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TOXICITY OF FIBERS AND PARTICLES—REPORT OF THE WORKSHOP HELD IN MUNICH, GERMANY, 26–27 OCTOBER 2000

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Fibers and particles can have primary genotoxic effects and also induce inflammation, fibrosis, and cancer in experimental animals and humans. The intensities of these adverse effects are dose dependent where inflammation is known to induce cell proliferation and secondary genotoxicity. Secondary genotoxicity may arise as a result of inflammation and cell proliferation and will not occur at exposure concentrations that do not induce inflammation and do not overcome the antioxidant and DNA-repair capacity in the lung (Greim et al., 2000). Thus, fibers and particles may act through a threshold mechanism. The exposure and the dose that results in a lung burden without adverse effects (no-observed-effects level, NOEL) during long-term exposure have to be defined. Optimally this no-effect exposure should represent a steady-state lung burden at which the rates of deposition and clearance are equal so that no further increase in lung burden will occur.

For primary genotoxicity caused by fibers and particles, the assumption is made that humans are at risk at any exposure, as any amount of a direct-acting carcinogen in theory can cause a mutation. For this reason, the exposure-response relationship for direct-acting carcinogens is generally described as low-dose linear. In any case, exposure reduction will reduce the risk of adverse effects. Thus, for risk assessment of fibers and particles the mechanism of genotoxicity (primary, induced by the inherent genotoxicity of the material, secondary, e.g., as a consequence of inflammation), the dose response of the critical effects and their NOELs have to be understood. Unfortunately, such information usually is minimal for the many fibers and particles.

To discuss the information necessary for a science-based risk assessment of fibers and particles, the MAK Commission, on behalf of the Deutsche Forschungsgemeinschaft, convened an international workshop, held in Munich on 26–27 October 2000. During the workshop the state of the art of fiber and particle toxicity was presented, research needs were identified, and the necessary information for risk assessment was discussed. The full-length contributions to the workshop will be published in this journal at a later date.

This communication presents summaries of the workshop discussions on specific issues, including any consensus views. It presents current scientific opinion, as conveyed by the workshop participants. This report is intended to provide guidance to the MAK Commission and other occupational health and safety organizations that interpret scientific data for the development of public health policy (e.g., exposure limits). Other interpretations may be possible, and conclusions could be modified in the future as new data are acquired. Table 1 offers the range of topics and questions discussed at the workshop.

THE MAK COMMISSION

The MAK Commission advises the German Ministry of Labor. It generates scientific recommendations for the establishment of MAK values (maxi-

TABLE 1. Topics and questions discussed at the DFG workshop, Munich, 2000

Inflammatory response after particle and fiber exposure
Is inflammation-mediated genotoxicity primary or secondary?
Is inflammation the appropriate parameter to determine the NOEL and dose response?
What are the relevant inflammation parameters in vivo?
Are there valid in vitro tests for predicting inflammation of fibers and particles?
The genotoxic mechanisms of fibers and particles
Do fibers and particles induce primary or secondary genotoxicity?
Can primary genotoxic effects of carbon black and diesel exhaust particles and specific fibers be ruled out?
What in vitro conditions are important to determine genotoxic hazard and what conditions of the in vitro system are predictive for in vivo genotoxicity and carcinogenicity?
The capacity and induction of the antioxidant system and DNA repair
Is determination of an oxidative mechanism of action supportive of a threshold in inflammation and genotoxicity?
Which components of the antioxidant system should be investigated?
Considering antioxidant, DNA repair, and other defense mechanisms, what is the most appropriate animal model for risk assessment of human exposure?
Toxicokinetics of particles and fibers
What dose metric should be used to evaluate toxicokinetics?
Are the biopersistence and effects of organic fibers comparable to inorganic fibers?
What parameters allow identification of a steady-state lung burden that does not induce critical effects at long-term exposure?
Can human toxicokinetics for particle and fiber retention and clearance be predicted from rodent data?
Is biopersistence (biosolubility) measurement enough to categorize fibers with respect to carcinogenicity?
What methods of biopersistence should be used?
Research needs

mal allowable concentrations at the workplace) and BAT values (biological exposure indices) for classification of carcinogens and of germ-cell mutagens, evaluates embryotoxic and fetotoxic effects of materials, and evaluates analytical methods for exposure control at the workplace and in biological materials. The results of the work are published annually in the "List of MAK and BAT Values" (e.g., DFG, 2000) and in "Occupational Toxicants" (e.g., Greim, 1999). These documentations are handed over to the Federal Minister of Labor. After examination and acceptance of the proposals by a tripartite committee, which also takes into account socioeconomic viewpoints, they become legal.

The MAK Commission cooperates closely with other international committees setting occupational exposure limits, such as the Threshold Level Values (TLV) Committee of the American Conference of Governmental Industrial Hygienists (ACGIH), the European Union (EU) Scientific Committee on Occupational Exposure Limits (SCOEL), the Dutch Committee "Updating of OELs," and other European national committees that are invited to the meetings.

To introduce quantitative criteria to the classification of carcinogens and to differentiate between primarily genotoxic and nongenotoxic thresholded effects, the MAK Commission has recently modified its classification concept (Neumann et al., 1997, 1998). If the underlying mechanism of tumor formation is cytotoxicity, inflammation, or increased cell proliferation, the effects are considered to be thresholded. The MAK Commission classifies such carcinogens as Category 4 carcinogens, provided an MAK value can be defined. Carcinogens with a defined but low genotoxic and carcinogenic risk at the MAK value are Category 5 carcinogens. Provided sufficient information on the mechanism, the dose response, and the NOEL is available, Category 4 may be applicable for certain fibers and particles (Greim et al., 2000).

STATE OF THE ART OF FIBER AND PARTICLE TOXICITY

General Concepts of Fiber- and Particle-Induced Carcinogenicity

It is generally accepted that both fibers and particles can induce carcinogenic effects in humans and experimental animals, although mechanisms and weight of evidence differ among the different types of fibers and particles. Several fibers including asbestos and erionite have been shown to be carcinogenic in humans, and their neoplastic responses include bronchogenic carcinoma and mesothelioma. Many other fibers have been shown to induce both responses in experimental animals, and fiber length and fiber biopersistence, and active metals are crucial in this respect. Biopersistence includes durability and its clearance from the lung; the latter is connected to fiber length again. All fibers do seem to produce inflammation at chronic inhalation beyond a certain exposure level, but not all of them are carcinogens. Therefore, inflammation is not considered the only crucial event in fiber carcinogenicity. Similarly, lung tumors in rats induced by poorly soluble low-toxicity particles seem to be caused by persistent inflammation, often caused by overload at chronic exposure (ILSI, 2000; Driscoll, 1996; Borm et al., 2000). Toxic particles, such as quartz, and ultrafine (<100 nm) particles of various materials are known to induce lung tumors at much lower mass doses and are probably associated with toxic effects (quartz) or with high surface area and interstitialization in the case of ultrafines. Evidence for human carcinogenicity of poorly soluble low-toxicity particles (PSP) is lacking, although some controversy exists in epidemiological reports on carbon black and coal-mine dusts (e.g., Morfeld et al., 1997). Diesel exhaust is considered as a primary carcinogen, based on its associated polycyclic aromatic hydrocarbons (PAHs), although its carcinogenic potency in rats does not seem to depend on PAHs (Heinrich et al., 1995). The relevance of the rat lung response to particle overload for human risk assessment has been the subject of a recent workshop (ILSI, 2000), and it has been concluded that not enough data for human are currently available to decide

that the concept of overload resulting in carcinogenicity would be invalid in humans.

Genotoxic Mechanisms of Fibers and Particles

Intrinsic physicochemical properties as well as reactive oxygen species (ROS) formed by inflammatory cells are implicated in genotoxicity of particles and fibers. Primary genotoxicity can be caused by direct formation of reactive oxygen species by particles or fibers, which may relate to surface properties, the presence of transition metals, processes of iron mobilization, and lipid peroxidation processes. Other important aspects are particle size and shape, crystalline structure and solubility, particle uptake by the target cell, and the presence of mutagens carried on the particle surface. Secondary genotoxicity is characterized by excessive and persistent formation of ROS by inflammatory cells during particle/fiber-elicited inflammation. Factors that determine the overall genotoxic response are the effectiveness and efficiency of intra- and extracellular antioxidant defense systems, and of DNA repair systems in those cells carrying premutagenic lesions. Similarly, direct and indirect processes are also believed to play a crucial role downstream in the initiation phase of fiber/particle carcinogenesis. In general, the following processes have been proposed for this phase with particles/fibers: (a) direct action of particles/fibers on activation of growth factors or their receptors, leading to activation of signaling pathways that are related to proto-oncogene activation; (b) indirect activation of the processes as mentioned earlier, via oxidants, cytokines, and growth factors released from inflammatory cells; and (c) compensatory proliferation in response to altered necrosis or apoptosis.

Appropriate In Vitro Test Conditions for Genotoxicity Testing of Fibers

With the exception of asbestos fibers, little information is available on genotoxicity testing of fibers (Jaurand, 1997). In contrast to standard genotoxicity testing of soluble substances, fibers pose specific problems. Fiber dimensions, surface properties, and biopersistence can influence test results. The mechanisms of fiber-induced genotoxicity are not yet clear, but interaction with the genetic material and effects via production of (ROS) have been proposed. Such effects can be determined with in vitro genotoxicity tests. The results from various genotoxicity tests show that asbestos did not significantly induce gene mutations in bacterial and mammalian systems but led to a clear induction of DNA strand breakage, with structural and numerical chromosome aberrations in cultured mammalian cells (Jaurand, 1997). Published positive in vitro genotoxicity data obtained for asbestos in the comet assay, the chromosome aberration test, and the micronucleus test indicate test conditions that are suited to the detection of asbestos-induced genotoxicity. Appropriate cell systems (e.g., mesothelial cell lines) are available for testing fiber-induced genotoxicity (Renier

et al., 1992). A combination of the micronucleus test and the comet assay seems to be well suited to detect genotoxic activity of fibers. Continuous treatment (without exogenous metabolic activation) seems to be appropriate to reveal fiber-induced genotoxicity. Furthermore, fiber samples have to be well characterized and phagocytosis and cytotoxic effects have to be determined for the correct interpretation of genotoxicity test results.

The Capacity and Induction of the Antioxidant System

Various mechanisms exist to protect cells and tissues against oxidants, and it is conceivable that genetic and acquired variations in these systems may contribute to, or account for, interindividual variation in the response to oxidative stress. Similarly, species differences in antioxidant defenses or the capacity of various defenses may underlie differences in response to xenobiotics that act, in whole or in part, through oxidative mechanisms.

Oxidative mechanisms of response to xenobiotics are especially relevant to the respiratory tract, which is directly and continuously exposed to an external environment containing oxidant pollutants. These include ozone and oxides of nitrogen, cigarette smoke that includes a variety of oxidizing molecules, and particles that may generate oxidants as a result of their chemical properties or by stimulation of production of cell-derived oxidants (Halliwell, 2000). Moreover, exposure to particles or other pollutants may produce oxidative stress in the lung by stimulating the recruitment of inflammatory cells. Regarding particulate exposure, there is considerable evidence that many pneumotoxic particles act, at least in part, through mechanisms that involve production of reactive oxygen species. For example, the toxicity of asbestos fibers, crystalline silica, and transition metals containing ambient particulate likely involves production of oxidants such as hydroxyl radical, superoxide anion, and hydrogen peroxide (Kamp et al., 1992; Janssen et al., 1993). Studies have also shown that these and other particulates may act by stimulating cellular production of reactive nitrogen species. Developing an understanding of the degree to which particulate materials can act via oxidative mechanisms and understanding the interplay between exposure, host antioxidant defenses, and interindividual or species variability in defenses may be very important for developing appropriate risk assessments on inhaled particulates.

The Capacity and Induction of DNA Repair

Lung injury after particle exposure is associated with the generation of ROS, leading to increased levels of oxidative DNA damage. Furthermore, both stable and unstable DNA lesions may arise after metabolism of polycyclic aromatic hydrocarbons (PAH) frequently bound to particles. ROS are also continuously induced due to oxygen metabolism, and even in normal cellular conditions in the absence of exogenous exposure cellular protection is not complete and a measurable amount of DNA lesions is detectable, representing a steady-state level derived from their induction

and repair. Oxidative DNA damage is predominantly removed by base excision repair (BER), which is usually comparatively fast and efficient. Nevertheless, an increase in ROS is expected to enhance the steady-state level, and repair capacities may be exceeded upon particle exposure (Nehls et al., 1997). Stable DNA adducts induced by PAHs are eliminated by nucleotide excision repair (NER), which is unequally distributed in the genome and incomplete in transcriptionally inactive DNA regions. Thus, even low adduct levels due to environmental exposure lead to persistent lesions in the human lung. Other factors that need to be considered are potential repair inhibitions by associated metal compounds, and interindividual differences in repair capacities.

Toxicokinetics of Fibrous and Nonfibrous Particles

Many nonfibrous and fibrous particles are known to cause lung tumors in rats upon chronic inhalation. Among those are low-toxicity particles such as TiO_2 and particles of greater toxicity such as crystalline SiO_2 or asbestos. In the absence of human data for many of these particles, a question arises as to whether the rat lung tumor responses can be extrapolated to humans, and if so, how such extrapolation should be performed. Dosimetric modeling based on toxicokinetic data is often one step in this extrapolation process. Toxicokinetic data can provide valuable information on potential mechanisms of inhaled particulate compounds as revealed by their dose-related buildup in the respiratory tract or by their clearance kinetics.

The hallmark of overload by PSP is an impaired clearance function of alveolar macrophages. This has a significant effect on the accumulation kinetics of inhaled PSP: Under normal conditions of unimpaired clearance the particle burden reaches a steady-state level after about five retention half-times have elapsed during a chronic exposure (the normal retention half-time for PSP in rats is about 70–80 days). Such steady-state levels of lung burdens will be reached as long as the deposition rate of inhaled particles in the lung is less than or at most equal to the clearance rate of those particles from the lung. If, however, the deposition rate is greater than the clearance rates, as seen with chronic exposures at very high exposure concentrations, then a continued accumulation of particles beyond the normal steady-state level (which is proportional to the exposure concentration) occurs, and a steady-state level will not be reached. Under these conditions of chronic exposure to PSPs, fibrosis and lung tumors have been observed in rats (ILSI, 2000). Other species such as mice and hamsters do not respond with lung tumors when exposed chronically to the same high dose levels, despite the fact that these species also develop impaired particle clearance with altered pulmonary accumulation kinetics of PSPs. The condition of impaired clearance due to lung particle overload can also be determined when in a 2-yr study the final lung burdens are normalized to the exposure concentration: If this normalized lung bur-

den (ratio of lung burden/exposure concentration) is greater than that from a low-level exposure, then an overload-induced impaired clearance is present. This applies only to PSPs and not to cytotoxic particles such as crystalline silica.

With respect to the mechanism leading to impairment of macrophage-mediated clearance function, Morrow (1988) developed the concept of volumetric overload of alveolar macrophages. It states that the volume of phagocytized particles in alveolar macrophages determines the state of functionality of these cells. If the phagocytized volume of PSPs exceeds a certain value, the alveolar macrophage-mediated clearance of these particles begins to be slowed down. A complete cessation of the alveolar macrophage clearance function occurs once the macrophage is fully loaded, which is defined by about 60% of their normal volume being occupied by phagocytized particles.

Another important parameter of PSPs is their surface area. The significance of the concept of particle surface area was demonstrated previously when it was shown that the surface area of PSPs retained in the rat lung correlated very well with lung tumor incidence in rats in chronic exposure studies (Driscoll, 1996).

The impairment of particle clearance and the accompanying continuous accumulation of particles in the lung lead to chronic inflammatory conditions under continued particle exposure (Tran et al., 2000b). This inflammation is characterized by a steady influx of neutrophils into the alveolar space, and these are activated to release significant amounts of reactive oxygen species. These have the potential to induce DNA damage (Driscoll et al., 1997; Knaapen et al., 1999), which means that the lung tumors observed in chronic rat inhalation studies with high-dose PSPs through this mechanism are due to a secondary genotoxicity (described earlier).

This secondary genotoxic mechanism of PSPs in rats operates only at high doses and high levels of neutrophils, and there is no evidence that this occurs at low exposure concentrations when the particle deposition rate in the lung is equal to or less than their clearance rate from the lung. Thus, the likelihood that such secondary genotoxic events are induced by PSPs depends on the chronicity and the level of retained PSPs in the lung. It also depends highly on the level of antioxidant defenses in the respiratory tract (see earlier discussion), which determines whether DNA damage is induced. Antioxidant defenses appear to be species dependent in that mice and hamsters may be better protected from pulmonary inflammation-induced oxidative DNA damage than rats, and therefore lung tumors are not induced in these species. It is unknown whether in humans secondary PSP-induced DNA damage could occur at extremely high retained particle levels in the lung. However, this appears unlikely, since even among coal workers who have been chronically exposed to very high dust levels rates of lung tumors are not significantly increased (IARC, 1997; Morfeld et al., 1997).

Based on our present knowledge regarding the importance of toxicokinetics of inhaled PSPs and adverse effects it appears that two thresholds with respect to PSP-induced chronic effects can be discerned:

1. A dosimetric threshold (related to alveolar macrophage clearance capacity): This is defined by the particle deposition rate being greater or lower than the pulmonary clearance rate. Its exceedence leads to particle lung overload and chronic inflammation. Staying below this threshold in terms of PSP lung burden of 200 cm^2/rat (Tran et al., 2000a) will prevent the induction of adverse chronic effects in the lung.
2. A mechanistic threshold determined by antioxidant defenses and DNA repair: This is related to and characterized by the presence of persistent inflammation and release of reactive oxidant species, which eventually overwhelm antioxidant defenses. Chronically PSP-exposed rats are more likely than other species to exceed this threshold and develop chronic lung lesions, including tumors. This concept implies that a no-observed-adverse-effect level (NOAEL) can be determined in an experimental rat study by a dose level that does not alter the toxicokinetics of the particles.

Fibrous particles The durability of inhaled fibers in the respiratory tract as determined by their biopersistence is a key parameter; fibers of a very low biopersistence are not carcinogenic. The slow phase of the retention kinetics of fibers longer than 20 μm should be the focus of a biopersistence assay for synthetic vitreous fibers (there are insufficient data to extend this statement to organic fibers). The outcome of such assay may allow the separation of fibers into two categories:

1. Biopersistent fibers: These would be classified as probably or possibly carcinogenic to humans, according to the IARC groups 2A or 2B.
2. Biosoluble fibers: These could be classified as probably not carcinogenic to humans, according to IARC groups 3 or 4.

Measurement of the biopersistence of fibers should not solely be used for their classification, but should be complemented by some toxicity tests before classification. With respect to organic fibers, different rules may have to be established, and more experimental data need to be generated. Short-term tests are unlikely to provide information on the role of biopersistence since the time scale of the test is too short, although tests could be developed that include a step to mimic long-term residence in the lungs.

DISCUSSION AND CONCLUSIONS

After intensive discussions of the state-of-the-art presentations, several questions have been formulated and answers concluded after detailed consideration.

Inflammatory Response After Particle and Fiber Exposure

Is inflammation-mediated genotoxicity primary or secondary? Fibers and particles cause polymorphonuclear leukocytes (PMNs) and macrophages to generate oxidants, so they have the potential to cause adducts and thereby mutations. This adds to the steady-state level of oxidative adducts in cells caused by normal respiration. The genotoxicity must be in proliferating cells, and genotoxicity in macrophages or other end-stage cells is not of relevance to neoplastic outcomes. Acute inflammation is protective and so should not be simply looked on as a disease risk; if inflammation is prolonged and severe, then the protective effect is overridden by the harmfulness of the chronic inflammatory milieu. There are no effects, even for toxic particles, if the dose is low enough such that no PMNs accumulate. There is always a mechanistic threshold (see earlier description), which is provided by the antioxidant defense and DNA-repair enzymes. Fibers are able to cause direct oxidative damage leading to clastogenicity in vitro and also cause aneuploidy and misaggregation independent of oxygen, which are presumed to result from direct interaction between fibers and the spindle at telophase. Thus, if particle-mediated genotoxicity is considered the consequence of inflammation, this effect is considered thresholded. Chronic exposure to PSP without inflammation will also not cause genotoxic and carcinogenic effects.

It was shown, by separating fibers into clear-cut sizes, that the toxicity is greater for longer fibers (mean length of 17 μm or greater) compared to shorter fibers (mean length of 7 μm or smaller); the inability of macrophages to completely engulf the longer particles may increase their toxicity (Blake et al., 1998). Deposition, durability, and clearance select out the thin, durable, and long fibers, which are difficult to clear and become the essential problem. However, a correlation between the occurrence of 5-μm fibers and lung tumor incidence in humans has been shown (Rödelsperger et al., 1999). In vitro studies show that there is increasing genotoxicity with increasing length, which plateaus at 20 μm. In vitro studies with cell lines may be misleading because human epithelial cells in situ undergo apoptosis when they are exposed to long fibers, in contrast to other cell lines, which survive and show the genotoxic effects.

Is inflammation the appropriate parameter to determine the NOEL and dose response of fiber and particle toxicity? For low-toxicity PSP, pathology in rodents indicates that if there is no inflammation there is no fibrosis, and if there is no fibrosis there is no cancer. Therefore, assuming these findings can be extrapolated to humans, then limiting exposures of nongenotoxic particles and fibers to levels that do not cause chronic inflammation would also prevent fibrosis and cancer in humans. The same can be considered for fibers, if a primary genotoxic effect can be ruled out. It was concluded that if inflammation is the underlying process that leads to cancer, it can be used to determine the NOEL. Application is further supported because it is a sensitive marker for fiber and particle toxicity in general.

What are the relevant inflammation parameters in vivo? The important and most used index is the PMN influx and surface-area dose of PSPs. It has been suggested that the 40% PMN level in bronchoalveolar lavage (BAL) fluid is a key level for cancer in rats. A lower, although statistically significant, increase in the percentage of PMNs has been reported in humans who were exposed to asbestos, coal, or silica and who had respiratory impairment (~4.5%, vs. 1.5% PMNs in controls) (Rom, 1991). This suggests that either the inflammation is also increased in the interstitium, or some other aspect of the inflammatory process is important, such as macrophage oxidants. However, high levels of PMNs have been observed in other human studies, recently in miners with simple coal workers' pneumoconiosis (31%, vs. 3% in controls) (Vallyathan et al., 2000), and in patients with acute silicosis (10-fold increase over controls) (Lapp & Castranova, 1993; Goodman et al., 1992). There is both alveolar and interstitial inflammation, and BAL may only detect the alveolar inflammation. A recent study using immunohistochemical detection of PMN and alveolar macrophages (AM) in lung sections of rats instilled with a high dose of various PSP showed a correlation between tumor rate and amount of both PMN and AM (Borm et al., 2000). A 90-day study may not be predictive because of confounding by the nonfibrous material, causing overload effects. BAL should be the gold standard–measuring granulocytes, proteins, and surfactant lipid and, as identified in later discussion, parameters of the antioxidant system like glutathione (GSH) and others, such as chemokines, NF-κB, or a global measure of antioxidant status such as TRAP or TEAC (Ghiselli et al., 2000). In humans there is a lymphocyte component in the BAL that is not seen in rats.

Are there valid in vitro tests for predicting inflammation of fibers and particles? Inflammation is a hierarchical and temporally sequential response with some components that occur early on in the scheme, such as NF-κB and tumor necrosis factor α (TNFα). Inflammation itself cannot be measured in vitro, but some changes that indicate proinflammatory effects such as chemokine production, TNFα, and NF-κB could be used as a screen after they are validated against known standards. In the meantime, since the in vitro tests are not standardized, they should be well controlled with the inclusion of positive and negative control particles, interpreted with care, and thoroughly described (review: Fubini et al., 1998). Cytotoxicity should not be the main endpoint.

The Genotoxic Mechanisms of Fibers and Particles

Do fibers and particles induce primary or secondary genotoxicity? Particles are considered to cause both primary and secondary genotoxicity. However, the only particle that has been agreed to induce primary genotoxicity is diesel exhaust. For quartz, primary genotoxic effects cannot be excluded at this time since genotoxicity has been observed in cellular systems with appropriate screening methods. It also needs mentioning

that among quartzes a large difference in inflammatory capacity is present, which is important in secondary genotoxicity (Donaldson & Borm, 1998). It is also clear that all PSP have a secondary genotoxicity at doses that induce inflammation in vivo. The answer is more complicated for fibers; in vitro genotoxicity tests can be used to detect fiber-induced genotoxic effects. However, appropriate test conditions have to be defined. In addition, since inflammation is present in every fiber in vivo study available, a secondary genotoxic effect is always involved.

Can primary genotoxic effects of carbon black, diesel particles, and specific fibers be ruled out? It is agreed that diesel particles have primary genotoxicity in appropriate test systems, although it is recognized that in vivo the particle core explains most of its carcinogenic outcome in rat studies. Studies to date have not demonstrated primary genotoxicity of carbon black with low PAH contamination using appropriate in vitro assays. DNA adducts related to associated organic compounds so far have not been found in lung tissue from rats exposed chronically to carbon black, although in the same studies adducts were found in diesel exhaust-exposed rats. There are various carbon black products with a range of associate PAHs. These need to be examined to further assure the lack of genotoxic potential for carbon black materials. It is recommended that appropriate testing includes determination of the bioavailability of the associated genotoxic materials.

What in vitro conditions are important to determine genotoxic hazard and what conditions of the in vitro system are predictive for in vivo genotoxicity and carcinogenicity? It is agreed that no current in vitro test is sufficient to predict in vivo carcinogenicity of fibers or particles. There is also consensus on the fact that in vitro testing should be performed under a dose regimen that allows direct extrapolation (based on fiber/particle number per cell) to in vivo exposure. One particular assay that deserves further investigation is the comet assay, since it is sensitive to both fibers and particles, and it can be applied after in vitro and in vivo exposure. During this assay particles should not come into contact with the isolated nuclei, since this can create false positive results.

However, apoptosis, which also causes DNA strand breaks, is caused by some particles in vitro, and this should be taken into consideration as this is unlikely to be a proneoplastic effect.

The Capacity and Induction of the Antioxidant System and DNA Repair

Is determination of an oxidative mechanism of action supportive of a threshold in inflammation and genotoxicity? Two conditions were discriminated: oxidative damage as a consequence of inflammation, and oxidative DNA damage induced by the particles themselves. With respect to the first case (oxidative damage as a consequence of inflammation), there was a consensus among the majority of participants that a threshold is given in the

absence of inflammation. If the amount of ROS is increased, there is a threshold at which, due to antioxidant defense systems, the damage will not significantly increase the background level of cellular damage. However, during persistent inflammation causing a permanent higher level of ROS, the dynamic relationship between particle exposure, induction of defense, and degree of oxidative stress needs to be considered. If oxidative cellular damage is induced by the particles themselves, the participants agreed that the practical threshold applies as well.

When considering risks to humans, it has to be emphasized that there are interindividual differences in antioxidant defense, for which at present there are insufficient data. With respect to different types of particles, quartz is one example where the underlying mechanisms of damage induction are not clear and differ between different quartz particles (Donaldson & Borm, 1998). Also, associated factors have to be taken into account, such as metal compounds, which have been shown to inhibit the antioxidant defense, for example, DNA repair.

Which components of the antioxidative system should be investigated? One frequently applied parameter is the glutathione (GSH) content. However, it is not clear whether GSH in lung lining fluid is representative for the relevant target cells. One other possibility is the use of a more comprehensive parameter for nonenzymatic antioxidant status, such as the TRAP assay, previously used in coal miners (Schins et al., 1994). One other important endpoint with respect to cancer prevention is the quantification of DNA damage due to oxidants. There are now sensitive techniques that are able to detect the background steady-state level as well as small increases of certain oxidative DNA base modifications (e.g., 8-oxo-guanine). Suitable methods are the measurement of Fpg-sensitive sites detected by different DNA strand break assays (comet assay, alkaline unwinding, alkaline elution) as well as immunostaining. Local increases in oxidative DNA damage in tissue should be investigated using immunohistochemistry.

Considering antioxidant, DNA repair, and other defense mechanisms, what is the most appropriate animal model for risk assessment of human exposure? The participants agreed that at present there is no better animal model than the rat for assessing lung cancer risk for poorly soluble particles (PSP). The rat appears to be the only laboratory animal species that develops lung tumors in response to PSP and therefore is the most sensitive species for this endpoint.

Toxicokinetics of Particles and Fibers

What dose metric should be used to evaluate toxicokinetics? Surface area appears to be an important dose metric for evaluating the toxicokinetics of particles, and possibly of fibers. Studies in rodents have shown that particle surface area is a better predictor than particle mass for both PMN (Oberdörster, 1996; Tran et al., 2000b) and tumor (Driscoll, 1996) responses. The biologically available surface area is important to consider

because the surface area of particles in the air and in the lungs may be different due to particle aggregation or disaggregation, or to adsorption or removal of substances on the particle surface (e.g., diesel exhaust particles). For fibers, particle number is generally considered a reasonable dose metric, in conjunction with the dimension and durability measures. A study by Timbrell et al. (1988) showed that surface area was also a better metric for asbestos fibers. It is recommended that particle surface area should be measured, along with number, mass, and diameter, to allow comparisons among studies of different dusts and sampling methods.

Are the biopersistence and effects of organic fibers comparable to inorganic fibers? Organic and inorganic fibers appear to be different with regard to biopersistence and response. For example, responses to certain organic particles include immune-mediated or eosinophil inflammation that is generally not observed with inorganic particles. There are insufficient data currently to evaluate the carcinogenicity of organic fibers based on biopersistence. For these reasons, organic particles were excluded from the remainder of the discussion.

What parameters allow identification of a steady-state lung burden that does not induce critical effects at long-term exposure? A steady-state lung burden and absence of critical effects may be identified in a chronic study that evaluates clearance kinetics and early responses (e.g., inflammation). However, the existence of a steady-state lung burden without significant inflammation at 3 months of subchronic exposure does not necessarily mean that an inflammatory response would not be observed after continuous 2-yr exposure. For example, in a study of rodents exposed to diesel particulate (Henderson et al., 1988), steady-state retention was observed at an exposure of 350 $\mu g/m^3$; although no PMN inflammatory response was observed at 3 mo, there was a significant increase in PMNs at 2 yr. Also, for fibers and particles that have long retention times or are cytotoxic (e.g., quartz), the lung burden continues to increase without reaching a steady state. Thus, identification of a steady-state lung burden is not always observable, and when it is, it must be evaluated in conjunction with the response data.

A research need was proposed to use existing data in rodents to identify no-observed- (adverse) -effect levels in subchronic studies of particles or fibers and then to use a toxicokinetic model to predict exposure levels at which the lung burdens after 2 yr would be below the no-effect levels in the subchronic studies. These predictions would be compared to the observations in the chronic studies. This information may be useful for determining threshold particle or fiber doses in the rodent studies, which could then be extrapolated to humans using accepted risk assessment procedures. For example, interindividual variability should be considered when extrapolating an average threshold value in animals to the human population.

Can human toxicokinetics for particle and fiber retention and clearance be predicted from rodent data? Although humans and rodents dif-

fer in their clearance and retention of particles and fibers, toxicokinetics can be used to quantify and calibrate the exposure–dose relationships across species. The pulmonary clearance rate in humans is approximately 10 times slower than in rodents (Snipes, 1996), while in both species the pulmonary clearance rate shows a continuous decrease with time. The retention patterns of particles in the lungs appear to be different in humans (coal miners) and in rodents (Nikula et al., 1997, 2000), although this finding is based on only one time point and thus does not permit an accurate assessment of the kinetics. Dose-dependent decline in pulmonary clearance due to particle overload has been well documented in rodents. Humans with occupational exposures to respirable particles (Freedman & Robinson, 1988; Kuempel, 2000) appear to have increased mean particle retention times in the lungs compared to humans without dusty jobs (Bailey et al., 1985; Philipson et al., 1985), although differences in measurement times and techniques introduce uncertainty. This finding of increased particle retention time is consistent with the prolongation of pulmonary clearance in humans due to particle overload and/or sequestration. In a human lung kinetic model, the inclusion of a sequestration process (thought to include particle interstitialization) was required to adequately predict the end-of-life lung burdens in U.S. and United Kingdom coal miners, whereas a rat-based overload model provided a poor fit to these human data (Kuempel, 2000; Tran & Buchanan, 2000). In a one-compartment kinetic lung model, based on both human and rat extrapolated data, overloading was predicted not to occur in humans at the estimated fiber exposures and observed lung burdens in three individuals (Yu et al., 1997).

Thus, species-specific toxicokinetic models should be used to predict particle and fiber clearance and retention in the lungs. In validating these models, the uncertainty and variability in model predictions and model structures should be evaluated. Toxicokinetic modeling enables estimation of equivalent doses across species and facilitates the use of the rodent data to predict disease risk in humans.*

Is biopersistence (biosolubility) measurement enough to categorize fibers with respect to carcinogenicity? It was agreed that biopersistence studies are useful as a screen for the carcinogenic potential of a fiber. However, these studies are not adequate to distinguish the carcinogenic potency. In addition to biopersistence data, it is recommended that in vivo toxicity data also be obtained to evaluate toxicological endpoints.

What methods of biopersistence should be used? The use of the weighted half-time was considered acceptable as a practical method for measuring biopersistence in both inhalation and instillation assays, as used

*The need for a toxicokinetic study in primates was discussed to evaluate differences in particle retention patterns and responses in the lungs of rodents and humans. However, a primate study was not recommended since species extrapolation to humans would still be required, existing data in baboons indicate differences in antioxidant capacity compared to humans, and other approaches (e.g., quantitative comparison of existing rodent and human data) may be adequate to address this issue.

in the EU fiber testing protocol. However, because the potential of a fiber to be retained in the lungs is of concern for adverse health effects, it is the slow phase of clearance that is the scientifically appropriate measure for computing the retention half-time. It is recommended that studies report both values, and their confidence intervals.

RESEARCH NEEDS

- To validate in vitro tests and conditions with regard to their ability to predict in vivo inflammation by PSP.
- To develop toxicokinetic models to predict the steady-state lung burdens during long-term exposure from short-term experiments showing a NO(A)EL.
- To quantitatively compare exposure, dose, and response relationships for particles and fibers for which both human and rodent data are available, including evaluation of relevant dose metrics, interindividual variability, and model uncertainty.
- To evaluate interspecies differences and interindividual differences in humans of antioxidant defense mechanisms.
- To dissect out the role of inflammation in cancer caused by particles/fibers by exposing animals to the particle/fiber in a long-term exposure study and giving concomitant antiinflammatory steroids to inhibit inflammation.
- To determine the level of PMN influx required to attain sufficient oxidative stress to produce indirect genotoxicity, by exposing rats to quartz at various exposures to obtain differing levels of PMN influx and assessing the relationship between the PMN influx adduct formation (8-OH-DG) and HPRT mutations.

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