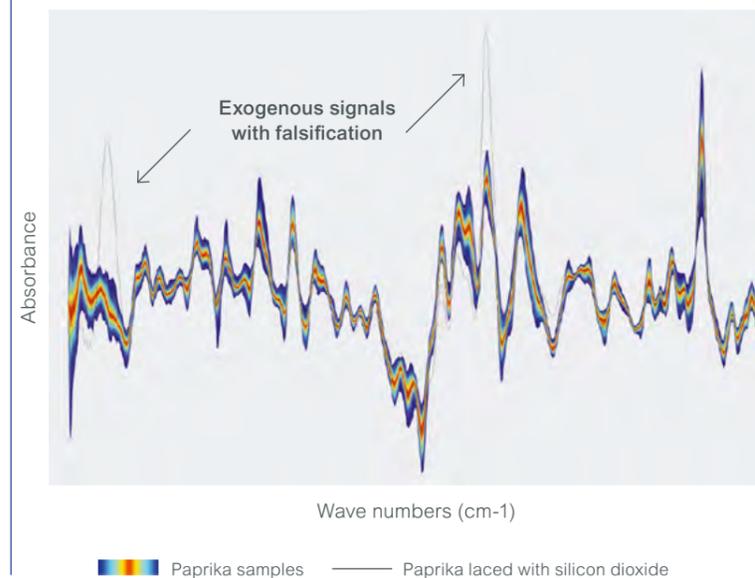
The BfR participates in several national and international joint projects to develop new approaches and strategies to detect food and feed fraud with which practices of this kind can be better discovered all over the world. The main focus of the BfR’s research in this field is on the designation of origin and identification of unknown additives by means of targeted and non-targeted analytics in combination with statistical methods and issues concerning data processing and interpretation.

“**The data points that lie outside the normal range or bandwidth can be very clearly recognised. A food that falls through this grid is suspect.**”

### Non-targeted analytics

A challenge in the search for the analytical characteristic of foods is the natural variation of ingredients. “That’s why a large number of unadulterated samples have to be drawn first, in order to find characteristic features in the data sets,” explains Dr. Carsten Fahl-Hassek, who heads various research projects on the authenticity testing of foods and feeds at the BfR. “Our results show that the idea of the unmistakable fingerprint of foods works”. The BfR has already recorded the physico-chemical

### The principle of “non-targeted methods”



### Relevant aspects during testing

Authenticity tests can confirm official product descriptions or prove statements to be false. Relevant aspects here are substitution with cheaper but similar additives, lacing with foreign substances (e.g. water, starch) or the use of unlisted or unauthorised methods such as radiation or extraction, confirmation of geographical origin, botanical or zoological species or of the manufacturing process, such as organic, conventional or genetically modified, as well as the declaration and statements on traceability (product labelling, process stages, batch identification).

fingerprint of various wines, edible oils and most recently herbs and spices by means of infrared and nuclear magnetic resonance spectroscopy. In doing so, the BfR laboratory was turned into a fraudster’s den: herbs and spices are mixed with various ingredients before processed samples of them are measured with the help of analysis equipment. “The data points that lie outside the normal range or bandwidth can be very clearly recognised. A food that falls through this grid is suspect”, says Dr. Fahl-Hassek. In this way, the spectra of oregano mixed with olive leaves, for example, or paprika powder laced with Sudan Red – a substance suspected of being a genotoxic carcinogen – look unmistakably different.

Accordingly, the new analytical methods that use the characteristic fingerprint of a food constitute a flexible and quick form of authenticity testing. Instead of looking specifically for falsified products, the focus here is on proving authenticity. This new approach is also known as non-targeted analysis.

### Fingerprint cloud

For the perspective, routine application of non-targeted analysis for food safety, however, joint utilisation of the databanks for reference food fingerprints of all actors involved in the field of food safety must first be enabled and it must also be ensured that the data collection and subsequent analysis of all users produce comparable results.

The BfR is conducting joint research on a simplified scenario for the future in which certified official and private laboratories make their food fingerprints available via a data cloud, with manufacturers, processing and refining businesses contributing the related product information. When monitoring authorities, wholesalers or retailers want to check food samples for authenticity, they compare the fingerprint of their own sample with those in the fingerprint cloud. If there are significant deviations, the food is initially classed as conspicuous and is earmarked for further examination. There are still many technical and organisational obstacles to be overcome.



The preparation of the cheese samples for testing involves homogenisation with liquid nitrogen.





A cheese fat sample the size of a pin head is put into the analysis device. To find characteristic features in the data sets, Dr. Carsten Faulh-Hassek (picture right above) and his team must analyse a large number of samples of this kind.

### The search for fingerprints

So-called non-targeted methods are used to build up standardised reference databases. In doing so, the physico-chemical fingerprints of foods are collected on the basis of spectrometric and/or spectroscopic data. These then serve as a comparative standard with which the data of unknown food samples are compared. This makes it easier to recognise deviations so that monitoring authorities no longer have to search specifically for particular undesired substances which are often unknown.

“First of all, uniform data standards, separately operating system solutions and organisational system approaches which guarantee the integrity and quality of data have to be developed if this vision in the field of food safety is to be realised,” explains Matthias Filter who coordinates tasks focusing on “Global Supply Chains” at the BfR.

### Networked reference databases

To bring this future scenario a bit closer, scientists at the BfR are conducting research to develop mathematical and statistical models for data evaluation and the harmonisation of data exchange formats, as well as harmonised sampling. These are essential prerequisites for the build-up and routine use of collaborative reference databases which

can be made available via the cloud. “At the moment, for example, all manufacturers of spectrometers use their own data formats so that the data have to be deciphered first before they can be used by everyone involved,” says Filter.

Until reference databases of this kind containing the typical chemical-analytical profiles of foods can be built up and made fit for widespread use in a network comprising monitoring authorities, laboratories, manufacturers and dealers will take at least another 10 to 15 years, in the opinion of the experts. All the more reason to lay the foundations now. ■

**More Information:**  
[www.bfr.bund.de/en](http://www.bfr.bund.de/en) > A-Z-Index:  
authenticity



## Original or fake – working together against food fraud

The BfR is involved in third party-funded projects to provide manufacturers and monitoring authorities with methods with which the authenticity of a food can be recognised quickly, reliably and cost effectively. A few of them are presented here.

### Food Integrity

#### EU consortium against faked foods

The EU consortium made up of 38 project partners aims to establish the structural and methodical prerequisites to recognise fakes uniformly and in a timely manner. To do so, a network involving authorities, industry and research is to be built up among other things, test processes are to be harmonised and knowledge databases created. The project is set to run until the end of 2018.



### FoodAuthent

#### Collaborative reference databases for chemical fingerprinting

The fundamentals for the routine use of fingerprinting analysis methods in the food sector and official monitoring are being created in this three-year project. In a project scheduled to end in April 2019, the BfR and its partners are developing joint fingerprinting databases and interfaces to privately operated product information systems. This is being done for hard cheeses, cooking oils and spirits, for example. The data is to be freely accessible to all parties involved in the food sector.



### Animal-ID

#### Proof of origin of animal proteins in feeds and foods

The project partners combine mass spectrometry with protein enrichment methods in order to develop analysis methods for the tissue-specific determination of the origin of feeds, as well as rapid tests on the basis of protein-based ELISA/dipstick tests for the most important animal species used for meat production, such as cattle, pigs, horses and chickens. The BfR is coordinating the four-year project (2016 to 2019).



### FoodRisk-Lab

#### Software solutions to support the assessment of risks

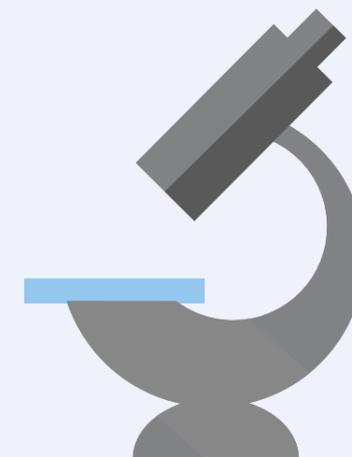
The BfR is developing freely available software solutions and web services for the assessment tasks of official institutions, as well as for science and research purposes. FoodChain Lab, for example, supports the traceability of feeds and foods along the supply chain and enables the interactive analysis of outbreak incidents. Other programs serve the mathematical modelling of hazards in the field of feed and food safety.



### SPICED

#### Safeguarding Europe's spice and herb supply chains

Spices and herbs are added to a great many foods. The results of this research project contribute towards the protection of Europe's spice and herb supply chains from natural, unintentional and intentional biological and chemical contamination. The project involving 11 partners from seven EU countries was promoted from 2013 to 2016 within the scope of the 7th EU framework programme and coordinated by the BfR.



# “We want to clamp down on fakes”

## Professor Wittkowski, which foods are usually falsified?

Fraud takes place wherever an economic advantage can be gained. That's why product piracy is practiced above all with high-quality, expensive branded products, such as olive oil, sheep's milk cheese, wine and spirits. A common trick is to sell inferior ingredients as high-quality produce, such as shrimps as prawns. Or, it is attempted to dissemble the origin of products with quality characteristics based on the geographical origin, such as Parma or Serrano ham, Irish whiskey or champagne.

## Can consumers trust food safety despite this?

Credibility, trust and safety are closely linked to one another. People want to shop, cook, eat and drink, stay healthy and enjoy the taste. They don't want to know how the wine was filtered or at what temperature the coffee was roasted, but they want to be able to trust that the information given is correct and that these technologies produce safe foods. This is only possible because standards exist for the food industry and monitoring authorities check for compliance.

## Just because Parma ham doesn't come from Parma doesn't mean that it poses a health risk. Isn't it much more about the economic interests of the manufacturers?

Scientists can recognise norm deviations but not what motivates falsifiers. It goes without saying that a risk assessment is conducted as soon as findings of this kind are made. After making further checks and tracing products back along the supply chain, it can then be decided whether it was a deliberate falsification or not.

## Can you give an example?

A few years ago horse meat was found in lasagne instead of beef. This case of fraud

didn't appear to pose a health risk at first, because horse meat is a high-quality food, but no one knew where the meat came from and whether it was contaminated with banned and/or health-damaging drugs. After this had been checked, the meat was declared safe. Then there were the pistachios from Iran which had increased mycotoxin levels for a while due to the traditional cultivation method and which were therefore subject to strict sanctions when they were imported into Europe. False declarations of origin then cropped up to get around these sanctions and Iranian pistachios were marketed as Californian produce.

## So it's always primarily about consumer protection and not about protecting against deceit or fraud?

In my view, yes. Some falsifiers simply accept that people can be harmed – through alcohol laced with methanol or milk powder thinned with melamine, for example. The falsifiers aren't toxicologists after all, so the question centres around the methods that authorities can provide to maintain trust in the safety of food.

## That sounds like a race: research against forgery. Is there always a right method?

The good thing about analysis is that if you use the right method, you will find what you are looking for. We don't always know though what new deceitful ideas falsifiers will come up with next. That's why we participate in international research projects to develop reference databases with the typical fingerprints of foods. Known as fingerprint analysis, it provides the opportunity of identifying not only foods as falsified through their deviation from the norm but also of discovering previously unknown adulterations and manipulations which could pose a health risk before the goods hit the shelf.

99  
**Our unique feature is that we conduct risk assessment and research.**

## Measures on this are bound to exist already in actual practice?

Private analysis firms have already specialised in the fingerprint method for certain products and established large databases, but there is a problem here, because they all work with their own data. The firms' results don't stand up in court either, because the data basis is not public. State institutions, on the other hand, work with transparent and comparable data so that complaints do stand up in court. That's why the measurements of the various labs have to be validated and the databases harmonised for the fingerprint methods to be successful.

## What does the BfR contribute to the verification of the authenticity of foods?

We have been involved for quite some time in all of the decisive EU research projects on authenticity testing. Our unique feature is that we conduct risk assessment and research. Unlike other assessment authorities, the BfR has its own laboratories, which is why we cast a very strict eye on assessment methods and ask how reliable and meaningful the data is. The core business of the BfR's 17 reference laboratories is the

BfR Vice-President Professor Dr. Reiner Wittkowski aims to ensure that research to find new methods for the authentication of foods is carried out at the BfR. As a young food chemist at the BfR's predecessor institution, the Federal Health Office, he already developed and established methods for verifying the authenticity of wine and began to build up a fingerprinting database.



validation of methods. On a national level, the BfR already works closely with the official food monitoring authorities of the federal states.

## What specific approach does the BfR use?

We combine analytics and data modelling. The institute has a long history when it comes to verifying the authenticity of foods, because research on this was already done in our predecessor institutions. One of the first databases for validating the authenticity of wine was built up in the 1990s. Nuclear resonance spectroscopic methods were used in food analysis for the first time. We then transferred the expertise acquired with wine to other food matrices. In addition to analytics, statistics is our second impetus. The BfR has gathered a lot of experience in the field of data analysis for upstream and downstream traceability along supply chains. Complex data modelling is possible with the FoodChainLab software that we developed in the course of the EHEC crisis.

## What are the next steps?

We have already established a national competence network between federal authorities, state investigations offices, universities and research institutions and we are driving ahead with the topic all over the world. The BfR organised a symposium in November 2016 at which around 100 experts from Germany, Europe, North America, Africa, Asia and New Zealand discussed the challenges of standardisation. In my view, a European reference laboratory must be set up at all costs – the EU Commission is already considering this – in order to establish a harmonised system of the applied methods and data collection for the authenticity testing of foods in Europe. Only this will help us to achieve our goal of clamping down on fraud! ■

# Keeping an eye on perceived risks

The BfR Consumer Monitor is one of the main instruments of consumer health protection. It shows how risks are perceived by the general public.

What topics interest consumers? What are they familiar with and what concerns them? The answers to these questions are given in the BfR Consumer Monitor, a representative population survey that has been conducted regularly since 2014 by the BfR to determine the public's general estimation of consumer protection topics. To supplement this, the BfR conducts representative surveys on individual subject matters which are of particular topical interest, such as antimicrobial resistance and plant protection products. The results of these appear as the "BfR Consumer Monitor Special". The Consumer Monitor is an essential indicator which allows the early recognition of possible misunderstandings or false estimations on the part of the general public. "It's very important for us to know specifically what the concerns of the population are so that we can decide what communicative measures are required," explains PD Dr. Gaby-Fleur Böl, head of the Risk Communication department at the BfR.

## Data collection method

To conduct the representative survey for the Consumer Monitor, approx. 1,000 persons living in private households in Germany who are at least 14 years old are interviewed over the telephone by market and opinion research institutions on behalf of the BfR. These computer-supported interviews use a programmed questionnaire and are conducted by trained interviewers in what is known for short as the CATI method – computer-assisted telephone interviewing. In the process, the questions on consumer health protection and other thematic areas are compiled into a so-called omnibus or multiple topic survey. The questions in the Consumer Monitor can have an open character (unprompted questions) or closed formulation (prompted questions). Each survey is structured in a similar way so that the results of the Consumer Monitor surveys can be compared with one another over time. The BfR dedicates itself on the one hand to topics which receive a lot of attention among the general public. On the other hand though, it analyses questions which have not so much been the focus of public attention but which are nevertheless relevant, such as health-damaging plant substances. Questions are also asked about the degree of trust in the structures of consumer health protection, as this can also influence the perception of risks.

”

**It's very important for us to know for sure what concerns the population and why.**



## Goals of the Consumer Monitor

- > Assessment and analysis of trends and topics in consumer perception
- > Over- or underestimation of health risks by the general public
- > Observation of changes in consumer behaviour
- > Rapid assessment of consumer perception in crisis situations

## Interests of consumers

The view of consumers that food in Germany is safe has hardly changed over the years. When the unprompted question is asked about the greatest health risks, the answers given most often in the most recent Consumer Monitor (08/2017) are still smoking, climate and environmental pollution and an unhealthy or wrong diet, followed by alcohol and unhealthy or contaminated foods. When specific prompted questions about selected topics are asked, salmonella, genetically modified foods, antimicrobial resistance and plant protection product residues are the topics most people are aware of. These are also the four topics which are a cause for concern with most respondents. It has been shown repeatedly that

consumers underestimate risks which are classified as relevant to health from the point of view of risk assessment, such as food hygiene in the home. The general public is largely unaware of a new method of genetic engineering which is currently a topic of discussion in scientific circles, so-called “genome editing”.

**Strengthen trust**

Different population groups perceive incidents or occurrences in different ways. How great a risk appears to be depends on a lot of factors. Perceptions of laymen and experts can differ in particular, as the intuitive risk estimation of the general public is often based on other principles and criteria than the scientific risk assessment. It is known from risk perception research, for example,

that concerns about synthetically produced substances are greater than concerns about substances that occur naturally, such as toxic plant substances.

The BfR Consumer Monitor special on plant protection products also shows that a majority of the general public wrongly assumes that plant protection product residues are not allowed to be contained in foods in general. In actual fact, however, residues of authorised plant protection products are permitted and classified as safe up to the maximum residue level. Risk communication can respond specifically to this discrepancy by providing the general public with comprehensive information on the legal and scientific foundations of the risk assessment of plant protection products through suitable communication channels.

The results of population surveys of this kind provide important insights into the risk perception of various population groups and show the differences linked with demographic factors such as age, educational background, profession and gender. Thus, for example, more women than men are aware of the occurrence of pyrrolizidine alkaloids in herbal tea. It has also been shown that men are less concerned than women about process contaminants in barbecued meat.

With the Consumer Monitor, the BfR has established an instrument with which a response can be given to information and communication requirements even earlier, thus further strengthening public trust in consumer health protection. ■

# Vegan and well-informed

**Cookery books with vegan recipes, blogs on the subject and animal-free supermarkets – vegan diets are en vogue. If you cut out animal products completely, however, you run the risk of an undersupply of certain nutrients. To guarantee efficient risk communication, the BfR questioned vegans about their levels of knowledge and attitudes.**

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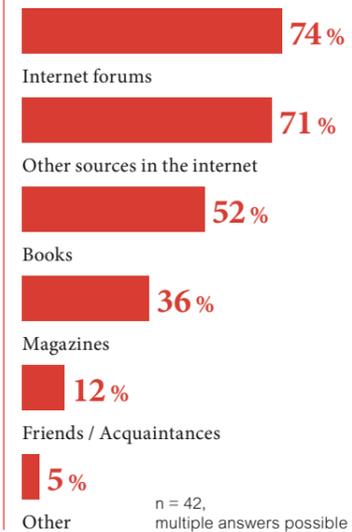
In 2016, there were 0.8 million people among the German-speaking population of Germany aged 14 years and over who described themselves as vegan or as someone who largely avoids animal-based products (2016, Allensbacher Markt- und Werbeträger-Analyse, IfD Allensbach). Several studies show that a vegan diet can have a positive influence on health, such as a reduced risk of Type 2 diabetes. Health risks due to nutrient deficiencies are also described, however, for groups like pregnant women and children. People who do not consume any animal products run the risk of a deficiency of vitamin B12, iron, calcium, iodine and zinc, as well as long-chain Omega 3 fatty acids.

Against this backdrop, the BfR aims to develop suitable risk communication strategies. One research project was dedicated to the individual and social influencing factors which lead to the motivation to switch to and maintain a vegan diet. With the help of focus group interviews, a total of 42 vegans were surveyed on their values, attitudes and opinions. As these deviate quite severely from those of the average population in some cases, some generalised statements can be made, but the survey data is not representative.

**Ethical decision**

Vegans almost always base their decision to adopt a vegan diet on ethical arguments; health motives are hardly ever mentioned. Most respondents do not belong to any religious confession and have an above-average level of education. In addition to this,

**What information sources do you use to acquire information on a vegan diet?**



responds with scepticism and aversion. To prevent their children from becoming social outcasts, some of the respondents permit compromises by allowing the children to choose by themselves whether or not to eat animal products when not at home, for example.

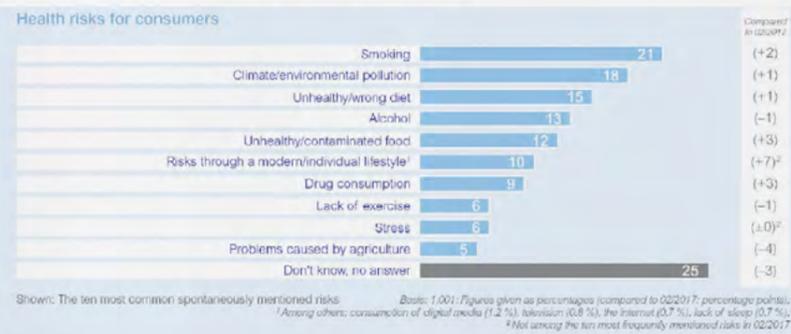
**Knowledge getting through**

People who choose a vegan diet usually have sound knowledge of nutrition. 40 of the 42 participants of the BfR study, for example, were aware that a vitamin B12 deficiency can result without animal products. Many of them take this vitamin as a supplement. When looking for information about all aspects of a vegan diet, the internet is the most important source of information (see chart).

**Risk communication strategy**

Some important link-up points for risk communication emerged in the course of the survey. Among other things, it became clear that a negative approach presenting a vegan diet as dangerous or abnormal can only have limited success. Effective risk communication should pick up on vegans' existing convictions with the goal of giving concrete tips for everyday life which can be combined with a vegan diet. Information on how a possible nutrient deficiency can be prevented with a vegan diet is fundamentally promising. Comprehensive information should be provided in particular with regard to alternatives or supplementation during pregnancy and with infants and young children. ■

**Which topics do you personally regard as the biggest health risks for consumers?**



**To what extent are you personally concerned or unconcerned about the following health and consumer topics?**



All previously published BfR Consumer Monitor surveys can be accessed at: [www.bfr.bund.de/en](http://www.bfr.bund.de/en) > Publications > Brochures > BfR Consumer Monitor

# Eat insects? More theory than practice



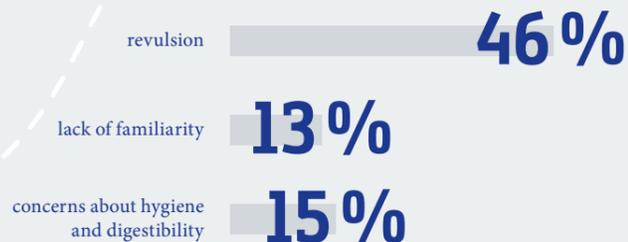
Crickets, mealworms and migratory locusts – insects are eaten by humans in many regions of the world. Since spring 2017, insects have been officially approved as food in Switzerland too, even though their consumption is not exactly commonplace in Europe in general and the public discussion is just beginning. How well known is the topic among the German population? What are the attitudes towards edible insects? How is the subject dealt with by the media? The BfR has found answers to these questions in two studies.

63 percent of Germans assume that eating insects does not pose a health risk, but they wouldn't try them.

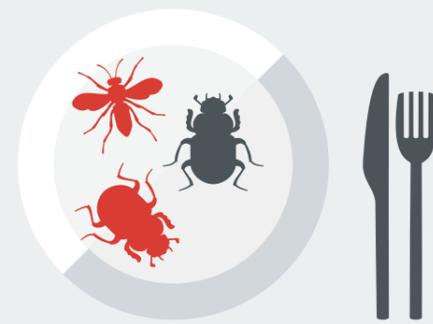
63%



The main reasons for this are revulsion, lack of familiarity and concerns about hygiene and digestibility.



**Insects as a food for humans** – 72 percent of the respondents have heard about this. Insects are considered to be rich in protein, nutrients and vitamins, and are seen as a key source of food in light of the problematic world food situation.



14 percent of the respondents have already eaten insects, mainly abroad. The majority are men, aged 18-29 years, well educated and city dwellers.

14%

Media reporting on insects as foods and feeds doubled from 2014 to 2015. The majority dealt with beneficial aspects: insects have been portrayed in the media as a “trendsetting, sensible food with which the benefits outweigh the risks”.

2015:  
60 articles



2014:  
27 articles



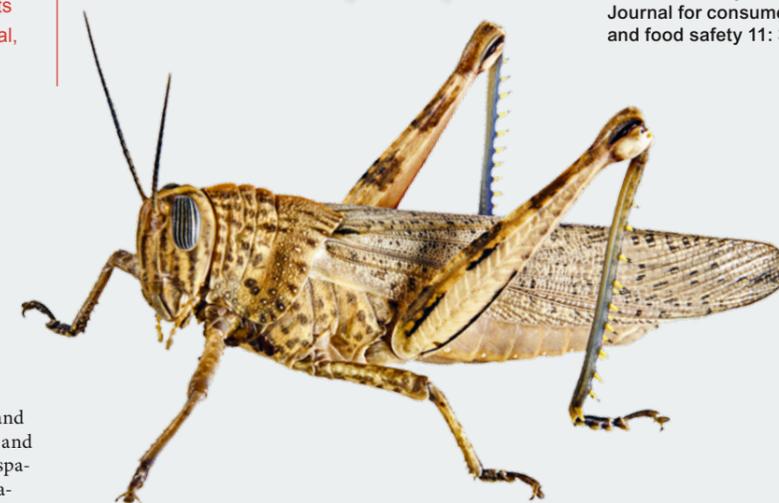
Consumers want more information on the possible health risks of insects as foods and feeds, and on their production and nutrient content. This could increase public acceptance of the consumption of insects. Insects that have been “rendered unrecognisable” in the form of processed foods would be more acceptable than insects in their original state.

### Insects as food

- > Are regarded as novel foods under EU food law and have to be approved
- > Unexplored aspects: toxicology, levels of contaminants and residues, allergenic potential, microbiological risks



Further reading (in German): BfR symposium “Insects as Foods or Feeds: A food of the future?” Report on the symposium on 24 May 2016 in: 2016. Journal for consumer protection and food safety 11: 3, 281–289.



**Underlying studies:** Representative telephone interviews of 1,000 persons (German-speaking population aged 14 years and over), media analysis of 17 German print and online media (supraregional quality newspapers, capital city press and weekly newspapers) in the period January 2014 to December 2015 on the type and frequency of reporting on edible insects.



## Undesirable in food

If pyrrolizidine alkaloids (PAs) enter the food chain, 1,2-unsaturated PAs in particular can harm human health. The BfR has been dealing for years with the question of what health risk 1,2-unsaturated PAs pose in foods. What is clear is that PA contamination in the food chain should be as low as possible.



Undesired substances which find their way into the food chain can pose a health risk to consumers. An example for this kind is 1,2-unsaturated pyrrolizidine alkaloids (PAs), which are formed by several plants as protection against predators. They were detected initially in honey and other bee products and later in teas, herbal teas and spices, as well as in plant-based dietary supplements.

### Toxic effects in humans and animals

In humans, sporadic cases of poisoning are known to have occurred after the pharmaceutical intake of herbal teas and products containing PAs. There are also reports of epidemic liver disease with fatalities in Pakistan, India, Afghanistan, Tajikistan and the former Soviet Union after the consumption of cereal contaminated with seeds of *Heliotropium* or *Crotalaria* species. In livestock farming, there are reports of the increased occurrence of liver cirrhosis in slaughtering cattle which had ingested alpine ragwort along with hay and silage. Seneciosis, a disease caused by PAs that is found together with liver degeneration, was also observed in horses.

The liver is the primary target organ for damage through 1,2-unsaturated PAs, although the lungs and other organs can also be affected. Acute hepatic veno-occlusive disease (HVOD) can occur in the liver or lungs, especially when higher doses are ingested. Developmental toxic effects have also been observed in animal tests involving higher doses of 1,2-unsaturated PAs. According to the latest available data from animal experiments, the carcinogenic effect of 1,2-unsaturated PAs is regarded as validated and a corresponding risk for humans is under consideration. Compounds that proved to be carcinogenic in animal experiments often showed a genotoxic effect in the corresponding mutagenicity tests.

### How 1,2-unsaturated PAs can find their way into foods

According to the latest level of available knowledge, 1,2-unsaturated PAs can find their way into foods via several pathways. In Germany, contamination with PA-forming wild herbs has occurred above all in areas where crop plants are cultivated, e.g. lettuce with ragwort or groundsel. Increased contamination in cereals is known from Afghanistan. The contamination of tea and herbal tea varieties with PAs is also attributed to contamination with certain wild herbs in the areas under cultivation. In addition to this, bee products such as honey and pollen can be contaminated with PAs if the flow originates from certain wild plants. 1,2-unsaturated PAs can also find their way into milk, eggs and meat through contaminated feeds. Dietary supplements can also be produced on the basis of plants which form 1,2-unsaturated PAs by themselves.

### Health risks through contaminated foods

The BfR made an assessment of the health risks of 1,2-unsaturated PAs in 2016. In the process, the contamination of all important food groups for which data on the occurrence of 1,2-unsaturated PAs is currently available was taken into account. The genotoxic-carcinogenic effect was used as the most sensitive endpoint in the assessment.

Accordingly, intake of 1,2-unsaturated PAs in children aged six months to under five years was essentially attributable to herbal tea including rooibos tea, black tea and honey. Fruit tea, milk, eggs and meat contribute only a little towards overall intake of 1,2-unsaturated PAs. Apart from certain dietary supplements, the picture is similar with adults, except that the contribution of honey to the overall 1,2-unsaturated PA intake is smaller and that of green tea greater than with children. Dietary supplements containing high levels can contribute to the overall intake of 1,2-unsaturated PA via food as an additional exposure source. It is also possible that spices and herbs, as well as flours, constitute a relevant additional exposure source, but there is insufficient data come to a final conclusion.

Although high levels of 1,2-unsaturated PAs have been detected in certain foods for normal consumption in individual cases, the BfR believes that, based on the current data, acute health impairments are unlikely even if high amounts of these foods are consumed. As is internationally customary, the BfR used the MOE (margin of exposure) approach as the basis for estimating the potential risk of the genotoxic-carcinogenic effects. On the basis of animal studies, it is assumed here that an MOE value of 10,000 or higher for genotoxic carcinogens can be regarded as being of only slight concern from the point of view of public health, so that the priority for risk management measures is correspondingly low. No threshold dose can be assumed for genotoxic-carcinogenic substances below which undesired effects can no longer be expected. For this reason, the recommendation applies in the European Union that exposure to substances with genotoxic and carcinogenic effects should be minimised to the greatest reasonably possible extent in line with the so-called ALARA principle (as low as reasonably achievable). Even low intake quantities can be connected with increased health risks, especially with regular consumption.

The assessment of the genotoxic and carcinogenic risk was made by comparing overall intake via various teas, milk and honey in children and adults on the basis of various consumption scenarios with a BMDL 10 (Benchmark dose lower confidence limit 10 %) value of 73 micrograms per kilogram body weight and day with calculation of the MOE. The BMDL10 value is

an estimate of the lowest dose that causes a cancer incidence no greater than 10 percent in animal experiments with 95-percent certainty. All calculation approaches for children as well as the adult population produced MOE values well below 10,000. As an MOE should only be regarded as being of slight concern for public health from a value of at least 10,000 in the view of the EFSA scientific committee, the BfR recommends that overall exposure to PAs from all foods be kept as low as possible and that, under consideration of PA intake from all sources, the MOE should not be allowed to drop below 10,000. To achieve this, efforts should continue to further reduce PA levels in foods.

### BfR research approaches

The BfR has been involved for several years with the problem of the contamination of foods with 1,2-unsaturated PAs and has been pursuing various research approaches in this regard. Due to their great structural variety and occurrence in many different foods, the analysis of 1,2-unsaturated PAs poses a special challenge. Over the last few years, the BfR has developed specific detection methods and validated them in ring trials. These methods are available at the BfR homepage and can be used in food and feed monitoring, for example.

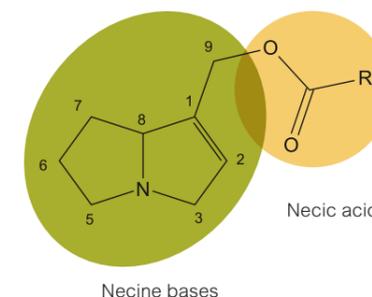
In addition to the development of analytical methods, the molecular mechanisms of the toxicity of 1,2-unsaturated PAs are also being examined at the BfR. The main focus here is on the examination of the structure-dependent intake of 1,2-unsaturated PAs via the intestinal barrier. The results show that the toxic potency of the 1,2-unsaturated PAs can differ due to differences in structure-dependent transport



Plants which form pyrrolizidine alkaloids are to be found above all in the compositae (Asteraceae), „forget-me-nots“ or borage (Boraginaceae) and legume (Fabaceae) families.

### The structure of PAs

From a chemical point of view, these are esters from a necine base and aliphatic mono or di-carbon acids (necic acids). Of particular importance from a toxicological point of view are pyrrolizidine alkaloids with a 1,2-unsaturated necine structure (1,2-unsaturated PAs). The 1,2-unsaturated necine structure as a common structural characteristic fundamentally permits toxicogenation through oxidation to dehydro pyrrolizidine, which is responsible for the genotoxic and carcinogenic effect of this substance group due to its alkylating properties.



via the intestinal barrier. The tested model substances also showed distinct structure-specific differences in metabolism in a cell culture model. Further examinations to the destruction of the sinusoidal endothelial cells through 1,2-unsaturated PAs should contribute towards understanding the mechanisms which lead to the veno-occlusive changes in the liver. Examinations of the metabolism of nutrition-relevant pyrrolizidine alkaloids are also being conducted. The research projects are being sponsored by the German Research Foundation (DFG).

### Recommendations to reduce PA levels

The BfR recommends that management measures should focus on the avoidance of the genotoxic-carcinogenic effects of the 1,2-unsaturated PAs as the most sensitive endpoint. Overall consumer intake of 1,2-unsaturated PAs should be as low as possible. To do so, PA levels in foods should be minimized as far as possible. This applies in particular to herbal teas, rooibos tea, black tea and green tea, as well as certain dietary supplements. Cultivation, harvesting and cleaning methods will have to be further improved to achieve this. The selection of the raw honeys can also contribute towards reducing levels of 1,2-unsaturated PAs in ready-to-eat honeys. In addition to this, monitoring by food companies in all affected food categories are still required.

Consumers can reduce the potential health risk of PAs by adopting a varied diet, thus avoiding general, slanted exposure to various potentially health-damaging substances. For children, expectant and nursing mothers in particular, the BfR recommends that the daily liquid requirement should not be met exclusively with herbal teas and tea.

When preparing salads, leaf vegetables and herbs, consumers should remove plant parts which they cannot identify as belonging to any known, edible plants.

Consumers of dietary supplements based on pollen or plants which form 1,2-unsaturated PAs should be aware that these products can contain 1,2-unsaturated PAs in higher concentrations. ■

**More Information:**  
BfR Opinion No. 030/2016  
of 28 September 2016

## MAIL FROM ... ZAMBIA



With the research project “SAD-Zambia”, the BfR helps to improve food safety and therefore helps to ensure food security in Southern Africa. Dr. Alexandra Fetsch, is the coordinator of the project. The Zambian PhD candidate Bruno Phiri talks about his daily work. Both work in the Unit “Microbial Toxins” at the BfR.

»

“The SAD Zambia project is a milestone for me and I am very grateful to the BfR for giving me this opportunity. I've learnt a lot already and have been able to improve my competence with regard to the diagnosis of disease pathogens. The project doesn't just help my personal advancement, however, my country can also benefit from it. My working day usually starts with visiting farmers and their herds. I take a look at how the cows are milked and take samples from the animals, the milk and the environment. I also go to milk collection points and markets to take samples. I analyse them in the laboratory at the University. I am already looking forward to conducting further examinations at the BfR. I was there already at the beginning of the project – a very exciting and impressive experience.”

(Bruno Phiri)

In Southern Africa, agriculture plays a key role in fighting poverty, but the increasing production of food of animal origin also poses new challenges in terms of food safety. The BfR research project “SAD-Zambia” (“*Staphylococcus aureus*” in the milk food chain in Zambia – combating food-borne diseases and antimicrobial resistance in humans”) aims to understand how the production and marketing of milk and dairy products in Zambia can be made safer. The objective of the project is to assess the extent to which the bacterium *Staphylococcus* (*S.*) *aureus* is transmitted to humans via milk and dairy products. In the first step, field studies in various provinces throughout the country will be conducted in order to analyse the prevalence of this pathogen and pinpoint possible weaknesses in the milk food chain in Zambia. The focus is on toxin-forming *S. aureus* strains. Likewise, the extent to which methicillin-resistant *S. aureus* (MRSA) – which are resistant to a certain class of antimicrobials – occur in the dairy food chain will be investigated. This

assessment work also includes a comparison of traditional and modern production systems. At the BfR, the characteristics of the bacterial isolates will be investigated and strain comparison analysis will be performed. In the next step, possible interventions to reduce transmission of bacteria as well as methods for the monitoring of zoonotic and antimicrobial resistant *S. aureus* in the dairy food chain in Zambia will be developed. The purpose of the project is to contribute to the understanding of food-borne risks along the dairy food chain in Zambia. The project has been launched on 1 July 2016 and will run for three years. Financial support is provided by the German Ministry of Food and Agriculture and by internal BfR funds. Alongside the BfR, the International Livestock Research Institute, the University of Zambia and the Central Veterinary Research Institute as part of the Zambian Ministry for Livestock and Fisheries are involved in the project. The research project “SAD-Zambia” will help to establish a long-term partnership in Southern Africa.



### Transmission pathways of the Hepatitis E virus

Infections with the hepatitis E virus (HEV) can result in acute liver inflammation. The number of reported cases of hepatitis E in humans in Germany has increased considerably in recent years. Research findings of the BfR in cooperation with other institutes have shown that HEV is widespread in domestic pigs and wild boars in Germany. The virus has also been sporadically found in deer. The distinct pathways by which the patients become infected with the virus are currently unclear. Direct virus transmission – for example from game to hunter – and indirect pathways via food made from infected animals are possible. Recent studies by the BfR show that HEV can be detected in various sausage products marketed by the German retail sector. What is still not known is whether the virus in these products is still infectious or whether it is destroyed during the production of the food. In the past, studies necessary to determine the infectivity were complicated by the fact that HEV is very difficult to cultivate in the laboratory. The initial results of tests performed using a novel cell culture system developed at the BfR indicate that HEV is heat-sensitive but can remain infectious for several weeks during cooling or at room temperature.



### Alkaloids in lupin seeds

Lupin seeds are in vogue, both for use in processed products and as a snack. However, these seeds can also contain toxicologically relevant bitter quinolizidine alkaloids (QA), which is why the BfR is assessing the corresponding health risks. The QA content of lupin seeds varies depending on their botanical and geographic origin. Due to their higher QA content, seeds from “bitter lupins” are not suitable for consumption unless they are treated accordingly by means of “debitting”, as these seeds can lead to poisoning symptoms in humans. Lupin varieties, from which seeds with low alkaloid content are derived from, are called “sweet lupins”. Sweet lupins are also suitable for consumption without debittering. There have been single cases of poisoning due to bitter lupin seeds in Germany. The BfR therefore advises producers to only bring whole lupin seeds on to the market that are suitable for consumption without additional debittering processes. Producers who make flour from lupin seeds should use low-alkaloid or adequately debittered seeds.

## SPECTRUM

### Algal toxins in tropical fish

Food poisoning caused by ciguatoxins – algal toxins that can occur in tropical edible fish like barracuda, snapper or grouper – used to be found predominantly in the Tropics. As a consequence of the globalised food chains, however, cases of ciguatera are now occurring with increasing frequency in Europe, and since 2012 also in Germany. One of the problems is that no analytical method is currently available for the routine testing of fish for ciguatoxins. As there are only few laboratories worldwide that are able to detect ciguatoxins in fish, the “EuroCigua” of the European Food Safety Authority (EFSA) was launched with the aim of, among other things, improving analytical methods and measuring the prevalence of ciguatoxic fish in Europe. The BfR is supporting the project by providing data on cases of ciguatoxin poisonings in Germany. The algal toxins that make people ill accumulate in predatory fish via the ecological food chain and are very stable. They cannot be destroyed by heating or deep-freezing. When consumers eat the contaminated fish, poisoning accompanied by sometimes severe or long-lasting impairments to the nervous system may occur. Patients suffering from ciguatera experience normal cold stimuli as being unusually painful.



# “Only a joint strategy can be effective”

**Bacteria that are resistant to antibiotics pose a public health risk. Professor Dr. Annemarie Käsbohrer is tasked with addressing this challenge at the BfR. In this interview, she explains which factors play a role in the spread of antimicrobial resistance.**

Ms. Käsbohrer, one of your major research interests is antimicrobial resistance. In the public debate, a connection is often made between the frequent use of antibiotics in livestock management and the occurrence of resistant bacteria in the field of human medicine. But aren't animals given different antibiotics than humans?

To explain the differences, we need to distinguish between the drugs themselves and the antibiotic substance groups. When treating animals, veterinarians use different drugs than those used by doctors treating human patients – but the active ingredient, the antibiotic, can be the same. Antibiotics can in turn be divided into active substance groups. In terms of these substance groups, the fact is that most of the antibiotics we use in humans belong to the active substance groups that are also used in veterinary medicine. There are only very few antibiotic groups that are used solely in the one or other field. These include carbapenems, for example, which may not be used for livestock. In the field of human medicine, carbapenems are above all used when other antibiotics are no longer effective.



If they can't be used in livestock, how is that you have found carbapenemase-producing bacteria in samples from livestock at the BfR during the RESET research project?

The emergence of antimicrobial resistance is a natural process with bacteria, and the use of antibiotics accelerates this process. Resistance to a certain active substance can also spread although the active substance group in question has not been used. Due to the selection process, the general rule is that if I use an active substance, then I favour those bacteria that are resistant to the substance. But carbapenemases, in other words the enzymes that are formed by carbapenem-resistant bacteria, can inactivate not just carbapenems but also almost all other  $\beta$ -lactam antibiotics. It is therefore theoretically possible that resistance to

**Professor Dr. Annemarie Käsbohrer** heads the „Epidemiology, Zoonoses and Antimicrobial Resistance“ Unit at the BfR, and this unit also comprises the National Reference Laboratory for Antimicrobial Resistance. In April 2016, she was also appointed to the position of Professor at the Vetmeduni Vienna, where she heads the „Public Veterinary Health Institute“.

“**If an infection pathogen collects multiple resistance mechanisms over time, it may be the case that there is no therapy at all that is ultimately effective.**”

carbapenems can spread although the veterinarian used a different active substance group in the livestock. Once a resistance to bacteria is present, there are many routes via which it can spread. A shed is not a sterile room but a living environment. Possible entry routes for resistant bacteria in a shed include live vectors such as newly introduced animals, mice, flies or birds as well as non-live vectors like feed, water, dust or equipment.

How has your work at the BfR changed since you found carbapenemase-producing enterobacteria in samples from livestock?

This finding had a wide range of consequences. Above all, we strengthened our targeted investigation activities. Since 2014, bacterial isolates from livestock and food have been routinely tested for carbapenem resistance within the framework of resistance monitoring at the BfR. In addition, we have also been able to draw on a further targeted detection method for carbapenemase-producing bacteria since 2015. This is an area in which there is a need for training in the laboratories. Moreover, the National Reference Laboratory for Antimicrobial Resistance at the BfR is testing bacteria suspected of producing carbapenemase for the characteristic resistance genes. To this end, various methods have been established at the BfR for analysis of *E. coli* and *Salmonella*. These kinds of resistance genes have only been detected very rarely to date – the case of the gene *bla*<sub>VIM-1</sub> in livestock, and the gene *bla*<sub>NDM-1</sub> in a wild bird.

It is often the case that genes encoding resistance to carbapenems are localised together with other resistance genes on mobile genetic elements. Why is particular caution advised in these cases?

First of all, the probability for selection is higher if multiple resistance genes are on one genetic element. In other words, the use of different antibiotics can create a “selection advantage”. The localisation of resistance genes on mobile genetic elements plays a key role in the transmission of resistance, because these resistance genes can be transferred to entirely different bacteria groups via horizontal gene transfer. This realisation triggered a kind of revolution. For a long time, a pathogen with all its properties was viewed as a single entity. But if resistance can be transferred back and forth between bacteria groups, then this possibly falsifies our perspective on a group. As

a result, the detection methods had to be adapted accordingly and the transferability of resistance also analysed. This is further complicated by the fact that a resistance gene can become a problem on an otherwise harmless bacterium. Some bacteria, such as *E. coli* are part of the normal intestinal flo-

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**Resistance to a specific active agent can also spread even if this substance group has not been administered.**

ra. If a bacterium of this kind possesses a resistance property, this is not dangerous in itself. But it can potentially pass this property on to a pathogen.

What are the specific potential consequences of this kind of transfer of resistance properties?

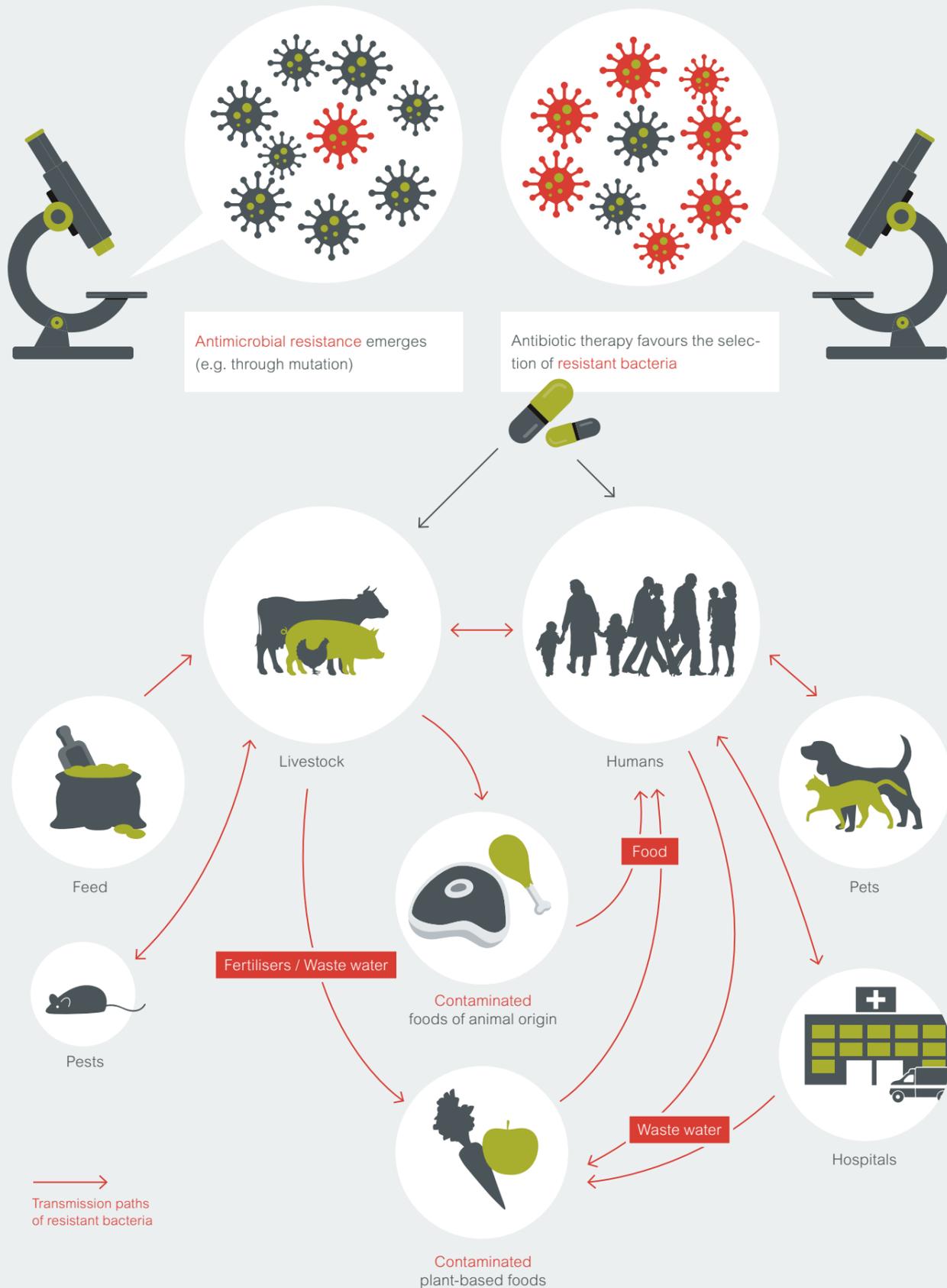
If a pathogen collects multiple resistance mechanisms over time, the final result may be that there is no effective therapy at all. Doctors are already resorting to active substance groups that had long been frowned upon in human medicine. This is the case with colistin, for example. It is not well tolerated by humans, and possible side effects include damage to the kidneys or the nervous system. Colistin has played a major role in veterinary medicine for decades, above all for the treatment of gastrointestinal infections in livestock and in order to avoid the use of other active substance groups.

Does the mobility of the resistance gene also play a central role in the case of colistin?

In the meantime yes. Up to the end of 2015, scientists agreed that colistin resistance is not transferable. In other words: if this kind of resistance occurs in a pathogen in livestock, it is not automatically transferable to a human pathogen bacterium. Then, however, the transferable *mcr-1* gene was described for the first time at the end of 2015 in China. Studies in Belgium subsequently led to the description of a further mobile gene for colistin resistance in livestock, namely *mcr-2*. This means that transmission of the resistance conferred by this gene to a human pathogen bacterium is theoretically possible. To date, however, this appears to happen only rarely.



# Spread of antimicrobial resistance



These insights underline the major need for more research. What do you do in the “Biological Safety” Department to assess the risk?

When it became clear in 2015 that there is a transferable colistin resistance, we naturally addressed this problem right away in our ongoing projects. In the RESET research project, for example, we analysed the existing bacterial material and the whole genome information from our cooperation partners for the occurrence of this new resistance gene. We soon realised that this gene was present in the sequences. And the analysis of colistin-resistant isolates from our strain collection confirmed that this resistance gene is already widespread.

Is it now necessary to initiate new research projects on this topic?

Yes, because there are still many unanswered questions: how often is the resistance actually transferred? What triggers this? There are also diagnostic problems. The standard method used in human medicine to test resistance does not generally cover resistance to colistin. There is a lot to do, in other words. We need to develop new diagnostic and typing techniques, expand our resistance monitoring activities, and routinely test phenotypically resistant isolates for the gene. We must then conduct a holistic assessment of the findings in order to pave the way for potential courses of action.

Which transmission paths play a role in colistin resistance for humans?

Actually, we can't say this with certainty. The Chinese working group has identified colistin resistance in both pork and humans, and this supports the hypothesis that this resistance is transmitted via foods. At the same time, what we are generally seeing is the nosocomial spread of resistant bacteria, in other words the spread of antimicrobial resistance in the hospital environment.

Resistances are spreading, the “silver bullets” are losing their effect, and we're not even safe in hospitals. Is there any effective strategy at all that can counter the spread of antimicrobial resistance? And who needs to step up?

If we are to prevent the spread of antimicrobial resistance, we need a joint strategy. At the end of the day, we are dealing with complex interrelationships between humans, animals and the environment. This is why we at the BfR advocate close cooperation with all the actors in the public health and veterinary system. This “one health approach” calls on both veterinarians and physicians to contribute their expertise. Consumers can also minimise their risk of infection with a resistant bacterium.

What do consumers need to be aware of?

They need to observe the same hygiene rules that apply to other disease pathogens that can be transmitted to humans by animals or food. People should wash their hands with hot water and soap after they have had contact with animals, for example. They should also wash their hands thoroughly after handling raw meat. Meat, eggs and raw milk must be heated prior to consumption, while raw vegetables and fruit should be washed thoroughly with drinking water or peeled before they are eaten. What is also important is to avoid direct or indirect contact between raw meat or raw eggs and ready-to-eat meals that will not be subsequently heated. By observing hygiene rules, people can prevent the transfer of resistant and / or pathogenic bacteria to other foods. ■

More information:  
Irrgang et al. 2017. Recurrent detection of VIM-1-producing *Escherichia coli* clone in German pig production. *Journal of Antimicrobial Chemotherapy* 72: 3, 944–946.

“**Consumers can minimise their risk of infection with a resistant bacterium.**”

## RESET

From 2010 to 2017, the RESET research network investigating antimicrobial resistance in animals and humans has been concerned with resistance to the particularly important antibiotic classes of  $\beta$ -lactam antibiotics and (fluoro-)quinolones in intestinal bacteria like *Escherichia (E.) coli*. If bacteria are resistant to both active substance classes, this dramatically restricts the range of therapeutic options. This kind of resistance has already been proven in *E. coli* and *Salmonella enterica*. The BfR is involved in the research network with two of its projects. The systematic merging of findings in the various research fields to create a joint database has made it possible to close the gaps in our knowledge and has paved the way for the assessment of risks. RESET was funded by the German Federal Ministry of Education and Research.

# The mixture is all important

World-wide, cumulative risk assessment is gaining ever more importance in toxicology. The BfR is working on a number of projects examining how mixtures can be assessed in the future.

Can the mixing of different pesticide residues cause hazardous exposure in the event of daily consumption?

When it comes to paradigm shifts, a fundamental change in thinking generally takes place. Some time ago, BfR scientists wrote that a paradigm shift that leaves traditional single substance assessment behind had taken place in the risk assessment of chemicals. Perhaps the Paracelsus adage in toxicology will need to be re-written in the future: Not just the dose, but also the sum of doses in a mixture, makes the poison.

## Cocktail of poisons or deadly cocktail – sensational media terms

It is true: in the last few years, ever more committees, political bodies and research projects have been dealing with the question of how the effects of mixtures on the environment and health can be assessed in the future. The handling of this subject is emotionally charged in the public sphere also: “mix of chemicals”, “cocktail of poisons” or even “deadly cocktail” – with the media using sensational terms to describe the phenomenon of multiple residues. Consumers are correspondingly scared. According to a representative perception survey conducted by the BfR in Germany, 80 % of respondents believe that the use of multiple different plant protection products can lead to health impairments through food.

## Mixing effects should not be a blind spot

Although various current monitoring projects show that a risk to health due to multiple residues of plant protection products is unlikely (see interview), the issue remains important in the scientific field. This is because of the need for reliable assessment of the risks to people and the environment is increasing even if mixtures have been subjected to scientific analysis and evaluation for decades. One reason is the large number of new substances, additives, chemicals, residues and contaminants to which people are exposed, combined with increasingly refined scientific methods of detection. Another reason is the increasing pressure from the public. Transparent and internationally harmonised procedures must be developed to avoid scientific divergences. Mixing effects should not be a blind spot in risk assessment.

## It is not possible to test all mixtures in animal experiments

How can this issue be explained in concrete terms? Mixtures are present whenever a person takes in different substances simultaneously at a specific time, e.g. residues, contaminants or chemicals that are registered according to the European chemicals regulation REACH. This happens when so-called intentional mixtures, such as plant protection products or biocides, are put on the market. However, the body also ingests unintentional mixtures. It may be that different substances are contained in one product, e.g. multiple plant protection product residues in an apple, or substances are consumed at the same time via different products. Mixing effects can occur in each of these cases. These effects can be more or less pronounced depending on the mode of action. Consequently, the effects of substances can potentiate one another or cancel one another out. Based on current knowledge, it is assumed that effects are generally additive if the ingested substances that possess the same mode of action are above the toxicological threshold and were ingested by the organism at a relevant point in time.

This is where the difficulties begin. There are too many intentional mixtures that enable an almost inestimably large number of combinations. Testing all these mixtures in animal experiments, as would be the usual procedure for individual substances, would be ethically unsustainable and practically unfeasible. For this reason, many research projects, including projects of the European Commission, are working on developing methods for cumulative that do not involve animal experiments. These methods assess the effects of mixtures *in vitro* or with computer-based *in silico* calculations (see interview and box).

## Throughout the world, countries are placing this issue on their agenda

This results in new challenges not only for science, but also for the harmonised implementation of laws. For example, the EU regulation on placing plant protection products on the market stipulates

## Step by step to a reliable assessment

Since March 2017, a method for assessing cumulative and synergistic effects during the health-related assessment of plant protection products within the approval process has been applied in Germany. The so-called hazard quotient (HQ), which is determined for each individual substance, forms the basis for the multi-step cumulative assessment procedure. The HQ is calculated from the ratio between the relevant health threshold and the estimated exposure.

In an initial step, the HQ is calculated individually for all substances in a mixture. If the HQ of each substance is less than 1, no risk to health is expected from the individual components of the mixture. If the HQ is more than 1 for individual substances, it is checked whether exposure can be reduced further using suitable measures, e.g. by wearing protective clothing.

If the HQ is below 1 for all substances, the so-called "hazard index" (HI) of the mixture is determined in a second step by adding together all hazard quotients of the substances used. If the calculated value is still less than 1, no risk to health is expected from the mixture.

If the HI is more than 1, a third assessment step takes place with further refined methods. This step checks whether a risk to health exists when the individual substances are grouped. To this end, the individual substances can be divided into cumulative assessment groups (CAGs), which may be defined according to such things as effects in the same target organ, the same mode of action, or structural similarity. The logic behind this grouping process is the assumption based on the current state of knowledge that only those effects that are comparable with respect to the target organs or the modes of action (MOA) are additive. The relative potency of the individual substances should also be considered in the refined assessment. Therefore, if the HI within a CAG is less than 1, no risk to health is expected. If it is above this value and neither the estimated exposure nor the hazard assessment can be further refined, the mixture is considered unsafe.

The multi-step process has the advantage that the more complex methods for refined assessment, for example, in comparable CAGs or MOAs, are only used if the initial, simpler assessment methods do not provide evidence that no risk to health is to be expected.



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that cumulative and synergistic effects must be taken into consideration, provided that scientific methods approved by authorities exist to measure such effects. To date, however, such methods do not exist. For this reason, technical guidelines for the new methods are now being created at all levels in order to guarantee harmonised procedures, transparency and traceability in the approval of products. Such technical guidelines are being developed by, for example, the EFSA (European Food Safety Authority), the ECHA (European Chemicals Agency), and the JRC (Joint Research Centre) of the European Commission.

These technical guidelines need to overcome other challenges in addition to scientific complexity. They should be valid for all sectors in which mixtures are placed on the market or could be created. Until now, mixtures were often only evaluated in one regulatory segment, for example, as a plant protection product, biocide or pharmaceutical. For other substances, such as food additives or contaminants, the assessment of mixtures was not clearly regulated. In the future, these substances should also be given more consideration in the assessment of mixtures. Furthermore, not only the chemical composition of an individual product should be systematically considered during the approval process, but also multiple residues in an entire shopping basket which an average consumer would be likely to consume at the same time. And finally, the new procedures must be harmonised worldwide in order to keep the cumulative risk to people and the environment low, also with respect to global product chains. For this reason, corresponding guidelines are currently the subject of discussion in politics and science not only at a European level. Other countries worldwide, as well as the World Health Organisation (WHO) and the Organisation for Economic Co-operation and Development (OECD), have placed cumulative assessment on their agenda and are coordinating their Guidance Documents with the other authorities.

### Germany already uses cumulative assessment

Scientists at the BfR are also participating in many of these international projects, involving both scientific research into cumulative

effects and the development of technical guidelines. Germany has already taken an important step in cumulative assessment in the European Union. On 1 March 2017, an announcement was published in the Federal Gazette stating that accumulation and synergy effects will be considered by the BfR in future in the health assessment of plant protection products. Germany is thus including systematic cumulative assessment in the zonal approval of plant protection products. A guideline for the cumulative assessment of plant protection products was compiled at the BfR based on the international discussions at the EFSA and the ECHA and was published in 2014. Since this time, the method has been applied, reviewed and refined at the BfR. In essence, its function is to cumulatively assess different active substances

in a plant protection product or in a proposed tank-mix in a multi-step procedure (see box). For this purpose, both the exposure of users and the acute exposure of consumers is examined so that the groups with the greatest exposure are taken into consideration.

### The aim: harmonised assessment of mixtures worldwide

The introduction of concepts for cumulative assessment of plant protection products also affects risk assessment processes in other EU countries. If Germany is the rapporteur member state in zonal approval procedures, the method will be used categorically. The cumulative assessment is also included as a German comment in assessment reports from other countries.

The concept has been subjected to a *peer review* in recent years through technical communications between the member states. To facilitate better european-wide harmonisation, international workshops are also currently being organised to discuss how mixtures are to be considered in the context of risk assessment moving forward. In November, the BfR is holding such a workshop on behalf of the European Commission, while the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) will hold another workshop with active involvement of the BfR in February 2018.

This represents a further step towards the aim of worldwide harmonised assessment of mixtures in order to better protect consumers and the environment. ■

## Combination effects: research projects of the BfR

A series of internal research projects on combination effects are being conducted in different departments at the BfR. These include research projects on, for example, cumulative effects in plant protection products and biocides, multiple residues in food, exposure estimates and alternative methods to animal experiments involving vertebrates. In addition, the BfR was or is involved in the following third party-funded projects performing research into combination effects.

### EuroMix

#### A tiered strategy for risk assessment of mixtures of multiple chemicals

EuroMix is a transnational project of the European Commission that is coordinated by the Dutch National Institute for Public Health and the Environment (RIVM). It has the aim of developing an experimental test strategy without the use of animal testing to better determine the toxicity of mixtures of different, toxicologically relevant food ingredients, food contaminants and plant protection product residues. A guideline for the future implementation of an experimental test strategy will be compiled based on the research results. This eight million euro project will run until May 2019.

More Information:  
[www.euromixproject.eu](http://www.euromixproject.eu)

### Combiomics

#### Analysis of combination effects of pesticides *in vitro*

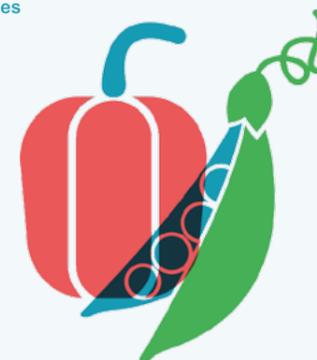
Combiomics was a two-and-a-half-year project of the German Federal Ministry of Education and Research (BMBF) which was concluded in the spring of 2016. In this project, coordinated by the BfR, possible combination effects caused by multiple residues were examined using the example of a group of fungicides *in vitro*. For this purpose, the molecular effects of the triazoles were studied using multi-level omics and predictive mathematical modelling. Based on the results, a prediction model for substance combinations was created, validated and extended to the pesticides of other substance groups or substance categories to make it possible to examine combination effects largely without animal experiments in the future.

More Information:  
[www.bfr.bund.de/en/home.html](http://www.bfr.bund.de/en/home.html) > Research > Third party projects of the BfR > Combiomics

### Combiomics 2

The second project phase of Combiomics is also being coordinated by the BfR. The aim is for the successfully tested methods and cell lines to be validated in the form of a standardised *in vitro* test battery and integrated into a standard procedure that will also comply with future regulatory requirements. The three-year project is being supported by Germany's Federal Ministry of Education and Research (BMBF) up to the end of 2019.

More Information:  
[www.fisaonline.de/en](http://www.fisaonline.de/en) > mixture effects of pesticides



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# “The goal is a step-wise improvement of the cumulative assessment”

Interview with Dr. Roland Solecki on the research activities of the BfR in the area of multiple residues



Dr. Roland Solecki heads the “Pesticides Safety” Department at the BfR

Mr. Solecki, the media often refer to a “cocktail of poisons” when it comes to multiple residues of plant protection products. Do you have an insight into how significant the health risk actually is?

The BfR together with the Federal Ministry of Food and Agriculture and the Federal Office of Consumer Protection and Food Safety has published a report on the cumulative effects of plant protection products. This is based on a method from the Dutch National Institute for Public Health and the Environment (RIVM). It has as its background a monitoring programme that was conducted in Germany over a period of six years. This monitoring programme examined the average dietary ingestion of plant protection product residues. The result was that a risk to health is unlikely, even assuming worst-case scenarios for mixtures of plant protec-

tion product residues. The European Food Safety Authority (EFSA) arrived at a similar result for all of Europe in its 2015 Monitoring Report.

You are also participating in the EuroMix project. What is the EuroMix project?

It is a large-scale research project of the European Commission on the cumulative assessment of mixtures in the context of Horizon 2020, the EU Framework Programme for Research and Innovation. The project has the aim of developing new assessment methods for mixture effects, particularly with respect to alternatives for animal experiments, innovative ways of estimating exposure and modelling of cumulative risks. It covers not only plant protection products, but also food additives and other chemicals.

How is the BfR involved in EuroMix?

Both the Pesticides Safety Department and the Food Safety Department within the BfR are contributing to the EuroMix project. While the department I head is involved in the process of regulatory assessment, the Food Safety Department is conducting experimental work in areas such as liver toxicity, which also involves the testing of combinations of pesticides with other food ingredients.

**“Cumulative risks come from not only plant protection products, but also from food additives and other chemicals.”**

What are you researching in the EuroMix project?

First, we performed research into which legal requirements exist and how different authorities throughout the world assess mixture effects. We also performed research into mixtures that cause certain adverse effects in the liver. In the process, particular key elements were examined in order to develop a concept for the assessment process. Omics analyses and other alternative test methods will now be supplemented by animal experiments so that we will be able to monitor effects within the entire organism. At the same time, the statistical tools as well as other electronic tools are being further developed. Additional studies on exposure will follow. The final results will be available when the project is concluded in 2019.

What other European activities is the BfR involved in?

The EFSA has already developed a number of methods for assessing combined exposure to multiple pesticides and contaminants in humans and multiple pesticides in bees. The EFSA is currently working on new methods and tools to harmonise the assessment of risks to people and the environment posed by exposure to multiple chemical substances in the food chain. I am a member of a working group of experts set up by the EFSA Scientific Committee to develop guidelines relating to combined exposure to multiple chemical substances. As part of this initiative, referred to as “MixTox”, the guidelines will be discussed in a public consultation in 2018.

In view of the involvement in all these different activities, what is your personal aim?

The aim is a step-wise improvement of the cumulative assessment of substances in health-based risk characterisation and its harmonised implementation within the legal process. This will still take some time. ■



What are the effects of aluminium on liver and intestine?

Aluminium is contained in food, cosmetics and consumer products and thus can be ingested. The initial findings of the German-French research project “SolNanoTox” – in which the BfR is one of the partners– show that orally consumed aluminium compounds temporarily accumulate in the intestine. Actual subjects of research are investigations on factors influencing the particle uptake and their related effects. Furthermore, the partners focused their research on the aluminium distribution within the body, in particular to the liver. The research group sees indications that a fraction of the accumulated particles are excreted with the stool. In this research project, BfR used biological samples to simulate the digestion of various types of aluminium from mouth to stomach to the intestine. During this process, physiological parameters like pH were controlled with the help of enzymes and salts. Throughout the simulated digestion, the aluminium compounds changed their reactivity and solubility. These findings are a first step towards a better understanding of the absorption, resorption and distribution of aluminium in the body. These findings allow the conclusion of data for the purpose of risk assessment. In high doses, aluminium compounds can have toxic effects on the nervous system and unborn life, and can influence fertility and bone development.



Insect stop in textiles: allergenic effect unlikely

The risk of an allergy following contact with clothing and products treated with the biocide permethrin is unlikely based on current knowledge. This is the outcome of a new BfR risk assessment. This finding is supported by the fact that, despite the widespread use of the active substance in medicines and consumer products, there are no indications that contact allergies caused by permethrin are on the increase. The BfR comes to the conclusion that the risk of carcinogenic systemic effects as a result of dermal and oral exposure to permethrin in textiles is negligibly low. Permethrin is a biocidal substance used as protection against insects in products like sleeping bags, picnic and yoga blankets as well as carpets and clothing. It is classified as “sensitising” under the chemicals legislation, and the possibility of low carcinogenic potential is the subject of discussion in various scientific bodies. The active agent can be released from textiles and absorbed via the skin. In the case of small children, who put all manner of products in their mouth, there is the added possibility of oral intake of the substance.

More information: BfR Opinion No. 006/2017 of 25 April 2017 (in German)

SPECTRUM

Identification of endocrine disruptors: from basics to practice

When it comes to active substances in pesticides and other chemicals, harmonised scientific criteria are necessary in order to identify endocrine-disrupting substances. In 2016, the BfR published a consensus paper as the scientific basis for the subsequent work of the EU authorities. On legislative level, EU-wide regulations are currently being drawn up to prepare scientific guidelines for the harmonised implementation of these regulations. The EU Commission has jointly commissioned EFSA and ECHA to handle this task. The BfR supports the authorities by providing scientific and technical expertise, through its participation in the relevant working group at EFSA, and by conducting research and surveys on the practicability of investigation methods. The corresponding guidelines are scheduled for publication in the spring of 2018. Following their implementation, they can then be used in approval practice on the basis of the adopted regulations.

More information: Solecki et al. 2017. Scientific principles for the identification of endocrine-disrupting chemicals: a consensus statement. Arch Toxicol 91: 1001–1006.



## Type of coating determines release

# Nanoparticles in functional clothing



**They make clothing water and dirt-repellent, they block UV radiation, and they reduce odours: nanomaterials have popular functional properties. It is, however, largely unknown whether they pose health risks on account of their miniscule size. In a research project on nanosilver, the BfR determined whether the tiny particles are released from textiles and can therefore pose a health risk.**

### Nanomaterial

is a natural material occurring in certain processes or an artificially produced material that contains particles in unbound state, in aggregate form or as an agglomerate, and in which the majority of particles have one or more outer dimensions in the range of below 100 nanometres. In nanosize, a substance can possess properties different from those that it would possess if the particles were larger.

In clothing the use of silver nanoparticles is particularly common. Silver has antibacterial properties and is supposed to reduce odour formation due to sweating. When nanosilver is used in items of clothing, the question of primary relevance is whether it is released into the sweat and therefore potentially comes into contact with the skin. Alongside the question as to whether and in what amount silver is released from textiles in general, the other issue researchers are looking into is the role played by the technology used to apply or incorporate the nanosilver on or into textiles. What was

also unclear to date is the extent to which the surface coating of the nanoparticles influences the release mechanism.

### Artificial sweat simulates release

In a research project, the BfR tested nine textile samples that were finished with nanosilver. The “control group” consisted of a piece of textile finished with conventional silver (in this case, silver salt with a particle diameter greater than 100 nanometres). Two of the textiles were so-called compos-

ites in which nanosilver is integrated in the fibre. In the other tested textiles, the fibre surface was coated with silver nanoparticles and in one case with conventional silver (textile coating). In order to simulate the release of silver due to sweating, the textiles were incubated in a simulated sweat solution for 24 hours. For evaluation purposes, the entire silver content in the sweat solutions was first determined by means of mass spectrometry. The samples in which silver was present were then differentiated by centrifuging and using a special method to measure individual particles of dissolved and particulate silver. In the final step, the particle sizes of the determined particulate silver were measured in order to identify nanoscale silver.

### Silver released from clothing

The tests performed by the BfR showed that silver migrated from each textile sample into the simulated sweat solution – with levels of between 7 and 75 percent of the silver originally contained in the textile depending on the tested textile. It was found that coated textiles released more silver than the composites. The BfR determined the highest share of released silver in the textile coated with conventional silver. The technology used for finishing therefore plays a greater role in the release of silver than the actual amount of silver contained in the textiles. Moreover, the release rate of silver from nanosilver appears to be not higher than from conventional silver. The released silver was mainly in dissolved form in the sweat solution. Only a small amount of silver was particulate silver, but this particulate silver was in the nanosize

range. Further investigations are necessary to establish whether these particles are individual nanoparticles or whether the silver is bound to larger particles. Based on current scientific knowledge, it is assumed that nanoparticles behave differently in the body than larger particles. Surprisingly, BfR researchers also detected particulate silver of nanosize in the simulated sweat of the textiles coated with conventional silver. In other words, the occurrence of silver particles does not appear to depend on whether the silver was applied to the textiles in nano form or in conventional form. In a parallel study, the BfR research group found that the consistency of the nanoparticles – in the form of a special coating, for example – also influences their solubility in the sweat solution.

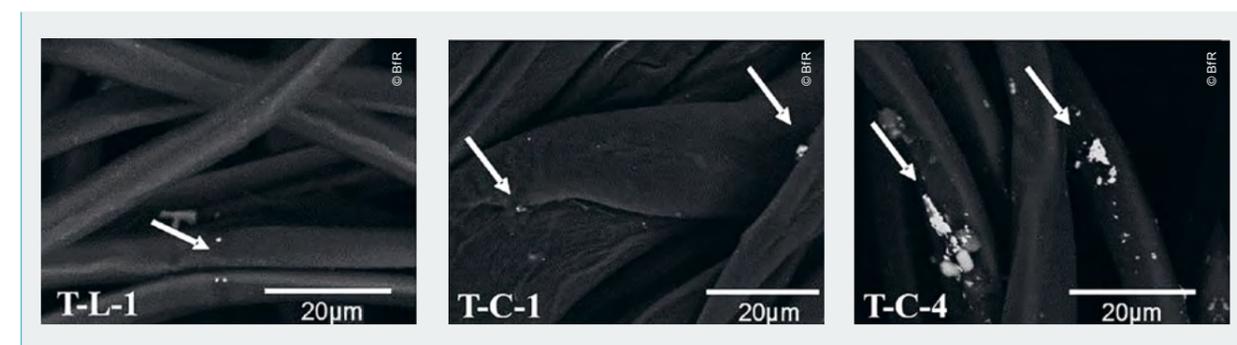
### Type of coating is relevant

It can be concluded that silver is released from textiles, and to a minor degree even in particulate form. The chosen form of textile functionalisation as well as the texture of the nanoparticles are key criteria for the release characteristics. Insights like these are particularly important with regard to the safe use of nanomaterials in everyday products, as human exposure to these kinds of substances should be minimised wherever possible. Moreover, they provide some indication as to the application form in which nanomaterials can be used safely in consumer products. ■

**More information:**  
Wagener et al. 2016. Textile Functionalization and Its Effects on the Release of Silver Nanoparticles into Artificial Sweat. *Environ Sci Technol* 50: 11, 5927–5934.



Silver can be detected on coated textile fibres using time-of-flight mass spectrometry.



Scanning electron microscope image of textile fibres

Left: nanocomposite in which hardly any silver particles can be found on the fibre surface.

Centre and right: textiles with silver coating; here, the textile surface is covered with silver more densely.



# Wanted: mirror image

## New approach to genome data comparisons of humans and animals

The German Centre for the Protection of Laboratory Animals (Bf3R) at the BfR indicates a new approach in order to identify the suitable animal model for basic research or specific clinical questions with the help of gene expression data. This means that it will be possible to avoid unnecessary animal experiments in future. Hamburg's scientific authorities presented a research award to the Centre in recognition of the insights gained during this process.

**Identifying suitable animal models**

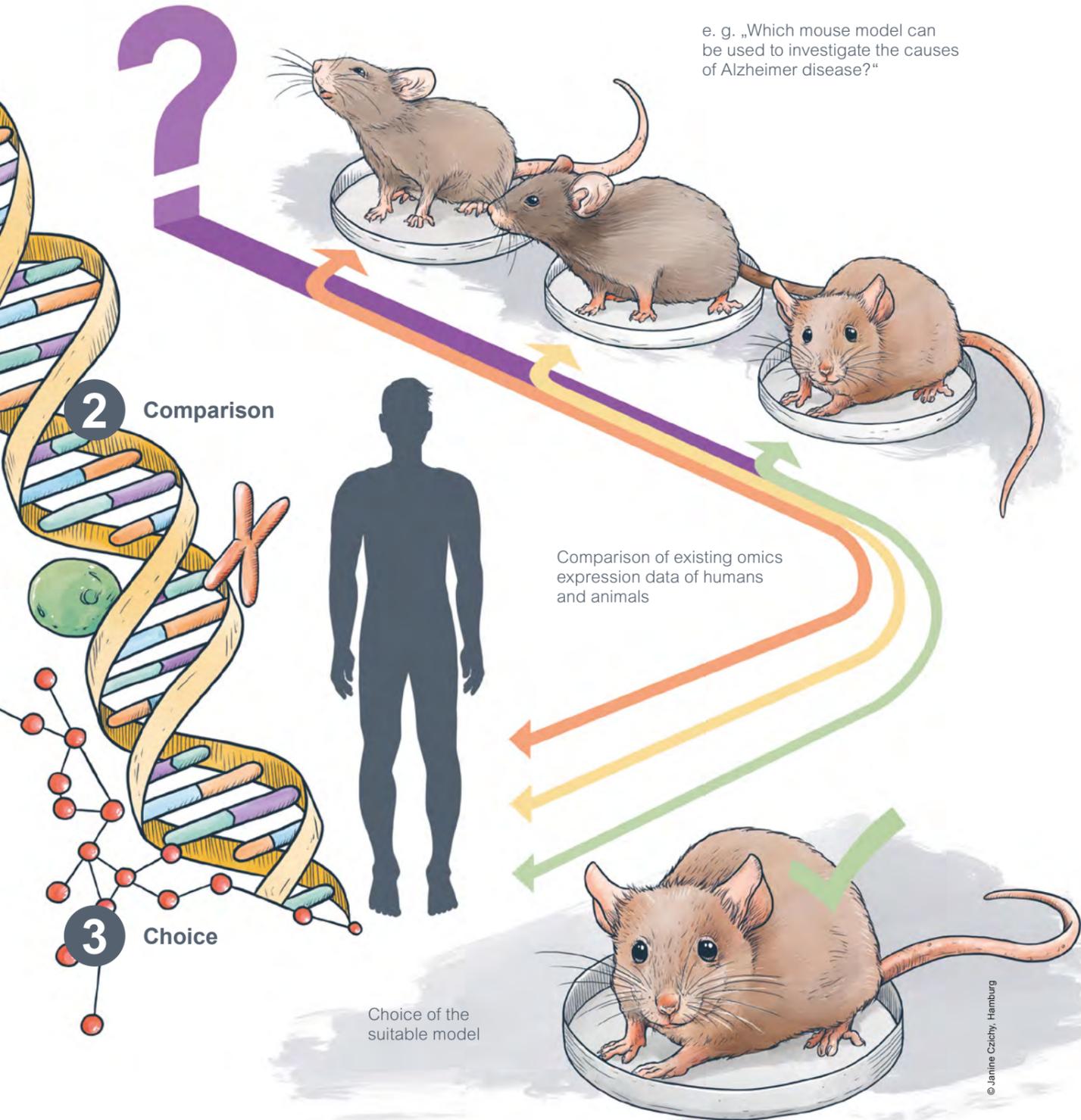
Animal models are used to investigate numerous biological processes and diseases such as wound healing, diabetes, tumours or inflammatory diseases. Comparison of the omics expression data in animal models and humans can help to identify suitable models for the issue in question.

e. g. „Which mouse model can be used to investigate the causes of Alzheimer disease?“

**1 Question**

**2 Comparison**

**3 Choice**



Is the course of an inflammatory process in mice similar to that in humans? Are mice therefore suitable for use in experiments to test anti-inflammatory substances? Scientific questions are often investigated in animal experiments in order to transfer research findings from basic research into daily medical practice – this is the field known as translational research. Whether and to what extent the results also apply to humans is regularly the subject of controversial debate. Suitable methods that scientists could use to select animal models that are fully transferrable to humans would underpin scientific progress and help to reduce the number of animal experiments. The key question in this respect is which animal model best reflects the “human system”.

**Comparison of genome data prone to error**

These considerations are based on data from modern, bioanalytical high-throughput techniques. The scientific community is increasingly using these so-called omics technologies that analyse the entirety of all genes and their products in both animals and humans. These technologies can comprehensively depict molecular processes in living systems – by showing the interaction of hundreds to thousands of genetic products, for example. However, as the corresponding procedure is not standardised, the analysis of this increasingly complex omics data sets presents major challenges for scientists. To date, the interpretation of the findings has mostly depended on the expertise of the scientist in question and the method used. As a result, the decision on the question of the transferability of findings to humans was prone to error, and this made the search for suitable animal models

more difficult. The low level of standardisation with regard to the interpretation of omics data has already led to contradictory results in research circles; this was the case with two studies conducted in 2013 and 2015 focusing on the comparability of inflammatory diseases in humans and standard mouse models. While one research group concluded in 2013 that inflammatory processes in humans and mice are not comparable, another group reached the opposite conclusion two years later based on the same omics data, claiming that mice react very similarly to humans on the molecular level and that they are extremely useful as animal models for investigating human diseases. Although it is not unusual for experts to differ in their assessment of the findings of studies, this is seldom the case when the underlying data is identical.

The motivation of the German Centre for the Protection of Laboratory Animals (Bf3R) at the BfR was to clarify these contradictions and develop a standardised and targeted analytical approach that simplifies the search for suitable animal models and therefore helps to avoid unnecessary animal experiments. One of the core tasks of the Bf3R is to coordinate all activities nationwide with the aim of limiting animal experiments to those that are absolutely essential and ensuring the best possible protection for laboratory animals. The tasks of the Bf3R are performed by the BfR.

**Targeted comparison of animal and human genome data**

The omics data from the two contradictory studies mentioned was systematically evaluated again using powerful computers by the scientists of the Bf3R. In this process, the genes to be compared were first as-

signed in groups to biological processes that are essential for an inflammatory process. This method, known as gene set enrichment analysis (GSEA), uses comprehensive genome data of common animal models and of patients stored in public databases. With their approach, the scientists of the BfR were then able to detect changes at the level of pathological signal pathways and identify differences between animal models and humans. Because the majority of the gene products evaluated were only changed slightly, it was possible to include all genes in the analysis at the level of complete biological signal pathways and evaluate changes in a biological signal pathway as a whole. This represents a significant difference as compared to previous analysis methods because, until now, genes from humans and animal models were often selected arbitrarily and subjectively for a direct comparison in order to address translational questions. The Bf3R's systematic evaluation showed that the results of this genome data comparison for certain mouse models were consistent with the data determined for humans. For other mouse models, on the other hand, this was not the case. For example, the inflammatory process in mice that are infected with *Staphylococcus aureus* (*Staphylococcus aureus* injection model) or are subjected to intestinal perforation (cecal ligation and puncture model) is similar to most clinical samples. In contrast, diseases caused by lipopolysaccharides (LPS) and *Streptococcus pneumoniae* show a different course in mice than in humans.

**Research approach helps to reduce animal experiments**

The GSEA approach to data analysis will make it easier for research groups to select the optimum animal model most closely

**Omics**

This term describes methods for the analysis of complex biological samples on the level of the whole genome, of transcripts, proteins or metabolites.

mirroring the human situation in a targeted and standardised manner in the future. A prerequisite for this is the existence of omics data relating to the clinical question and to corresponding animal experiments. This is already the case for a large number of animal models and human illnesses, including not only inflammatory diseases but also, for example, cardiovascular diseases, cancers, respiratory diseases, metabolic disorders and neurological diseases. Due to the increasing use of omics methods, it can be assumed that additional systems biology data will be steadily acquired and published. For this reason, the method applied by Bf3R will continue to be useful in the targeted selection of animal models. This method can be used in basic research as well as translational and applied research. In 2015, according to the German Federal Ministry of Food and Agriculture, the majority of animal experiments (a total of 73 %) took place in these research fields. Many of these experiments were performed on mice, rats, and, increasingly, fish. In principle, the new method can be used to select the suitable laboratory animal model and/or exclude unsuitable animal models for all animal experiments in these fields. Similar to the numbers for animal experiments, most omics data is currently available for mice and rats. For example, data is listed for approximately 340,000 mouse samples and 75,000 rat samples in the public NCBI / GEO (National Center for Biotechnology Information/ Gene Expression Omnibus) database alone.

### Method identifies suitable cell culture models

The Bf3R's method is not restricted to human and murine data sets, but can be applied to all other species for which extensive databases on biological signal pathways or gene groups exist. The new research method can also be used for the evaluation of cell-based alternative methods. A systems biology comparison of the clinical data with omics data from, for example, modern 3D cell cultures or organ-like microstructures enables the characterisation and verification of cell-based alternative methods.

### Award-winning research work

In 2016, the scientists of the BfR received the Hamburg Research Award for promoting the development of alternative and complementary methods. The research

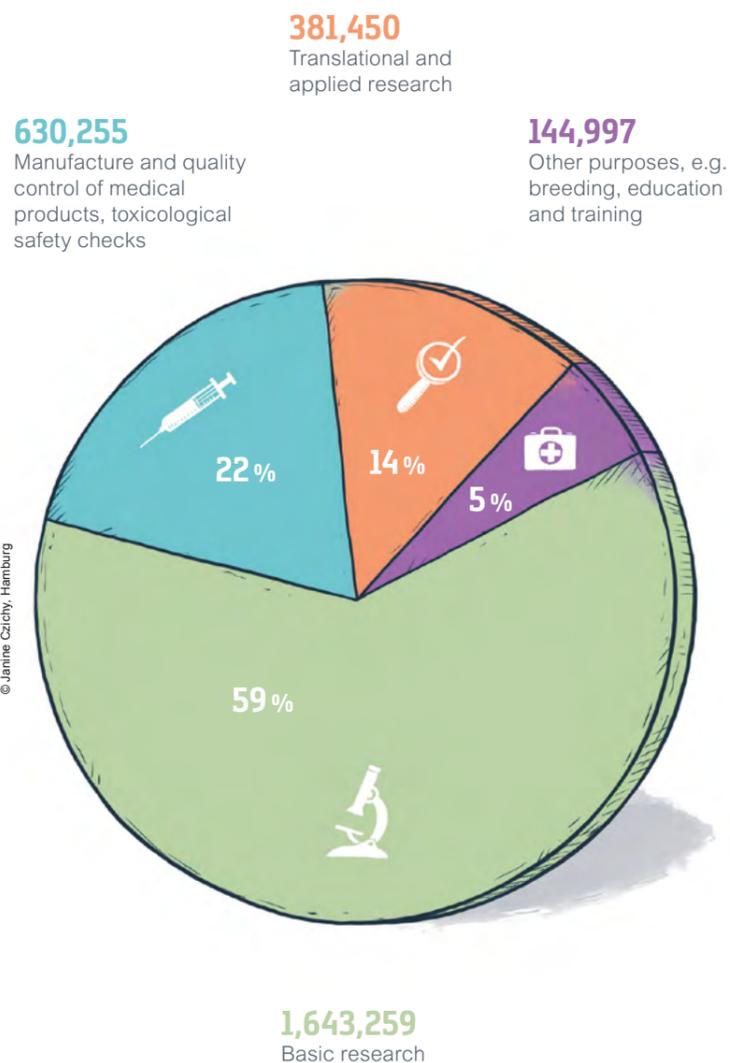
prize with a value of €20,000, was awarded for the first time by the Hamburg Agency for Health and Consumer Protection and the Hamburg Agency for Science, Research and Gender Equality. The prize is awarded for work that contributes towards replacing or minimising animal experiments. ■

**More information:**

Weidner et al. 2016. Defining the optimal animal model for translational research using gene set enrichment analysis. *EMBO Molecular Medicine* 8: 8, 831–838.

Weidner et al. 2017. A protocol for using gene set enrichment analysis to identify the appropriate animal model for translational research. *J Vis Exp.* Aug 16;(126). DOI: 10.3791/55768.

### Purposes for which laboratory animals are used in Germany



© Janine Czichy, Hamburg

Source: German Federal Ministry of Food and Agriculture, laboratory animal statistics 2015

## SPECTRUM

### Testing the inhalation toxicity of nanomaterials

Inhalation is considered the most significant path of entry for nanomaterials. In the context of BB3R research, the BfR is reproducing this type of exposure using *in vitro* aspiration epithelium models at their interface to the air. This approach to testing inhalation toxicity helps to reduce the number and scope of animal experiments.



Test setup: three tubes conduct a test aerosol with airborne nanomaterials to epithelial cells.

### Alternatives to animal experiments: pooling regional research

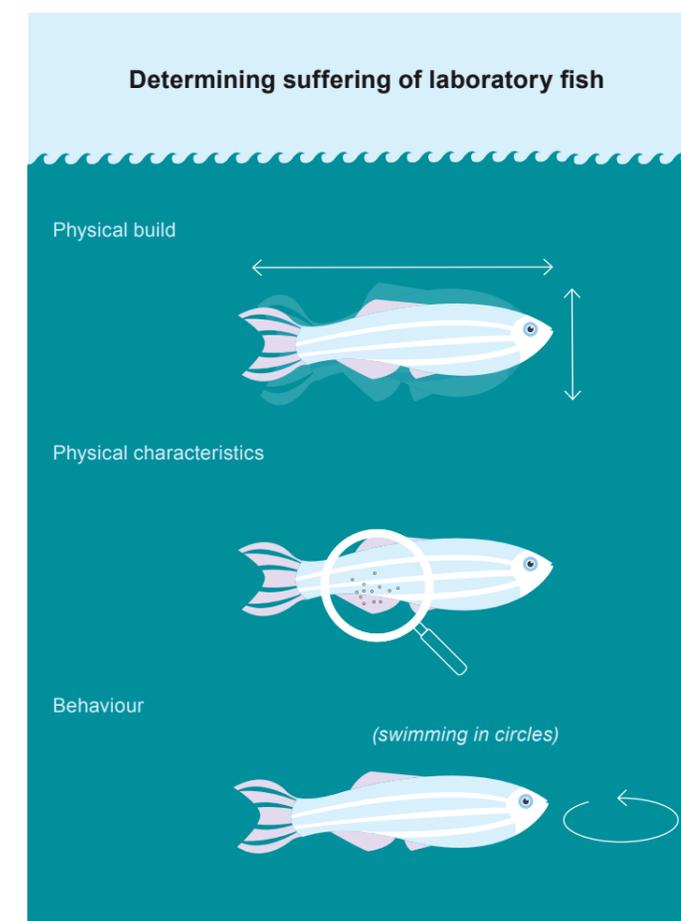
Individualised pain therapy for laboratory mice and a reduction in animal experiments for *testing the inhalation toxicity of nanomaterials* (see column on the right) – the BfR's initial work results in the Berlin-Brandenburg cooperative project BB3R will contribute to these topics in the future. The network of universities and federal institutes performs research on avoiding animal experiments (replacement) or reducing their scope (reduction) and on decreasing the suffering of laboratory animals (refinement). As one of six project partners, the BfR is involved in the refinement and replacement research fields. The German Federal Ministry of Education and Research is supporting the project, abbreviated to BB3R, for four years until the spring of 2018; it is designed as a platform for scientific communication and includes a graduate education programme.

More information:  
[www.bfr.bund.de/en](http://www.bfr.bund.de/en) > Research  
[www.bb3r.de/en](http://www.bb3r.de/en)

### Determining suffering of laboratory fish

The question of whether laboratory fish feel pain or suffer can be answered based on specific criteria. Together with external experts and representatives of approval authorities, the "National Committee for the Protection of Animals Used for Scientific Purposes" at the BfR coordinated the preliminary work on this topic. The criteria, defined for the first time, evaluate anomalies in the physical build and behaviour of the animals, for example. In this way, pain and suffering can be treated and alleviated in a targeted manner. Because the criteria clearly address the question of the animals' distress, they also aid decision making for authorities responsible for approving the breeding of genetically modified animals that could experience pain or suffering. After mice and rats, fish (particularly zebrafish) represent the third most frequently used laboratory animal type. The number of laboratory fish has increased steadily over the past few years.

More information:  
 Bert et al. 2016. Considerations for a European animal welfare standard to evaluate adverse phenotypes in teleost fish. *EMBO J.* 35: 11, 1151–1154.  
 Recommendations of the National Committee TierSchG Nr. 001/2015



## EVENTS



### Anniversary celebration and international symposium

On 29 November 2017, the BfR will celebrate its 15th anniversary with an official ceremony. The event is for invited guests only and will take place at the Akademie der Künste in Berlin. From November 30th to December 1st 2017 a Joint International Symposium hosted by the BfR and its sister institutes in France (ANSES), Denmark (DTU) and South Korea (NIFDS) will take place. According to the motto “Past, Present and Future Challenges in Risk Assessment – Strengthening Consumer Health Protection”, attendees will look back on the last one and a half decades of risk assessment. In addition, current activities and future issues in consumer health protection on national and international levels such as microbiological agents, chemical substances, methodologies and harmonisation will be discussed.

## PERSONNEL

### Supporting young scientists

To support the new generation of scientists, the BfR sets up junior research groups on specific topics. In the autumn of 2017, another doctoral student will join the “Authenticity Along the Product Chain” junior research group, which was established in 2015.

### Support in the protection of laboratory animals

The appointment of university professor Dr. Lars Lewejohann on 1 April 2017 reinforces the competence area “Reduction of severity and improvement of living conditions” at the German Centre for the Protection of Laboratory Animals (Bf3R). In addition to heading the “Animal Protection and Laboratory Animal Science” unit at the BfR, Lars Lewejohann will pass on his expertise and newly acquired knowledge to students and post-graduates in his capacity as professor for “Animal Welfare and Refinement” at the Faculty of Veterinary Medicine at the Freie Universität Berlin.



### Alternative method to animal experiments: “Bone-on-a-chip”

A summer school on alternative methods to animal experiments for risk assessment took place in Ispra (Italy) at the Joint Research Centre (JRC) of the Directorate General of the European Commission in May 2017. There, Dr. Ing. Frank Schulze from the BfR unit “ZEBET – Alternative Methods to Animal Experiments” was awarded a poster prize (first place) for his presentation and the accompanying poster on the topic “A bone-on-a-chip for basic science and the identification of bone-harming substances”.

## INTERNATIONAL NEWS

### Expansion of international capacities

Cooperation with the partner institutions takes place through reciprocal visits, joint symposiums, the exchange of information, and the training of scientists at the BfR. Furthermore, thanks to Germany’s endeavours, the EFSA Advisory Forum has established the Discussion Group on Capacity Building, which has already met in Berlin twice this year. The aim is to expand joint international capacity building activities of EFSA and food safety institutions in Europe through policy advice, scientific cooperation, exchange of information, joint scientific meetings and training activities.



### Visitor groups to the BfR

The BfR currently has cooperation agreements with 47 partners in 28 countries. This year, for example a cooperation agreement was signed with Portugal and Russia. Moreover, the BfR

receives many visits from delegates from around the world who are interested in collaborative activities. In 2017, the BfR received visits from, among others, the Nepalese Ambassador, a delegation from the Ministry of Agriculture in Argentina and a representative of Nanyang University in Singapore.

### Vice-President in Japan

In April, the Vice-President of the BfR, Professor Dr. Reiner Wittkowski, travelled to Japan within the context of a joint initiative of EFSA, BfR and ANSES on the topic of the future of international scientific cooperation in the field of food safety. During his visit, he also held a presentation on “Global Aspects of Risk Assessment in Food Safety”.

### EU-FORA

The scholarship initiative EU-FORA (The European Food Risk Assessment Fellowship Programme) operated by EFSA helps to develop the scientific assessment resources and the knowledge community of the EU. The BfR supports this initiative through its involvement in the programme committee. The programme was launched in September 2017, and the BfR hosts four EU-FORA fellows.



### Expansion of cooperation with Africa

At the beginning of May 2017, the acting General Director of the International Livestock Research Institute (ILRI) based in Nairobi, Kenya, Dr. Dr. h. c. Dieter Schillinger (Back row: second from left), visited the BfR. The two institutions entered into a cooperation agreement in November of last year. For over 20 years, the ILRI has been studying illnesses in livestock in developing countries and working on diagnostic methods and vaccines in order to increase the productivity of livestock and retain their genetic diversity. The ILRI’s work also places a focus on improving food safety and on issues relating to antimicrobial resistance.

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