

# Characterization of Reactive Chemicals Based on their Primary Mode of Action

Dead fish washed on the shore “belly up”: who has not seen these pictures? They illustrate dramatically the fatal effects on aquatic life that accidents with environmental pollutants can have. However, our environment is also continuously influenced by chemical pollutants in low concentrations which unfortunately remain unperceived in most cases. It is important therefore to find out how exactly pollutants react in organisms. Thus, the goal of our research is to identify and classify the various primary modes of action of reactive chemicals by means of a bacterial test system.

The ecotoxicological risk of reactive chemicals may be only insufficiently assessed by classical test methods. The reasons for this are that reactive chemicals hydrolyze rapidly and that traditional testing methods often assess only a small part of the broad spectrum of effects that reactive chemicals may cause. Recognizing the mode of action is crucial, however, particularly for reactive chemicals, since the reaction mechanism is one of the major factors determining the risk potential. For this reason, we are currently developing a comprehensive bacterial test system that will cover a wide spectrum of reactive chemicals and their particular modes of action.

## Pollutants Damage Biomolecules

Ultimately, all toxic effects can be traced back to primary interactions of the pollutants with three groups of biomolecules: membrane lipids, proteins and genetic ma-

terial (DNA) [1]. Interactions span the entire range from weak van der Waals forces to specific interactions, such as the formation of hydrogen bonds or mutual attraction between charges, all the way to the formation of chemical bonds (Fig. 1). Weak interactions typically cause nonspecific, reversible effects and are only relevant for hydrophobic pollutants. Specific interactions are observed, for example, in enzyme inhibition, where a pollutant competes in the role of “key fitting” the lock, thus keeping out the actual substrate. Our special interest, however, is focused on reactive chemicals that form covalent – usually irreversible – bonds with a specific target region of the affected biomolecule. Among these reactive chemicals are a large number of compounds with different functional groups such as the reactive oxygen species (see article by B. Fischer, p. 15) and the so-called electrophilic chemicals, which is the focus of this article.

## The Cell Arms Itself against Electrophilic Compounds

Electrophilic chemicals are molecules that are electron-poor, due to their electron configuration, and therefore prefer to react with nucleophilic (= electron rich) groups in peptides, proteins or DNA. Preferred targets are the thio groups in proteins and peptides as well as certain oxygen and nitrogen groups in DNA (Fig. 2). In the extreme case, proteins can be damaged by electrophilic compounds to such an extent that they can no longer perform their functions, while reactions between electrophilic compounds and DNA usually cause instability and mutations of the DNA, leading to cancer at the worst. Reactions at both targets may lead to death.

But the cells defend themselves against such attacks. Glutathione, an intracellular tripeptide (Fig. 2), intercepts electrophilic compounds which will subsequently be transported out of the cell. There are also a

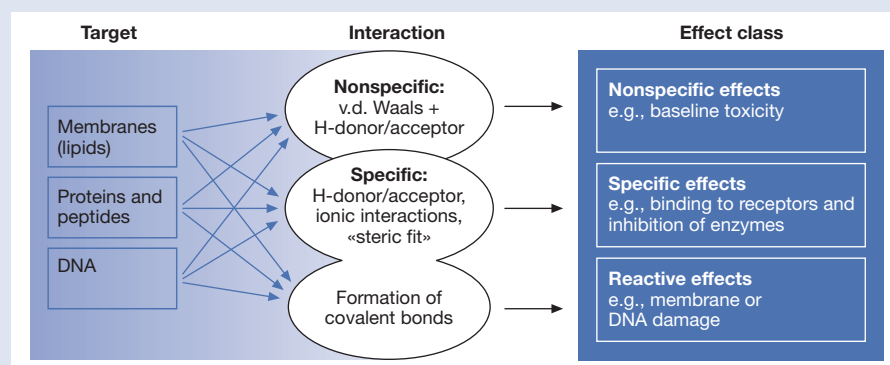


Fig. 1: Classification of toxic effects according to the mode of interaction with biomolecules at the target site.

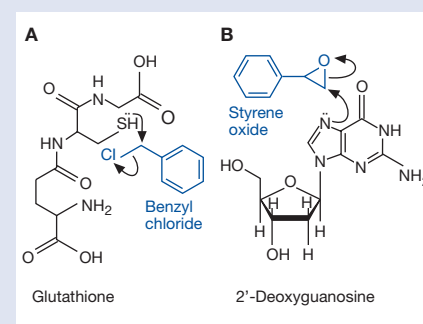


Fig. 2: Two examples of primary modes of action of reactive pollutants. Chemical reactions with proteins (A) or DNA (B) cause toxic effects.

number of repair mechanisms for damage to the DNA, such as proteins that are able to recognize and repair errors in the DNA sequence. However, when pollutants are present in high concentrations and/or over long periods of time, defense mechanisms are overwhelmed and toxic effects begin to manifest themselves.

### Evaluation of Different Mutants of *E. coli* and Effect Parameters



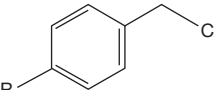
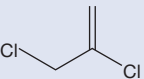
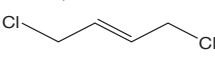
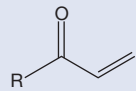
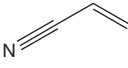
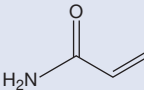
Methods showing the activity of the defense systems are particularly suitable test systems to identify the primary mode of action of electrophilic compounds. One has to take into consideration, however, that the toxicity of electrophilic compounds is not only determined by their chemical reactivity, but also by their concentration at the intracellular target site. The concentration at the target site, in turn, depends on how many electrophilic molecules enter the organism, how the molecules are distributed within the organism, and whether the organism is able to transform the molecule into a nontoxic form. These processes determine the bioavailability of the compound in question. In single-celled organisms, however, one can assume in case of hydrophilic substances that the pollutant concentration at the target site is the same as the extracellular concentration. We therefore have chosen to work with the bacterium *Escherichia coli* as our test organism. An additional advantage of this organism is that numerous mutants of *E. coli* are available.

We evaluated a wide range of *E. coli* strains and a total of 17 electrophilic compounds exhibiting different modes of action (Tab. 1). We measured the following effect parameters: growth inhibition, intracellular glutathione concentrations as well as the occurrence of DNA strand breakage and the activation of various DNA repair mechanisms [2].

### Using Two Pairs of *E. coli* Strains as Biosensors

Two pairs of *E. coli* strains have proven to be especially successful for our test purposes. The strains MJF276 (glutathione<sup>+</sup>) and MJF335 (glutathione<sup>-</sup>) are genetically identical except for their ability to synthesize glutathione. The second pair is also genetically identical except for the ability to repair DNA damage: in MV4108 (DNA<sup>-</sup>), several genes encoding for DNA repair systems are mutated, while these genes are intact in MV1161 (DNA<sup>+</sup>).

Different concentrations of electrophilic compounds were added to liquid cultures of these strain pairs and growth inhibition

| Structure  | Damaged biomolecules |
|--|----------------------|
| <b>Epoxides</b>  |                      |
|                                   |                      |
| Styrene oxide                      R = phenyl  | DNA                  |
| 2,3-Epoxypropyl benzene        R = benzyl  | DNA                  |
| 2-(4-Nitrophenyl)-oxirane       R = p-nitrophenyl  | DNA and proteins     |
| 1,2-Epoxybutane                  R = C <sub>2</sub> H <sub>5</sub>   | DNA                  |
| Epichlorohydrin                  R = CH <sub>2</sub> Cl  | DNA and proteins     |
| 2-Methyl-2-vinylloxirane<br>     | DNA and proteins     |
| <b>Reactive organochlorides</b>  |                      |
|                                 |                      |
| Benzyl chloride                    R = H   | DNA and proteins     |
| 3-Methylbenzyl chloride        R = m-CH <sub>3</sub>   | DNA and proteins     |
| 4-Nitrobenzyl chloride         R = p-NO <sub>2</sub>   | DNA and proteins     |
| 2,3-Dichloro-1-propene<br>      | DNA and proteins     |
| trans-1,4-Dichloro-2-butene<br> | DNA and proteins     |
| <b>Compounds with activated double bonds</b>   |                      |
|                                 |                      |
| Acrolein                              R = H  | Proteins             |
| Ethyl acrylate                      R = O-C <sub>2</sub> H <sub>5</sub>  | Proteins             |
| 2-Hydroxyethyl acrylate        R = O-C <sub>2</sub> H <sub>4</sub> -OH   | Proteins             |
| Isobutyl acrylate                  R = HO-sec-C <sub>4</sub> H <sub>9</sub>  | Proteins             |
| Acrylonitrile<br>               | Proteins             |
| Acrylamide<br>                  | Proteins             |

Tab. 1: The 17 pollutants examined in this study and their primary mode of action.

chemicals that primarily target the DNA clearly cause growth differences in the DNA<sup>+</sup>/DNA<sup>-</sup> strain pair, significantly inhibiting the growth of the DNA<sup>-</sup> strain (Fig. 3B). These compounds, which include three of the examined epoxides (Tab. 1), do not induce any growth differences between the glutathione<sup>+</sup> and the glutathione<sup>-</sup> strain. In addition to these two groups of chemicals, we identified a third group of compounds, characterized by non-specific reactivity since they attack at the protein as well as at the DNA level (Tab. 1). This group of compounds causes growth differences in both strain pairs (Fig. 3C).

these compounds obtained in the *E. coli* studies are compared to the EC<sub>50</sub> values obtained from experiments on aquatic organisms. There is a linear correlation between the different sets of EC<sub>50</sub> values (Fig. 4). The EC<sub>50</sub> value is the concentration of a pollutant where a 50% effect occurs – in our case growth inhibition for *E. coli* and algae as well as lethality for daphnids and fish.

The study described here is a first step in assessing reactive chemicals based on their primary mode of action. The goal of our further work is to find ways to incorporate the strain pairs used in this study into ecotoxicological test batteries and to expand the concept to other toxicity mechanisms. Differentiated ecotoxicological risk assessments will only become feasible, however, when we succeed in construction of the complete chain of “cause and effect” from the primary interaction at the molecular level to the observable effects on the population or ecosystem level.

was measured. Reactive chemicals that primarily attack at the protein level caused differences in growth between the glutathione<sup>+</sup> and the glutathione<sup>-</sup> strain, while the growth of the DNA<sup>+</sup> and the DNA<sup>-</sup> strains was not affected (Fig. 3A). Six of the examined compounds, characterized by activated double bonds, fall into this group of chemicals (Tab. 1). In contrast, reactive

### Validation and Further Development of the Test System

The results from this study are not only useful in classifying modes of action for environmental pollutants, but they can also be used to describe the effects these pollutants have on aquatic organisms. This becomes evident when EC<sub>50</sub> values for

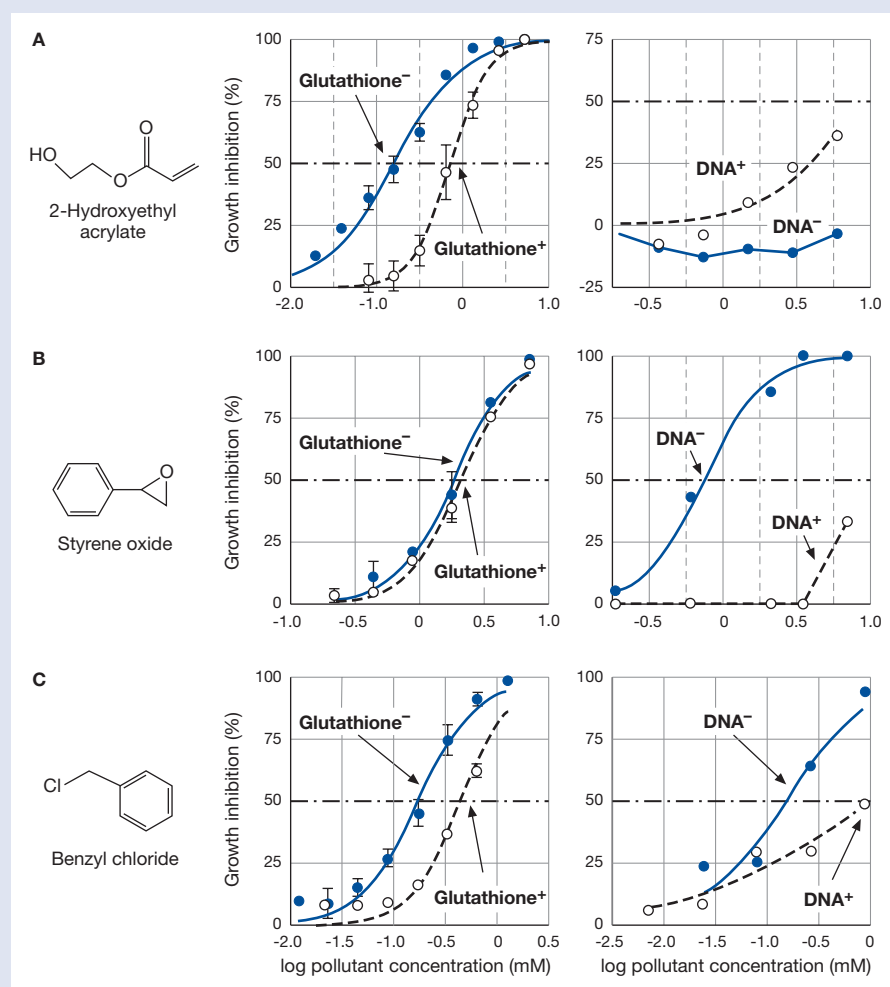


Fig. 3: Growth of the two *E. coli* strain pairs glutathione<sup>+</sup>/glutathione<sup>-</sup> and DNA<sup>+</sup>/DNA<sup>-</sup> in the presence of different pollutant concentrations.

A: 2-Hydroxyethyl acrylate as an example of a toxin causing protein damage.

B: Styrene oxide as an example of a toxin causing DNA damage.

C: Benzyl chloride as an example of a nonspecific reactive chemical, reacting with proteins and DNA.



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Research interests: Uptake and distribution of pollutants by organisms, mechanisms of toxicity, methods for hazard and risk assessment.

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[2] Harder A. (2002): Assessment of the risk potential of reactive chemicals with multiple modes of toxic action. Dissertation ETH Zurich, 78 p.

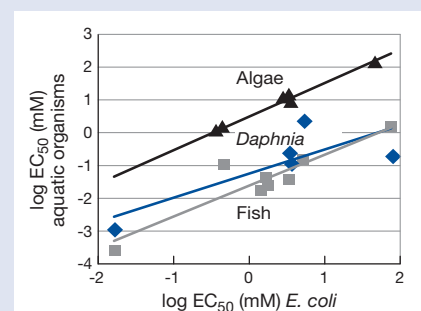


Fig. 4: Toxicity data (EC<sub>50</sub> values) for pollutants examined in this study. Comparison between EC<sub>50</sub> values for *E. coli* and EC<sub>50</sub> values for algae, *Daphnia* and fish.