Introduction
Titanium dioxide (TiO$_2$) has been produced industrially for more than 100 years$^1$; this explains why nano-titanium dioxide (nano-TiO$_2$) is the best-investigated nanomaterial. The question of health risks of TiO$_2$ both in its regular and nano-form is therefore highly relevant. Numerous epidemiological as well as in-vivo and in-vitro studies have been conducted in order to determine the risks. This dossier provides a brief overview. Details and compilations of studies along with more comprehensive risk assessments are available from international bodies (EU, IARC, OECD, FDA, CDC).

Uptake of TiO$_2$: Exposureroutes
Nanomaterials and nanoparticles (NP) can be incorporated directly into the body through inhalation or by swallowing$^1$. One topic of discussion is that the skin is a natural barrier against NPs, but that these substances can enter via the pores. For the general public, the two relevant routes are exposure of the skin – by using TiO$_2$-NP-containing sunscreens – and swallowing of nano-containing TiO$_2$ in the form of a food additive. For those persons in the NP-production industry, the main route into the body is inhalation and swallowing.

Epidemiological studies
No epidemiological studies explicitly designed to examine nano-scale titanium dioxide particles are available. Nonetheless, numerous studies have been conducted on the effects of the TiO$_2$ particles released during industrial production and processing on employees. All such epidemiological studies concluded that there is no evidence of an increased risk of lung cancer or other types of cancer$^2$-$^4$. Nonetheless, one should be careful because the absence of a clear evidence of a cancer risk is not a definitive conclusion to the potential carcinogenic capacity of nano-TiO$_2$. In a statement in 2010, the German Federal Institute of Risk Assessment (Bundesinstitut für Risikobewertung, BfR) it is explicitly stated: "Conclusions about a cancer risk associated with exposure to nano-TiO$_2$ cannot be drawn based on the available epidemiological investigations"$^5$.

Tests on animals:
in-vivo studies
Uptake via the lungs: inhalation
Numerous studies on animals have investigated damage to the respiratory organs by nano-TiO$_2$. The effects are heterogeneous and depend on the experimental animals, with considerable differences between rats, mice and hamsters. Nonetheless, these studies uniformly show that inhaled TiO$_2$-NPs are deposited in the lungs. There, they can trigger inflammatory reactions; these, however, are temporary as long as the exposure is not chronic$^6$$^7$. After administration of high doses of TiO$_2$-NP (5 days, 50 mg/m$^3$), the inhaled particles are deposited in the lung as agglomerates; they were found inside the lymph nodes associated with the lungs (in the ma-
The use of TiO$_2$-NPs as components of sunscreens is one of the key applications. More than one half of the synthetically produced nanoparticles of this type are used in this sector. In sunscreens, TiO$_2$-NPs function as physical filters. This approach is gradually replacing chemical filters because the latter can cause skin irritation or allergies (see1). A range of calculations are available on the amount of nano-TiO$_2$ used in sunscreens. In the case of a suntan lotion containing 5 % nano-TiO$_2$, the assumption is that 0.5 to 2.3 g/applicat-

**Uptake via the gastrointestinal tract: ingestion**

Regular TiO$_2$ is acertified food additive (E171). The addition of TiO$_2$-NPs to foods or food packaging is not uniformly regulated EU wide. In Germany, however, it is not permitted. One study shows that the production process that more than one third of the E171 in foods contains nanoparticles (diameter < 100 nm)$^{19}$. TiO$_2$-NPs can therefore enter the gastrointestinal tract. The authors calculated the daily intake of adults to be ca. 1 mg TiO$_2$/kg body weight. Accordingly, 1/3 of this (ca. 300 µg/kg) could potentially be present in nano-size. Moreover, small amounts of TiO$_2$ from the lung can enter the gastrointestinal tract through re-swallowing. Unfortunately, only few studies are available on the uptake of nano-TiO$_2$ via the gastrointestinal tract$^{13, 20}$. It is known, however, that TiO$_2$ can be reabsorbed into the human body from the gastrointestinal tract. In a study TiO$_2$ (diameter 160 and 380 nm, 3 and 46 mg) in gelatine capsules was administered for test persons, after which a size-dependent resorption into the blood was detected. The smaller the particles, the quicker the resorption$^{21}$. In another study, female rats were orally administered 12.5 mg rutile TiO$_2$/kg (500 nm) for 10 days. The authors found small amounts of translocated TiO$_2$-NP in the liver, spleen, lung and peritoneum, but not in the heart and kidneys$^{22}$. The assumption is that most of the particles taken up were excreted with the feces.

Another study investigated the genotoxic effect in pregnant mice that consumed nano-TiO$_2$ in drinking water in a (very high) dose of between 60 and 600 µg over a period of five days. These mice took up nano-TiO$_2$ in the second half of their pregnancy, and their offspring showed special DNA damage, oxidative stress and/or inflammatory reactions attributable to secondary effects$^{23}$. In summary, the in-vivo studies on inhalation/installation have shown that nano-TiO$_2$ can trigger dose-dependent effects in the lungs. In rats, changes in lung clearance and an increase in particle retention due to overload were observed at very high doses. These can trigger inflammatory reactions, oxidative stress, fibres and even lung cancer. It remains unclear whether the studies involving highly dosed NPs are relevant. Studies on mice showed lung impairment and inflammation but not fibroses or cancer; hamsters showed the least impairment. That particles and dust can cause lung disease is a well-known phenomenon which is not related to nano-TiO$_2$ as such.
of nanoparticles that potentially reach deeper skin layers and possibly also the bloodstream. Nonetheless, when the applied dose of nanoparticles is very high, small amounts could accumulate and trigger adverse health effects.\textsuperscript{23} This mechanism is conceivable, but there is currently no scientific evidence or verification to support this statement.

Investigations on cells – in vitro

In-vitro studies are typically used to investigate mechanistic questions. The tested parameters include so-called biological endpoints such as survival rate, cell death, formation of free radicals etc. in order to determine potential mechanisms of action leading to cell damage. The choice of cells is important because, depending on the organ from which they stem, cell-type-specific effects can occur. Typically, in-vitro studies can only reveal acute effects. The development of multi-cell models that closely approach in-vivo conditions is a promising approach that is currently undergoing research.

In order to successfully expose cells to TiO\(_2\)-NPs, the NPs must undergo complex treatment because they show a strong tendency to agglomerate. This also means that the number of free NPs is rather low. Very high doses of nano-TiO\(_2\) (> 50 µg/cm\(^2\)) are known to cause cell damage in vitro and to affect almost all biological endpoints. Nonetheless, cells react differently to different doses. Such high doses, however, never occur in everyday life or when regulations are adhered to in the case of occupational exposure.\textsuperscript{34, 35}

TiO\(_2\) belongs to the class of substances known as granular bio-persistent dusts. This means they are not degraded in the body but either remain there or are excreted. In high concentrations, inhaling such dusts can cause inflammatory or fibrotic changes as well as lung tumors in rats. The tumor formation develops indirectly due to the inflammation in the lung region. This, in turn, reflects the increased uptake of particles by macrophages and other immune-relevant cells, whereby increased amounts of free oxygen radicals are formed. These can damage adjoining cells and the DNA (see\textsuperscript{36}), which can ultimately end in inflammation or even malignancy. Numerous studies have been conducted with various lung, blood and epithelial cells to investigate this mechanism. In all cases, only high doses of TiO\(_2\)-NPs showed toxic effect. Although high doses in vitro also cause genotoxic effects, these are attributed to the secondary genotoxicity due to the persistent inflammation\textsuperscript{37, 38}.

In-vitro studies using human intestine cells show similar results, albeit only few studies are available. Applying high doses (20 and 80 µg/cm\(^2\)) of TiO\(_2\)-NPs triggered significant membrane damage and vitality losses, but no relevant genotoxic effects were detected\textsuperscript{39}.

Skin cells are also an object of research. Numerous studies have been conducted in an attempt to determine potential gene-damaging or other effects, with contradictory results. One clear conclusion is that, that the dose, the size, shape, as well as the physical and chemical properties of the NPs are relevant when particles enter the interior of the cell (see overview\textsuperscript{40}).

Expert assessments of the risks

A number of expert groups are involved with estimating the human health risks of substances. Often, the focus is on the carcinogenic effects. These assessments use evidence-based results of scientific investigations with predetermined criteria to enable comparability between the studies.

Table 1: Categorization of substances that can be carcinogenic in humans (after IARC/WHO\textsuperscript{41})

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Number of agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carcinogenic in humans</td>
<td>107 agents</td>
</tr>
<tr>
<td>2A</td>
<td>Probably carcinogenic in humans</td>
<td>63 agents</td>
</tr>
<tr>
<td>2B</td>
<td>Possibly carcinogenic in humans</td>
<td>271 agents</td>
</tr>
<tr>
<td>3</td>
<td>Not classifiable as to its carcinogenicity to humans</td>
<td>509 agents</td>
</tr>
<tr>
<td>4</td>
<td>Probably not carcinogenic to humans</td>
<td>1 agent</td>
</tr>
</tbody>
</table>

The World Health Organization’s International Agency for Research on Cancer (IARC/WHO) has assessed TiO\(_2\) – without consideration of size – as “possibly carcinogenic in humans”\textsuperscript{41}. This classification into the risk category 2B is based on what is seen as “sufficient” evidence of increased rates of lung tumors in rats that have inhaled TiO\(_2\)-NP over lengthier periods (see Table 1).

The CDC/NIOSH (USA) came also to the assessment that ultrafine TiO\(_2\) particles represent a potential occupational carcinogen at the workplace\textsuperscript{13}. This is because these particles, similar to other poorly soluble ultrafine particles with low toxicity (PSLT), consistently triggered dose-dependent lung damage and lung tumors in rats.

The so-called “MAK-Kommission” (commission dealing with maximum allowable concentrations at the workplace) of the Senate Commission, German Science Fund (DFG-Senatskommission) now groups the inhalable dust fraction (see Fig. 1 and below) of TiO\(_2\) dusts in category 3A, representing “demonstrated carcinogenic substances in animals”. In this case as well, ultrafine (nano-scale) particles were not considered in the assessment\textsuperscript{15}.

All three organizations based their categorizations on the results of experiments with animals because no data and no cases are known that point to tumor development in humans due to nano-TiO\(_2\).

![Figure 1: Classification of particle sizes.](image)

In some cases, particles are considered to be fine particulate matter only at a diameter < 2.5 µm. Accordingly, particles with a diameter > 2.5 µm are already considered to be coarse particulate matter (after\textsuperscript{51})
On the regulation of nano-titanium dioxide

The question whether TiO2 dusts originating during the production process entail health risks has long been a topic of epidemiological research. Numerous measurements have been conducted at workplaces and the overall exposure has been calculated for days, years and the entire working life. The measured dust concentrations – the so-called inhalable fraction – amounted to 0.1 mg/m³ air in the period 1976-1980. This value dropped to ca. 1.0 mg/m³ air between 1996 and 2000. The alveolar/respirable (smaller particle size) fraction was calculated to lie between 0.1 and 0.7 mg/m³ air, with peak exposures of up to 6.0 mg/m³ air. The epidemiological studies4-6 found no evidence of an elevated lung cancer risk. Animal experiments, however, have shown that inhaling TiO2 – as well as other granular and bio-persistent dusts without known specific toxicity – can trigger dose-dependent inflammatory lung damage and even lung tumors. This mechanism is valid for all granular bio-persistent dusts, not only for TiO2. Current regulations or recommendations on legally binding workplace concentrations do not consider the specific risks of small (ultrafine and nano-scale dusts (see Fig. 1). Equally, no agreement exists on the potentially carcinogenic effects of granular bio-persistent dusts. Accordingly, lower limit values are currently under discussion.

Regulations for ultrafine and nano-scale titanium dioxide particulate matter at the workplace

The term (inhalable) particulate matter generally refers to particulate matter with a particle size smaller than 10 µm (PM10); (alveolar) ultrafine particles, in contrast, are smaller than 0.1 µm (see Fig. 1). In Austria the limit value for TiO2 is 6 mg/m³ regardless of size. The internationally valid guide values for the permissible concentrations of TiO2-containing particulate matter at the workplace range between 3 mg/m³ in Switzerland and Germany, and 10 mg/m³ in numerous other countries11. In most cases these values refer to average exposure values over an 8-hour workday (TWA, time weighted average).

In Germany the “Allgemeine Staubgrenzwert” (general airborne dust limit) is valid; it is directed at avoiding “unspecific effects of dusts on the respiratory organs of employees”. Up until 1997, this pertained only to the smaller particle fraction, the alveolar/respirable fraction. Since then, however, the potential effects of the components deposited in the bronchial tubes, the inhalable components, have been assessed based on a separate limit value (see Table 2). An expert group (Senate Commission) of the German Science Fund (DFG) has been tasked with developing reasoned proposals for the limit values. The recommended limit values will become legally binding only after the official assessment by the Committee on Hazardous Substances (“Ausschuss für Gefahrstoffe”, AGS) and the publication as a “technical rule for hazardous substances” (“Technische Regel für Gefahrstoffe”, TRGS). The TRGS rules explicitly state that the general dust limit value is not applicable for ultrafine particle fractions43.

Beyond the “general airborne dust limit”, which is equivalent to the “maximum allowable concentration at the workplace” (MAK), a second value is often defined, namely the “workplace limit value” (“Arbeitsplatzgrenzwert”, AGW). Since TiO2 is a “demonstrated carcinogenic substance in animals” (category 3A). For TiO2 dust the DFG has classified TiO2 as a “demonstrated carcinogenic substance in animals”. For TiO2 dust the DFG has therefore recommended a drastic reduction in the limit value for the alveolar component from 1.5 to 0.3 mg/m³. In the USA the limit value for dust exposure involving TiO2 is 15 mg/m³ air. In 1988 the US National Institute for Occupational Safety and Health (NIOSH) classified TiO2 as a potentially carcinogenic substance and recommended keeping the exposure to the lowest level possible. The revised TiO2 assessment recommends stricter limit values of maximally 2.4 mg/m³ for fine particulate matter and maximally 0.3 mg/m³ for ultrafine and nano-scale particulate matter for a 10-hour workday13.

### TiO2 in consumer products, cosmetics, foods etc.

Principally, the handling of titanium dioxide – like all chemicals in the EU – has been regulated since 2007 by the REACH regulation and the CLP regulation (for the classification, labeling and packaging of substances)44-46. The determining property – both in REACH and in CLP – has been chemical structure, not shape or size. Nonetheless, in the nano-regulation sector, things are apparently changing.

- Commission Regulation (987/2008) already rescinded the exemptions for several substances that were previously classified as “non-hazardous” in REACH Appendix IV. Higher risks can be involved due to “inherent substance properties”, requiring a special risk analysis for nano-scale forms of carbon and graphite (carbon nanotubes and C60).
- In 2010, changes were made to the REACH regulation (453/2010). In relation to the publication of the “safety data sheets”, they specify for the first time that nanomaterial-relevant information is also required, e.g. grain size distribution, surface chemistry and physico-chemical properties that govern reactivity.

In the future, stricter regulations will be in place for market approval of cosmetics, foods and biocides as well as for electronic wastes. This will include mandatory classification with the label “nano” and clear regulations on safety assessments.

In the USA the use of TiO2 is regulated by the Food and Drug Administration (FDA) without consideration of size. Regarding its use in the food additive E 171, the FDA has since 1966 specified a maximum proportion of 1 %

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Table 2: Limit values or suggested limit values for fine particulate matter containing TiO2, but not for ultrafine or nanoparticles

<table>
<thead>
<tr>
<th>Limit value or suggested limit value</th>
<th>Expert group (and year)</th>
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<tbody>
<tr>
<td>Particulate matter (MAC value)</td>
<td></td>
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<tr>
<td>Alveolar component (A):</td>
<td>1.5 mg/m³</td>
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<tr>
<td>Inhalable component (E):</td>
<td>4 mg/m³</td>
</tr>
<tr>
<td>Shift average (AGW-TRGS 900)</td>
<td></td>
</tr>
<tr>
<td>Alveolar component (A):</td>
<td>3 mg/m³</td>
</tr>
<tr>
<td>Inhalable component (E):</td>
<td>10 mg/m³</td>
</tr>
<tr>
<td>Recommended new MAC value for GBS-dusts</td>
<td></td>
</tr>
<tr>
<td>Alveolar component (A):</td>
<td>0.3 mg/m³</td>
</tr>
<tr>
<td>(except ultrafine particles)</td>
<td></td>
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</tbody>
</table>

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on a per weight basis; TiO₂ is also included in the list of permitted color additives in medications, cosmetics and contact lenses. With the new FDA regulations that came into force in June 2012, the testing specifications and classification of sunscreens are newly regulated, yet without restrictions for nano-scale particles consisting of TiO₂.

Conclusions

see Dossier 035en

Notes and References

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