Transformation products – relevant risk factors?

Chemical substances can be transformed in the environment into products that are more stable, more readily water-soluble and even, in certain cases, more toxic than the parent compound. Eawag is developing methods that will allow the risk of these transformation products to be better assessed.

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The degradation of a chemical substance in the environment does not always lead to rapid and complete mineralization. Instead, in the course of the degradation process, relatively stable transformation products may be formed. This is the case in particular for many active substances – such as pesticides, pharmaceuticals and biocides – which have relatively complex molecular structures that are only gradually degraded. This is confirmed by extensive measurements of stable pesticide transformation products in surface and ground waters in the US [1], but also by findings in Switzerland [2].

Detecting unknown transformation products

For most conceivable transformation products, no chemical standards are available. Normally, however, such reference standards are required to allow a compound in an environmental sample to be unequivocally identified. We therefore developed a detection method that provides sufficient information to enable a measured signal to be confidently assigned to a possible structure. In high-resolution mass spectrometry, compounds are detected on the basis of their exact mass, which is determined with such a high degree of accuracy that only a small number of molecular formulae having precisely this mass are even conceivable. However, further evidence is needed to confirm that a measured signal actually corresponds to the postulated transformation product. In our method, we therefore introduced two additional parameters alongside high-resolution mass spectrometry: the liquid chromatography retention time, which is a measure of the polarity of the compound, and fragment spectra from tandem mass spectrometry, which provide further information on the molecular structure.

The structure of transformation products is often very similar to that of the parent compound. However, they are usually somewhat more polar and enter natural waters more easily on account of their greater solubility. Generally speaking, toxicity is only rarely increased as a result of these structural changes, and similar or lower toxicity than the parent compound would normally be expected. This means that the stable transformation products of polar compounds such as pesticides, pharmaceuticals and biocides not only contribute to chemical water pollution but also, in combination with the parent compound, may lead to increased overall toxicity in the aquatic environment.

These points would need to be taken into account both in the assessment of chemical water quality – e.g. in the context of the EU Water Framework Directive – and in prospective chemical risk assessment. However, no clear requirements are specified in the relevant EU regulations, except in the case of the authorization of pesticides. Accordingly, we have only a fragmentary knowledge of what transformation products we should expect to find in our water resources, and to what extent they contribute to overall chemical pollution.

Within the KoMet project (combined modelling and measurement approach for the identification of relevant transformation products in water resources), we have therefore developed a toolbox of methods to address this problem. KoMet is part of the MicroPoll strategy initiated by the Federal Office for the Environment (FOEN).

Can the formation of possible transformation products be predicted? Transformation products are formed in the environment in many different ways. In addition to chemical processes such as hydrolysis, redox reactions or photolysis, microbial, enzyme-catalysed degradation plays an important role. Compounds of aquatic relevance in particular, which usually enter the environment via the soil or a wastewater treatment plant, are frequently degraded by bacteria and/or fungi in these compartments.

In a joint project involving the University of Minnesota and the Technical University of Munich, we are therefore developing a computerized expert system [3] that can predict typical products of microbial degradation. The system relies on biotransformation

rules, defined on the basis of experimentally studied biodegradation pathways. These rules recognize existing structural units in the compound and simulate the formation of transformation products. However, the multiplicity of applicable rules rapidly generates numerous predicted products – including some that have never been observed for a given chemical. In order to restrict the number of predicted transformation products, we defined a series of higher-level rules that determine priorities among the various applicable rules.

For example, without rule priorities, the expert system predicts 12 products for atenolol, a beta blocker used to slow the heart rate (Fig. 1). However, when the higher-level rules are applied, only 6 possible products remain. One product in particular is found experimentally – atenololic acid, which is formed by enzyme-catalysed hydrolysis. For 47 other pharmaceuticals and pesticides, it was likewise possible to reduce the number of predicted products by an average of 16 % without known products being lost in the process [4].

Tracking down transformation products. The predictions of the expert system can now be used to search specifically for possible transformation products, either in a biodegradation experiment in the laboratory or in an environmental sample. As a general rule, the methods of trace analysis can only be applied when one knows what one is looking for. Also required are reference standards – pure forms of the target compounds which are used to calibrate the analytical procedure, so that a target compound can actually be identified in an environmental sample. Typically, however, no reference standards are available for predicted transformation products. To get round this problem, we developed an analytical method based on high-resolution mass spectrometry – a relatively new technique – which does not require the use of reference standards (see Box on p. 15). With this method, for three pharmaceutical substances studied, we have already identified four previously unknown transformation products, which – as predicted by the expert system – were formed in biodegradation experiments carried out in reactors containing sewage sludge (Fig. 2).

Non-negligible amounts in surface waters. Even more crucial, however, than the question of what transformation products are formed in laboratory experiments, is what products actually occur in water resources – and in what quantities. To investigate this, we also applied our combined method. For a total of 52 pesticides, biocides and pharmaceuticals used in significant quantities in Switzerland, and representing different chemical classes, a list of approximately 1800 possible transformation products was generated using the expert system. Of these candidates, 19 were actually detected in 6 representative samples from medium-sized Swiss watercourses. As regards the pesticides, these included not only several well-known and frequently observed transformation products (desethylatrazine or metolachlor ethanesulfonic acid) but also various pesticide transformation products that had previously only been detected in laboratory studies and rarely or never in environmental samples (products of the fungicide azoxystrobin and of the herbicides chloridazon, metamitron and metribuzin). In the case of pharmaceuticals, the transformation products found were generally known human metabolites. It is, however, possible that some of these also partly arise as a result of biodegradation in wastewater treatment plants, since they are also predicted as microbial products. Another 10 transformation products – though not directly identified by our method since they were present in relatively low concentrations – could nonetheless be detected because reference standards were available for these products.

Overall, our study shows that, in Swiss surface waters, transformation products do not occur with unexpected frequency or in very high concentrations. However, for around half of the active

Fig. 1: Transformation products predicted by the expert system for the beta blocker atenolol. The main environmental transformation product, atenololic acid, is highlighted in orange.
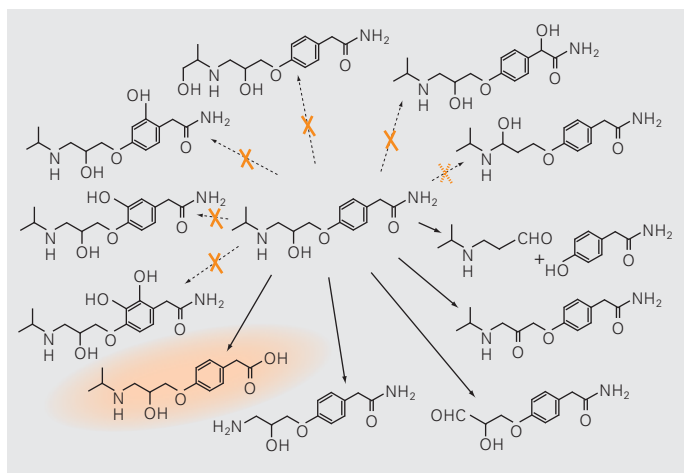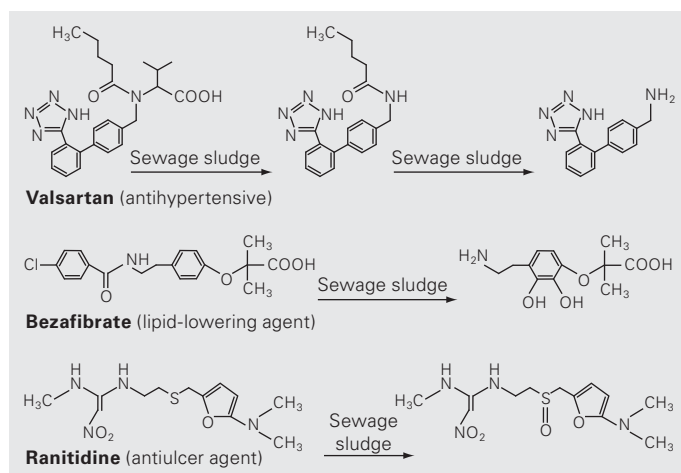

Fig. 2: Four newly discovered transformation products of pharmaceuticals, which were initially predicted by the expert system and then actually detected in degradation experiments.

Prediction of transformation products using a computerized expert system.

substances studied, one or two transformation products were found, which must therefore be relatively stable and mobile. Transformation products are thus not negligible as additional contaminants in water resources, but they most probably do not represent a problem of unsuspected proportions.

A relevant contribution to overall toxicity? It is often argued that transformation products are only really relevant if their toxicity is equal to or greater than that of the parent compound. This is only the case for about 30 % of the transformation products from 37 pesticides for which data on the relative toxicity of transformation products and parent pesticides was compiled [5]. However, as the transformation products generally occur in a mixture with the parent compound and at least in some cases have the same mode of action, their contribution to the overall effect cannot be neglected.

In the part of our project dealing with toxic effects, we are therefore investigating how knowledge about the effects of the parent compound can be used to estimate the toxicity of transformation products. Many parent compounds exert what is known as specific toxicity on particular organisms (e.g. interaction with

enzymes or reactions with DNA or proteins). This means that their toxicity is higher than the minimum (baseline) toxicity which every chemical substance exerts by accumulating in the cell membrane of an organism and disrupting its function. The baseline toxicity of a compound is associated with its lipophilicity (degree of solubility in fats) and can be readily estimated on the basis of its structure. The toxicity of transformation products can be expected to lie between baseline toxicity and the specific toxicity of the parent compound. On the basis of the lipophilicity of the transformation products, it is thus possible to delimit a range of possible effective concentrations, expressed as the EC_{50} (concentration at which a response is observed in 50 % of the study population).

The procedure is illustrated here by the example of the herbicide diuron and two of its main transformation products – DCPMU and DCPU (Fig. 3A). As a herbicide, diuron is specifically effective against algae, and the risk assessment was therefore carried out for this organism. From the spring to the autumn of 2008, we determined the concentrations of diuron and its transformation products during major rain events in a tributary of Lake Murten (La Petite Glâne). It was shown that, during one of the later events in September, the transformation products evidently contributed
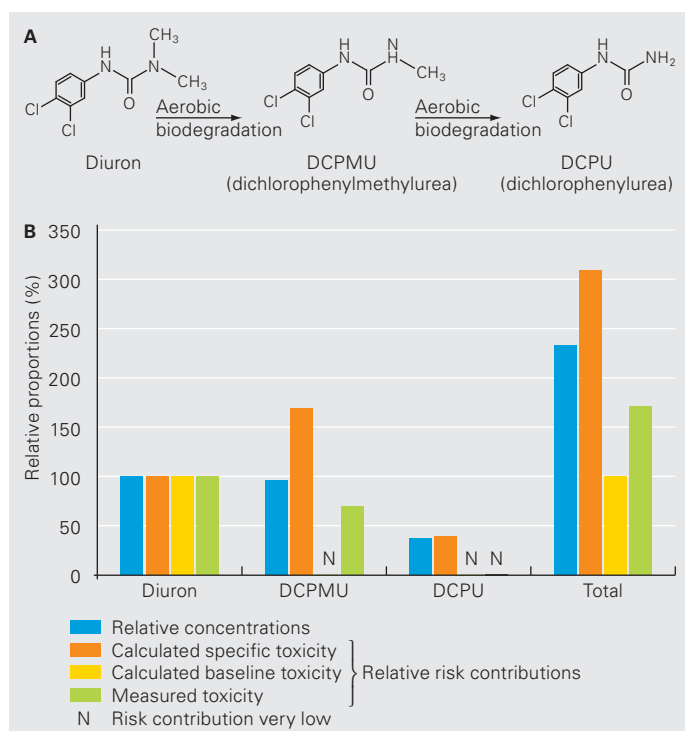
Fig. 3: (A) Formation of the transformation products DCPMU and DCPU by microbial degradation of the pesticide diuron. (B) Measured concentrations and predicted and observed risk contributions of diuron, DCPMU and DCPU during a late-summer rain event. The values for DCPMU and DCPU are scaled to those for diuron (= 100 %).

the example of diuron – may well contribute to an increase in chemical-related effects in water resources. Measures to reduce input of transformation products are difficult to adopt, as these products are of course formed only when the parent compound is degraded (as desired). In addition, they often exhibit higher mobility than the parent compound and are thus distributed relatively efficiently in surface and ground waters. It would therefore appear to be all the more important to take transformation products into account in the assessment of chemicals. While this is already the case for pesticides, such requirements and specific procedures have yet to be included in the assessment of industrial chemicals and pharmaceuticals. A key prerequisite for this is that models for the prediction of transformation products and for estimation of their environmental concentrations and effects should be further developed for implementation in practice. ○ ○ ○

more than 50 % to the total concentration (blue bars in Fig. 3B). Their contribution to the risk can now be estimated via the quotient of the measured concentration and the predicted EC_{50}. We thus calculated that, if the two transformation products are taken into account in the risk assessment, a 210 % higher risk is to be expected (orange bars), on the assumption that they also act as specific inhibitors of photosynthesis. However, if the transformation products lose their specific activity and only exert baseline toxicity, the risk would not be significantly increased (yellow bars). To test the plausibility of these maximum and baseline estimates, we also charted the risk contribution using EC_{50} values determined experimentally by our group, which revealed a 70 % increase in the risk contribution (green bars). This example thus demonstrates that transformation products potentially relevant to the environmental risk can be reliably identified with the aid of our method, and that, in individual cases, transformation products may indeed substantially increase the overall risk.

Taking transformation products into account in chemicals assessment. Overall, our studies indicated that transformation products of pesticides, biocides and pharmaceuticals do not occur with unexpected frequency or in very high concentrations in Swiss surface waters. Even so, for around half of the compounds studied, one or two products were found, which – as shown by

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[2] Kilchmann S., Reinhardt M., Schürch M., Traber D. (2009): Ergebnisse der Grundwasserbeobachtung Schweiz (NAQUA). Zustand und Entwicklung 2004–2006. Umwelt-Zustand Nr. 0903. Bundesamt für Umwelt (Bafu), Bern, 144 S.

[3] University of Minnesota Pathway Prediction System, http://umbbd.msi.umn.edu/predict/

[4] Fenner K., Gao J.F., Kramer S., Ellis L., Wackett L. (2008): Data-driven extraction of relative reasoning rules to limit combinatorial explosion in biodegradation pathway prediction. Bioinformatics 24, 2079–2085.

[5] Sinclair C.J., Boxall A.B.A. (2003): Assessing the ecotoxicity of pesticide transformation products. Environmental Science & Technology 37, 4617–4625.