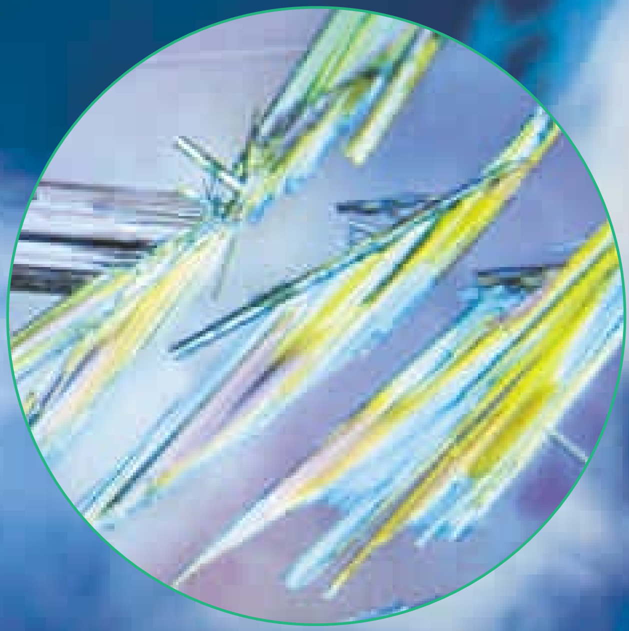
Just one tiny crystal from the synthesized sample (inset) is required for structural analysis with the X-ray diffractometer.

Structural analysis for crop protection research

Detective with X-ray vision

X-ray structural analysis gives scientists detailed information on the structure of molecules, making it an essential tool in the development of new active ingredients. The experts at Bayer Industry Services now only need tiny crystals for their work. The most recent success with their elucidation work was in mapping the precise structure of a new antifungal agent for grapevines.



Dr. Jordi Benet-Buchholz uses a disco ball to explain the diffraction patterns produced by X-rays striking crystals.

Search for active ingredients with X-ray eyes

Two types of X-ray equipment – known in technical jargon as "diffractometers" – are available to Bayer researchers for the structural elucidation of small molecules such as the crop protection agent iprovalicarb.
The star among the machines – the "single crystal diffractometer" – is used when the researchers want to determine the structure of a new substance for the first time. Only a single tiny crystal is needed for this. With the "X-ray powder diffractometer" the researchers examine crystalline powders. It provides information on whether the substance forms different crystal shapes. An important detail, as characteristics such as solubility or stability of a compound change along with the crystal form. This phenomenon, known as polymorphism, occurs with the production of almost every chemical substance. If the individual crystal forms can be identified, they can be separated more easily or selectively produced.

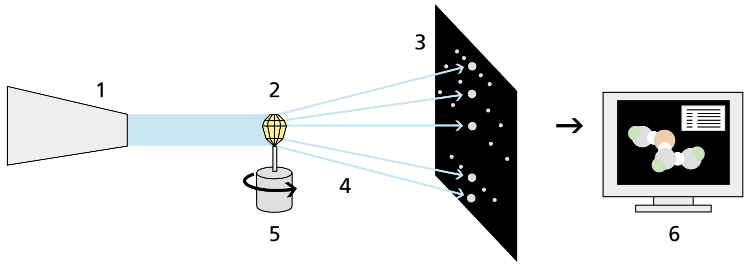
Perhaps another amino acid here or a methyl group there? It takes the Bayer CropScience researchers a long time to put a chemical compound together, because there are so many painstaking steps involved in the optimum development of a molecule from an initially promising substance to an effective and environmentally friendly active ingredient. Detailed information on the structure of the molecule is also needed.
Because the molecular builders' "components" cannot be seen with the naked eye, Dr. Jordi Benet-Buchholz, project leader in Bayer Industry Services' Analytics Department in Leverkusen, and his team provide support for their colleagues in crop protection research with what is known as X-ray structural analysis. This not only allows the precise molecular formula of an unknown substance to be determined – i.e. which elements are present in what proportions – but also the precise three-dimensional structure – i.e. the arrangement of the individual atoms. The process is thus one of many important tools in the development of new pesticides.
"At the start of the development process, test substances are always in very limited supply," explains Benet-Buchholz. For their analyses, Bayer's researchers need only the tiniest amounts of a chemical compound to elucidate its structure using X-rays. In extreme cases, as little as 30 micrograms of a substance is sufficient to enable researchers to identify its three-dimensional "blueprint". Explains Benet-Buchholz: "There are not many laboratories in the world that can manage with such small quantities. This saving on materials is a great advantage in the development of new substances."
Each structural modification is followed by testing on plants: does the substance have a broader spectrum of action and is it biologically more active than its predecessor? Tests on its effects on the environment and for the protection of the user also take up a considerable amount of time. Ultimately, after a great deal of effort and a good bit of luck, a new commercial product will be ready after many stages of refinement. "On average, the development of a new crop protection substance takes eight years," says Dr. Thomas Seitz, Fungicides Group Leader at Bayer CropScience in Monheim.

Tiny crystals are big enough for analysis

Benet-Buchholz and his team ensure that the analyses provide meaningful results from ever-smaller quantities of test substance. There have been quite a few changes in the field of X-ray structural analysis since the chemist arrived at Bayer. Tiny crystals with a diameter of a few micrometers are now sufficient for him to do his work. And the miniaturization of this application is advancing particularly rapidly in his team. "We're hooked on records – we

Thomas Felkel places synthesized crystal samples in a glovebox for analysis.

A look inside a crystal
The three most important elements of X-ray structural analysis are a radiation source **1**, a crystal **2** and a detector **3** on which the X-rays **4** depict the structure. The "goniometer" **5**, as it is termed, accurately measures the angle when the crystal being analyzed is positioned. The researchers use the results of the analysis as a database for computer simulations **6** of the crystal structure.



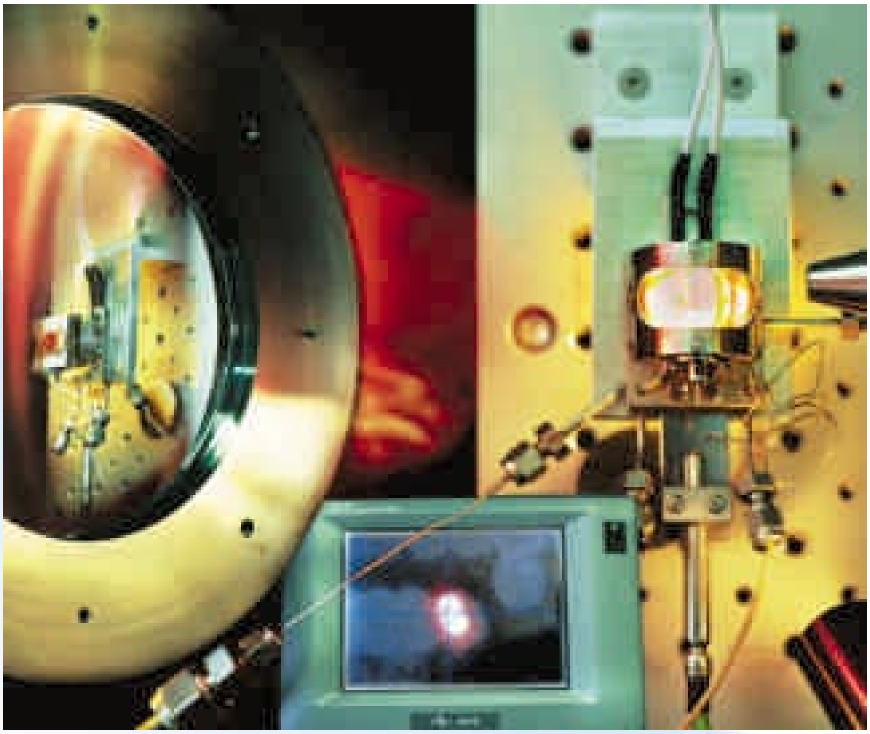
1 nm = 10^{-9} m

want to see how much further we can go," says the chemist.
These efforts are not blind competitive zeal, however; after all, it was only the miniaturization of the dimensions which made many things possible in the first place – for instance, the precise structural elucidation of iprovalicarb, a fungicide that protects grapevines against oomycete infestation. This disease known as downy mildew is extremely aggressive and a few decades ago destroyed entire vineyards. "Unlike other fungicides, as a modern pesticide iprovalicarb not only covers the surface of the plants, but also penetrates inside them. There it circulates with the sap flow and protects the plant from the inside against penetrating fungi," says Seitz. In 2001, the crop protection researcher was awarded the Otto Bayer Prize for outstanding research at Bayer for the invention of iprovalicarb.

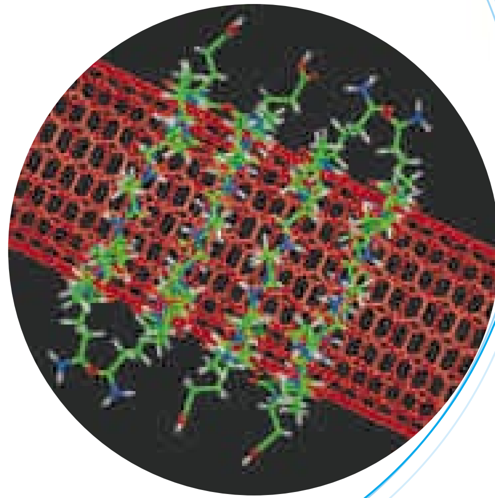
Active substances identified with modern X-ray technology

The researchers were faced with a major problem in the development of the new pesticide, which could only be solved with new equipment. This was because iprovalicarb only crystallized in the form of very fine needles with a diameter of about 10 micrometers and a length of 150 micrometers. The standard equipment in the 1990s could not cope with such precise identification and differentiation.
The new generation of X-ray equipment with which Benet-Buchholz carries out his elucidation work has one crucial benefit: unlike earlier equipment, it does not depend on the strong signals of the largest possible crystals. Even tiny crystals measuring only a few micrometers can be handled by the high-performance equipment, so researchers can now identify the new active substance more precisely.
Taking a disco ball in his hand, Benet-Buchholz explains how the X-ray structural analysis works. In the darkened laboratory, the countless tiny mirrors on the surface of the ball reflect a thin ray of light and project an armada of dancing spots of light on to the wall. "Imagine this as how the results of an X-ray structural analysis might look," says the chemist.
Of course, the image is greatly simplified, because the scientist uses X-radiation instead of light and does not illuminate disco balls with it, but irradiates chemical substances. Nor are the results of his analysis light reflexes, but "diffraction patterns". They occur when the X-ray is deflected by the electrons of the crystal – in a similar way to the light by the mirrors.
"Each substance creates a unique pattern of reflexes," says Benet-Bucholz

Using a micro-reaction chamber, any changes in miniaturized processes can be observed under real-life conditions.



Nanotubes
The tubular carbon molecules are about 1 to 30 nanometers in diameter and possess extreme mechanical strength. They are used in composite materials and electronic components such as diodes.



= 10 Å

The analysis team comprises Christian Hipler, Dr. Jordi Benet-Buchholz, Anja Luederitz and Thomas Felkel (left to right).

as the diffraction pattern of iprovalicarb shines on the screen. From this distinctive fingerprint, computer programs calculate how the molecules are constructed. A very strong radiation source allows the crystals to be bombarded with a considerably higher level of radiation, and highly sensitive detectors identify even the weak reflexes of minicrystals. "With the aid of these techniques, a small crystal today provides us with just as much information as a large one used to," Benet-Buchholz says.
The fact that researchers such as Seitz usually only have to wait one night for their structural results rather than years is possible because the countless reflex points now no longer each have to be recorded and evaluated in turn by a detector. Instead, the equipment collects the whole pattern at once, stores it as a file and leaves the evaluation to a high-speed computer. The limits of what is feasible have not yet been reached as far as Benet-Buchholz is concerned. At present he is attempting to outdo his own record of ten micrometers with even smaller crystals: one to two micrometers is his next goal. "But even that will not exhaust the possibilities," he says. With even stronger X-radiation, such as can be created by particle accelerators, for example, miniaturization could be pushed several orders of magnitude further forward – into the realm of crystals measured only in nanometers.

www

www.matter.org.uk/diffraction/x-ray/default.htm
Further information on X-ray diffractometry can be found on the University of Liverpool's website.

Dr. Thomas Seitz was the first to synthesize iprovalicarb, a substance used to protect grapevines against downy mildew.