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

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.

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

© Tatomm/istock.com

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

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.

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

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 > 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 > mixture effects of pesticides

© TATYANA Yamshanova/shutterstock.com

"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 protection 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. ◘