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TOXICITY AND OCCUPATIONAL HEALTH HAZARDS OF COAL FLY ASH (CFA). A REVIEW OF DATA AND COMPARISON TO COAL MINE DUST

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Abstract—Coal fly ashes (CFA) are complex particles of a variable composition, which is mainly dependent on the combustion process, the source of coal and the precipitation technique. Toxic constituents in these particles are considered to be metals, polycyclic aromatic hydrocarbons and silica. The purpose of this review was to study the *in vitro* and *in vivo* data on coal fly ash and relate the studied endpoints to the role of (crystalline) silica, considering its recent classification as a human carcinogen. For most of the effects coal mine dust was chosen as a reference, since it contains up to 10% of crystalline silica (α-quartz) and is well studied both *in vivo* and *in vitro*. Most studies on fly ash toxicity were not designed to elucidate the effect of its silica-content nor did they include coal mine dust as a reference. Taking this into account, both *in vitro* and *in vivo* experimental studies show lower toxicity, inflammatory potential and fibrogenicity of CFA compared to silica and coal mine dust. Although *in vitro* and *in vivo* studies suggest genotoxic effects of fly ash, the data are limited and do not clarify the role of silica. Epidemiological studies in fly ash exposed working populations have found no evidence for effects commonly seen in coal workers (pneumoconiosis, emphysema) with the exception of airway obstruction at high exposure. In conclusion, the available data suggest that the hazard of coal fly ash is not to be assessed by merely adding the hazards of individual components. A closer investigation of 'matrix' effects on silica's toxicity in general seems an obligatory step in future risk assessment on fly ashes and other particles that incorporate silica as a component.

INTRODUCTION

Fly ash particles are released into the environment in effluents from the combustion of fossil fuel and these particles provide a matrix for the interactions of a great variety of substances during the combustion of coal and the emission process. Combustion temperature is an important factor that determines the physical properties of the particles. At conventional, high temperature (> 1400°C) combustion, the major aluminosilicate melts and condenses to form small, spherical particles. Fluidized bed combustion (FBC) offers the potential of burning at lower temperatures (800–1000°C) and does not require additional fuel gas desulfurization. The fly ash particles that arise are mostly irregular in shape, but both types contain four major elements: aluminium, silica, iron, and calcium. Trace elements like metals (Ni, As, Cr, Pb, Cd) can differ between fly ash types and also between days and seasons.

Depending on their toxicity, chemical properties and concentration in the air, fly ash particles may pose an inhalation hazard to exposed workers. When fly ash particles are inhaled and deposited in the lung they can impose health risks by leaching genotoxic compounds, and through the alteration of immunological

mechanisms. More specifically, as the lung burden of particles increases, alveolar macrophages and epithelial cells become activated leading to the release of inflammatory mediators, reactive oxygen species (ROS), enzymes (elastase, proteases, collagenase), cytokines (TNFα, MIP-1) and growth factors (TGFβ) that control and stimulate fibrosis (Heppleston *et al.*, 1984; Borm, 1994; Vanhée *et al.*, 1995) and genotoxic events (Borm and Driscoll, 1996; Driscoll *et al.*, 1996, 1997). In this review some of these key-events will be discussed as markers of the toxicity or activity of coal fly ash in experimental systems. Studies are discriminated into experimental studies, including *in vitro* and animal research, and human studies.

The background of this review was provided by several meetings or reports that put forward crystalline silica as a non-genotoxic human carcinogen (DECOS, 1996; IARC, 1987). A renewed evaluation of silica's carcinogenicity by the International Agency for Research on Cancer (IARC) in October 1996 decided on crystalline silica (quartz, cristobalite) as a human carcinogen. This opens the general question of whether exposure to all dusts that contain crystalline silica should comply with new national threshold levels based on their silica content. No adequate evidence is available for the carcinogenicity of inhaled coal mine dust, despite the millions of coal miners exposed worldwide and the high levels of exposure, especially in the past. Coal mine dust is known to contain up to 10% quartz, but the quartz is part of a carbon matrix that clearly influences its intrinsic properties. Coal mine dust therefore seemed a good reference in the discussion and evaluation of the available data on coal fly ashes. No data on residual oil fly ash (ROFA) were reviewed since this type of fly ash contains (almost) no silica, and was therefore considered to beyond the scope of this risk evaluation. The general term coal fly ash is used throughout the document, since in the available literature it is not always clear which type of ash is involved.

METHODS

A search of available literature on fly ash was done in 1996 using references on CD-ROM (DOE Energy Science and Technology and CIMI) and on-line searches in the following databases: CAPLUS, RTECS, CSNB, BIOSIS, EMBASE, MEDLINE (STN International). The search was done retrospectively until 1980, using the items quartz, silica, silicon dioxide, silicates, fly ash(es), flyash(es), health, safety, toxic(ity) and equivalent terms in Dutch, French and German. Language restrictions were set to English, German, French and Dutch. Abstracts were screened by the author and the original publications retrieved from national libraries. TNO reports (R85/156; R83/182a and V 93-008) and an ICI report (Pigott, 1983) were provided by H. W. Hoeksema (EPON, Zwolle). The literature on coal dust was available in the authors' collection and complemented with an IARC-search on coal dust in all available literature. The data were reviewed with special emphasis on: documentation of treatment and exposure, type of ash, use of appropriate postive and negative controls, particle size data, data on silica (quartz) content, and effect parameters measured. The results are critically reviewed in a conventional approach discriminating experimental (*in vitro*, animal) studies and human studies.

NOMENCLATURE, CHEMICAL AND MINERALOGICAL CHARACTERIZATION OF COAL FLY ASH

The term fly ash is used both in the literature and in daily practice for the ash that remains after the complete combustion of any material. In many publications concerning properties, and sometimes toxic aspects, only the term fly ash is used and no more details are given. The best known combustion processes are municipal waste combustion and coal combustion, the latter for energy production. The properties of the fly ash generated during these processes are different and are illustrated by the concentrations of heavy metals. This review deals mainly with fly ash originating from coal combustion although (for the above reasons) the literature search was done with the keywords 'fly ash' and 'coal firing'.

Coal can be fired in different ways including stoker fired (in grid boilers) combustion, in fluidized bed combustion (FBC), as pulverized coal in dry-bottom boilers, slag-tap boilers and cyclone boilers. To a large extent the type of boiler influences the properties of the fly ash. A second important parameter is the flue gas cleaning equipment, especially the particulate control devices such as the hot- or cold-side electrostatic precipitator ESP, wet scrubbers, baghouses etc. Both the size and the chemical composition of the fly ash, therefore depend upon the type of boiler and the type and degree of efficiency of the control devices. Of course the coal itself is also a major parameter that determines fly ash properties by its degree of carbonization, geological origin, and previous washings.

In The Netherlands, the energy sector uses only pulverized coal-fired dry bottom boilers. In all cases fly ash is removed from the flue gases by high efficiency cold-side electrostatic precipitation (ESPs). Furthermore, in all cases additional flue-gas cleaning is established using wet flue-gas desulphurization (FGD) of the lime/limestone–gypsum process. The coal used is bituminous and is from mainly the U.S.A. (eastern), Australia, Columbia, South Africa, Indonesia and to a lesser extent Venezuela, China, Poland. It has an average ash content of 11%. At this type of power plant, three or four types of ash stream need to be considered, each with its characteristic properties. The most important ash stream, making up about 88% of the total, is the ash removed from the flue gases in the ESP, specified as ESP-ash or pulverized fuel ash (PFA).

PFA is spherical (solidified droplets), and the average particle size is characterized by $D_{(m)}10 = 5$ μm, $D_{(m)}50 = 20$ μm and $D_{(m)}90 = 60$ μm. PFA consists of 60–80% amorphous silicon–aluminium–glass with small amounts of mullite ($Al_6Si_2O_{13}$) and α-quartz (SiO_2) (Si: 23.1% and Al: 13.8%). Other major components are iron (4,2%), calcium (2,4%) and unburned carbon (few per cent). Typical average concentrations of trace elements are arsenic 36 ppm, barium 1503 ppm, bromine 4 ppm, cadmium 0.9 ppm, cobalt 47 ppm, chromium 149 ppm, copper 115 ppm, fluorine 145 ppm, mercury 0.5 ppm, molybdenum 22 ppm, nickel 91 ppm, lead 59 ppm, antimony 5 ppm, selenium 15 ppm, vanadium 231 ppm and tungsten 163 ppm. Secondly, about 12% of the ash is removed at the bottom of the boiler and this type of ash is labelled bottom ash (sometimes called slag). The ash is coarser and relatively low in heavy metals with respect to the PFA. The third source of ash is the proportion that remains in the flue gases downstream of the ESP (about 0.1%) and which is released into the air via the chimney if no other flue gas cleaning

equipment is present. This is labelled fly ash. Fly ash is finer than PFA and the concentrations of heavy metals are relatively higher. When an FGD-plant is present (standard in many countries) another 90% of the ash is removed from the flue gases and the remaining dust which is released into the air is labelled fly dust. Fly dust is finer than fly ash and also consists of gypsum particles.

IN VITRO STUDIES

Red blood cells, haemolysis

Red blood cell haemolysis has been used for decades as a simple test for indicating mineral dust toxicity and surface activity. Using red blood cells as a target it is well-known that all crystalline silica varieties induce haemolysis in the range of 1–15 mg/ml. Studies with coal-mine dusts in this system have shown varying results. Haemolysis by coal mine dust from low-rank mines in the U.K. did not correlate with its total or individual components, while lysis by dust from high-rank pits increased with the amount of noncoal minerals and quartz present but not with the kaolin or mica levels (Gormley *et al.*, 1979). Seasonal variations in fly ash constituents, including silica and metals, were not correlated with their haemolytic activity (Srivastava *et al.*, 1986). CFA (Si-content, 53%) haemolytic activity (EC_{50}, 1 h: 5.5 mg/ml) was about one fifth that of pure silica (EC_{50}, 1 h: 1.0 mg/ml) at equal particle size (Liu *et al.*, 1986). Suprisingly, calcium-rich CFA (and low silica, 34%) induced similar haemotoxicity in red blood cells compared to pure silica (Liu *et al.*, 1987). The authors of the latter study checked for a possible effect of pH change of this Ca-enriched CFA. A number of fly ash samples from thermal power stations in India (< 5 μm) all showed similar haemolysis (10–15% at 16 mg/ml) compared to quartz which gave 50% hemolysis at 2 mg/ml (Kaw *et al.*, 1988). No data on the quartz content of these dusts were given. However, results from haemolysis are in general poorly correlated with results of cytotoxicity in macrophages (Gormley *et al.*, 1979). In addition, extensive studies in the coal mining industry revealed a poor correlation between the outcome of this test and various measurements of pneumoconiosis risk (Reisner and Robock, 1975; Davis *et al.*, 1983; Bruch and Rehn, 1994).

Alveolar macrophages or other phagoctytic cells

Both fresh macrophages from different animal species (rat, guinea-pig, dog, rabbit, bovine, hamster) and a cell line of Chinese hamster ovary cells (CHO) have been used in cytotoxicity assays of coal fly ashes and various coal mine dusts, using proper positive (for example quartz) and negative controls (for example TiO_2). Gormley *et al.* (1979) conducted a study with coal dust using P388D1 cells, viability counts (trypan blue exclusion) and several biochemical indices of cytotoxicity (release of lactate dehydrogenase LDH, glucosaminidase, lactic acid, total protein) and failed to demonstrate a correlation between quartz and cytotoxicity. Actually, they demonstrated that the role of rank and non-coal mineral content was more important. Similar studies with model particulate compounds and fly ash particles were done using rabbit alveolar macrophages (RAM) and Chinese hamster ovary cells (CHO). Garrett *et al.* (1981) tested silica, silicic acid, titanium oxide and size-

fractionated fly ash particles with and without coating with various metal oxides, and found silica to be highly toxic (EC_{50} in viability-assay: 0.1 mg/ml) and uncoated fly ash relatively non-toxic ($EC_{50} > 5$ mg/ml) in RAM. However, a marked toxicity of uncoated fly ash was seen in the CHO clonal assay at 1 mg/ml. In general, coating with metal oxides increased the toxicity in both assay methods (Garrett *et al.*, 1981). Using three different assays for cell function in RAM and CHO cells, Mumford and Lewtas (1982) showed that FBC fly ash (6% silica) was more toxic than fly ash obtained by conventional combustion (13.7% silica). Typically, in their study the CHO cells were less senstive to fly ash toxicity than RAM. Unfortunately, interpretation of these data is hindered by the fact that the fly ash samples were of different particle sizes (FBC contained smaller, < 1 μm, particles), no silica reference was studied, and the FBC ash came from an experimental miniplant and therefore was not representative.

Another *in vitro* study using (bovine) alveolar macrophages compared different fly ash samples to three coal dust samples using appropriate positive (Min-U-Sil and quartz) and negative (glass beads) controls (Hooftman and Arkesteijn, 1983; Hooftman *et al.*, 1985). Using cell survival, leakage of lactate dehydrogenase (LDH) and phagocytosis as tests for cell viability and function, Mount St Helens fly ash and glass beads were relatively inert. All fly ash samples ($n = 8$) from a plant in Apeldoorn (NL) showed similar data, that is a significant effect on macrophage phagocytosis at 0.1–0.3 mg/ml in the absence of lethality. Less toxicity was seen in two fly ashes sampled by electroprecipiation in a powder-coal combustion plant from Nijmegen (NL). Among the positive silica-controls α-quartz (DQ12) was more toxic than Min-U-Sil, both EC_{50}-levels for survival not exceeding 0.03 mg/ml. Monolayers of quinea-pig macrophages showed significant loss of viability (LDH release, dye uptake) after incubation (3 h, 20 h) with 300 μg but not 75 or 150 μg of fly ash (Kaw *et al.*, 1988). However, no differences were seen among samples from four different thermal power stations in India, and quartz exerted significantly higher toxicity than fly ashes. Unfortunately, the data in this study do not allow extrapolation to other *in vitro* studies (mg/ml) or *in vivo* work. In a similar approach Hill and Hobbs (1982), using dog alveolar macrophages, showed no differences in toxicity between fly ashes from a number of coal combustors and types of coal. The tested types included three types of pulverized coal combustor (all ESP), two fluid bed combustors (Baghouse) and one stoker-fed combustion (ESP), the positive control used was quartz. Whereas quartz reached 50% toxicity at 100 $\mu g/10^6$ cells, coal ashes induced only 5% viability loss at this level. Similar results were obtained by incubation of RAM with ex-Australian coal fly ash supplied by Dutch PGEM at different samples sizes (< 5, < 10 μm), but with similar α-quartz content (18, 15%). Using dye exclusion, after 2 h exposure to fly ash (5–25 mg) 'inert-type dust responses' were found (Pigott, 1983).

Genotoxicity assays

The *in vitro* mutagenic potential of fly ashes has been evaluated mainly in the Ames test and in human blood lymphocytes (SCE, aberrations). Most studies show that the ESP fraction fly ash from pulverized coal combustion is not mutagenic, but that other fractions can contain mutagenic activity, which is related to ash content of (nitro-containing) aromatic hydrocarbons (Fisher *et al.*, 1978; Harris *et al.*, 1984).

A marked mutagenicity of FBC ash (6% silica) but not of CC fly ash (13.7% silica) was observed in the Ames test (TA98, TA1538) between concentrations of 3 and 20 mg fly ash or 20 and 600 μg of extractable mass (per plate). The mutagenicity of FBC ash was enhanced by metabolic activation and/or solvent extraction and therefore was concluded to be due to organic compounds absorbed on the surface (Mumford and Lewtas, 1982). Later studies using the more sensitive tester strains TA97 and TA102 observed a minor mutagenic activity of PCC fly ash (median size: 2.4 μm, silica content unknown). Lytag-dust (PCC fly ash: humidified, heated to 1100°C), however, caused marked mutagenic effects in TA97 and TA102 (Kleinjans *et al.*, 1989) at concentrations of between 1 and 10 mg per plate, while no mutagenic activity was detected in the extracts of either dust. The same study showed a significant increase of SCE in human blood lymphocytes after 48 h incubation with Lytag and PCC fly ash (ECP fraction) at 0.01 mg/ml. Interestingly, the number of SCEs was also increased in workers exposed to Lytag dust (no exposure data) but the effect was only significant in non-smokers (9.5 SCE/cells in exposed vs 8.0 SCE/cell in controls).

Formation of reactive oxygen species (ROS) in guinea-pig alveolar macrophages by fly ash and other pollutants was measured by Hatch *et al.* (1980), showing that uncoated fly ash (2–5 μm) was relatively inactive (155% of control) compared to latex microspheres (400%), silica (1180%) and crocidolite asbestos (2600%). Adverse effects of ROS in the lung may include (1) damage to cell membranes through lipid peroxidation, (2) oxidation of proteins, and (3) DNA damage. Oxidative DNA damage may play a role in carcinogenesis, most likely through hydroxyl-radicals formed in Fenton-like reactions (Janssen *et al.*, 1993). Basically, two mechanisms by which mineral dust exposure causes formation of ROS have been demonstrated. First, direct formation of ROS through the intrinsic properties of particles such as silanol groups on the surface of silica, surface charge properties (Brown and Donaldson, 1989) and the iron content of asbestos fibers. Also mechanical processes, such as the grinding and cleavage of dust, including coal dust, are believed to result in the generation of radicals on 'fresh' surfaces (Dalal *et al.*, 1989, 1995). Since fly ash is known to contain trace metals that can induce Fenton-like reactions, and these metals vary considerably from sample to sample, the negative results from this study should be interpreted with caution. A second, indirect pathway for the excessive formation of ROS is by the oxidative burst of macrophages and neutrophils during particle phagocytosis and inflammation. However, no data were found on the ROS production of AM or PMN (*ex vivo*) after exposure to coal fly ash.

IN-VIVO ANIMAL STUDIES

Coal dust and fibrosis

Early studies by Ray *et al.* (1951) were aimed at determining the effects of coal dust (or graphite) and supplemented α-quartz (2–40%) in rats after intratracheal doses of 100 mg of each dust. They observed typical fibrotic lesions and concluded that coal dust (anthracite) had no inhibitory action on quartz-induced fibrosis. Later studies typically using intratracheal administration of 50 mg coal dust in rats

confirmed that coal dust was less fibrogenic than quartz or hard rock dust, but did suggest that coal dust had an attenuating effect on the quartz-induced effect. An intratracheal dose of 50 mg coal dust containing 4, 7 or 18% quartz showed significant development of fibrosis 3–18 months after installation, whereas the high-quartz dusts (7 and 18%) always led to more fibrosis (Martin *et al.*, 1972). Rosmanith *et al.* (1982) injected 50 mg of each of 30 different coal mine dusts into rats. The study showed focal or diffuse fibrosis in parenchyma and lymph nodes in five groups of rats at 6 and 12 months after administration. The corresponding samples were those that caused the highest dust and ash content in the lymph nodes. A similar i.t. dose (50 mg) of coal dust supplemented with quartz to 10% (final ratio) caused an increase in the numbers of cells in the tracheobronchial lymph nodes of rats after 90 days follow-up (Sahu *et al.*, 1988).

Several subchronic and chronic inhalation studies have evaluated the pathologic changes associated with the experimental exposure of rats and some other species to coal dust. SPF–Wistar rats exposed for 20 months (6 h/day, 5 days/wk) at levels of 6.6 and 14.9 mg/m^3 developed lesions similar to simple CWP in humans (Busch *et al.*, 1981). No advanced lesion types (micro- or macronodules, infective granulomas) were observed in these animals, but focal bronchiolization occurred after 20 months of exposure. The importance of quartz in coal dust fibrogenicity was demonstrated by Ross *et al.* (1962) and Martin *et al.* (1972) who exposed rats to different coal-quartz mixtures. Fibrosis was apparent in all groups exposed to coal dust (300 mg/m^3, 6 h/day, 5 days/wk, 3 months) supplemented with quartz, but only at 18 months for the lowest concentration (4%). With higher quartz levels (7 and 18%) collagen formation was already increased at 6 months, and above 10% nodules appeared and collagen production was five times greater than with coal alone (Martin *et al.*, 1972). Previously similar experiments using rats at dust levels of 60 mg/m^3 (16 h/day, 10 months) and quartz percentages from 5 to 40%, showed little fibrosis in the rats exposed to mixtures with 5 and 10% quartz (Ross *et al.*, 1962). However, rats exposed to 20 and 40% quartz–coal mixtures had at the end of exposure considerable pathology in fibrosis-grade and collagen content. Both parameters appeared to be correlated with the total silica remaining in the lung after exposure (420 days).

Coal fly ash and fibrosis

Few inhalation studies have been done using (coal) fly ash. MacFarland *et al.* (1971) and Alarie *et al.* (1975) generated fly ash aerosols for exposure of monkeys and rodents and reported no unique biological effects. Raabe *et al.* (1982) exposed rats to two concentrations of fly ash (0.6 mg/m^3 and 4.25 mg/m^3) for 8 h/day for up to 180 consecutive days, and found no health effects in the lower exposure group and only minor effects in the high exposure group. These effects included more and larger macrophages in BAL, and minor changes in glycoprotein secretion (mucus), or viability, adherence and phagocytic index of macrophages. The authors conclude that these mild responses are natural to inhaled particles and not unique to coal fly ash (Raabe *et al.*, 1988), although no negative or positive controls were studied. Another inhalation study was done by Bice *et al.* (1987) who exposed male and female F344 rats for 20 days (4 weeks, 5 days/week, 7 h/day) to levels of 36–38 mg/m^3 of α-quartz, CFA from a fluidized bed combustor (FBC) or CFA from a

pulverized coal combustor (PCC). The data indicate that the lung-associated lymph nodes accumulated fly ash and quartz particles, that were cleared in the lung lymphatics, resulting in an increased number of cells in these tissues. Although the (massive) exposure was only short (4 weeks), the increased number of cells was still seen at 52 weeks both for CFA and quartz. At this time point, however, the lungs from rats exposed to FBC fly ash had minimal lesions. The lesions from rats exposed to PCC fly ash were more severe and included some small granulomas and refractive particles in epitheloid cells. The lesions were most severe in quartz-exposed rats, with granulomas up to 0.5 mm scattered throughout the lung and associated with the typical interstitial inflammation and fibrosis (Table 1).

Various studies have used intratracheal administration of coal fly ash, although this technique easily leads to (initial) overload and, therefore, should be interpreted to identify the hazards from the administered agent. An excellent study is described by Schreider *et al.* (1985) using intratracheal installation of three different doses (5, 15 and 45 mg) of six types of dust, including three different coal fly ashes (Schreider *et al.*, 1985) with data on particle size and elementary composition. After 90 days the most severe fibrosis was found in quartz-treated rats (Sprague–Dawley, 300 g), followed in order of intensity by, heated clay, Mount St Helens volcano ash, hopper-collected CFA, stack-collected CFA and baghouse filter-collected coal–oil mixture fly ash (COM). In hopper CFA mild interstitial inflammation and fibrosis was seen at the low- and mid-dose (5 and 15 mg), and clear fibrotic lesions were seen only after the high dose. Stack CFA showed no fibrosis at the lower dose and multifocal, moderate fibrosis at mid- (15 mg) and high (45 mg) doses. COM fly ash showed minimal to mild accumulation of macrophages, thickening of alveolar septa and mild, loose fibrosis. The fibrotic activity of the stack and hopper CFA were found to be approximately equal, in line with their total silica content of 26.8 and 26%; however only 1.3 and 1.7% of the total amount is quartz, the rest are alumina-silicates. COM contained 19.2% silica, but no data on quartz were given. The authors relate the lower activity of the COM fly ash to the abundance of large particles (Schreider *et al.*, 1985).

A single administration of 30 mg of fly ash from an experimental fluidized bed combustor (FBC) to Syrian Golden hamsters was carried out by Lantz and Hinton (1986), with morphometric analysis of the lungs 1, 3, 6, 9, and 30 days post-administration. They observed an initial (<9 days) recruitment of PMNs and AM which returned to control levels at 30 days post-administration. At this point, however, an increased volume of the noncellular interstitium was observed. Unfortunately, no histology or biochemistry was done to confirm interstitial fibrosis. Intratracheal adminstration of 2, 10 and 50 mg fly ash (low-temperature combustion) to male Wistar rats, using silica (Min-U-Sil, 5 μm) and TiO_2 as positive and 'negative' controls, was reported by Arts (1993). No significant differences between the effects of fly ash and TiO_2 in general were detectable up to 4 weeks post-administration, except for: (i) a dose-related increase in granulocytes at days 14 and 28 after inoculation of fly ash, and (ii) a small increase in lymphocyte numbers in the high dose (50 mg/kg) CFA on day 28. The histopathology did not show fibrosis. From the data, the author suggests a non-observed effect level for CFA of 2 mg/kg, corresponding to a 14 mg/m^3 exposure level in man (Arts, 1993).

An interesting report, which is only useful for the ranking of acute toxicity of

Table 1. Overview of *in vivo* animal studies either using inhalation or intratracheal administration of coal fly ash (CFA)

Type ash[a]	Exposure	Species	Effect(s)	Ref
CFA, not specified	8 h/day, 180 days 0.6 and 4.25 mg/m^3	Rat	Mild response, No fibrosis	Raabe *et al.*, 1982
CFA (FBC and PCC)	4 weeks, 5 days/week, 7 h/day 36–38 mg/m^3	Rat (F344) male/female	Chronic cell influx Sil > PCC > FBC	Bice *et al.*, 1987
CFA (2 types) and COM	Intratracheal (5, 15, 45 mg) quartz positive control	Rat (SD)	Fibrosis at 15 mg Mild inflammation all doses of CFA. COM less toxic.	Schreider *et al.*, 1985
CFA (FBC)	Intratracheal (30 mg)	Hamster (SG)	Transient (30 days) recruitment of PMN and AM	Lantz and Hinton, 1986
CFA (PFA)	Intratracheal (2, 10, 50 mg) TiO_2 and quartz as controls	Rat (Wistar) male	Dose-related influx of PMN, NOEL of 2 mg/kg	Arts, 1993
CFA	Intratracheal (10 mg), other dusts incl. quartz as controls	Rat (Wistar) female	Ranking: quartz > CFA > mica	Bajpai *et al.*, 1992[b]
CFA (PCC?)	Intratracheal (12.5 mg), 60 days; quartz controls	Rat	No fibrosis; dust laden AM and cells dusts including quartz	Kaw *et al.*, 1988

[a]FBC: fluidized bed combustion; PCC: pulverized coal combustion; COM: coal oil mixture fly ash; PFA; pulverized coal fuel ash; NOEL: No observed effect level; AM: alveolar macrophages; PMN: polymorphonuclear neutrophils.
[b]Fibrosis was not studied as an endpoint.

industrial dusts, is by Bajpai *et al.* (1992), who studied several markers of damage in bronchoalveolar lavage (BAL) of female Wistar rats (165 g) instilled with 10 mg of various dusts. After 8 days BAL was recovered and markers of toxicity (LDH, total protein, β-glucuronidase) were determined. The dusts tested were ranked in the following order of fibrogenic potential: quartz > CFA > mica > corundum. A few years earlier the same research group (Lucknow, India) published some papers on the modifying capacity of CFA on the fibrogenic action of quartz (Kaw and Khanna, 1988; Kaw *et al.*, 1990). After 60 days a single dose of CFA (intratracheal, 12.5 mg) alone induced mostly intracellular dust without much change in morphological characteristics, although dust-laden macrophages were seen in lymphoid aggregrates surrounding the blood vessels and airway walls. Interestingly, in rats first exposed to silica (10 mg, 60 days) fly ash had an attenuating effect on the fibrogenicity of quartz. These data are less relevant from the point of view of risk assessment, but should be interpreted as a contribution to mechanisms involved in pulmonary defence. In fact, this model could be similar to the attenuation of inflammation and fibrosis in mice, when administered silica and FMLP, a protein chemotactic for PMN (Adamson *et al.*, 1992)

Effects on immune system and inflammatory cells. Studies aimed at the mechanism of action of coal dust and quartz have focused on inflammatory cells or precursors from bronchoalveolar lavage (BAL) and spleen after *in vivo* exposure of animals to coal dust and/or silica. Many studies done using coal dust show an increase in the number of alveolar macrophages and neutrophils. However, the duration of this effect seems to be strongly dependent on the exposure-route and regimen, and the total dose. In rats exposed to 10 mg/m^3 coal dust (7 h/day, 5 days/week, 32 days) the number of neutrophils and lymphocytes is still increased (15 vs 0.5%) 64 days after recovery, while the total cell number is back to normal (Brown and Donaldson, 1989). However, after a single intratracheal instillation (Adamson and Bowden, 1978) the AM-yield increases for the first 6 days after instillation, returning to control levels by 28 days, while neutrophils increased after 1 day and return to normal after 3 days. The NIOSH long-term inhalation study with coal dust and/or diesel (2 mg/m^3, 7 h/day, 5 days/week, 2 yr) resulted in a chronic elevation of AM in rats (Castranova *et al.*, 1985), no effect on influenza infection in mice (Hahon *et al.*, 1985) and failed to demonstrate any effects on immunocompetence. AM function, measured by phagocytosis and bacterial killing was shown to be affected in rats after inhalation exposure to two coal dust types (Utah, Pensylvania) at levels of 2 mg/m^3 (6 h/day, 5 days/week, 4 months) (Bingham *et al.*, 1975).

Typically, few studies have investigated the functional properties of alveolar macrophages after inhalation or intratracheal inoculation of coal fly ash. After intratracheal administration of quartz, mica, corundum or coal fly ash to rats (50 mg/kg, 8 days), animals were sacrificed, the lungs were perfused and cells harvested (Dixit *et al.*, 1990). CFA caused a significant cell-influx (three-fold increase) compared to saline-instilled rats, whereas silica caused a five-fold increase in total cells. Phagocytosis and adherence of AM, as well as the appearance of antibody forming cells in lymph nodes were moderately but significantly affected by *in vivo* exposure to both fly ash and silica (Kaw *et al.*, 1988; Dogra *et al.*, 1995). Inhalation studies with Syrian Golden hamsters using silica (Min-U-Sil, 1 mg/m^3,

2 weeks) or fly ash (CFA, 1.6 mg/m^3, 6 weeks) showed a depressed ability of alveolar macrophages from BCG-primed and rechallenged hamsters to mediate *ex vivo* tumor cell lysis. Moreover, fly ash, and not silica, suppressed the ability of BCG-activated macrophages to lyse target cells through antibody-dependent cell-mediated cytotoxicity (Burns and Zarkower, 1982). Acute inhalation exposure of mice (C57BL/6) to fly ash or silica (200 mg/m^3, 100 min) showed a significant depression of *ex vivo* AM phagocytosis after 6 and 15 days of exposure. Although macrophage colony-forming efficiency was initially depressed (2 days), a marked increase was seen at day 15. In summary, their data showed recruitment of blood cells into the lung. Inhalation of fly ash (15 days, 6 h/day, 0.27 mg/l) significantly elevated pulmonary and hepatic (surfactant) lipids at 1 and 15 days after cessation of exposure (Chauhan and Misra, 1991).

HUMAN DATA

Pneumoconiosis

Diseases caused by coal (mine) dust exposure have been reviewed previously (Heppleston, 1992; Wouters *et al.*, 1994). Apart from simple coal workers' pneumoconiosis (CWP), characterized by the presence of small opacities (< 10 mm) on the chest X-ray (ILO, 1980), complicated CWP (PMF, progressive massive fibrosis), pleural abnormalities, emphysema, chronic bronchitis, accelerated lung function loss, lung and stomach cancer have also been reported in (ex) coal miners and some occupations other than mining. CWP and PMF are highly correlated with estimates of cumulative dust exposure or dust components remaining in the lung (Hurley *et al.*, 1982, 1987; Attfield and Seixas, 1995). The amount remaining is the net result of deposited dose minus long-term clearance. Several post-mortem studies have been done in which the whole lung is digested or ashed and the total or specific dust in the lung measured (Nagelschmidt *et al.*, 1963; Rossiter, 1972; Douglas *et al.*, 1986). These studies show that in coal workers, 40–60 g of total dust may be found in the lungs, and suggest that the lung dust burden is not simply a reflection of cumulative exposure, but that deposition and/or clearance might be fine-tuning factors. No generalization can be made about the effects of quartz content and coal rank on the induction of fibrotic endpoints (review: Heppleston, 1988) and the no-CWP-level of between 50 and 100 mg yr/m^3, is associated with an 2 mg/m^3 coal dust limit in a number of countries (U.S.A., Germany). Data on the pneumoconiotic effects of coal fly-ash can be derived from studies done in U.K. workers employed by the electricity supply industry throughout 1950 to 1977, and reviewed by Bonnell *et al.* (1980). Studies in more than 1465 workers in power stations in South Wales and the Midlands have failed to demonstrate the existence of pneumocionosis caused by working in coal-combusted power plants, other then in men with previous exposure in underground coal mining. Two of these studies were surveys of entire populations of power stations and boiler makers (1960–1961). Two others concentrated on 'high-risk groups', meaning those employed in dusty environments, as identified by station managers. Although no firm deductions can be made from these studies, since they were done asystematically and on a voluntary basis, the (total) number of screened workers

and the (negative) outcome do not argue for a high fibrogenic potential of CFA. Interestingly, a recent case-report (Cho *et al.*, 1994) documented fairly well a man with acute silicosis following a massive, accidental, exposure to fly ash when (un)loading his truck. Both chest-X ray and CT-scan, supported by open lung biopsy showed silicotic nodules. A disease course like this is usually only seen after massive exposure to ground silica.

Chronic obstructive pulmonary disorders (COPD)

Another characteristic, though controversial, component of simple coal dust lesions is localized (focal) emphysema. Post-mortem analyses of coal miners lungs have demonstrated an association between dust exposure (Ruckley *et al.*, 1984) and dust content (Leigh *et al.*, 1994), but failed have to reveal the role of silica and pre-existing dust-related fibrosis. The basic mechanism suggested is the protease–antiprotease balance in which activated neutrophils and coal dust play a role through their release of oxidants inactivating α1-antitrypsin on the one hand (Zay *et al.*, 1995; Li *et al.*, 1997) and releasing elastases/proteases on the other (Rom, 1990; Huang *et al.*, 1993). No data are available that show the ability of fly ash to induce emphysema in humans.

Chronic bronchitis and airflow obstruction are common effects of inorganic dust exposure in the workplace (review: Oxman *et al.*, 1993). The extra loss of lung function has been estimated from both cross-sectional and longitudinal studies to be somewhere between -0.5 and -1.2 ml FEV_1 per g h/m^3 of exposure which is equivalent to 40–100 ml at current standards of 2 mg/m^3. Chronic bronchitis is also increased among smoking and non-smoking coal miners and is associated with a greater loss of FEV_1 (Rogan *et al.*, 1973; Marine and Gurr, 1988). Respiratory effects of prolonged exposure to pulverised fuel ash (PFA) were shown by Schilling *et al.* (1988), in a survey among 268 men with a history of more than 10 yr exposure to PFA. The workers were grouped using their occupational histories into high-, medium- and low exposure categories. Lung function tests showed a modest effect on FVC, FEV_1, PEF and gas transfer, associated with heavy exposure to PFA. Workers with prolonged heavy exposure also showed higher prevalence of all respiratory symptoms. This study suggests that prolonged inhalation of PFA caused effects in line with other inorganic dusts (Oxman *et al.*, 1993) including coal dust.

Genetic risks

Although several fly ashes, and also nitrosated coal dust, have been described as exerting genotoxicity in bacterial and human cells *in vitro*, few data are available on the *in vivo* genotoxicity of coal dust- or coal fly ash-exposed workers. In a study among 38 retired coal miners and 24 age-matched controls, oxidative DNA-damage was determined in blood lymphocytes (by the ratio of 7-hydroxy-8-oxo-2′-deoxyguanosine versus deoxyguanosine) and was found to be significantly higher in coal miners (Schins *et al.*, 1995). Cumulative exposure of these miners was between 93 (reference miners) and 145 g h/m^3 (miners with CWP), but no difference in oxidative damage was seen between these groups. These data can be explained by the presence of coal dust containing stable coal dust radicals (Dalal *et al.*, 1989) that could affect the circulating lymphocytes' DNA. In workers exposed to fly ash increased frequencies of chromosome aberrations have been observed (Bauman and

Horvat, 1981; Léonard *et al.*, 1984), although radioactivity seems the main source of risk. Two other studies have described genetic effects in a population of workers exposed to Lytag-dust (a humidified and heated ECP-collected fly ash from pulverized coal combustion). In a first survey, the frequency of sister-chromatid exchanges (SCE) was compared between 22 exposed workers and 22 controls (from the flour-processing industry), and the frequency of SCEs was different (that is, higher in Lytag workers) between the non-smokers in both groups (Kleinjans *et al.*, 1989). After exposure-reducing measures, a follow-up of 18 Lytag workers and 18 controls (from the same groups) was carried out using SCE and micronucleus frequencies, as well as mutations in the HPRT gene, as effect parameters in blood lymphocytes (Stierum *et al.*, 1993). No differences in these markers were detected between the groups, although it was noted that a downward drift of SCE counts had occurred over the years. Unfortunately no exposure data are listed in either study, limiting their use for risk assessment or comparison with coal miners.

DISCUSSION

Coal fly ashes constitute complex particles with associated chemicals, which are not always homogeneous and depend on the combustion proces, source of coal and the type of precipitation. Unfortunately, not all studies on coal fly ash have documented these variables to allow comparison of different toxicity studies. Nevertheless some general trends are noted in the work on fly ashes.

First, *in vitro* studies show that coal fly ash (CFA)—independent of type of coal combustion, origin or precipitation—exerts cytotoxicity in a number of conventional tests using either animal lung cells, human red blood cells or cell lines such as hamster ovary cells. In general, CFA is less toxic than crystalline silica (when used as positive control) but significantly more toxic than negative controls (TiO_2, latex beads, methacrylate-polymers). *In vitro* data do not support the importance of the silica content in toxicity as demonstrated in similar studies using samples of coal dust (Davis *et al.*, 1982; Robock and Reisner, 1982). However, it should be mentioned that studies with fly ashes have not really adressed this hypothesis. An obvious reason for this is that silica's potential carcinogenicity only became known in 1987, when most of the *in vitro* studies on coal fly ashes had already been carried out. Both *in vitro* and *in vivo* studies indicate slight differences between various types of coal fly ashes. The data suggest a moderately higher fibrogenicity of pulverized coal combustion compared to ash from fluidized bed combustion; however, the latter showed mutagenic activity *in vitro*, probably due to associated polycyclic hydrocarbons. Apart from the relevance of such a comparison, the poor description of fly ashes in many studies at least questions the reliability of such comparison.

Almost no *in vitro* or *in vivo* data are available on the effect of CFA on markers recently demonstrated to be crucial in dust toxicity, including pro-inflammatory cytokines (TNF, IL-8), growth factors (TGFβ, fibronectin) or the generation of reactive oxygen species (Piguet *et al.*, 1991; Janssen *et al.*, 1993; Vanhée *et al.*, 1995; Schins and Borm, 1995). In contrast, these studies have been carried out with other fly ashes including residual oil fly ash (ROFA) and particles including coal mine dust, silica and others. It should be stated, however, that most studies using ROFA applied this as a model particle for PM10, PM2.5 or total suspended particles in

relation to environmental air pollution (Lindroos *et al.*, 1996; Meng *et al.*, 1996). Interestingly, these studies have shown that ROFA are able to induce growth factors (TGFβ, PDGF), cytokines (IL-1) and reactive oxygen species *in vitro* and upper airway inflammation *in vivo* (Hauser *et al.*, 1995). Associated soluble transition metals such as vanadium, nickel and iron seem to play an important role in these effects (Dreher *et al.*, 1997). Factors investigated in the ROFA studies as well as other mineralogical factors that can affect the biological effects of silica (Guthrie, 1995) should be studied in fly ashes in order to better understand the toxicity and health risks. *In vivo* exposure of various animal species to coal fly ashes shows only very mild to moderate fibrosis, and in rats the 'no observed effect' levels are between 2 and 10 mg/kg (intratracheal dose) and 4 mg/m^3 (inhalation). The observed pathology is similar to other 'nuisance' dusts in the same dose regimen. Unfortunately, no *in vivo* studies on CFA have used coal dust as a reference dust. Such studies would allow comparison of (crystalline) silica content in both types of dust. However, *in vivo* data suggest that silica is less fibrogenic in inhaled CFA than in coal mine dust (Martin *et al.*, 1972; Bice *et al.*, 1987). Extensive human epidemiological studies on fly ash exposed populations are lacking, but studies based on U.K. electricity workers fail to show any convincing evidence of pneumoconiosis other than in ex-coal miners or emphysema. However, these studies do show lung function impairment and respiratory symptoms in workers with prolonged, heavy (>5 mg/m^3) exposure to CFA. This is, however, an effect which is also caused by other inorganic dusts at prolonged, high exposure. Other small scale *in vivo* studies have indicated a possible genetic risk in workers exposed to fly ash or its products, based on *ex vivo* genotoxicity assays (Kleinjans *et al.*, 1989). However, these data are not confirmed by an increased specific cancer mortality among coal fly ash exposed workers.

In conclusion, although most studies have not been designed to test fly ash toxicity in relation to its content of crystalline silica, there are no available data that suggest that coal fly ash is merely an addition of (crystalline) silica and other components. There is minimal knowledge on the effect of the mineralogical properties of fly ash on the activity of (crystalline) silica. The lack of evidence for fibrosis in humans exposed to fly ash combined with the strong association between silicosis and lung cancer (McDonald, 1995) in silica-exposed cohorts also forwards a careful evaluation of other silica-containing materials. In the context of silica's recent denomination as class 1 carcinogen by the IARC and the consistently negative data for coal mine dust as a lung carcinogen, a closer investigation of 'matrix' effects, masking silica's toxicity in particles with complex composition, seems obligatory.

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