Toxicological Assessment of Respirable Fibre-shaped Particulates (RFP) derived from p-ARAMID

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AED	aerodynamic equivalent diameter
AEL	acceptable exposure limit
ALP	alkaline phosphatase
ATP	adenosine triphosphate
BAL	bronchoalveolar lavage
β-gal	beta-galactosidase
BrdU	5-bromo-2-deoxyuridine
CKSCC	cystic keratizining squamous cell carcinoma
CKSCT	cystic keratizining squamous cell tumour
D_LCO	carbon monoxide diffusing capacity
DNA	deoxyribonucleic acid
FEV_1	forced expiratory volume in 1 second
FVC	forced ventilatory capacity
GC	gas chromatography
GM	geometric mean
LDH	lactate dehydrogenase
LOAEL	lowest observed adverse effect level
LOEL	lowest observed effect level
MMAD	mass median aerodynamic diameter
MMOF	man-made organic fibres
MMVF	man-made vitreous fibres
MS	mass spectrometry
MTD	maximum tolerated dose
NAG	N-acetyl-beta-glucosaminidase
NOAEL	no observed adverse effect level
NOEL	no observed effect level
ODC	ornithine decarboxylase
OEL	occupational exposure limit
PAF	para-aramid fibres
PCNA	proliferating cell nuclear antigen
PCOM	phase contrast optical microscopy
PKC	proliferative keratin cyst
RFP	respirable fibre-shaped (or 'fibrous') particulate (or 'particle')
SEM	scanning electron microscopy
STEL	short time exposure limit
TEM	transmission electron microscopy
TGA	thermogravimetric analysis
TWA	time weighted average
UICC	Union Internationale contre le Cancer
VME	valeur limite de moyenne d'exposition

1. SUBSTANCE IDENTIFICATION, PHYSICAL- AND CHEMICAL PROPERTIES

1.1 SUBSTANCE IDENTIFICATION

1.1.1 Name and Synonyms

1.1.1.1 Chemical Name poly (para-phenylene terephthalamide)

1.1.1.2 Synonyms aramid, p-aramid, para-aramid, PPT-A, PPDT, poly (1,4-phenylene terephthalamide), poly (para-phenylenediamine terephthalate), poly (imino-1,4-phenylene iminocarbonyl-1,4-phenylenecarbonyl)

1.1.1.3 Trade names Kevlar®*
Twaron®**
Terlon

1.1.2 Identification numbers

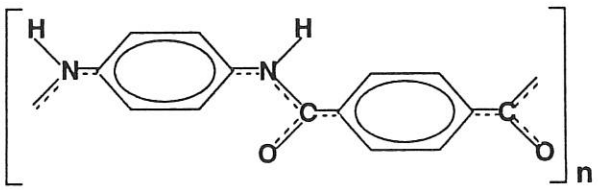
1.1.2.1 CAS numbers 24938-64-5; 25035-37-4; 26125-61-1

1.1.2.2 EINECS number not applicable

1.1.3 Chemical structure

1.1.3.1 Formula $(C_{14}H_{10}O_2N_2)_n$

1.1.3.2 Structural formula



para-aramid or PPDT or poly-(para-phenylenediamine terephthalate)

1.1.3.3 Isomers meta-aramid (Nomex®*)

1.1.4 Classification and labelling (Directive 67/548/EEC)

Symbols, Risks phrases, Safety advice:
None [not quoted in Database ECDIN (1993)]

* Registered trademarks of the DuPont Company
** Registered trademark of Akzo Nobel

1.2 PHYSICAL AND CHEMICAL PROPERTIES

1.2.1 Physical status

p-Aramid fibres are spun as continuous yarns with individual filaments, which are too large in diameter to be respirable - nominally 12-15 µm. They are commercially available as continuous filament yarn, cut fibres (staple), floc, pulp (short fibres with attached fibrils) and fabrics. Due to the highly oriented crystalline structure of p-aramid fibres, which gives them their properties (such as: heat and flame resistance, dimensional stability, ultra-high strength and modulus, electrical resistivity, chemical inertness and permselective properties), sub-fibres (fibrils) with a diameter of the order of 0.1 µm can under circumstances be peeled from their surface.

1.2.2 Thermal characteristics

p-Aramid fibres begin to lose strength at 180°C, but heat resistance is reasonable up to 320°C. Slow pyrolysis and oxidation set in above 300-350°C.

1.2.3 Molecular weight

20,000 (n = 80)

1.2.4 Density (at 20°C)

1.44 g/cm^3

1.2.5 Melting point

Does not melt, pyrolyses

1.2.6 Odour

Odourless

1.2.7 Colour

Golden yellow

1.2.8 Water solubility

Insoluble

1.2.9 Solvent solubility

Negligible

1.2.10 Conversion factor

For RFP's suspended in an aerosol, there is no reliable conversion between RFP's/ml and mg/m^3. As a very rough approximation, the concentration in RFP's/ml is usually numerically of the order of 100 times the concentration in mg/m^3 (HSE, 1995).

1.3 Respirable Fibre-Shaped Particulates (RFP's) derived from p-Aramid Fibre: Structural Characteristics, Properties and Biological Relevance in Comparison with RFP's derived from other Fibres

As stated above, p-aramid fibres are spun as continuous yarns with individual filaments, that are too large in diameter to be respirable - nominally ranging 12-15 µm. In a broad sense, the term "respirable" means "capable of being carried into the respiratory system by breathing". For a particle, this capability is largely determined by its aerodynamic characteristics, which are generally expressed in terms of "aerodynamic equivalent diameter" (AED) or "mass median aerodynamic diameter" (MMAD). These are determined by comparing the sedimentation velocity in air of the particle to that of a spherical particle of known diameter and a density of 1.0 g/cm^3. As a rough guide, particles with an AED up to 100 µm can enter the upper parts of the respiratory system (the nose and throat) and particles with an AED of less than 5 µm may be carried all the way into the lower parts of the respiratory system - including the gas exchange areas of the human lung (respiratory bronchioles and alveoli). It should be noted, that the physical dimensions of a particle can be much larger than the AED (ECETOC, 1996). For respirable fibre-shaped particulates (RFP's), the AED is primarily determined by the particle diameter, rather than by its length. Fibrils having an AED above 12 µm for humans or 6 µm for rodents are generally considered to be "non respirable", i.e. large numbers are not likely to reach the gas exchange regions of the lung (Schlesinger, 1985). Conversely, fibrous particles with physical diameters less than or equal to 3 µm are considered "respirable" - even when their length reaches 100-200 µm (Timbrell, 1965, 1983).

In most countries, "respirable fibrils" (i.e. RFP's) have been defined for regulatory purposes as "fibrous particles with length > 5 µm, diameter < 3 µm and a length/diameter ratio > 3" with only minor variations (ECETOC, 1996). Such fibrils will be referred to throughout this document as "respirable fibre-shaped particulate(s)" or "respirable fibrous particle(s)"- abbreviated RFP. To ensure consistency we have used this "RFP" nomenclature throughout - regardless of the terminology used in the various publications cited. The term "fibrils" has been used for somewhat larger (but still mostly microscopic) fibre-shaped particles, that can become airborne, but that do not penetrate into the deep lung. The word "fibre" has been reserved for generic purposes and to describe macroscopically visible fibre-shaped products - such as filament yarn, staple, pulp or fly from textile processing.

It has been observed that, due to surface abrasion, cutting or fracturing, sub-fibres (fibrils, RFP's) of about 0.3 µm in diameter can be peeled from the surface of p-aramid base fibre. It has been shown, using electron microscopy techniques, that shear forces can create fibre-shaped particulates of respirable size. Most of these are ribbon-like, branched and/or curled. Their surface can carry a high electrostatic charge, especially in dry atmospheres. Mechanical entanglement and electrostatic attraction predispose such p-aramid particles to agglomerate into non-respirable clusters. Pulp material seems less prone to release RFP's than staple fibre, but this could be primarily due to the nature of the mechanical work that the material is subjected to during processing - rather than to an inherent difference in dustiness of the product (HSE, 1995). Taking into account the above observations, it can be assumed that workplace exposure to p-aramid involves fibres as such, as well as fibrils and RFP's.

The fibrillation propensity of p-aramid fibre is related to its chemical structure. An accurate and comprehensive overview of structural, physical and chemical properties of p-aramid fibre, that are useful in the understanding of its toxicological properties and behaviour, has been provided by Yang (1993). Fibre properties, including excellent mechanical stability and strength and durability, can largely be attributed to the linear structure of the amide-linked benzene rings - substituted throughout in opposing para positions (for the fibre structure, see the figure at paragraph 1.2). The amide linkages are essentially coplanar with the aromatic rings - which permits a quasi-linear structure of the polymer backbone. When dissolved in the spinning solution, the rodlike macromolecules align and stick together by hydrogen bonding and *Van der Waal*'s forces. They form tiny crystal-like sheets, which subsequently become highly ordered "domains" in the "liquid crystalline" state. Initially these domains are randomly oriented. The spinning solution is subsequently extruded through the small holes of a "spinneret". The solution near the spinneret wall is slowed down by friction, while the solution in the centre of the holes makes up for this by flowing faster. The resulting liquid shear aligns the elongated crystalline domains along the extrusion (fibre) axis. The key manufacturing technology involves obtaining optimal conditions for dissolving the polymer, for spinning it and for removing the solvent. In the end, one obtains a unique fibre structure consisting of quite rigid macromolecules, that are to a very high degree aligned in small crystallites - which are in turn efficiently aligned along the fibre axis (Yang, 1993, Jackson, 1994).

It is worth stressing, that the more common synthetic organic fibres are essentially amorphous and, therefore, have a totally different structure. Thus, nylon, polyester, acrylic and polyolefin fibres all have highly flexible, intertwined polymer backbones, that can be twisted and rotated at will. Although the spinning process also tends to align the macromolecules of these fibres to some extent, spinning them does not involve a liquid crystalline solution and the alignment is rudimentary at best. Modulus and strength also remain comparatively low, since the polymer chains can never be solicited all at the same time and in the same direction. Most important, fibrillation propensity of such amorphous materials is low.

Inorganic fibres are also very different, since they normally do not have a polymer structure. They either consist of elongated crystals - many of which cleave fairly easily along a crystal axis (e.g. asbestos, whisker fibres) - or they are amorphous (glassy). Since the latter have no crystalline domains and no long polymer chains, they also have no clearly defined structural faults: they fracture randomly. Fragments that are too large to be taken up by macrophages can only be resolved in the lung by a leaching- or dissolution-process, which leads to a progressive reduction of particle size. The fibril-shortening break-up process, that seems to be characteristic of p-aramid RFP's, cannot occur.

When abraded, asbestos tends to split longitudinally into RFP's of much smaller diameter. The profile of fractured ends reveals a stepwise fracture, which releases numerous needle-shaped subfibres from the fibre bundles. In contrast, p-aramid does not show this tendency to separate into thinner fibre particles, but discloses an axially aligned internal structure that allows the peeling of free and/or attached fibrils during mechanical treatment. Airborne p-aramid RFP's have been found to be markedly curled or branched and tend to form fluffy clumps (Lee et al., 1983).

Typical p-aramid macromolecules have a molecular weight of about 20,000, which corresponds to a degree of polymerisation of about 80 and a chain length of about 0.1 µm. Crystallites and defects in the fibre have a periodicity of about the same order of magnitude (a little smaller) in both axial and lateral directions (Yang, 1993). This compares to p-aramid RFP's that are typically a few µm long and 0.1-1 µm wide. Such particles have been forcefully ripped off from whole fibre and they have been substantially damaged in the process. The original coherence between domains has thus been disturbed in many places. Because such abraded RFP's are at best a few domain sizes wide, each damaged transition area between adjacent domains represents a serious "weak spot" in their cross-section. There are many such weak spots for every µm of length.

The shear forces in the spinning process impart a sheath/core effect to the fibre. The sheath has a comparatively high degree of crystallinity and the core has a less dense, slightly pleated structure. The sheath/core effect can be demonstrated dyeing the fibre. Only the core structure is "open" enough to allow dye access and penetration. Undamaged whole fibre is virtually impossible to dye, but if the sheath is damaged (abraded) the core can be dyed (Yang, 1993). This implies that very large dyestuff molecules can penetrate damaged fibrils and the same probably holds for enzymes capable of hydrolysing the amide linkage (e.g. peptidases). A combination of mechanical damage and hydrolytic enzyme access may explain the apparent paradox, that whole p-aramid fibre is resistant to hydrolysis, while RFP's derived from the same fibre are readily broken down *in vivo*.

Virtually all of the information currently available on fibre toxicity mechanisms is based on experience with asbestos and man-made mineral fibres (or man-made vitreous fibres, MMVF). Whether the toxic activity and the toxicity mechanisms of organic RFP's are similar or identical to those of inorganic RFP's remains to be determined. However, due to the disparity in chemistry and physico-chemical characteristics between these two general fibre types, it is quite likely, that also significant toxicological differences exist (ECETOC, 1996).

Several aspects of surface chemistry have been implicated as contributing to mineral fibre toxicity, but are not applicable to (man-made) organic fibres. These include the presence of atoms or ions with unsaturated valences or unpaired charges (especially on fresh surfaces), polar surfaces, electrical vacancies, exposed cations and anions, the presence of transition metals that participate in redox-reactions and the formation of active oxygen species (Fubini, 1993).

As stated above, by convention, fibres and RFP's are defined as having an aspect ratio (length:diameter ratio) > 3:1. Several other factors have been identified as important in influencing the pathogenesis of fibre-related lung disease: RFP dimensions, chemical composition and structure, physical characteristics, deposition patterns and translocation, biopersistence and biodegradability, surface chemistry, particle overload conditions, and interspecies-, interstrain- and gender differences.

The importance of particle dimensions (for penetration into the lung) has already been discussed, together with the different tendencies to RFP formation during mechanical abrasion of asbestos and p-aramid fibres. p-Aramid RFP's, artificially generated for inhalation toxicity testing, typically have lengths up to 100 µm (mode 10-20 µm) and a diameter of < 1 µm (Lee et al., 1988). However, the tangling and branching of these particles leads to the formation of agglomerates with a MMAD between 2.5 and 10 µm, and a mass median aerodynamic diameter of 3.6 µm (Warheit et al., 1992).

Together with chemical composition, other factors such as degree of crystallinity and polymerization, orientation and cross-linking of the molecules can influence the toxicological characteristics of fibres.

The flexibility of RFP's may have an important influence on the pulmonary deposition pattern and on physiopathological processes (Pott, 1977). The properties and mechanical analysis of p-aramid RFP's were discussed by Knoff (1993), who concluded that these deform more easily than asbestos- or glass based RFP's. The p-aramid particles also showed so-called "kink bands" (areas of compressive yield, visible under the microscope), which makes them more vulnerable to physico-chemical attack (Kelly et al., 1993).

Inhalation and deposition are the initial events that lead to fibre-related lung disease. Deposition can occur by impaction, sedimentation or interception. Deposition of inhaled material has been shown to occur according to a unique pattern, irrespective of the material's nature. Little is known about the possible role of electrostatic precipitation and diffusion in fibre deposition (Lippmann, 1988). Impaction and sedimentation are governed by the AED of the particles. Impaction is favoured by a high flow velocity and occurs in the larger airways. In contrast, sedimentation is favoured by low flow velocity, long residence times and small airway size. The likelihood of interception increases with particle length (Timbrell, 1965). The early pathogenesis of fibre-induced lung disease is probably determined to a large extent by the initial pattern of particle deposition in the pulmonary tree. It is generally believed that the larger bronchial airway bifurcations are the preferred deposition sites in humans and it is there, that fibre-related bronchial cancer often occurs (Lippmann, 1988). Little is known about deposition at the alveolar level. However, it has been demonstrated in rodents, that inhaled RFP's and other particles small enough to pass through the conducting airways, deposit primarily at alveolar duct bifurcations. This is perhaps a consequence of airflow characteristics (Brody & Roe, 1983; Warheit & Hartsky, 1990). Alveolar deposition decreases rapidly with increasing RFP length and increasing aerodynamic diameter. This is in conformity with observed increases in the proportion of tracheobronchial deposition with increasing RFP lengths (Morgan et al., 1980; Morgan & Holmes, 1984).

Translocation refers to the movement of RFP's after their initial deposition. Particles may translocate to foci at the respiratory bronchioles, onto the ciliated epithelium at the terminal bronchioles, or into and through the epithelium to interstitial storage sites, along lymphatic drainage pathways or to the pleura. They can also migrate from the initial deposition sites to the lung periphery and to other tissues distant from the lung. The pathways and processes involved in most of these translocations are poorly understood (ECETOC, 1996).

Biopersistence is defined as the period of particle retention in the lung or in other tissues. Biopersistence can be influenced by the number of particles present, their dimensions, surface characteristics, chemical composition, surface area and other factors. Differences in any of these parameters could alter their toxicity. Biodegradability refers to the breakdown and/or dissolution of particles inside the lung or other tissues - mechanical, chemical and/or enzymatic factors may be involved (Muhle et al., 1991).

The biopersistence of a particle in the lung is dependent upon the site and the rate of deposition, as well as on rates of translocation, clearance, dissolution and biomodification. Following inhalation, long asbestos particles are selectively retained in the rat lung: chrysotile RFP's progressively split into thinner ones, but crocidolite does not show a reduction in diameter (Roggli & Brody, 1984). p-Aramid RFP's quickly break into shorter

fragments (< 5 μm), which are then more rapidly removed from the lung (Warheit et al., 1992). This process is considerably slower after high dose exposures, suggesting a dose-dependent mechanism. It also suggests that the breakdown process is macrophage-mediated and slows down under overload conditions (Kelly et al., 1993).

The term "particle overload" was introduced to describe the accumulation of particles (including RFP's) in the lung of experimental animals. Under overload conditions, particle retention increases substantially (and may exceed the lifespan of the animal), resulting in lung burdens far in excess of what one would expect on extrapolation of low-dose experiments. Under such circumstances, the observed effects are to a large extent no longer specific for the tested materials (ECETOC, 1996).

As stated above, interspecies-, interstrain- and gender differences may also play an important role in conditioning the toxicological behaviour of inhaled fibres. Rodents are commonly used in fibre inhalation toxicity studies, but important anatomical and physiological differences exist between these experimental animals and humans. These differences must be taken into account when extrapolating deposition and corresponding lung clearance responses. Thus, RFP's tend to deposit on alveolar duct bifurcations in rodents, whereas in humans they tend to concentrate near the final respiratory duct bifurcations (Brody & Roe, 1983). Anatomical differences also influence the size of deposited particles. The maximum size of inhaled particles in rodents is somewhat smaller than in humans, as is the size of particles deposited in the alveoli (Schlesinger, 1985). There are also differences between humans and rodents concerning the preferential deposition sites for RFP's (Craighead et al., 1982 [as quoted by ECETOC, 1996]). In this context, it is worth noting that the development of early asbestos-induced lesions occurs primarily at the sites of initial deposition (Warheit et al., 1984; Brody et al., 1984).

As far as particle clearance is concerned, transport from the deeper lung regions towards the tracheobronchial mucociliary escalator is a slow, macrophage-mediated process, that appears less effective by an order of magnitude in humans and other large animals than in rodents (Kreyling, 1990). The size of particles, their solubility and locus of deposition may all influence their dissolution rate, possible translocation to the interstitium and/or lymphatics, and reaction with lung fluids. Interspecies differences have not been studied. However, it has been demonstrated, that there are large differences in macrophage responses to chemotactic factors and phagocytic activity between various rodent species - with consequent differences in the cellular inflammatory responses (Warheit et al., 1988; Warheit, 1989; Warheit & Hartsky, 1994). Which species best simulates the human response remains to be determined.

Finally, a review of fibre toxicity and the health effects of exposure to man-made fibres (including p-aramid RFP's) and non-asbestos fibre-shaped silicates was recently published by Lockey (1996). The main determinants of fibre toxicity were size, durability and dose. Fibrils less than 3.5 μm in diameter and up to 200 μm in length were reported to be respirable. Studies reviewed noted that RFP's between 0.25 and 1.5 μm in diameter and above 4 to 8 μm in length were the most carcinogenic and that toxicity increased with increasing durability.

2. PRODUCTION AND USES

Aramid fibres are defined as fibres in which the base material is a long chain synthetic polyamide in which at least 85% of the amide linkages are attached directly to two aromatic rings. They are produced in a two-step process involving polymerisation followed by spinning. The polymer is typically produced by the reaction of aromatic di-amines and aromatic di-acid chlorides in an aprotic amide solvent. Kevlar® and Twaron® (para-aramids) and Nomex® (meta-aramid) fibres are examples, that differ primarily in the substitution positions on the aromatic ring. The meta-aramid fibre is spun from the polymerization solution of dimethylacetamide after neutralization. The para-aramid polymer must first be neutralized and isolated from the polymerization solution. It is then re-dissolved in a spinning solution of concentrated sulphuric acid. This gives a liquid crystalline solution, which is extruded through a spinneret followed by acid extraction and neutralization to form a highly-oriented fibre (IPCS, 1993).

p-Aramid fibres are commercialised as continuous filament yarn, cut fibre ('staple' of 30-100 mm in length), short fibre (6-12 mm in length) and pulp (2-4 mm in length), all with a nominal diameter of ca. 12 μm. However, the generation of RFP's during production, use and disposal of man-made organic fibres (MMOF), including p-aramid fibre, has been reported. As described by several authors (Lee et al., 1988; Verwijst, 1990; Cherrie et al., 1995), p-aramid RFP's are typically curly, ribbon-shaped and branched, with a strong tendency to form non-respirable agglomerates. As was pointed out earlier, the physico-chemical properties (e.g. solubility, biodegradability) of p-aramid whole fibre and derived RFP's are quite different - with relevant consequences for their toxicological characteristics.

p-Aramid fibre is made from an aromatic amide polymer and as such is closely related to the nylon family. Currently, 1/3 of production is staple fibre and pulp, the remaining 2/3 being filament yarn (HSE, 1995).

2.1 QUANTITATIVE DATA

USA	(Kevlar®)	20,000 tonnes (annual production capacity)
Holland-Germany	(Twaron®)	5,000 tonnes (annual production capacity) [from: Hodgson (1989)]
Northern Ireland	(Kevlar®)	5,000 tonnes (annual production capacity)
Japan	(Twaron®)	5,000 tonnes (annual production capacity) [from: IPCS (1993)]

In terms of world markets, the demand for p-aramid fibre is currently around 17,000 tonnes of which some 5,000 are used in Europe (HSE, 1995).

2.2 TYPES OF USES

Due to their excellent physical properties, such as heat and flame resistance, dimensional stability, ultra-high strength and modulus, electrical resistivity, chemical inertness and permselective properties, p-aramid fibres are useful in many applications (Preston, 1979). They are used principally as strengthening and reinforcing material in composite structures - making use of their low density, high specific strength and stiffness, as well as better vibration damping, resistance to crack propagation and fatigue resistance than obtainable with typical inorganic fibre-shaped materials. p-Aramid fibres are used primarily for tire cords, protective clothing, industrial fabrics, high performance (sports and aerospace) composites, high-strength ropes, cables, friction materials and gaskets (ILO, 1989).

Finally, p-aramid fibres are sometimes combined with other fibrous materials such as carbon- and graphite fibres to reduce costs or to increase impact strength (Delmonte, 1981)

3. OCCURRENCE

p-Aramid fibres are synthetic industrial products; therefore, they do not occur spontaneously in the environment.

p-Aramid RFP's, as well as other synthetic organic fibre dusts, can be released in the workplace during operations such as fibre forming, winding, chopping, weaving, cutting, machining and composite processing.

3.1 HUMAN EXPOSURE

3.1.1 Environmental exposure

Virtually no data are available with respect to environmental fate, distribution, and general population exposure (IPCS, 1993). However Knoff (1994) reported that the rate and extent of polymer degradation and decrease in mechanical properties of p-aramid RFP's due to solar ultraviolet radiation in the environment could be estimated with a mathematical model. p-Aramids absorb ultraviolet radiation strongly, with a maximum near the 330 nm absorption band. Using polymer absorption data, the solar energy spectrum and the Beer-Lambert absorption law, the solar ultraviolet energy absorption rate for a circular cross-section p-aramid fibre was computed as a function of fibre diameter. The calculations show that as the diameter decreases, the energy absorption rate per unit volume of polymer increases dramatically. This is due to a transition from predominant surface absorption (at the larger diameters) to uniform (in-depth) penetration and absorption at the smaller diameters. Fibrils of 0.3 μm diameter are calculated to absorb ca. 1.3 x 10^{20}quanta/(cm^3.-s) (~70 W/cm^3) of solar ultraviolet radiation - a significant energy absorption density.

The strength of fibres with high molecular orientation is highly dependent on the molecular weight of the polymer: therefore, theoretical computations were also carried out to estimate the ultimate strength of p-aramid fibres as a function exposure. The author estimated that after ~160 days (3,840 hrs.) in the environment, the molecular weight of 0.3 μm diameter p-aramid RFP's would degrade from ~20,000 to less than 400 due to the effect of ultraviolet solar radiation. The tensile strength would be reduced from 3,500-4,500 to 40 MPa, the elongation from > 4% to 2-3% and the modulus from 80-180 to 1-2 GPa. With tensile strength and modulus reduced to ~1% of their initial values, such a degraded RFP will be weak and brittle "with properties similar to those of uncooked *spaghetti*". Finally, Knoff (1994) concluded that an experimental verification of the computed degradation rate curves and estimates of size reduction of degraded RFP's in the environment would be desirable.

3.1.2 Occupational exposure

3.1.2.1 Fibres and RFP's

A survey was undertaken among a selection of manufacturers of p-aramid-containing products to assess the 8-hr time weighted average (TWA) exposure to RFP's in the work-place (Cherrie et al., 1995). Representative sites were selected for the full spectrum of p-aramid uses in industry, including yarn spinning, weaving, production of gaskets and friction materials, production and machining of thermoset composites and manufacture of sporting goods. Samples collected on these sites were counted by phase contrast optical microscopy (PCOM) and they were sized by scanning electron microscopy (SEM). p-Aramid RFP's were separately identified by means of fluorescence microscopy. The exposure level was expressed as a geometric mean (GM) of the 8-hr TWA for each job class, and was reported to be generally low, ranging 0.005-0.4 RFP/ml. Assuming a log-normal distribution, less than 1% of industry-wide exposure levels would be expected to exceed 0.5 RFP/ml, and only about 0.002% would be above 2 RFP/ml. The authors expected that exposures might be higher without the sophisticated ventilation systems in use at the sites examined.

A summary of typical exposure levels to p-aramid RFP's with respect to different types of raw material, end uses and operations was reported by HSE (1995). The data, summarised in table 3.A, suggests that levels of p-aramid RFP's in air rarely exceed 0.3 RFP/ml on an 8-hr TWA basis across a large range of processes and raw materials (HSE, 1995).

Occupational exposure to p-aramid fibrils and RFP's could also occur from friction by-products. Grünthaler et al. (1985) concluded that no significant quantities of p-aramid RFP's are released from p-aramid-containing brake pads under normal friction conditions. The extent of airborne release of fibrils from asbestos substitute materials (including p-aramid RFP's) used in friction products was also evaluated in another experiment, which can be criticised for the atypical method of dust generation used (Jaffrey et al., 1992). Brake and clutch linings containing man-made mineral fibres and p-aramid pulp were rubbed with emery paper and four air samples were subsequently collected and examined by transmission electron microscopy (TEM) and energy dispersive X-ray analysis. At 3,000x magnification, TEM examination showed p-aramid RFP's presumably capable of deep penetration into the lung. Of these particles, 96% had diameters below 2.5 µm and all were more than 80 µm in length. The bulk of the fibrous particles released from the car brake shoes was glass. Significant amounts of dust were produced by clutch plates; samples included carbon- and inorganic fibres of silicon (Si), aluminium (Al), calcium-sulphate, and calcium (Ca), as well as some metallic dusts - e.g. copper. Truck brake shoes were also found to contain inorganic fibres with Si, Ca, Al, sulphur, magnesium and sodium. A small amount of chrysotile was found, while p-aramid RFP's were not detected. The authors concluded that replacement fibres generate substantially fewer RFP's than asbestos, even if these RFP's are in the same diameter and length range (Jaffrey et al., 1992).

3.1.2.2 Decomposition products

The burning or pyrolysis of organic fibres produces a wide variety and quantity of off-gases varying widely with decomposition or oxidation conditions.

Razinet et al. (1976) reported that the products of fast pyrolysis/decomposition of p-aramid fibres at 650°C - as identified by thermogravimetric analysis/gas chromatography/mass spectrometry (TGA/GC/MS) - were mainly carbon monoxide and benzene, but toluene was also seen. Slow pyrolysis (at 650°C for 15 min.) yielded nitrogen, carbon monoxide, carbon dioxide, methane, ethylene, propene, benzene, toluene, benzonitrile, methyl-aniline, aniline, phenylacetonitrile, phthalonitrile, phenylenediamine, biphenyl, benzimi-dazole, fluorene and benzanilide. However, it was not clear whether pyrolysis was performed on p-aramid alone, or on p-aramid in combination with other polymers.

An evaluation was undertaken at the Ebtec East Corporation (Agawam, Massachusetts) for possibly hazardous working conditions caused by emission products from cutting of a p-aramid reinforced composite materials with a carbon-dioxide laser (Moss & Seitz, 1990). The data collected indicated the presence of low levels of several volatile organic compounds, including aliphatic, aromatic, and chlorinated hydrocarbons, alcohols, and aldehydes in air samples taken both inside and outside the laser booth. These levels were low enough not to be considered hazardous to workers. Gases such as carbon-monoxide (30 to 35 ppm) and nitrogen oxides were also detected inside the booth during the cutting operations, but were far from the breathing zones of the workers.

Chemical by-products from laser operations were reviewed by Doyle (1991). In all previous studies where the off-gases of polymer cutting with carbon-dioxide lasers were analysed, polycyclic aromatic hydrocarbons had been reported. The formation of nitrogen-containing compounds together with polycyclic aromatic hydrocarbons, was also observed during laser cutting of p-aramid.

The results of a NIOSH evaluation of airborne emissions from carbon-dioxide laser cutting operations were discussed (Tharr, 1991). In a study documenting breathing zone exposures during cutting of p-aramid, the laser technicians who performed the cutting operations complained of eye- and skin irritation. Carbon-monoxide concentrations ranged up to 35 ppm TWA and nitrogen oxide concentrations of around 5 ppm were found. Short-term hydrogen-cyanide concentrations of 0.03 to 0.08 mg/m^3 were generated in the laser cutting area, resulting in a TWA exposure of 0.05 mg/m^3.

Table 3.A Typical occupational exposure levels to p-aramid RFP's with respect to different types of raw materials, end uses and operations (HSE, 1995)

Type of raw material	End-use	Operation	Number of samples	Exposure level range (RFP/ml)
Continuous filament	Woven fabrics	Twisting, rewinding and weaving	6	0.01-0.08
	Composites	Drilling	9	0.03-0.12
Staple fibre	Spun yarns	Blending, carding, drawing, spinning	17	0.02-2.16
	Spun yarns	Carding, spinning	4	0.01-0.32
	Spun yarns	Winding	1	0.05
	Yarns	Reclamation	1	0.06
Pulp	Brakes	Mixing, mixing pulp, pressing, cutting, grinding	7	0.01-0.14
	Gaskets	Mixing, stamping, grinding	7	0.06-0.14
	Brakes	Mixing, stamping, grinding	4	0.01-0.16
	Brakes	Mixing, stamping, grinding	6	0.02-0.10
	Gaskets	Mixing, calendering, grinding	6	0.01-0.04

4. MEASUREMENT TECHNIQUES AND ANALYTICAL METHODS

HSE (1988)

Health and Safety Executive, Occupational Medicine and Hygiene Laboratories.

Man-made mineral fibre. Airborne number concentration by phase-contrast light microscopy. Methods for Determination of Hazardous Substances (MDHS) 59. HSE, Bootle.

HSE (1990)

Health and Safety Executive, Occupational Medicine and Hygiene Laboratories.

Asbestos fibres in air. Light microscopy methods for use with the Control of Asbestos at Work Regulations. Methods for Determination of Hazardous Substances (MDHS) 39/3. HSE, Bootle.

INSERM (1993)

A flotation-based method for preparing p-aramid RFP's for biological study was developed (Schins et al., 1993). Optimum results were obtained when 2 g of p-aramid pulp were added to 800 ml distilled water containing 0.125% ethanol, the suspension stirred for 15 hours and then allowed to settle for 5 hours. Settling produced two phases of different colour, the upper phase being lighter. Gravimetric and scanning electron microscopic analysis of fibre-shaped material from the upper phase indicated that about 0.5% of the mass of the starting material was suspended in that phase and showed that this fraction consisted primarily of RFP's with a mean length of 6 µm and a mean diameter of 0.4 µm. More than 90% of these particles had an aspect ratio of 3:1 or more. The average yield of RFP's was 4 x 10^6 fibres per µg of material. Experiments leading to optimization of the procedure were also described. The authors concluded that the method is a simple and rapid technique for obtaining p-aramid RFP's of relevance for both *in vitro* and *in vivo* toxicological studies.

5. TOXICOLOGY

5.1 TOXICOKINETICS

No information is available on the absorption or distribution of p-aramid RFP's in experimental animals by any other route of exposure than by inhalation. Due to its insoluble nature, no absorption is expected to occur through the skin or gastro-intestinal tract wall (HSE, 1995).

There is also no information concerning the pulmonary disposition and clearance of p-aramid RFP's in human beings.

5.1.1 Experimental data

5.1.1.1 Inhalation exposure

The pulmonary response to inhaled p-aramid RFP's derived from Kevlar® pulp was studied by Lee et al. (1983; 1988), who exposed rats to a specially prepared aerosol of ultrafine fibrils. To produce a reasonable quantity of RFP's (diameter < 3 μm, length < 100 μm), a special pulp batch was prepared, which contained many more fine fibrils than normal commercial product. Attempts to obtain substantial atmospheric RFP concentrations (> 1 mg/m^3) with conventional dust generation techniques were unsuccessful, because the particles became mechanically entangled and electrostatically held back in the pulp matrix. A high-pressure air impingement device (Micro-Jet, Fluid Energy Co., Hatfield PA) was then linked to a $5^{1/4}$-in. cyclone to separate RFP's from larger fibre clumps. The theoretical cyclone 50 % cut-size was 5.5 μm for a unit density sphere at an airflow of 1,000 litres/min. A twin-screw bin feeder (K-tron Co., Glassboro NJ) delivered a continuous supply of the special pulp to the Micro-Jet. Only about 5% of the bin-fed pulp reached the chamber; the rest was cyclone collected and recycled once through the system. A maximum chamber concentration of 18 mg/m^{3}was obtained (Lee et al., 1983; 1988).

Male Sprague-Dawley rats (number of rats in each group not specified) were exposed to these specially prepared p-aramid RFP's (60-70 % length 10-30 μm, diameter 1 μm) at concentrations of 0.1 mg/m^3 (1.3 RFP/ml), 0.5 mg/m^3 (26 RFP/ml), 3 mg/m^3 (280 RFP/ml), or 18 mg/m^3 (number concentration not determined) for 6 hrs./day, 5 days/week, for 2 weeks (Lee et al., 1983). Five rats in each group were killed 0, 2 weeks, 3 months and 6 months post-exposure, except for the rats exposed to 18 mg/m^3, which were killed at 0, 4, and 14 days and 1, 3, and 6 months post-exposure. Rat lungs were stained for histological and microscopic examination. The authors reported that 6 months post-exposure, inhaled RFP's had accumulated mainly at the bifurcations of alveolar ducts and adjoining alveoli, with only a few RFP's being deposited in peripheral alveoli of the acinus (Lee et al., 1983).

Lee et al. (1988) also reported that Crl:CD(SD)BR rats (100 males and 100 females per group) were exposed to p-aramid RFP's (source as in the previous study by the same authors) at concentrations of 0, 2.5, 25, or 100 RFP/ml, 6 hrs./day, 5 days/week for 2 years. Another group was exposed to 400 RFP/ml for 1 year and allowed to recover for 1 year.

Selected rats at 3, 6, and 12 months, any moribund rats and all rats surviving after 2 years were killed and necropsied. The pattern of deposition and persistence of aramid RFP's in this 2-year study was similar to that of the previous study by the same authors. p-Aramid RFP's, which are more curled than chrysotile fibres, were retained mostly in the respiratory bronchioles and alveolar duct region, particularly in the ridges of alveolar duct bifurcations. A year after termination of the 1-year exposure to 400 RFP/ml, the length of the RFP's in the lung appeared reduced. At the 3 highest exposure levels (25, 100, and 400 RFP/ml), there was a minute amount of dust accumulation in alveolar macrophages and in tracheobronchial lymph nodes, resulting from the transmigration of intrapulmonary RFP's. Most dust particulates in the alveolar macrophages were less than 1 µm long (Lee et al., 1988).

Crl:CD-BR rats were exposed to p-aramid RFP's at concentrations ranging 600-1,000 RFP/ml (gravimetric concentrations of 2 to 13 mg/m^3), 6 hrs./day for 3 or 5 days (Warheit et al., 1992). The RFP's used were prepared from the same batch that was used by Lee et al. (1988). Evaluations were performed 0, 24, 72 or 96 hours, 1, 3, or 5 weeks and 6 months after exposure. A preferential deposition pattern of RFP's on alveolar duct bifurcations nearest the bronchio-alveolar junctions was seen by scanning electron microscopic (SEM) examination. RFP's were observed within alveolar macrophages within 24 hours after exposure; however, they did not appear to have translocated to epithelial or interstitial cells. Three months after exposure only a few alveolar macrophages containing RFP's were identified. RFP clearance studies demonstrated a transient increase in the numbers of retained RFP's 1 week post-exposure, with rapid clearance of RFP's thereafter. This transient increase in the number of RFP's is thought to be due to transverse breaking of the longer RFP's, since the average length continued to decrease over time. A progressive decrease of the mean length (12.5 µm to 7.5 µm) and diameter (0.33 µm to 0.24 µm) occurred over a 6-month post-exposure period. More importantly, the number of long (i.e. > 5 µm) RFP's showed a sharp decrease, that set in after 1 week: particle counts declined to 4% of the original values after 24 weeks. The number of short RFP's (i.e. < 5 µm) was stable up to 12 weeks and then also dropped sharply. These data show that the longer and potentially more hazardous RFP's are quickly broken into shorter fragments. The generation of short fragments keeps up with clearance as long as there are sufficient long RFP's present. The authors concluded that inhaled p-aramid RFP's have reduced durability and persistence in the rat lung (Warheit et al., 1992).

Kelly et al. (1993) reported on the deposition, clearance and shortening of p-aramid RFP's in young Crl:CD-BR-rats after acute, subchronic, and chronic inhalation. The rats were exposed in inhalation chambers to air containing RFP's of up to 400 RFP/ml for periods of 1 day, 3 weeks and up to 2 years at 6 hours of exposure/day, 5 days/week. Membrane filter samples were taken at least weekly for RFP counting and for measurement of RFP lengths and widths. Rats were necropsied at the end of each period and lungs were sectioned for RFP counting and measurement. *In vitro* durability of RFP's in the presence of different proteolytic enzymes was also determined (see below, paragraph 5.1.1.2). The mean initial dimensions of inhaled RFP's were 12 µm length and < 0.3 µm diameter. Results showed that the median length of RFP's deposited in the lungs decreased for successive sacrifice times. After a 1 day exposure, the recovered RFP's decreased rapidly from about 11 µm to about 5 µm after 20 days. The time to reduce median RFP length to half the original length was about 18 days. After 3 weeks of exposure, median RFP length dropped to about 2 µm after 2 years of recovery. The lower the exposure concentration or

the shorter the exposure time, the faster the reduction in RFP length. As the total number of RFP's declined during recovery, the fraction of long RFP's decreased dramatically, while that of short RFP's actually increased. After 2 years, RFP's longer than 20 µm decreased to less than a few percent, whereas those shorter than 5 µm were at nearly 70 %. The build-up of RFP's in the lungs was lower than the expected rate in the lowest exposure group of 2.5 RFP/ml. *In vitro* durability of RFP's was shortened with all commercial enzymes, but this decrease was statistically significant only with pancreatin (see below, paragraph 5.1.1.2). The authors concluded that RFP's deposited in the rat lung become shorter with residence time under every exposure condition and that this appears to be enzyme mediated. They also concluded that RFP's are less durable in the lungs of rats and humans than one would expect from the chemical and physical properties of the original material (Kelly et al., 1993).

A short-term study was performed on the biopersistence of p-aramid RFP's and wollastonite in Crl:CD-BR rats (Warheit et al., 1994a). Groups of 8-week old males were exposed 6 hrs./day for 5 days to p-aramid RFP's (900 or 1,344 RFP/ml) or wollastonite (800 RFP/ml). Rats were examined after specific time intervals, up to 6 months post-exposure. The p-aramid RFP clearance data exhibited a half life of less than 30 days. A transient increase in the number of RFP's at the 1 week interval suggested fibre shortening. Over 6 months, the mean RFP length decreased from 12.5 to 7.5 µm and the diameter from 0.33 to 0.23 µm. Wollastonite RFP's had a half life of less than 1 week, with a decrease in their mean length from 11 to 6 µm, but an increase in mean diameter from 0.5 to 1 µm. The authors proposed that the thin wollastonite particles were readily solubilised, while the thicker ones were more difficult to clear. They concluded that the low durability of p-aramid and wollastonite RFP's in the lungs might account for their low toxicity in comparison with more durable fibrous materials.

The pulmonary clearance of inhaled p-aramid RFP's was also compared with that of chrysotile at similar concentrations (Warheit et al., 1995). Crl:CDBR rats (total group size not specified) were exposed to aerosols of p-aramid RFP's at concentrations of 772 and 419 RFP/ml (median length and diameter 9.0 and 0.3 µm, respectively) for 6 hrs./day, 5 days/week for 2 weeks. Final chrysotile mean concentrations were 782 and 458 RFP/ml (Canadian chrysotile: median length 6 µm, diameter not provided). Groups of rats (n = 3) were killed at 0 days, 5 days, 1 month, 3 months, 6 months or 1 year (aramid only) post-exposure for lung fibre burden analysis. p-Aramid RFP's were recovered from rat lungs following a 10-min. digestion of the lungs in 1.3% of NaOCl. This method was found not to contribute to fibre breakage and therefore it was claimed that it was validated. Asbestos was recovered by incubating lung tissue with a 5.25% hypochlorite solution for 3 hrs. Subsequent fibre counting by optical microscopy (only RFP's of length > 5 µm were counted) and fibre size analysis by SEM were performed. Immediately after the end of the exposure, the following lung fibre burdens were found: for p-aramid 7.6×10^7 ($\pm$ 1.9×10^7) RFP/lung at the higher exposure level and 4.8×10^7 ($\pm$ 2.1×10^7) RFP/lung at the lower exposure level; for chrysotile this was 3.7×10^7 ($\pm$ 7.4×10^6) RFP/lung and 1.3×10^7 ($\pm$ 4×10^6) RFP/lung, respectively. These findings are consistent with those described by IOM (1995) for their study - in which different fibre recovery techniques were used. Initial median fibre lengths were 8.6 µm for p-aramid RFP's, and of 3.5 µm for chrysotile. An early transient increase in the total number of p-aramid RFP's recovered was reported, and attributed to RFP breakage and a corresponding increase in the number of short particles. At 6 months, the median length of recovered p-aramid RFP's was reduced to about 4 µm.

Conversely, at 3 months, the median length of chrysotile RFP's had increased to 11 µm - suggesting that the shorter particles were selectively cleared from the lungs by macrophage phagocytosis, while pulmonary clearance of the longer ones was apparently insignificant. In conclusion, long chrysotile fibres are retained in the lung or cleared at a slow rate, whereas p-aramid RFP's have low biodurability. Median lengths of chrysotile recovered from exposed lung tissue increased over time, while median lengths of p-aramid RFP's decreased (Warheit et al., 1995).

The rapid pulmonary clearance of p-aramid RFP's reported by Warheit et al. (1992) has recently been reproduced in an independent study (IOM, 1995; Searl, 1997), in which male Wistar rats were exposed to p-aramid RFP's, to code 100/475 glass microfibres and to chrysotile RFP's. An important aspect of lung burden studies is that the residual test material must be recovered from lung tissue without loss or damage. For this reason, a series of validation experiments was undertaken to investigate the reactivity of the test fibres with the reagents used for tissue digestion. The combined ethanolic KOH-Clorox digestion procedure was compared with an enzymatic procedure - involving a mixture of collagenase, papain, DNA-ase and lipase). The author concluded that none of the digestion methods tried was without reproach for the recovery of p-aramid RFP's, but that the ethanolic KOH-Clorox technique was satisfactory, caused minimal loss of RFP's and little change in their dimensions (IOM, 1995; Searl, 1997). For a detailed description of this study, see below (par. 5.3).

A multifunctional study was carried out to compare the pulmonary effects of inhaled p-aramid RFP's in hamsters to the effects previously seen in similarly exposed rats (Warheit et al., 1997). Male Syrian golden hamsters were exposed whole-body to aerosols of size-separated p-aramid RFP's for 2 weeks at design concentrations of 350 and 700 RFP/ml. The lungs of sham and fibril-exposed hamsters were evaluated immediately after exposure, as well as 10 days, 1 month and 3 months post-exposure. Mean aerosol RFP concentrations over the 2-week exposure period were 358 and 659 RFP/ml. Mean lung burdens for the high-dose group was 1.4×10^6 RFP/lung. During the 3-month post-exposure period, the mean numbers of retained p-aramid RFP's decreased from 1.4×10^6 to 5.0×10^5 and biopersistence/RFP dimensional determinations demonstrated breakage of inhaled p-aramid particles. Mean lengths of RFP's recovered from the hamster lungs at time 0 were 10.4 µm and subsequently decreased to 6.3 and 6.1 µm (respectively) at 1 and 3 months post-exposure. Reductions in length of retained RFP's over time signified a shortening consistent with the results of earlier studies with p-aramid RFP-exposed rats (Warheit et al., 1997).

5.1.1.2 *In vitro* studies

Durability of fibres and RFP's has been recognised to be an important property in determining toxicity, and therefore studies of the solubility and biodegradability of p-aramid RFP's are useful in predicting possibly hazardous properties (HSE, 1995).

p-Aramid fibres, carbon fibres and chrysotile were kept for 8 weeks in a static Gamble solution and for 2 weeks in a flow-through system. Whereas chrysotile split into many RFP's the two other fibre types were virtually unaffected (Förster, 1984). p-Aramid fibres and polyester fibres were incubated in a saline solution with and without lysosomal enzymes at pH 5, 6, and 7.2. Polyester quickly broke down at pH 5 in the presence of enzymes, but p-aramid fibres were unaffected (Mieschental et al., 1987). Wening & Lorke

(1992) reported that incubation in human plasma for up to 26 weeks had no effect on p-aramid fibre diameter or surface morphology.

Larsen (1989) reported on the solubility of various natural and synthetic fibres (including aramid fibres) in physiological Gamble's solution (at 37°C or more, for 1 hr. to 20 weeks and 1 hr. to 20 weeks for closed and open system conditions, respectively; pH not specified) as determined by atomic absorption spectrometry. Carbon and p-aramid fibres were found to be "practically insoluble", and there was no evidence of alteration of the surface during examination by SEM with energy dispersive spectrometry.

The vulnerability of p-aramid RFP's to proteolytic enzyme digestion was investigated by Kelly et al. (1993). The scientific background of this investigation is related to the above considerations about the structure of the p-aramid fibre (see paragraph 1.3). Whole p-aramid fibres are notoriously weak under compression and can, under certain circumstances, develop numerous microscopically visible damage zones, where the crystal structure has been disrupted. These zones are called kink bands. p-Aramid RFP's are, by definition, subfibres peeled from the whole fibre surface and normally they have been severely damaged in the process. They show many visible kink bands - the presence of which drastically reduces their tensile strength. The disrupted crystal structures near the kink bands are also much more accessible to chemical attack than they would be in undamaged whole fibres. RFP's are typically about 1/100th the thickness of whole fibre, so the time for chemicals to diffuse through the structure is also orders of magnitude shorter. Consequently, RFP's could be vulnerable to enzymatic attack. Kelly et al. (1993) reported a reduction in median RFP length after 3-month exposures to flowing proteolytic enzymes (pancreatin, papain, trypsin, collagenase). However, a statistically significant change from the saline control was only observed for RFP's exposed to pancreatin ($p < 0.05$). It is conceivable that lysosomal enzymes could produce a similar cutting effect, if RFP's in the lungs are subjected to macrophage attack. The involvement of macrophage attack could also account for the dose dependence of RFP shortening, since the finite number of lung macrophages would limit the rate at which the inhaled particles could be engulfed and broken down(Kelly et al., 1993).

5.2 TOXICODYNAMICS

5.2.1 Experimental data

5.2.1.1 Acute toxicity

An acute oral study in male rats at doses from 670 to 7,500 mg/kg of p-aramid fibres was reported (Du Pont, 1991; 1994). The p-aramid was administered by gavage as a 15% suspension of pulp in corn oil, and the observation period was 14 days. No fatalities were observed.

Reinhardt (1980) reported that rats (number and sex not stated) were exposed to 150 mg/m^3 p-aramid dust (generated from raw polymer) for 4 hours. Particle size and count were not stated, although a "low" (but undetermined) proportion of the dust was reported to be respirable (diameter less than 1.5 μm and length ranging 5-60 μm). No deaths were reported. Decreased activity during exposure was reported as the only clinical sign. The same author also reported that a group of 40 rats (sex not stated) were treated by a single intratracheal instillation of 25 mg of p-aramid dust (as above) in physiological saline,

while 40 rats in a control group received saline alone. Five animals from each group were sacrificed at intervals from 2 days to 21 months post-exposure, and a complete gross pathological examination with histopathology of the respiratory tract was performed. Over the 21 month period, mortality rates, clinical observations and gross autopsy findings were comparable between test and control groups. In the treated animals, an initial inflammatory response in the lungs subsided within a week. The terminal bronchioles contained large, non-respirable particles around which foreign body granulomas later formed. Particles of smaller size (5 μm) were found in alveolar ducts. All tissue responses decreased over time, and collagen deposition was negligible (Reinhardt, 1980).

It has been reported that skin contact tests in experimental animals did not cause "toxic reactions" (DuPont, 1996).

5.2.1.2 Short-term and chronic toxicity

Lung effects in rats exposed by inhalation to p-aramid RFP's have been described in the studies by Lee et al. (1983; 1988), already described.

In the Lee et al. (1983) study using Sprague-Dawley rats - see section 5.1.1.1 for conditions -, inhaled p-aramid RFP's were seen to have been quickly phagocytised by alveolar macrophages. Dust deposition and dust cell response were dose related. The lungs appeared to be normal 2 weeks post-exposure, and most dust cells were eliminated by 3 months. There was no collagen fibre deposition. Some alveolar ducts were thickened by giant cells and chronic inflammatory cells. Dust accumulation was particularly prominent at alveolar duct bifurcations. The epithelium of terminal bronchioles was desquamated locally. There was some fibrotic thickening of alveolar duct regions with dense reticulum fibre networks. p-Aramid RFP's tended to be markedly curved or formed fluffy clumps [for this reason, the authors concluded that inhaling p-aramid RFP's does not appear to pose a hazard in the workplace] (Lee et al., 1983).

In the subsequent chronic exposure study (Lee et al., 1988 - see section 5.1.1.1 for conditions), exposures of 2.5, 25, and 100 RFP/ml did not cause any clinical signs of toxicity, body weight changes, or excess mortality. Twenty nine males and 14 females exposed to 400 RFP/ml died from obliterative bronchiolitis. After 1 year of recovery, no signs of toxicity, body weight changes, or excess mortality were seen in surviving 400 RFP/ml rats. The 100 and 400 RFP/ml exposures caused significantly increased lung weights. Exposure related pathological changes were confined to the lungs. The 2.5 RFP/ml exposure caused no pathological changes in the alveolar architecture of the lungs except for a few scattered RFP-laden alveolar macrophages (this was considered by the authors to be the no-observed-adverse-effect level, NOAEL). In the 25 and 100 RFP/ml groups, a dose-related increase in lung weight, and significant particle accumulations in the respiratory bronchioles of the alveolar ducts were observed. Slight type-II pneumocyte hyperplasia, alveolar bronchiolarization, and mild alveolar collagenous fibrosis were also observed.

From this study, a NOAEL could be concluded at exposure level of 2.5 RFP/ml [Crl:CD(SD)BR-rats, inhalation, 6 hrs./day, 5 days/week for 2 years, lung histopathology].

The immediate next tested concentration of 25 RFP/ml could be considered as representative of the LOAEL in this study [Crl:CD(SD)BR-rats, inhalation, 6 hrs./day, 5 days/week for 2 years, lung pathology (increase in lung weight; significant particle accumulation in the respiratory bronchioles of the alveolar ducts; slight type-II pneumocyte hyperplasia; alveolar bronchiolarization; mild alveolar collagenous fibrosis)].

In the previously discussed (section 9.1.1.1) study by Warheit et al. (1992), the initial pulmonary toxicity of inhaled p-aramid RFP's in Crl:CD-BR-rats indicated an early transient pulmonary inflammatory response, which was evidenced by an increased number of neutrophils in the broncho-alveolar lavage (BAL) fluids following exposure. Transient increases in lactic dehydrogenase (LDH) levels and BAL fluid protein were noted 3 and 5 days post-exposure. No differences were observed in the percentages of ruffled macrophages, the phagocytic capacities of macrophages, or in the labelling index of lung parenchymal cells in exposed animals compared with controls. No pulmonary lesions could be seen 3 or 5 days after exposure.

Pulmonary cellular changes were investigated in Crl:CDBR rats exposed to both p-aramid and chrysotile RFP's in the already mentioned study by Warheit et al. (1995) - see section 5.1.1.1 for exposure conditions. Following p-aramid RFP inhalation, minimal to mild centriacinar inflammation and fibrosis (with particle accumulation) were observed during histopathological examination. The severity of the inflammatory response was similar in both the high (772 RFP/ml) and low (419 RFP/ml) exposure groups. Lesions were most prominent up to 1 month post-exposure and severity generally diminished with time. By 6 months post-exposure, only occasional centriacinar regions with slight thickening of the alveolar duct bifurcations were noticed. No differences were reported between the lungs of exposed rats and controls at 12 months post-exposure..

Lesions observed subsequent to chrysotile exposure were similar to those observed in p-aramid-exposed animals. However, in the early stages, the inflammatory response was less marked than that observed after p-aramid-exposure. Six months post-exposure, centriacinar lesions were mild or absent in the chrysotile-exposed rats. Fibre- and sham exposed rats were given 5-bromo-2-deoxyuridine intraperitonally at various time points post-exposure for subsequent analysis of cell proliferation in the terminal bronchiolar, proximal lung parenchymal (alveolar duct and adjacent areas), subpleural and mesothelial tissues. In the low-dose p-aramid-exposed rats, no statistically significant increases in cell labelling indices were observed in any tissue at any time point. In the high-dose group, only transient increase in cell proliferation in the terminal bronchiolar (statistically significant) and visceral pleural/sub-pleural (not statistically significant) regions occurred up to 5 days post-exposure, although no increases were measured in lung parenchymal or mesothelial cells. No increases were measured in tissues of animals exposed to the lower doses of p-aramid RFP's. In the chrysotile-exposed rats, cell division was reported substantially increased compared to controls in all regions (terminal bronchiolar, parenchymal, visceral pleural/subpleural and mesothelial surfaces). Many of these effects were sustained through 3 months post-exposure, with return to control levels by 6 months. Moreover, cell viability, cell numbers, and differential cell counts were measured, and biochemical analyses were performed on cells obtained from the BAL fluids of both controls and fibre-exposed rats; a transient inflammatory response in all exposed animals was reported, with no substantial differences between p-aramid RFP- and chrysotile-exposed animals.

Finally, an ultra-structural morphometric analysis was performed (at 0 and 30 days post-exposure) of tissues from the first alveolar duct bifurcation regions, the preferential sites of fibre deposition in rodents and the sites where the earlier pulmonary lesions occur. Significant morphological changes were observed at the alveolar duct bifurcations in both p-aramid- and chrysotile exposed animals. Increases in alveolar and interstitial macrophages were also seen in both exposed groups, but the increase was greater in the p-aramid-

exposed rats. Substantial increases in the volume and number of Type II epithelial cells (reflecting a compensatory hyperplastic response to Type I cell injury) were measured at the alveolar duct bifurcations in both groups. Interstitial thickening at duct bifurcations was seen 0 days as well as 30 days post-exposure in the RFP-exposed rats, but only 30 days post-exposure in chrysotile-exposed animals. No differences between high- and low-dose groups were seen in these studies. It was concluded, that the proliferative effects and enhanced biodurability of chrysotile, that are associated with the induction of chronic disease, do not occur with p-aramid RFP's. Therefore, inhalation of chrysotile asbestos fibres is likely to produce greater long-term pulmonary toxic effects than p-aramid RFP's (Warheit et al., 1995). More recently, the same conclusions have been confirmed (Warheit et al., 1996).

In the previously mentioned multifunctional study by Warheit et al. (1997), male Syrian golden hamsters were exposed whole-body to aerosols of size-separated p-aramid RFP's for 2 weeks at design RFP concentrations of 350 and 700 RFP/ml. At the time of publication of data, the study was ongoing and was to last for an additional 9 months. Histopathological analysis was conducted to asses the following major endpoints: 1) RFP deposition and clearance; 2) biopersistence of inhaled RFP's; 3) cellular proliferation of terminal bronchiolar, pulmonary parenchymal and subpleural surfaces and 4) broncho-alveolar lavage fluid parameters. Broncho-alveolar lavage studies demonstrated a transient influx of neutrophils which persisted through 1 month post-exposure. Lavage biomarkers such as LDH and protein were not significantly different from controls. Histopathological analysis revealed minor lesions characterised by increased numbers of alveolar macrophages (with or without RFP's) admixed with lesser numbers of neutrophils and some cellular debris. The lesions were similar for most high- and low-dose animals. As is typical for dust/fibre inhalation studies, lesions were most prominent in the alveolar duct regions (Warheit et al., 1997). The results of cell proliferation studies of p-aramid RFP- and sham exposed hamsters demonstrated a small, but transient and statistically not significant, increase in immunostaining of terminal bronchiolar cells relative to controls. In addition, labelling indices of cells in pulmonary parenchyma and subpleural regions were not significantly different from unexposed sham controls. The transient nature of this response is similar to the cell labelling data reported in rats exposed to p-aramid RFP's for 2 weeks (Warheit et al., 1995; 1996).

A recent review by Lockey (1996) discusses health effects of exposure to man-made fibres (including p-aramid RFP's) and non-asbestos fibre-shaped silicates. The main determinants of toxicity are size, durability and dose. Fibre shaped particles of less than 3.5 μm in diameter and 200 μm in length were reported to be respirable. Studies under review noted that particles from 0.25 to 1.5 μm in diameter and above 4 to 8 μm in length were the most carcinogenic, and that fibre toxicity increased with increasing durability. In the framework of the materials under consideration, p-aramid RFP's were considered to provide minimal risk for man (Lockey, 1996).

Reinhardt (1980) briefly described a study of intratracheal administration of p-aramid polymer dust in rats, but it is unclear whether fibrous dust or unspun, non-fibre-shaped polymer dust was used. A 21-month follow-up of an unknown number of rats showed an early, non-specific inflammatory reaction, subsiding within a week, followed by foreign-body granuloma development with negligible collagen formation. All tissue reactions decreased over time.

5.2.1.3 Irritation and sensitisation

Kevlar®(fibre as such has only slight potential for skin irritation. Skin sensitisation has not been observed (DuPont, 1996)

5.2.1.4 Carcinogenicity studies

5.2.1.4.1 Intraperitoneal/Intrapleural administration studies

Intraperitoneal and intrapleural injections of fibril dispersions can produce a high incidence of mesotheliomas in experimental animals. The technique has been extensively employed to determine the influence of fibril dimensions on carcinogenic potential (Pott et al., 1980; Stanton et al., 1981). intra-cavity models have been advocated as relatively cheap and highly sensitive tests to predict the carcinogenicity of fibres. However, these routes of administration bypass all natural defences, and the single dose (or few repeated doses) early in life is also un-physiological (ECETOC, 1996). It is important to stress that these exposure routes, although non-physiological, are intended to mimic conditions, where fibres have transmigrated from the lung or gut wall into the intrapleural or intraperitoneal cavity. These studies focus especially on mesothelioma incidence, as fibre transmigration is thought to be the mechanism by which asbestos produces mesothelioma in humans (HSE, 1995). However, there is considerable concern that intra-cavity models may give false positive results - even for the prediction of mesothelioma risk - and there is no consensus over their predictive value for lung cancer (ECETOC, 1996). A WHO consultation (1992) concluded that the intraperitoneal model cannot be used for quantitative risk assessment or for comparing the relative hazard of different respirable fibres.

The carcinogenicity of p-aramid was assessed in rats intraperitonally injected (Davis, 1987) with pulp primarily ranging from 0.5 to 8 millimeters in length (NIOSHTIC database abstract). As noted earlier, abrasion can peel respirable fibre-shaped particles from the surface of larger pulp fibres. In this particular study, the p-aramid preparation was a pulp previously subjected to violent desegregation and contained both filaments and large aggregates. Length measurements proved impossible because the fibres were too tangled, but 96% were < 1 µm diameter and 56% < 0.25 µm diameter. AF/HAN-rats were injected intraperitoneally with a single dose of 25, 2.5, or 0.25 mg/kg (groups of 44, 32, 48 rats, respectively). Twelve top dose animals were killed at intervals up to 9 months after injection for histological examination. The remainder were killed as they aged (40% survived for nearly 3 years). All animals were subjected to full gross autopsy, with histological examination of abnormal tissues. No difference in survival was noted when treated rats were compared to control rats (48 animals, untreated). Cellular reaction to the fibres was vigorous with large cellular granulomas developing in which injected p-aramid was embedded. The granulomas consisted mainly of macrophages and fibroblasts, but also held many foreign body giant cells. Eventually fibrosis developed and both collagen and reticulin were identified. A gradual increase in fibrosis was described over 2 to 3 years. Peritoneal mesotheliomas (each consisting of numerous small tumour nodules) were found in 2 of 32 rats in the group receiving the highest dose (25 mg/kg), and although this was not a significant increase, the author concluded that the preparation possessed a low, but definite carcinogenic potential when administered intraperitoneally (Davis, 1987). No mesotheliomas were seen in other groups. Historically no mesotheliomas had been seen in "several hundred" (apparently uninjected) controls over a 12-year period. Peritoneal sarcomas were found at low levels in all groups including controls (3.1-6.3%, no dose response) (Davis, 1987).

Pott et al. (1987) administered 10 mg of p-aramid fibrils, prepared by ultrasonic treatment of pulp, to 5-week old female Wistar rats in 3 weekly intraperitoneal injections of 2, 4 and 4 mg. Surviving animals were killed 2.5 years after treatment, and incidence of tumours (sarcoma, mesothelioma or carcinoma of the abdominal cavity) was reported in 12.9% (4/31) of test rats compared with 6.3% in controls. In an further study in which there was an attempt to obtain finer fibres and better suspension by drying, milling and ultrasonic treatment, 8-week old female Wistar rats were administered 20 mg p-aramid fibrils (50% < 3.4 μm in length and 50% < 0.47 in width) in saline, 5 injections of 4 mg weekly. At 28 months post-treatment, the percentage of animals that developed tumours was 5.8%. The authors pointed out that it had not been possible to produce a homogeneous suspension of p-aramid fibrils and that, as a result, these fibrils were more likely to be present in clumps in the peritoneal cavity than were other dusts. In these studies, tumour incidences in rats intraperitoneally administered with 0.25 to 0.5 mg actinolite, chrysotile, crocidolite or erionite were in the 50-80% range, while 2.5% of controls (5 out of 204 rats intraperitoneally administered with saline alone) had malignant tumours of the abdominal cavity.

In a subsequent study performed by Pott et al. (1989), female Wistar rats were administered a total dose of 20 mg of milled bulk p-aramid fibrils (length 10% < 2.2μm, 50% < 4.9 μm, and 90% < 12 μm; diameter 10% < 0.28 μm, 50% < 0.48μm, and 90% < 0.76 μm) in 4 weekly doses of 5 mg. The number of fibrils administered was 1,260 x 10^6 and the fibre mass was 88% of the total mass injected. At 130 weeks after treatment, survivors were killed. In the 53 rats necropsied, there was no statistically significant increase in peritoneal tumours (mesothelioma/sarcoma - the 2 tumour types were not differentiated) in test animals (5.7%, 3/53) compared with controls, that were administered saline alone (2%, 2/102). For comparison, following administration of UICC chrysotile (202 x 10^6 fibres, fibre mass 55% of the total mass injected; length 10% < 0.28 μm, 50% < 0.67 μm and 90% < 2.1 μm; diameter 10% < 0.03 μm, 50% < 0.05 μm, and 90% < 0.12 μm) at doses of 0.05 mg, 0.25 mg, and 1 mg, tumour incidences of 33% (12/36), 68% (23/34) and 83% (30/36) were found.

Brinkmann & Müller (1989) reported that in a study based on Pott's protocol (as reported above) 8-week old Wistar rats were intraperitoneally injected weekly for 4 weeks with 5 mg of p-aramid fibrils suspended in 1 ml physiological saline (fibre size distribution or sample preparation method not specified). 28 months after the first injection, the rats were sacrificed. The great omentum, with pancreas and adhering lymph nodes was removed and examined histologically by light- and scanning electron microscopy. Animal numbers and tumour incidence were not reported. Lesions from 2 animals were presented and discussed, and different stages of events following treatment were described. In an initial stage, multinucleated giant cells with phagocytosis of the p-aramid particles and inflammatory reactions were observed. In a second stage, granulomas with central necrosis developed indicating the cytotoxic nature of the fibres. In a third stage, a "mesenchymal activation with capsular structures of collagenous fibres as well as a slight submesothelial fibrosis" were observed. Finally, the reactive granulomatous changes in the great omentum of the rats were accompanied by proliferative mesothelial changes which, in 1 of the 2 rats examined, led to development of a "multilocular" mesothelioma. The authors commented that the reaction to p-aramid fibrils in the intraperitoneal test resembled the well-studied reaction to similar injections of glass or asbestos fibrils. It was also noted that, as in the case of mineral fibres, fragments of p-aramid fibrils were transported through lymphatic pathways and stored in lymph nodes where they caused inflammatory reactions (Brinkmann & Müller, 1989).

Minardi & Maltoni (1988), reported (NIOSHTIC abstract) on the carcinogenic activities of natural and modified asbestos in Sprague-Dawley-rats (groups of 40 animals, 20 each sex). Test materials were administered in a single intraperitoneal, intrapleural, or subcutaneous injection of 25 mg. Rats were observed until spontaneous death or sacrifice at 2 years. Types of natural asbestos tested included crocidolite, chrysotile (Canada, Rhodesia, California), amosite, anthophyllite, and asbestos-cement. Modified materials were asbestos-latex paper or asbestos fibres treated by phosphatation. Included were rockwool and p-aramid RFP's. All natural materials induced mesotheliomas on pleura and peritoneum. The peritoneum was generally more sensitive to induction. Crocidolite was the most potent inorganic material tested, and asbestos-cement was the least potent in terms of incidence and average latency time. Except for p-aramid fibres, modified materials induced mesotheliomas in pleura and peritoneum or in peritoneum only. The asbestos product with the lowest potency was a short chrysotile fibre treated with phosphoryl-chloride at 300°C. The authors concluded that asbestos of different types and origins are mesotheliomatogenic, that the peritoneum is more responsive than the pleura, that carcinogenic effects vary depending on type and origin of material, and that the effect can be altered by changing physical and chemical properties of materials. The validity of long term bioassays in qualitative and quantitative carcinogenic risk assessment was demonstrated (Minardi & Maltoni, 1988).

Maltoni & Minardi (1989) reported that in an additional study different types of natural, modified natural and man-made materials were tested in a highly standardised manner by peritoneal injection into 8-week-old Sprague Dawley rats and Swiss mice in order to assess their carcinogenic potential. They were: crocidolite, chrysotile, amosite, anthophyllite, asbestos cement, crystalline silica, amorphous silica, alumina, wollastonite, talc, kaolin, bentonite, natural zeolites, man made zeolites, rock wool, carbon fibres, and synthetic fibres [2 types, including p-aramid fibres]). Animals were examined 3 times daily for general behaviour and were weighed and examined for gross changes every 2 weeks. They were kept under observation for 104 weeks, at which time the survivors were sacrificed. A complete autopsy was performed on all animals, whether dying naturally or sacrificed. A histopathological examination was carried out on the peritoneum (site of injection), brain, thymus, lungs, liver, spleen, kidneys, adrenals, stomach, uterus, gonads, mesenteric, mediastinal, and subcutaneous lymph-nodes, and all pathological organs and tissues. Groups of 40 Sprague Dawley rats (20 each sex) were intraperitoneally injected with 25 mg p-aramid fibrils (the material being suspended in 1 ml water) and kept for 104 weeks thereafter, but no peritoneal mesotheliomas were seen. Groups of 40 rats (20 each sex) were also intraperitoneally injected with 1, 5, or 10 mg of p-aramid fibrils suspended each time in 1 ml water. At 76 weeks post-treatment, no peritoneal mesotheliomas were observed (Maltoni & Minardi, 1989).

5.2.1.4.1.1 Assessment of intraperitoneal/intrapleural injection studies

As far as studies involving intra-cavity injection are concerned: most of them caused either no incidence or only a low incidence of peritoneal mesothelioma. It is generally assumed, that following intra-cavity injection, any increases in mesothelioma incidence smaller than 10% indicate that the test substance is unlikely to possess a mesothelioma-inducing potential of relevance to human health (Pott, 1987; Pigott, 1991). Only Pott et al. (1987) reported a tumour incidence more than 10% (12.9%) following intraperitoneal administration of p-aramid fibrils, but this percentage represented the combined incidence of mesotheliomas, sarcomas and carcinomas in the abdominal cavity.

In other studies, following intraperitoneal injection of p-aramid fibrils (at doses up to 25 mg) a granulomatous response was observed, but no significantly increased incidence of neoplasms. The authors suggested that the lack of neoplastic response was possibly due to the agglomeration of the p-aramid particles in the peritoneal cavity.

Generally speaking, these studies failed to show any potential for p-aramid fibrils to cause mesotheliomas, even though it is worth stressing in this connection, that due to the physical properties of the material, it was not possible to inject a stable homogeneous suspension.

5.2.1.4.2 Inhalation exposure studies

Inhalation testing using laboratory animals has obvious advantages over other test systems for assessing the toxicity of man-made organic fibres. In fact, this route of exposure is the same as in humans and exposure can be directed to the intact pulmonary system, involving all natural defence, metabolic and exacerbation mechanisms. Warheit & Hartsky (1994) [as quoted in ECETOC (1996)] reported that fibrosis, lung cancer and mesothelioma resulting from asbestos inhalation are similar in rats and human beings - even if quantitative differences exist. However, animal inhalation studies also have disadvantages, which can be summarised as follows: inter-species differences in respiratory anatomy and function; species-specific pathology both in control animals and in treated animals (in the latter, especially as the result of "overloading"); and relatively low sensitivity. Moreover, the value of this approach to fibre toxicity screening is limited because it is time-consuming, expensive and cannot elucidate the details of cellular and molecular events. In addition, it may prove to be extremely difficult to generate sufficient amounts of RFP's to test the more common man-made organic fibres (ECETOC, 1996). Despite these limitations, the WHO concluded that inhalation studies are currently the best available laboratory model for assessing human health risks of fibre exposure (WHO, 1992).

In the study cited earlier by Lee et al. (1988) with Crl:CD(SD)BR rats exposed to p-aramid RFP's, the authors reported pulmonary lesions, that they initially described as "cystic keratinising squamous cell carcinomas" (CKSCC's) in 4 females (6%) at the 100 RFP/ml exposure level (6 hr./day, 5 days/week for 2 years). CKSCC is a tumour not observed spontaneously in this strain or in human beings. More prominent foamy alveolar macrophages, cholesterol granulomas and alveolar bronchiolarization were reported in female rats: this was related to the development of CKSCC. Twenty nine males and 14 females exposed at 400 RFP/ml died from obliterative bronchiolitis resulting from dense accumulation of inhaled RFP's at the ridges of alveolar duct bifurcations during 1 year of exposure. In rats that survived the 1 year at 400 RFP/ml, the extent and severity of pulmonary lesions were significantly reduced 1 year post-exposure. After a year of recovery, no signs of toxicity, body weight changes, or excess mortality were seen in these rats. However, slight centriacinar emphysema and minimal alveolar duct fibrosis were observed. CKSCC's were reported for 1 male (1/36, 3%) and 6 females (6/56, 11%) of this group. The lesions progressively advanced and often occupied a large portion of the lung. Since there was no evidence of malignancy on the basis of biological behaviour and morphological characteristics, the authors stated that CKSCC can be interpreted as benign neoplastic lesions. The lung tumours observed in this study were classified as CKSCC because there was no benign type of squamous cell tumour that was widely accepted in human or animal tumour classification (Lee et al., 1988). The CKSCC's have been classified as metaplastic or dysplastic rather than neoplastic lesions: the authors stated that "it appears appropriate to designate new diagnostic nomenclature indicating a benign type of

squamous cell tumour such as keratinising squamous epithelioma in order to distinguish it from squamous cell carcinoma". In fact, Lee (1989) later changed his own diagnostic terminology to "cystic keratinising squamous cell tumour" (CKSCT). Due to some specific properties, i.e. species- and gender specificity, histopathological characteristics and mechanism of tumorigenesis, it has been speculated that these lesions have little relevance for humans (Lee et al., 1988; Lee, 1989). No mesotheliomas were seen. Given that the p-aramid RFP's were of what is generally considered to be "the most carcinogenic size" (length > 8 μm and diameter < 1.5 μm) for natural and man-made mineral fibres, the authors concluded that the lack of mesothelioma induction by p-aramid RFP's probably is related to their branched and curled morphology (Lee et al., 1988). Another important finding was the absence of transmigrating RFP's in pleura and other vital organs (Lee et al., 1981). The unique physical configuration of p-aramid RFP's appears to prevent them from penetrating lung tissue. The amount of transmigrated p-aramid RFP's in the tracheobronchial lymph nodes was minimal compared to that seen with asbestiform RFP's (Lee et al., 1981).

5.2.1.4.2.1 Nature of p-aramid RFP induced cystic pulmonary lesions in the rat

On October 5-6, 1992, an attempt was made to obtain a suitable descriptive diagnostic term for and a consensus about the significance of cystic keratinising pulmonary lesions induced in rats by p-aramid RFP's and pigment grade TiO_2 particles. A panel of medical and veterinary pathologists under the chairmanship of Dr. L.S. Levy, University of Birmingham addressed the morphology of these lesions (Carlton, 1994). All participants agreed, that these cystic keratinising lesions were not malignant neoplasms. The majority was of the opinion that they were not neoplasms. A minority (3/13) considered the lesions to be benign tumours. The participants had not seen similar cystic keratinising lesions in humans and concluded that the most appropriate morphologic diagnosis for the lesions was "proliferative keratin cyst" (PKC). In addition, the panel agreed on the following descriptive text: "The lesions are cysts lined by a well-differentiated stratified squamous epithelium with a central keratin mass. Growth appears to have occurred by keratin accumulation and by peripheral extension of the metaplastic change into the adjacent alveolar spaces. The lesions are sharply demarcated except in those areas in which there has been an extension of metaplasia into adjacent alveoli. The squamous epithelium has few mitotic figures and dysplasia is absent."

The conclusions of this 1992 workshop was also summarised by Levy (1994). All participants agreed that the overall morphological appearance of the lesions from the TiO_2 and p-aramid RFP studies were indistinguishable. Thus, any comments on the pathology would be equally valid for both substance-induced lesions. There was also a consensus regarding certain key pathological features, that was summarised as follows:

i- all participants felt that the lesions were not malignant neoplasms;

ii- the majority (10/13) felt that they were not neoplasms and could best be described as some form of cystic keratinising lesion;

iii- a minority (3/13) felt that they were best described as benign neoplasms;

iv- the key diagnostic features that led to a final and unanimous diagnosis were the absence of invasion, the absence of metastases, orderly squamous metaplasia and keratinisation, paucity of mitotic figures, and absence of dysplasia;

v- the final unanimous morphological diagnosis that was acceptable to all participants and the accompanying descriptive text was "proliferative keratin cyst" (PKC).

The pathogenesis of the lesions was further discussed by the panel. Firstly, a majority agreed that the observed squamous lesions arose primarily as a consequence of or alongside a severe particle-induced inflammation and that the lesions would not occur without the background of such inflammatory changes. Secondly, it was noted that irregular growth into free spaces, such as alveoli, is common in metaplastic lesions. In the slides viewed, the epithelial boundaries of the lesions became more regular around solid surfaces, such as pleura, vessels, and interlobular septa. Thirdly, although the cystic lesions appeared to enlarge over time, they remained similar in nature by increasing central accumulation of keratin and by proliferation at the squamous epithelial margins. As far as the relevance of these lesions to humans is concerned: none of the participants (and, more significantly, none of the human pathologists), had seen such lesions in humans. There was unanimous agreement, that it was not possible to use these induced squamous lesions in rats to predict the possible carcinogenicity of TiO_2 or p-aramid RFP's for humans.

Other relevant points made were that:

i- these PKC lesions are rare as spontaneous lesions in the rat, but have been seen in a number of different strains;

ii- one of the participants had induced such lesions with other substances and had not been able to transplant them into nude mice;

iii- rats are highly susceptible to keratin formation in the respiratory tract, and there may be a hormonal relationship;

iv- true keratin squamous carcinomas can be induced in the rat lung by carcinogens. These are invasive, can metastasize, are fatal, and are transplantable;

v- sequential studies of the PKC lesions and serial sectioning might permit better characterisation of the biological activity (Levy, 1994).

The conclusions of this *ad hoc* international pathology panel are consistent with evaluations made by a Pathology Working Group who peer-reviewed a National Toxicology Program sponsored study on talc in rats and mice (NTP, 1993). Inhalation of talc produced a spectrum of pulmonary lesions in female rats including inflammatory, reparative and proliferative processes. Alveolar/bronchiolar adenomas, and carcinomas were observed in the lungs of female rats. Squamous cysts were observed with greater frequency in the lungs of female than male rats. The Pathology Working Group noted that squamous metaplasia of the alveolar epithelium was usually associated with inflammation and was characterised by the replacement of alveolar Type I and Type II epithelial cells by well-differentiated keratinised squamous cells. Squamous cysts had outer walls of well-differentiated, stratified squamous epithelium without cellular atypia and central lumens often contained sloughed keratin. While there was a consensus in the Group that the squamous cysts represented a form of squamous metaplasia (and not a neoplasm), there was some uncertainty regarding the biological potential of these lesions. Although these squamous cysts were considered pre-neoplastic, there is little known about their potential for autonomous growth or for progression to malignancy (NTP, 1993).

A re-evaluation of the pulmonary lesions in rats from the study by Lee et al. (1988) was also performed by Haskell Laboratory (1993). The review pathologists were in essential agreement with the conclusions of the study as to morphologic changes produced in the rat lung and with respect to the NOAEL of 2.5 RFP/ml for non-neoplastic lesions. The most significant compound-induced morphologic changes were dust cell granuloma

formation, bronchiolarization, and foam cell accumulation. Fibrosis, though present, was minimal to mild and was a sequela of the inflammatory response. The review pathologists concluded that the cystic keratinising lesions in female rats were non-neoplastic, and diagnosed them as "proliferative keratin cysts" (PKC's). Further, there was some question as to whether the single squamous cell carcinoma found in 1 male rat was compound-related. This proliferative squamous lesion, unlike those seen in female rats, was not cystic. In addition, the lesion was poorly keratinising. Though difficult to assess with absolute certainty, this lesion was considered not treatment-related. This opinion was based upon the nearly complete absence of precursor metaplastic lesions in male rats and the absence of squamous cell carcinomas in female rats (the sex more prone to particulate induced proliferative squamous lesions). Though rare, squamous cell carcinoma of the rat lung can occur spontaneously. Therefore, under the conditions of this study, p-aramid RFP's were not considered carcinogenic in rats. Finally, the review pathologists considered respiratory insufficiency/failure as the best designation for the cause of death in high-dose animals (400 RFP/ml) found dead or moribund prior to the 1-year-interim sacrifice. Respiratory insufficiency was likely the result of multiple factors including a heavy lung burden of RFP's and pulmonary granuloma formation. With pulmonary function thus compromised, continued exposure to high RFP concentrations likely resulted in acute respiratory failure (Haskell Laboratory, 1993).

On February 15-17, 1995, another pathology workshop composed of an international panel of experts, was held in Hannover (Germany). This "Pathology Workshop on Keratinous Lesions in the Rat Lungs" was organised by the German Research Society (*Deutsche Forschungsgemeinschaft)* primarily to address the diagnosis of dust-induced proliferative squamous lesions in the rat lung. Diagnostic terminology and criteria for these lesions were developed by the panel. The pathologists agreed on diagnostic criteria for: 1) simple metaplasia (transition of alveolar epithelium into squamous epithelium); 2) pulmonary keratinous cysts (thin-walled hollow lesions filled with keratin [horn-like substance] without signs of autonomous growth); 3) cystic keratinising epitheliomas (as above, but with signs of benign peripheral autonomous growth); 4) squamous cell carcinomas, of which 2 types are acknowledged: a) the keratinising type, which may or may not arise from the wall of an epithelioma, b) the non- or poorly keratinising type, which occurs independently. These lesions appear to be unique for the rat lung; even epitheliomas occur only at a late stage, and they do not necessarily (or often) progress to squamous cell carcinomas; thus, it should not be considered as a pre-cancerous lesion. The chairman of the panel expressed as his personal opinion that a malignant degeneration would be more substance-dependent than time-dependent. The panel was of the opinion that, as long as lung pathology was limited to epitheliomas, they had probably little relevance for humans (Wagner, 1995; Boorman, 1996).

Using the criteria established at the Hannover workshop, another review at Haskell Laboratory concluded that all lesions in female rats that were originally diagnosed by Lee et al. (1988) as CKSCC really were keratin cysts, while the squamous lesion in the male rat was an authentic squamous cell carcinoma (Brockmann et al., 1995; Haskell Laboratory, 1995). Thus, the diagnosis of the squamous lesions based on the criteria established at the Hannover workshop confirmed the results of the 1992 Haskell pathology workshop reported by Carlton (1994). These cystic keratinising lung lesions appear to be unique to the rat. The panel concluded that if the only evidence of tumorigenicity is the presence of cystic keratinising epitheliomas, then it may not have relevance for human safety evaluation (Brockmann et al., 1995; Boorman et al., 1996; Frame et al. 1997).

Finally, the squamous cystic keratinising lesions from the p-aramid 2-year inhalation study by Lee et al. (1988) were re-evaluated by 4 pathologists (3 participants of the panel) according to the criteria established by the 1995 Hannover workshop. Using these criteria, unanimous agreement was reached for a diagnosis of pulmonary keratin cyst for 9/10 cystic keratinising squamous lesions produced in female rats. The one remaining cystic squamous lesion was more difficult to classify, with one pathologist considering it to be a cystic keratinising epithelioma, and three others considered it a pulmonary keratin cyst. The squamous lung lesion, which occurred in the one male rat was unanimously diagnosed as squamous cell carcinoma. The authors concluded that the cystic keratinising lung lesions produced following exposure to p-aramid and many other dusts appear to be unique to the rat, and that the keratin lesions are probably not relevant for human risk assessment of pulmonary cancer (Brockmann et al., 1995; Frame et al., 1997).

As stated, the diagnosis of the cystic keratinising squamous cell lesions induced in rats following chronic exposure to high concentrations of a variety of dust particles has been controversial. To compare the proliferative activity of keratoacanthomas (KA) from untreated rats and the cystic keratinising lung lesions (KL) from rats following chronic exposure to p-aramid RFP's, examples of each of these lesions were evaluated for cell proliferation by immunostaining for the proliferating cell nuclear antigen (PCNA, 6 KA and 5 KL) and enumeration of silver-stained nucleolar organiser region proteins (AgNOR, 5 KA and 5 KL) (Frame et al., 1996). Positive immunostaining of nuclei on PCNA was present within the squamous lesions of both the skin and lungs. In those areas of KL where the margins of the cyst wall were distinct, labelled nuclei were primarily limited to segmental areas along the basal-most cell layer or, in the cases of epithelial nests, to the peripheral layer of the cells. More numerous immunopositive nuclei were present within areas of inflammation immediately adjacent to the epithelial wall of KL. Cells in these areas included inflammatory cells, fibroblasts, alveolar and bronchiolar epithelium, and small clusters of squamous epithelium. Definitive identification of labelled cells was not always possible in these areas.

Most margins of all KA evaluated were well circumscribed, but compared to analogous areas in KL, intensely-stained nuclei were usually present along a greater extent of the basilar margin. In focal areas of most KA evaluated, intense nuclear labelling extended above the basal-most layer of cells. AgNOR numbers within nuclei were variable both within and between KA and KL, but were increased in KA as compared to KL. The mean AgNOR count for KL was 1.76 ± 0.21 (ranging 1.50-2.04). The mean AgNOR count for KA was 2.51 ± 0.41 (ranging 2.13-2.95). This increase in mean AgNOR counts was statistically significant (Student's t test, $p < 0.05$). The results of PCNA labelling and AgNOR counts suggest, that the proliferative activity of the KL was less than that of KA, especially at those margins of the KL that were not associated with severe inflammation or expanding against dense barriers. Indeed, when abutting structures more analogous in density to dermal collagen, the group of KL evaluated resembled an epidermoid cyst and in the absence of criteria of malignancy, are best designated as cysts (Frame et al., 1996). Such a designation is also supported by Nolte et al. (1993), who established that the KL has intense immunopositivity for cytokeratin expression - suggesting a high grade of differentiation. Evan et al. (1993) also reported that the finding of some limited cell proliferation activity in the basal epithelium of KL is not inconsistent with the designation of the lesion as a cyst. Frame et al. (1996) concluded that assessment of cell proliferation in archived tissues using PCNA immuno-histochemistry and AgNOR staining should prove

useful for comparing the proliferative activity of KL induced by different dusts and for comparing the proliferative activity of KL with other proliferative squamous lesions.

All the results of inhalation toxicology studies associated with p-aramid RFP's have been recently summarised by Warheit (1995). The review is subdivided into 2 categories: inhalation toxicity studies and mechanistic inhalation studies. Keratin-associated lesions were observed in the lungs of female rats following chronic exposure to high concentrations of p-aramid RFP's. These lesions were originally interpreted as cystic keratinising squamous cell carcinomas (CKSCC, Lee et al., 1988). In recent years, this keratinising lesion has been observed in the lungs of rats with great regularity in numerous chronic inhalation studies with a variety of dusts. To reach a consensus on an appropriate diagnosis for this lesion, an international panel of pathologists was convened. This panel considered that the most appropriate diagnosis for this lesion was "proliferative keratin cyst" (PKC); the biological potential of the PKC remains controversial, but it appears to be unique to the rat species and has little relevance for humans.

Mechanistic studies with p-aramid have demonstrated that acute inhalation of high concentrations of RFP's produces a potent but transient pulmonary inflammatory and cell labelling response. Inhaled RFP's have low durability in the lungs of rats as evidenced by a progressive decrease in median fibre lengths with increasing residence time in the lung. In contrast, size-separated chrysotile asbestos produced a sustained increase over controls in cellular proliferation responses of terminal airways, parenchyma, subpleural and mesothelial regions. In addition, while the short chrysotile fibres were cleared at a normal or rapid rate, the longer ones (i.e. > 5 μm) were retained in the lung or cleared slowly, suggesting that this sub-population of fibres is durable in the lung of exposed rats. The author concluded that these results point out the differences in lung responses to p-aramid and chrysotile RFP's: chrysotile produces enhanced and sustained cell proliferation responses and long chrysotile fibres are retained in the lung (Warheit, 1995).

5.2.1.4.2.2 Assessment of inhalation studies

The study by Lee et al. (1988) appears to be the key investigation for assessing the carcinogenic potential of p-aramid RFP's by inhalation exposure. However, this 24-month study has come under criticism for different reasons, despite its apparent compliance with EPA and OECD GLP's. First, the results are of limited value for assessing the potential of p-aramid RFP's to induce mesothelioma, because mesothelioma normally requires at least 28-30 months to develop in rats (HSE, 1995). It has also been commented that the study might well have been terminated too early for an eventual evolution of PKC's into other malignancies. A fortuitous degeneration of a small percentage of PKC lesions can of course never been excluded, but the observed nature of the lesions does not support such speculation (ECETOC, 1996). However, as far as methodology is concerned for evaluation of the effects of RFP's by inhalation, it should be noted that at the time that it was carried out, this study fully met EPA and OECD standards and that the natural lifespan of the Sprague-Dawley rat did not seem to justify its prolongation.

A more important criticism concerns the exposure levels used. The exposure level of 400 RFP/ml clearly exceeded the MTD, with consequent excessive mortality and forced discontinuation of exposure after 1 year. To assist further in the interpretation of the results of this study, a comparison of the effects obtained with p-aramid RFP's to those produced by chrysotile asbestos is considered to be potentially worthwhile. A study by Davis et al.

(1978), in which 48 rats were exposed by inhalation to a chrysotile concentration of 390 RFP/ml for 1 year seems to be suitable for comparison purposes. At concentrations of 400 RFP/ml, both p-aramid RFP's and chrysotile produce extensive pulmonary damage, but it is worthwhile stressing that the detailed toxicological profile is quite different. For example, occurrence of PKC's and early deaths due to obliterating bronchiolitis were reported in p-aramid-exposed rats, while in chrysotile-exposed rats PKC's were not been seen and long-term survival was unaffected. After chrysotile exposure, a far more severe degree of pulmonary fibrosis occurred than after p-aramid exposure. In chrysotile-exposed rats one peritoneal mesothelioma (not useful for comparison purposes with p-aramid RFP's) and two malignant lung tumours (one squamous cell carcinoma and one adenocarcinoma) were observed. Both lung tumour-types developed against a background of marked pulmonary fibrosis. We have already discussed the study by Lee et al. (1988), in which a gender specificity in the occurrence of PKC's (described only for female rats) was reported, and a single squamous cell carcinoma in 1 male rat (not PKC-related) was found. These developed against a background of non-neoplastic lung damage, for which a NOEL of 2.5 RFP/ml was identified. PKC's are widely considered as benign lesions of no relevance to malignant tumour development, and the result of a common, non-specific reaction of the rat lung to the particle overload. Furthermore, the one lung carcinoma that occurred (not statistically significant: 1/36) appears more likely to be a spontaneously occurring tumour than a compound-specific event. Given these wide differences in the spectrum of pulmonary toxicity produced by p-aramid and chrysotile RFP's, it is doubtful that a good overall comparison of pulmonary/pleural toxicity on a fibre-for-fibre basis can be made. It would seem more appropriate to make comparisons with respect to particular end-points. As for mesothelioma occurrence, it would appear that the degree of hazard must be very low for p-aramid RFP's - likely much lower than for chrysotile fibres.

A low potential of p-aramid RFP's to produce mesothelioma is also suggested by the inconclusive evidence for the penetration of the pleura provided by Lee et al. (1988). In a more recent study (Warheit et al., 1995), no increases in cell proliferation in the sub-pleural and mesothelial tissues have been observed in rats following short-time high-level inhalation exposure, in contrast with chrysotile tested under the same conditions. Finally, taking into account that durability of fibres is an important property with respect to mesothelioma development, the lack of durability for RFP's in the lung reported by different investigators also suggests that the degree of mesothelioma hazard for p-aramid RFP's is very low.

5.2.1.5 Mutagenicity

p-Aramid fibres, as well as other many synthetic fibres and yarns, are sometimes "dipped" after spinning to apply an epoxide coating, which improves abrasion resistance or bonding qualities. Acrylates and polyurethanes are also used. The exact composition of these dips may be confidential. Coated p-aramid yarns, which are non-respirable, have been used in skin sensitisation and *in vitro* genotoxicity tests (HSE, 1995). Wilmer et al. (1984) reported that Ames tests were carried out on extracts from coated and uncoated p-aramid yarns. The extraction procedure involved extracting yarn with methanol or tetrahydrofuran for 4 hours (temperature not stated), with subsequent evaporation of the extract. p-Aramid fibres have been shown to be stable (no loss of tensile strength) for up to 300

days storage in various solvents including methanol (at room temperature) (Enka, 1984). Therefore, the Ames test probably measured the mutagenicity of the coating. Two components of a dip used by Akzo were also tested. The tests were conducted on *Salmonella typhimurium* strains TA 1535 and TA 100, with and without metabolic activation (rat liver S9 mix). The methanol extracts of coated and uncoated p-aramid fibres (Twaron®, Akzo) showed no mutagenic activity. However, the 2 components of the dip showed a strong, positive dose-response with and without metabolic activation. The tetra-hydrofuran extract of a coated p-aramid fibre (Kevlar®, Du Pont) was positive with metabolic activation in strain TA 1535. These results suggest that solvent extracts from uncoated p-aramid are not mutagenic in bacteria; however, some activity may be present in extracts from coated fibres.

Wening et al. (1989) briefly reported that p-aramid fibres were not mutagenic in the Ames test (with a preincubation step of 30 min. to 4 hrs.) with the *Salmonella typhimurium* strains TA 97, TA 98, TA 100, TA 102, TA 104, TA 1535, TA 1537, and TA 1538. p-Aramid fibres were also not cytotoxic in V79 Chinese hamster ovary cells. Fibres, ethanol extracts and chloroform extracts were all tested. Fibre coatings were not mentioned. No information was provided about the dimensions of the tested fibres and there is no indication that p-aramid was also tested in the form of RFP's.

5.2.1.5.1 Summary of mutagenicity studies

p-Aramid fibril extracts were not mutagenic to *Salmonella typhimurium* or to Chinese hamster V79 fibroblasts.

5.2.1.6 Reproduction effects

This topic is not relevant to p-aramid RFP toxicology since the distribution pattern precludes effects on the reproductive system (HSE, 1995).

5.2.2 Human data

No information is available on the effects of acute exposure to p-aramid RFP's, or on repeated exposure by routes other than inhalation. Genotoxic, carcinogenic, and reproductive effects have not been studied in human beings.

5.2.2.1 Inhalation exposure

Pal et al. (1990) reported that lung function of a group of 167 workers regularly co-exposed to p-aramid RFP's and sulphur dioxide was monitored, with a follow-up after one year. The subjects were compared to a control group of 142 workers involved in polyester fibre processing. No data were provided about exposure length or previous exposure history. It was unclear whether variables such as sex and age were taken into consideration when comparing lung function of the 2 groups. In the initial study, a slightly higher carbon monoxide diffusing capacity (D_LCO) and declined forced expiratory volume in 1 second (FEV_1) were observed in exposed workers compared to controls: this evidence has been attributed by the authors to "healthy worker selection" or controls not being a "real not exposed group". The presence of respiratory symptoms did not correlate with

FEV_1 values. In the follow-up study D_LCO, FEV_1, and forced ventilatory capacity (FVC) had all declined in both "exposed" and "exposed plus control" groups, but wide variability in the data make it difficult to assess the significance of the decline. The FEV_1/FVC ratios were not stated. The SO_2 co-exposure and unclear reporting make it impossible to draw any conclusions from this study as to the effect of p-aramid RFP's on human lung function.

5.2.2.2 Dermal irritation and sensitisation

Reinhardt (1980) reported that skin irritation and sensitisation by aramid fibres was assessed in panels of human volunteers. No skin sensitisation, but some minimal skin irritation following dermal contact with fabrics containing Kevlar® or Nomex® were reported. It was also reported that, because these fibres, especially Kevlar®, are stiff, there is a potential for abrasive skin irritation under restrictive contact (i.e. during the wearing of tight clothes).

In a briefly reported human volunteer study, 100 individuals wore patches with squares of p-aramid yarn, 5 day/week for 3 weeks on their skin. This was followed by a challenge patch at 5 weeks. No sensitisation was seen to either coated or uncoated yarn. Earlier studies on 20 and 22 subjects, with an induction period of 6 days, did not induce sensitisation to knitted patches of coated aramid yarns. Slight erythema was seen following induction in a few subjects (2/20 and 4/22) (Du Pont, 1991). These studies indicate that, in humans, p-aramids have no potential to induce skin sensitisation and cause only slight irritancy in some individuals.

Similar to the results obtained with experimental animals, skin contact tests on humans showed no toxic reactions, although mechanical skin irritation by short fibres at clothing chafing points has been observed occasionally. Airborne fibres can also cause mild irritation of the eyes. A single case is known where a skin wound (cut), caused by p-aramid yarn, encapsulated fibre debris. This resulted in a sterile inflammation with persistent scale formation - until the fibre particles had been surgically removed (letter of Braun, C.L.J. (Akzo) to Du Pont, 1989).

5.2.3 An overall evaluation by the IARC

In an evaluation by the IARC (1997), the available data were summarised as follows: no human carcinogenicity data are available; the biological significance of proliferative keratin cysts (PKC) observed in rats exposed by inhalation for 2 years to p-aramid RFP's is unclear; no intra-abdominal tumours were observed in 2 experiments in rats treated by single intraperitoneal injection of p-aramid RFP's; inhalation exposure to p-aramid RFP's in rats for 2 years produced minimal pulmonary fibrosis, and PKC; chronic inhalation studies demonstrate that inhaled p-aramid RFP's are biodegradable in the lungs of rats. Similarly, 2-week inhalation studies in rats and hamsters demonstrate transient pulmonary inflammatory and cell proliferative response and biodegrability of inhaled RFP's in the lungs of exposed animals. p-Aramid fibrils demonstrate some cytotoxic activity to cells under *in vitro* conditions, but p-aramid fibre extracts were not mutagenic to *Salmonella typhimurium* or to Chinese hamster V79 fibroblasts. Therefore, it was concluded that there is inadequate evidence in both humans and experimental animals for the carcinogenicity of p-aramid RFP's, and that p-aramid RFP's cannot be classified as to their carcinogenicity [Group 3] (IARC, 1997).

5.3 Comparative Toxicological Behaviour: Respirable Fibre-Shaped Particulates (RFP's) derived from p-Aramid, Asbestos and Man-Made Vitreous Fibres

Because of the tendency of fibres to align parallel to the direction of airflow, the deposition of fibre-shaped particles in the respiratory tract is largely a function of fibre diameter. Length and aspect ratio are of secondary importance. Electrostatic charges may also have an effect on deposition (Davis et al., 1988).

A study of lung burdens represents an important step for the evaluation of clearance of inhaled RFP's. However, a crucial methodological aspect is that the material of interest must be recovered without loss or damage from the lung tissue. In order to accurately assess changes in lung burden for each fibre type following the cessation of exposure, a series of validation experiments were undertaken by different authors to determine the reactivity of the tested fibres with the reagents used for tissue digestion.

The hypothesis that caustic materials used for digesting lung tissue in fibre clearance studies might produce alterations in the physical dimensions of the recovered particles was tested by Warheit et al. (1991). A simulation investigation was carried out in which bulk samples of six different fibre types were digested by two different caustic materials, bleach and potassium-hydroxide (KOH). Fibre samples included chrysotile, crocidolite, code 100 glass, wollastonite NYAD-G, polyacrylonitrile-based carbon fibre, and p-aramid. Aliquots of these materials were ground and incubated with either bleach, KOH, or saline and the dimensions of the incubated fibrils were subsequently quantified by SEM. Among the observed results were decreased mean and median lengths of carbon fibrils after bleach treatment, increased mean and median lengths of carbon fibrils after KOH treatment, a decreased median length of glass fibrils after KOH treatment, a decreased mean diameter of wollastonite after bleach or KOH treatment, decreased mean and median diameters of p-aramid fibrils after bleach treatment, increased mean and median diameter of glass fibrils after KOH treatment, a slightly decreased mean diameter of chrysotile after bleach treatment, an increased median diameter of wollastonite after bleach treatment, slightly decreased median diameters of crocidolite and chrysotile after bleach treatment, and an unchanged mean diameter of carbon fibrils after either bleach or KOH treatment. The length of bulk sample p-aramid fibrils could not be measured, due to the curly nature of the particles, but the observed decrease in diameter of bleach-treated p-aramid fibrils was statistically significant ($P < 0.05$) - unlike what was found for saline or KOH-treated fibrils. The results of a follow-up study with wollastonite and glass fibres indicated that the direct application of bleach or KOH may have dissolved some of the smallest particles. The authors concluded that lung digestion methods should be further assessed before initiating fibre clearance studies (Warheit et al., 1991).

Franz et al. (1984) compared p-aramid fibrils (length not specified) with UICC B crocidolite and found a comparable degree of cytotoxicity as measured by LDH and beta-galactosidase (β-gal) release and ATP-content of the guinea pig alveolar macrophages.

The toxicity of aramid fibrils for pulmonary alveolar macrophages was investigated in rats (Dunningan et al., 1984). Short aramid fibrils extracted from commercial grade aramid fibres were incubated at doses from 0 to 250 µg/ml with pulmonary alveolar macrophages obtained from male Long-Evans-black-hooded-rats by BAL. After an 18-hr. incubation period, LDH and β-gal added to the incubation medium were measured in freshly harvested

macrophages and those maintained 24 hours in culture prior to the aramid challenge. ATP cell content was also measured using bioluminescence. Chrysotile fibres were used as a positive control. The response of freshly harvested macrophages to chrysotile or p-aramid RFP's was essentially identical, although in cultured cells the cytotoxic response was higher with p-aramid than with chrysotile fibres. In both freshly harvested and cultured macrophage cells LDH and β-gal in the culture medium increased in a dose dependent manner following p-aramid exposure. LDH in the medium increased 35, 50, 60, and 60 % in freshly harvested alveolar macrophages at 25, 50, 100, and 250 µg/ml p-aramid, respectively. In the same preparation, β-gal increased 20, 30, 35, and 45 % with increasing p-aramid doses. Results with cultured cells were similar. ATP decreased to 60, 40, 30, and 20 % of untreated control values in freshly harvested cells following increasing doses of p-aramid. In cultured cells ATP values decreased in a dose dependent manner although the toxicity was not as severe, 250 µg/ml producing a 50 % decrease in cellular ATP. The authors concluded that man-made organic fibres can induce a cytotoxic response in pulmonary macrophages - suggesting that such fibres should not be considered biologically inert (Dunningan et al., 1984). These conclusions were confirmed by Dunningan (1987) in a review of literature concerning the biological effects of selected asbestos substitutes, including p-aramid fibres. The author concluded that the available evidences indicate that p-aramid fibres (as well as all the asbestos substitutes discussed) have some level of biological activity, at least in experimental animals.

The cytotoxicity of p-aramid RFP's to respiratory cells was studied *in vitro* (Marsh et al., 1994). RL90 rat lung fibroblasts and Syrian-hamster tracheal epithelial (HTE) cells were cultured and incubated with 0 to 50 µg/cm^2 p-aramid RFP's for 4 hours to 7 days. The RFP's had mean lengths and diameters of 6.0 and 0.4 µm, respectively. Other cultures were incubated with chrysotile (mean lengths and diameters of 3.21 and 0.06 µm, respectively) and crocidolite (mean lengths and diameters of 3.14 and 0.13 µm, respectively) for comparison purposes. Cytotoxicity was assessed by measuring changes in intracellular protein content after 24 hours and ornithine decarboxylase (ODC) activity after 4 hours. Changes in colony forming activity were assessed in HTE cells after 7 days. Changes in cellular proliferation were determined in both cell types by measuring uptake of tritiated thymidine after 24 hours. p-Aramid caused a dose dependent decrease in intracellular protein concentration in both cell types. The decreases were comparable to those induced by chrysotile and crocidolite. All three materials caused a significant decrease in HTE cell colony forming activity at concentrations above 0.025-0.05 mg/m^3. Lower concentrations stimulated colony formation and a significant dose related induction of ODC activity only in HTE cells. RL90 fibroblasts had very low levels of ODC and enzyme induction could not be detected. All three materials caused comparable and significant increases in cell proliferative activity. The authors concluded that p-aramid RFP's are as cytotoxic *in vitro* to HTE cells and RL90 fibroblasts as asbestos and that they induce similar effects (such as increasing cell proliferative activity) even if the length of p-aramid RFP's used in this study was substantially longer than the length of either crocidolite or chrysotile RFP's (Marsh et al., 1994).

The development of a short-term inhalation bioassay for assessing the pulmonary mechanisms and toxicity of inhaled fibres was described (Warheit, 1993). Crl:CD-BR-rats were exposed by inhalation either to crocidolite fibres for 1 day at 12,800 RFP/ml (41 mg/ m^3, diameter average 0.15 µm), p-aramid RFP's at 600 to 1300 RFP/ml (9-11 mg/ m^3, from a special preparation of p-aramid RFP's as previously utilised by Lee et al. [1988]) or wol-

lastonite fibres at 835 RFP/ml (115 mg/ m^3, diameter range 0.2-3 µm) for 5 days. Following cessation of exposures, rats were killed and lung cells and fluids of sham-exposed and fibre-exposed rats were recovered by broncho-alveolar lavage (BAL). Biochemical assays were performed on BAL fluid for the presence of LDH, alkaline phosphatase (ALP), N-acetyl-beta-glucosaminidase (NAG), and lavage fluid protein. Macrophage cell cultures were established and incubated with carbonyl-iron particles for an *in vitro* phagocytosis assay. SEM was used to assess morphology and *in vitro* phagocytosis. Lung cell labelling studies were conducted to measure the effects of p-aramid RFP or wollastonite inhalation after a 5-day exposure. Lungs from rats were prepared for SEM analysis. p-Aramid RFP's were recovered from digested lung tissue for fibre counting and dimensional analysis by SEM. Increased numbers of the various RFP's were deposited on the alveolar duct bifurcations nearest the bronchiolar/alveolar junctions. Five day exposures to p-aramid or wollastonite RFP's elicited a transient granulocytic inflammatory response with concomitant increases in ALP, NAG, LDH, and protein in the BAL fluid. Biochemical parameters returned to control levels at time intervals between 1 week and 1 month post-exposure. Macrophage function in wollastonite or p-aramid RFP's exposed alveolar macrophages was not significantly different from controls. Crocidolite inhalation exposure elicited a sustained pulmonary inflammatory response that did not return to control levels after 1 month post-exposure. Exposed macrophages were impaired in their phagocytic responses. p-Aramid RFP exposure resulted in retained particles 1 week after exposure, but these cleared rapidly with a half time of about 30 days. Inhaled wollastonite fibres were also cleared rapidly with a half time of 1 to 2 weeks. The author concluded that lung toxicity of p-aramid RFP's is similar to that of wollastonite and carbon fibre and differs significantly from that of silica or asbestos (Warheit, 1993).

A detailed study was performed to examine the post-exposure lung burdens and size distributions of three fibre types (i.e. p-aramid RFP's, chrysotile, and code 100/475 glass) following high concentration inhalation exposure (IOM, 1995; Searl, 1997). Groups of 40 male Wistar rats were exposed by inhalation for 2 working weeks (i.e. 2 x 5 days with an intervening weekend) for 7 hours/day to one of the three test fibres: the exposure levels (expressed as mean and range [daily means]) are summarised in table 5.A, while table 5.B shows the estimated mean concentrations of long (> 20 µm) and very short (< 5 µm) RFP's. Figure 5.1 gives the percentage length distribution of the various RFP's in their aerosol clouds and figure 5.2 shows diameter distribution of the particles in these aerosol clouds as a percentage. The concentration of the three dust clouds was very similar as determined by optical fibre counting (for RFP's > 5 µm) and confirmed by SEM. However, the concentration of very short p-aramid RFP's (< 5 µm) was significantly lower than that of the other two fibre types and the glass fibre aerosol had a lower concentration of long (> 20 µm) RFP's. After exposure, groups of 5 animals were killed at each of 8 time points: 0, 3 days, 1 and 4 weeks, 3, 6, 12 and 16 months post-exposure. The lung fibre burdens were recovered and analysed by optical and electron microscopy to determine RFP dimensions and size distributions as well as the total number of RFP's/lung for each animal.

As pointed out earlier, validation of lung burden studies requires that test materials are recovered without loss or damage. A series of validation experiments were undertaken as part of this study to determine the reactivity of the test fibres with the reagents used for tissue digestion. Length distributions were obtained by SEM of p-aramid RFP's recovered from lung samples 3 days, 12 weeks, 6 months and 12 months post-exposure. The results obtained with the combined ethanolic KOH-Clorox digestion procedure is

given in figure 5.3, while the results obtained with the enzymatic digestion procedure (using collagenase, papain, DNA-ase, and lipase) are reported in figure 5.4. The ethanolic KOH-Clorox digestion technique appears to have a minimal effect on p-aramid RFP's in these experiments with spiked lung tissue, but may have a small effect on p-aramid RFP's recovered from lungs after inhalation experiments. The enzyme digestion method can be seen to be inappropriate for recovery of p-aramid RFP's. It yields different data from the ethanolic KOH-Clorox digestions. These data may provide useful clues to the mechanism by which p-aramid RFP's is cleared from animal lungs. In conclusion, these validation experiments indicate that none of the digestion methods available to recover p-aramid RFP's is ideal, but that the ethanolic KOH-Clorox digestion technique causes minimal particle loss and little change in RFP dimensions (IOM, 1995; Searl, 1997).

Figure 5.5 shows for each of the three fibre types, what percentage of RFP's is retained in the lung as a function of time over the 16-month post-exposure period. The results are given for all particle lengths combined and for short (< 5 μm), intermediate (5-15 μm) and long (> 15 μm) RFP's. The number of long RFP's derived from p-aramid and glass fibres diminishes rapidly - much more so than is the case for chrysotile. p-Aramid shows a slightly slower reduction in the number of shorter and intermediate length RFP's than the other two fibre types, although the long term retention of short and intermediate length RFP's is similar for all three fibre types. Table 5.C shows the lung burdens (all fibres > 0.4 μm) retained after 3 and 16 months recovery relative to the burden after 1 week recovery for each of the three fibre types. Table 5.D shows how the amount of RFP's in different length categories changes between 1 week post-exposure, 3 month post-exposure and 16 months post-exposure. Table 5.E compares clearance half-times in weeks for the same data set. It must be noted that the authors questioned the value of these clearance half-time estimates, because clearance rates did not conform to first-order kinetics (IOM, 1995; Searl, 1997).

The study findings have been summarised as follows: 1) p-aramid RFP's: the rapid pulmonary clearance reported by Warheit et al. (1992) was confirmed. In particular, the longer RFP's, that would be outside the normal size range for macrophage clearance, were rapidly removed through breakage into shorter fragments that could then be removed by the macrophages. RFP breakage appears to have occurred along susceptible zones within the RFP microstructure. 2) Chrysotile: after an initial increase in the RFP count due to fibre splitting, facilitated by wetting in lung fluids, there was a rapid reduction in the total chrysotile lung burden, particularly of particles in the macrophage size range. However, there was relatively little clearance of long fibres and after 1 year, the residual long fibre burden was still about 30% of that present immediately after cessation of exposure. 3) Code 100/475 glass microfibres: these were rapidly removed from rat lungs following cessation of inhalation exposure. The longest RFP's appear to have been most rapidly cleared, probably through breakage into shorter fragments as a result of surface hydration. The main mechanism in the reduction of the total lung burden is believed to be macrophage-mediated clearance. Dissolution was not a primary mechanism by which glass was cleared, although its partial dissolution may have promoted breakage of the longer RFP's.

In a general comparison, the authors (IOM, 1995; Searl, 1997) reported that immediately after exposure, the total lung burdens of the animals exposed to glass and chrysotile RFP's were about 10 times greater than that of animals exposed to p-aramid RFP's, while the number of retained long RFP's was actually somewhat smaller. All three fibres types were

largely cleared (more than 90%) from the animal lungs during the subsequent 4 months. The relative reduction in total lung burden over 16 months, however measured, was found to be similar for all three fibre types. Significantly, however, a strong preferential retention of long (> 15 μm) chrysotile RFP's was observed, whereas both the p-aramid RFP's and the glass RFP's showed preferential clearance of the longer RFP's. Most of the removal of the long RFP's occurred during the first 6 months of recovery for all three fibre types. This left only a small residual population of long RFP's. The rate of removal of long p-aramid RFP's and glass RFP's was much greater than that of chrysotile and the residual population was much smaller. A comparison of the relative reduction in number of short (< 5 μm) and intermediate length RFP's (5-15 μm) for the three fibre types was found to be very different to that of longer RFP's. Only a small reduction in the number of short and intermediate length p-aramid RFP's was observed during the first 3 months post-exposure - in contrast to vast reductions in the number of short and intermediate length chrysotile and glass RFP's (IOM, 1995; Searl, 1997). The lung burdens for all three fibre types appeared to fluctuate dramatically during the first week post-exposure and it may be more meaningful to compare clearance rates only subsequent to the first week of recovery, rather than from time zero. The authors stated that it seems unlikely that RFP's that are only in the lung for a matter of a few days are as hazardous as those that are retained for prolonged periods of time.

In summary: the data for all three fibre types suggest that macrophage clearance was the most important mechanism responsible for reduction of total lung burdens. Macrophage-mediated clearance did not appear to be equally effective for different fibre types within any one given size category nor for particles of the same type, but of different size. This suggests that there may be differences in macrophage response to different fibre types and that RFP length influences the ease with which macrophages are able to ingest and transport them. The biopersistence of RFP's that are too long to be readily cleared by macrophages was distinct from the biopersistence of the total lung burden - regardless of fibre type. This is probably related to the unequal durability of the longer RFP's in the lung environment. The most important mechanism in the removal of the longer RFP's from rat lungs appears to have been breakage into shorter fragments, which could be more readily cleared by macrophages. Different mechanisms of breakage have been proposed for each fibre type. p-Aramid RFP's appear to break along intrinsic discontinuities in the structure, possibly where the amide bonds in the polymer chain are more exposed (and therefore more readily hydrolysed) than similar bonds in the more crystalline parts of the polymer structure. Breakage of the glass fibre was probably largely due to a reduction of its mechanical strength caused by dissolution of certain components - leaving a much weakened silica skeleton. The relatively limited removal of long chrysotile may have been due to longitudinal splitting into diameters below the detection limit or into lengths slightly shorter than those of the parent RFP's. The authors concluded, that in terms of total lung burden (counting all fibres longer than 0.4 μm) all three fibre types had similar, limited biopersistence during the months following the cessation of the exposure. In terms of durability in lung tissue, however, the chrysotile was considered much more durable than either p-aramid or the code 100/475 glass RFP's - which appear to have fairly similar durabilities (IOM, 1995; Searl, 1997).

Another study was designed to compare pulmonary toxic effects of inhaled, size separated preparations of chrysotile asbestos with p-aramid RFP's at similar aerosol concentrations and particle length distributions (Warheit et al., 1994b). Size separation techniques were used to raise the median length distribution of the chrysotile particles from 3.3 μm to 6.2 μm. The median lengths and diameters of p-aramid RFP's (obtained by the same method of aerosol generation described by Lee et al. [1988]), were 9 and 0.3 μm, respectively. Crl:CD-BR-rats were exposed by inhalation to these chrysotile and p-aramid RFP's at concentrations of 750 and 400 RFP/ml for 2 weeks. The post-exposure recovery time periods used for evaluation were: 0, 5 days, 1, 3, 6 and 12 months. The initial pulmonary cellular reaction, studied by ultrastructural morphometry of the alveolar ridges was similar for both fibre types and reversible within 6 months. Pulmonary cell labelling studies demonstrated substantial increases in lung parenchymal, airway, and pleural/subpleural cell labelling indices following chrysotile exposure. Fibre biopersistence and durability results indicated that long chrysotile asbestos RFP's are cleared at a slow rate. As a result, median lengths of chrysotile RFP's increased over time, while median length of p-aramid RFP's decreased. The authors concluded that inhalation of chrysotile fibres is likely to produce greater long-term pulmonary toxic effects than inhalation of p-aramid RFP's (Warheit et al., 1994b).

	Concentration (RFP/ml)		Concentration mg/m^3	
	mean	range (daily means)	mean	range (daily means)
para-aramid	654	418-901	1.3	1.1-1.5
chrysotile	685	406-1553	4.2	2.8-8.4
code 100/475	596	138-870	3.9	0.1-6.3

Table 5.A Mean and range of daily mean dust concentrations measured in the inhalation chambers by optical microscopy (RFP's) and gravimetric sampler (mass) over the 2-week period (IOM, 1995)

	Estimated concentration of short RFP's (< 5μm)		Estimated concentration of long RFP's (>20μm)	
	from optical and EM data	from TEM data only	from optical and EM data	from TEM data only
para-aramid	1138 (436*)	1926	115 (149*)	118
chrysotile	1644	5532	126	378
code 100/475	2283	6278	27	68

* The figures in brackets for para-aramid were derived using SEM analysis of MRE samples, whereas the other combined EM/optical estimates were derived using TEM data.

Table 5.B Estimated mean concentrations of long and very small RFP's in the chamber clouds. The RFP concentrations from the electron microscopy (EM) data scaled to match the mean concentrations of optically counted fibrils are probably a better estimate than those based on transmission electron microscopy (TEM) alone (IOM, 1995)

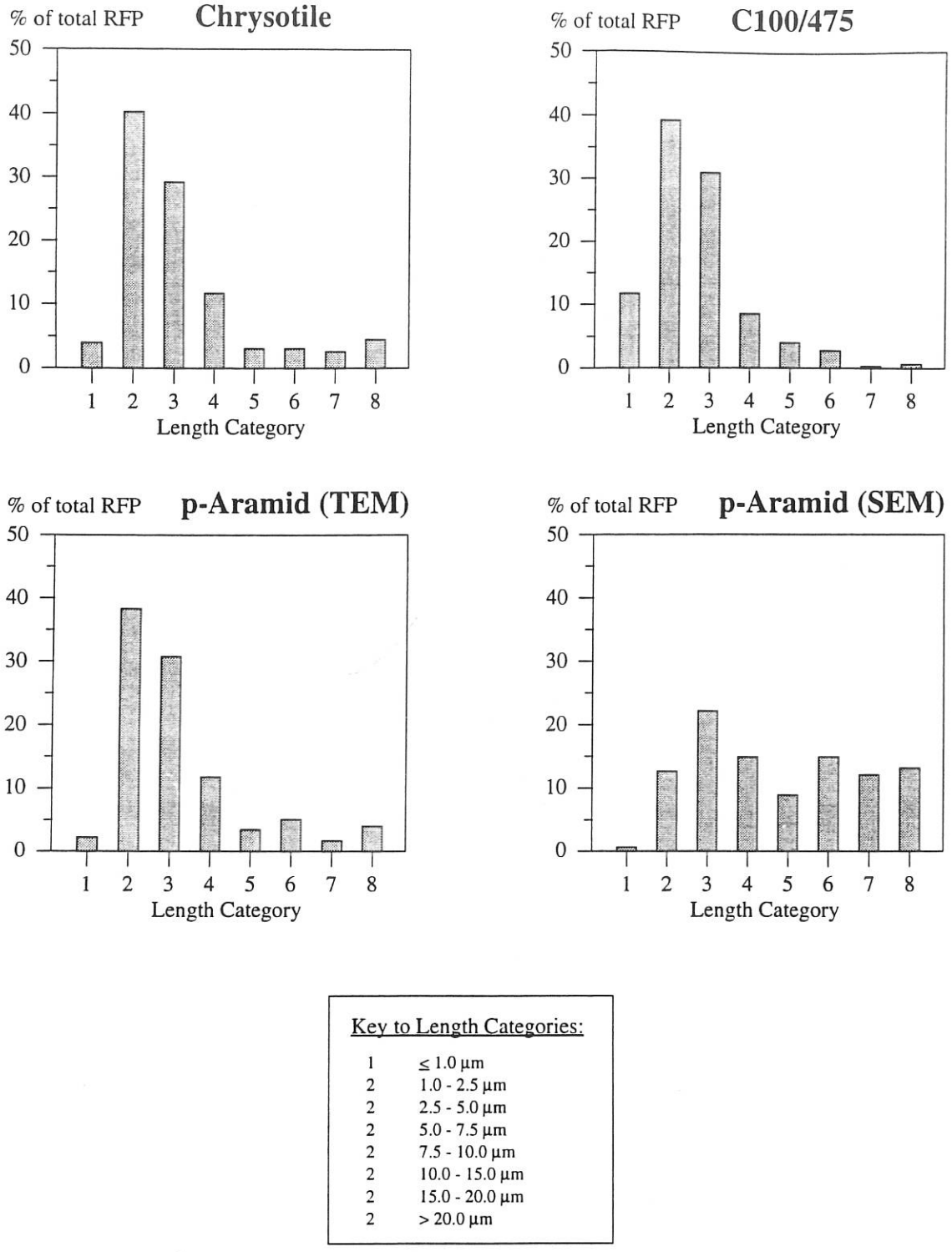


Figure 5.1 Percentage length distribution of RFP's in aerosol clouds as measured by transmission electron microscopy (TEM) for each fibre type and as measured by scanning electron microscopy (SEM) for the pooled gravimetric samples of respirable p-aramid. The length distributions as determined by TEM are similar for all three fibre types whereas the length distribution of respirable p-aramid as determined by SEM includes a smaller proportion of short RFP's and a greater proportion of longer RFP's (IOM, 1995)

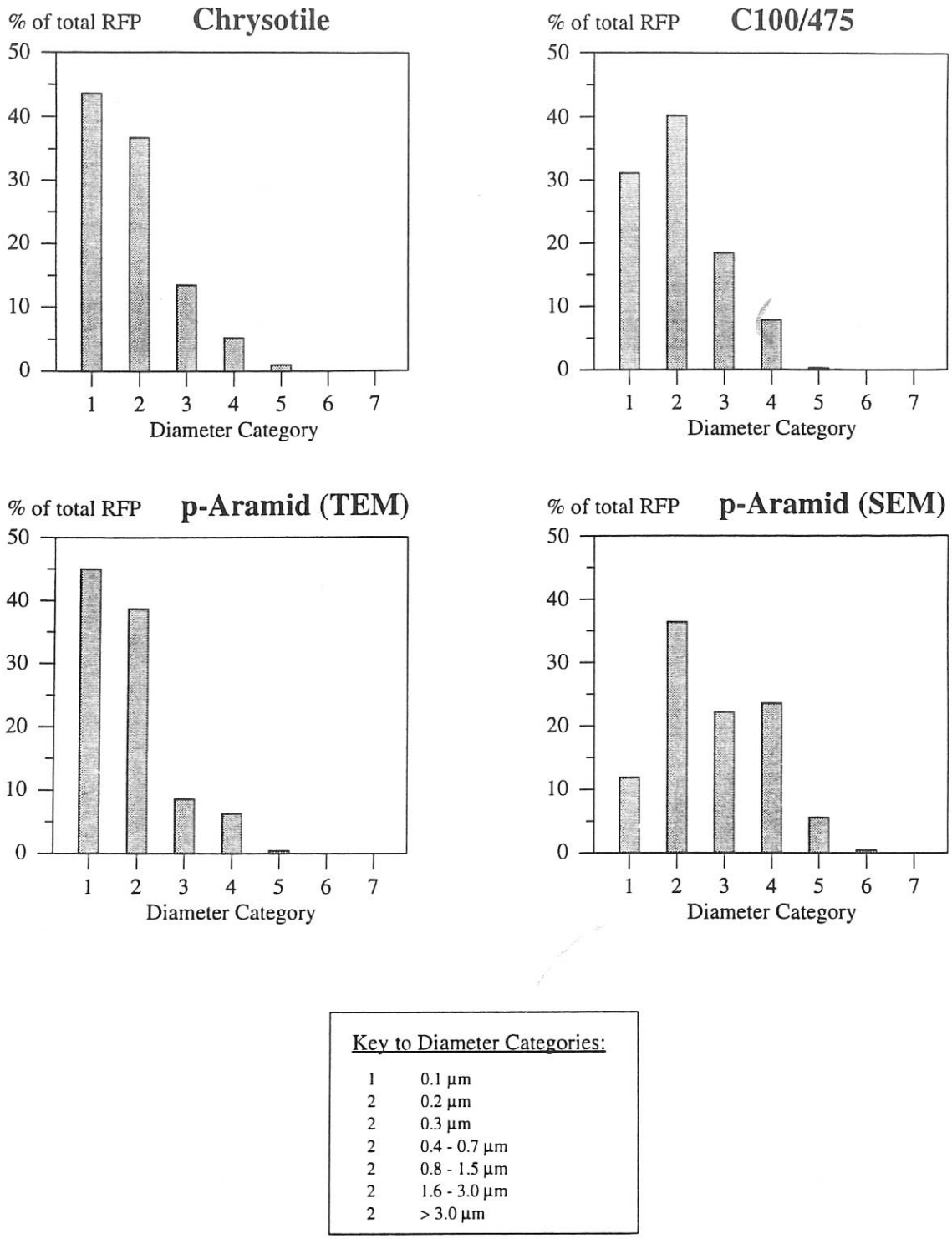


Figure 5.2 Percentage diameter distribution of RFP's in the aerosol clouds as measured by transmission electron microscopy (TEM) for each fibre type and as measured by scanning electron microscopy (SEM) for the pooled gravimetric samples of respirable p-aramid. The diameter distributions as determined by TEM are similar for all three fibre types whereas the diameter distribution of respirable p-aramid as determined by SEM includes a smaller proportion of very thin RFP's and a greater proportion of thicker ones (IOM, 1995)

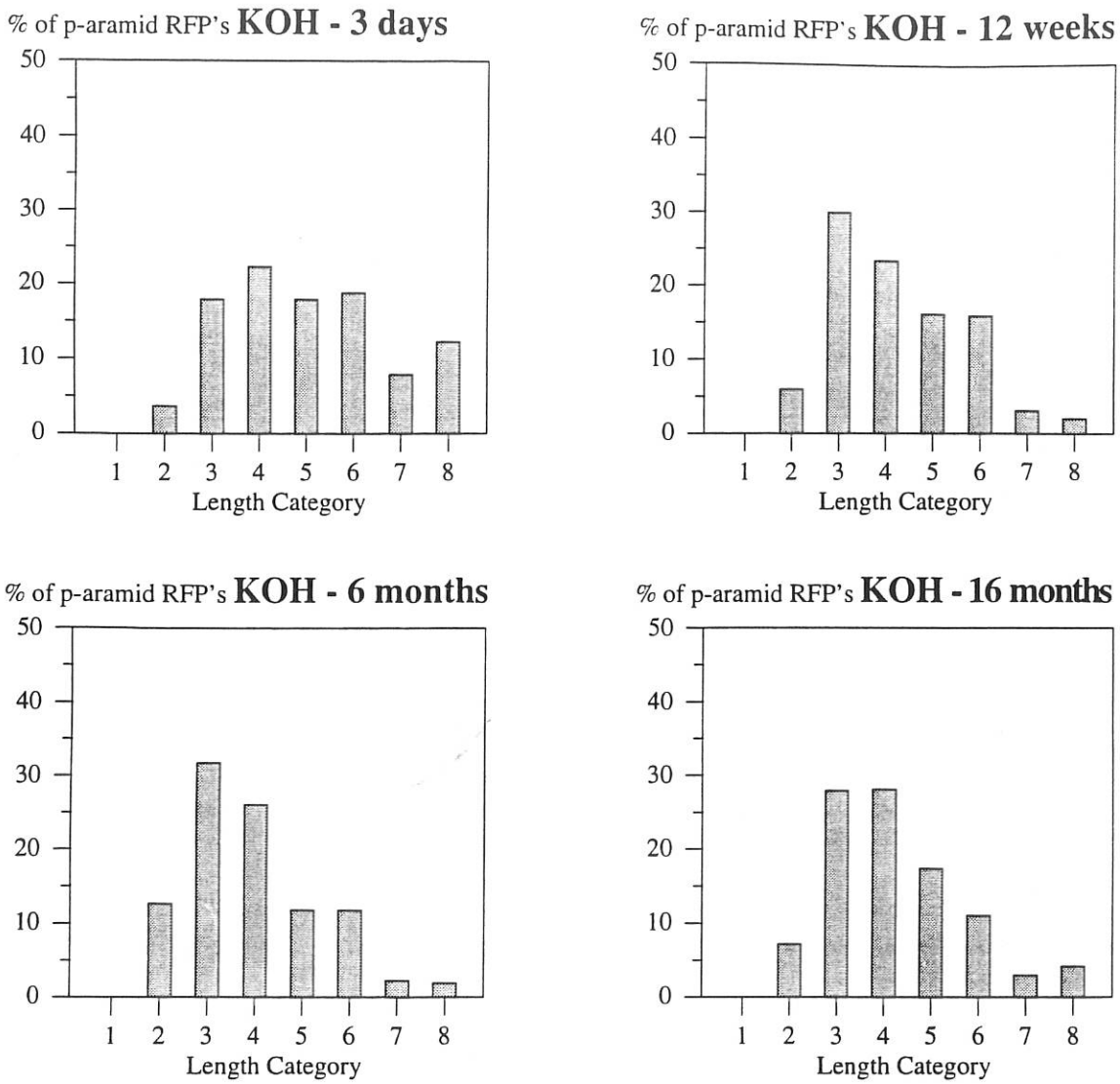


Figure 5.3 Percentage length distribution (SEM data) of p-aramid RFP's recovered from lung samples at 3 days, 12 weeks, 6 months and 12 months post exposure using the ethanolic KOH-Clorox digestion technique. RFP lengths show a marked shift towards shorter length classes through time (IOM, 1995)

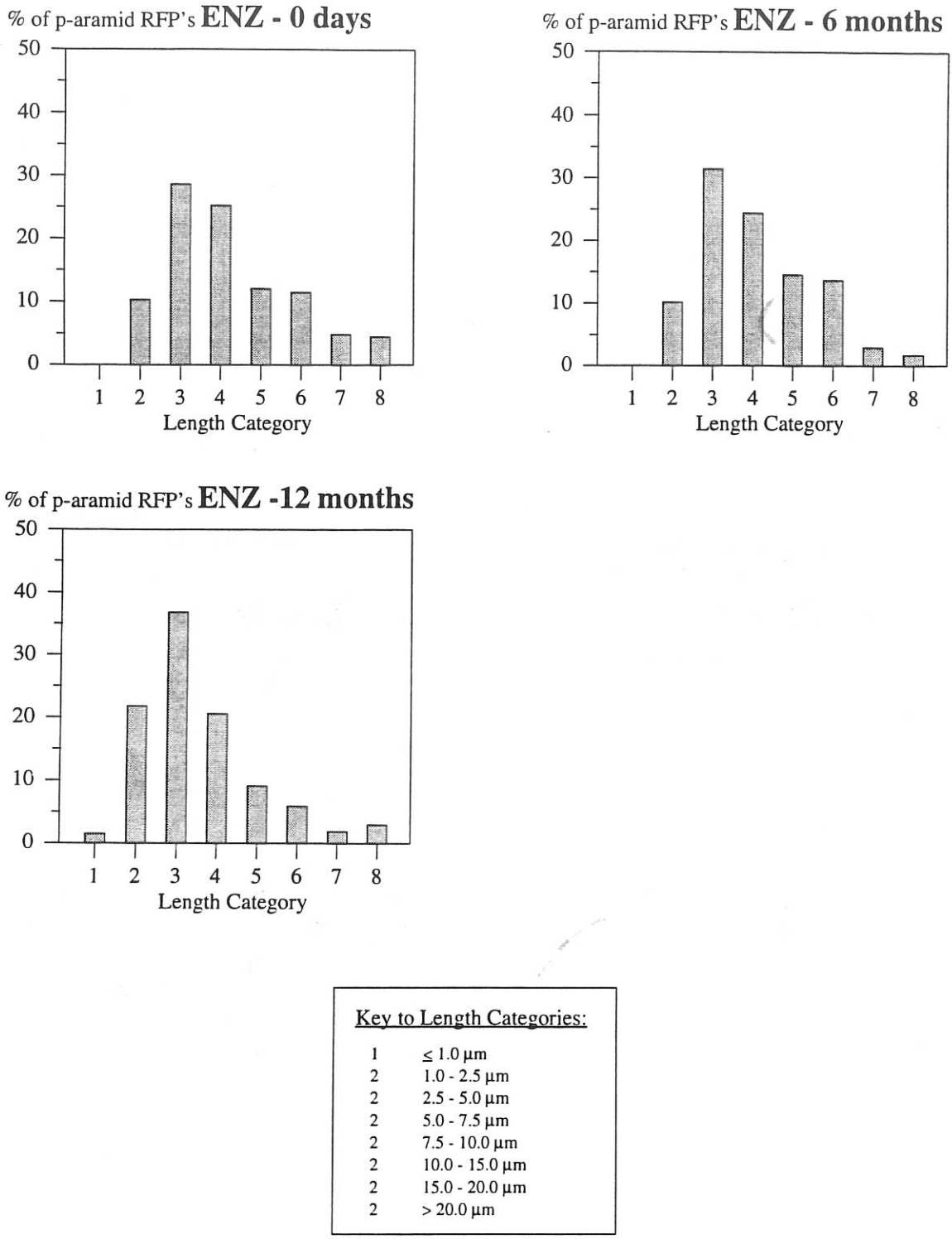


Figure 5.4 Percentage length distribution (SEM data) of p-aramid RFP's recovered from lung samples at 0 days, 6 months and 12 months post exposure using the enzymatic digestion procedure. The data show a greater proportion of RFP's in the shorter length categories at time zero than those recovered using the KOH-Clorox method and a small shift towards shorter length classes with time (IOM, 1995).

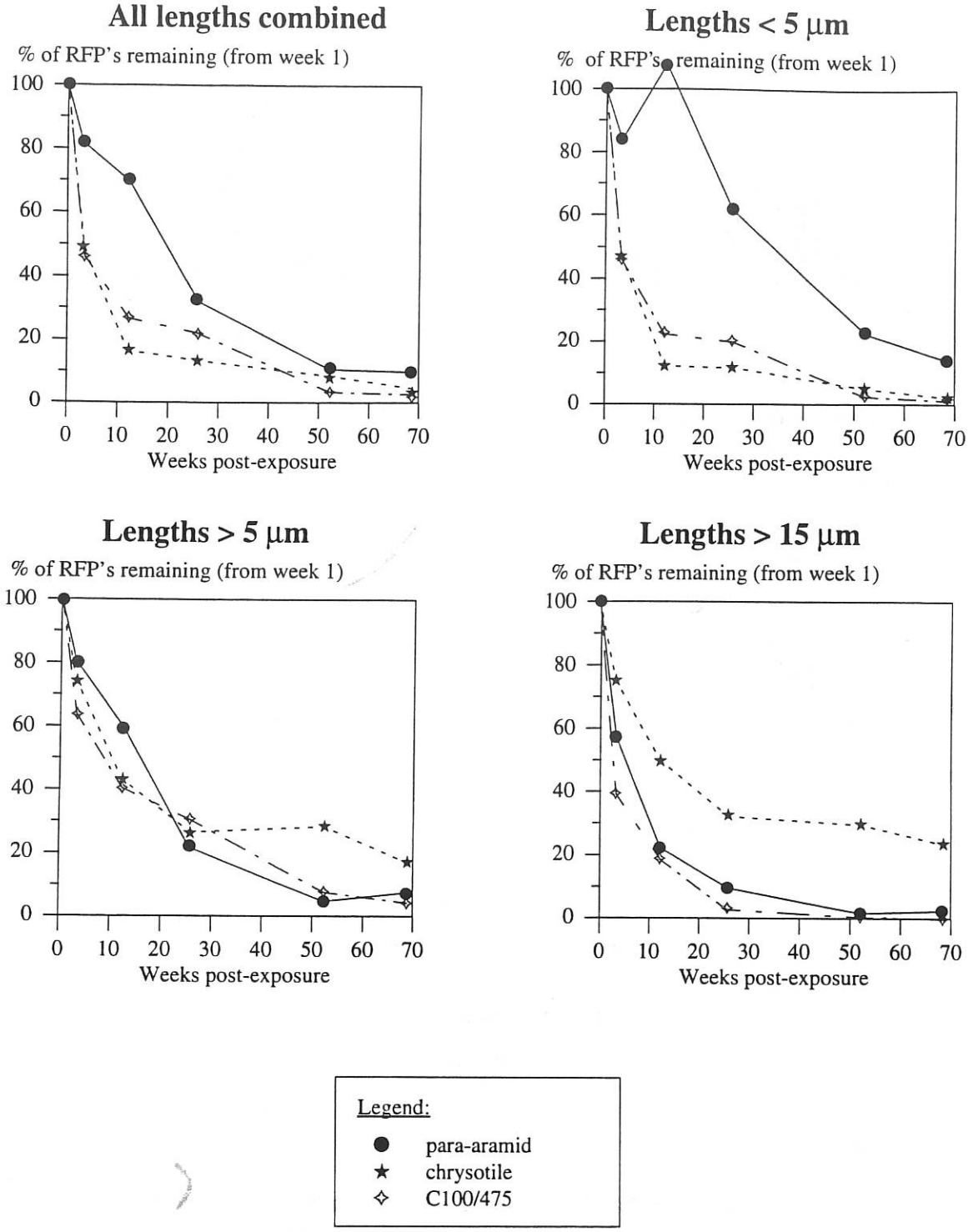


Figure 5.5 Comparison of clearance and mean retained RFP counts in the rat lung for different particle length categories and for each of the three fibre types during a 16 months post-exposure period (IOM, 1995).

	Lung burden remaining after 3 months (%)			Lung burden remaining after 16 months (%)		
	number	volume	length	number	volume	length
para-aramid	70.6 (74.3)	46.8	50.8	8.3 (11.6)	1.8	6.2
chrysotile	16.5 (43.8)	32.6	29.2	3.2 (5.3)	7.7	9.1
code 100/475	26.5 (41.3)	30.7	32.9	1.9 (5.4)	2.7	3.2

Table 5.C Comparison of the relative proportions of the lung burden (all RFP's > 0.4 μm) retained after 3 and 6 months of recovery relative to that retained after 1 week of recovery from the three fibre types (IOM, 1995).

	Lung burden remaining after 3 months (%)			Lung burden remaining after 16 months (%)		
	0.4-5 μm	5.1-15 μm	> 15 μm	0.4-5 μm	5.1-15 μm	> 15 μm
para-aramid	108.3	81.8	19.0	12.5	9.4	3.4
chrysotile	12.5	20.0	49.1	1.4	14.4	24.5
code 100/475	24.2	42.3	18.7	2.3	5.6	0.5

Table 5.D Comparison of the proportion of RFP's of different length categories at 1 week recovery left after 3 months recovery and 16 months recovery for each of the three fibre types (IOM, 1995).

	All fibrils > 0.4 μm			Long fibrils > 15 μm	
	1-4 weeks	4-24 weeks	6-16 months	1-24 weeks	6-16 months
para-aramid	4.5	7.0	9.9	3.1	*
chrysotile	1.3	5.5	9.2	6.8	46.2
code 100/475	1.1	10.1	5.1	2.5	6.6

* too few fibres were analysed to make a valid estimate of clearance half time

Table 5.E Estimated clearance half times (expressed in weeks) for the three fibre types for different time periods during recovery after exposure (IOM, 1995).

6. GROUPS AT EXTRA RISK

The available toxicological information does not suggest that any particular group would be at extra risk from exposure to p-aramid RFP's (HSE, 1995).

However, as far as occupational exposure is concerned, it is reasonable to suggest that workers with existing severe chronic respiratory disease should avoid exposure to p-aramid RFP's, as well as to other fibres and/or particulates.

7. GAPS IN KNOWLEDGE

No information is available on pulmonary deposition and clearance of p-aramid RFP's in human beings exposed by inhalation.

No data are available concerning health effects following both short- and long-term occupational exposure in man. Epidemiological studies in occupationally exposed human populations are also lacking. Even if not crucial for the hazard and risk assessment processes related to p-aramid RFP's, these data could be of importance both in Italy and in other European countries.

8. EXISTING OCCUPATIONAL EXPOSURE LIMITS

In the United States, occupational exposure to p-aramid fibres are currently regulated by the general inert or nuisance dust standard (permissible exposure limits of 15 mg/m^3 for total dust and 5 mg/m^3 for respirable fibre-shaped dust) (Yang, 1993).

It is not advisable to exceed a concentration of 2.5 RFP/ml air, nor a weighted average concentration over 8 hours (standard working time/day) of 2 RFP/ml (Akzo, 1993).

DuPont (1996) has set an Acceptable Exposure Limit (AEL) of 2 RFP/ ml of air (as 8-hr. TWA) for dust derived from Kevlar® - not to be exceeded in its internal operations.

Dutch Ministry of Labour, The Netherlands: a concentration of 2.0 RFP/ml was recommended as a interim occupational exposure limit (OEL) by the Expert Committee for Occupational Exposure Limits (WGD-MAC). The public draft of the Dutch DECOS health-based recommendations for occupational exposure limits proposes a MAC (8-hr. TWA) of 0.5 RFP/ml.

In France, the Valeur Limite de Moyenne d'Exposition (VME, 8-hr. TWA) for p-aramid fibres was originally set at 1.5 RFP/ml and became 1.0 RFP/ml in 1997.

An 8-hr. TWA occupational exposure standard (OES) of 0.5 RFP/ml ("respirable dust") was adopted by HSE in the U.K. (1995). The 2-year rat inhalation study by Lee et al. (1988) was considered key for deriving this OES, since it showed that 2.5 RFP/ml was a NOAEL for pulmonary toxicity in the rat. The adopted OES of 0.5 RFP/ml thus allowed for inter-species differences (HSE, 1995).

In Germany, p-aramid (fibrous dust) is provisionally classified in section III A2 of the list of MAK values by the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Report No. 32. The III A2 classification applies to substances considered to be carcinogenic (only) in animal studies, but is currently under review by the Commission on the basis of substantial new scientific information. Meanwhile, the regulatory advisory panel to the German Ministry of Social Affairs and Worker Protection has *not* adopted this classification, which remains without binding legal implications. A more definitive decision is not expected before end 1997.

9. SUMMARY EVALUATION

9.1 SUBSTANCE IDENTIFICATION

9.1.1 Name and Synonyms

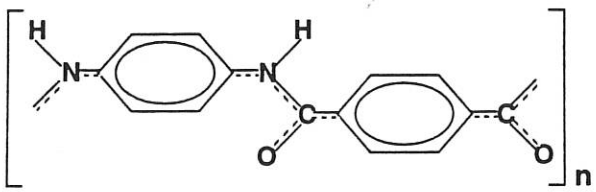
9.1.1.1	**Chemical Name**	poly (para-phenylene terephthalamide)
9.1.1.2	**Synonyms**	aramid, p-aramid, para-aramid, PPT-A, PPDT, poly (1,4-phenylene terephthalamide), poly (para-phenylenediamine terephthalate), poly (imino-1,4-phenylene iminocarbonyl-1,4-phenylenecarbonyl)
9.1.1.3	**Trade names**	Kevlar®* Twaron®** Terlon

9.1.2 Identification numbers

9.1.2.1	**CAS numbers**	24938-64-5; 25035-37-4; 26125-61-1
9.1.2.2	**EINECS number**	not applicable

9.1.3 Chemical structure

9.1.3.1	**Formula**	$(C_{14}H_{10}O_2N_2)_n$
9.1.3.2	**Structural formula**	



para-aramid or PPDT or poly-(para-phenylenediamine terephthalate)

* Registered trademarks of the DuPont Company

** Registered trademark of Akzo Nobel

9.2 Occurrence and Use

p-Aramid fibres are synthetic industrial products; therefore, they do not occur spontaneously in the environment.

Aramid fibres are formed by reaction of aromatic diamines and aromatic di-acid chlorides. They are produced as continuous filaments, staple and pulp. There are two main types of aramid fibres (para- and meta-aramids), both with nominal filament diameters of 12-15 μm. p-Aramid fibre may have fine, curled, tangled fibrils of respirable size (< 1 μm in diameter) attached to the surface of core fibres. Such fibrils can break loose and become airborne (RFP's) upon abrasion during manufacture or use.

p-Aramid RFP's, as well as other synthetic organic fibre dusts, are released in the workplace during operations such as fibre forming, winding, chopping, weaving, cutting, machining, composite fabrication and handling. Available data from environmental monitoring campaigns in workplaces suggest that levels of p-aramid RFP's in air rarely exceed 0.3 RFP/ml on an 8-hr. TWA basis across a large range of processes involving p-aramid fibres in any form.

Due to their properties, such as heat- and flame resistance, dimensional stability, ultra-high strength and high modulus, electrical resistivity, chemical inertness and permselective properties, p-aramid fibres have many applications. They are used principally as strengthening and reinforcing material in composite structures - making use of their low density, high specific strength and stiffness, as well as greater vibration damping and better resistance to crack propagation and fatigue than can obtained with typical inorganic fibre-shaped materials.

p-Aramid fibres are used primarily for tire cords, protective clothing, industrial fabrics, high performance (sports and aerospace) composites, high-strength ropes, cables, friction materials and gaskets. The material is sometimes used in combination with other fibre-shaped materials, such as carbon and graphite fibres, to reduce costs and increase impact strength.

9.3 HEALTH SIGNIFICANCE

No human data are available concerning disposition and clearance of inhaled p-aramid RFP's. As far as the toxicity of p-aramid RFP's to human beings is concerned: no information is available on the effects of acute exposure, or repeated exposure by routes other than by inhalation.

Following inhalation exposure in rodents, the main sites of pulmonary deposition of p-aramid RFP's are in the regions of the terminal bronchioles and alveolar ducts and particularly primary alveolar duct bifurcations - this is similar to what has been described for a variety of other dusts and RFP's. Macrophages containing short p-aramid RFP's (mainly < 1 μm) were observed in the tracheo-bronchial lymph nodes in a 2-year rat study. However, no conclusive evidence for translocation of p-aramid RFP's to the pleura has been obtained in more recent studies. It is not anticipated, that there would be any systemic absorption or distribution of inhaled p-aramid RFP's, nor is any absorption predicted to occur across the skin or gastro-intestinal tract. Following short-term inhalation exposures to high concentrations of p-aramid RFP's, a substantial clearance from the lung over a 6-month period was reported by different investigators. Over this 6-month period, the total number of RFP's recovered from the lungs of exposed animals declined to about one half to one quarter of the initial burden, together with a progressive reduction in the median RFP length to almost half of the initial value. These data are in contrast to those obtained with chrysotile, for which the median particle length shows an increase, due to selective macrophage-mediated clearance of the shorter RFP's, over the same 6-month period. In summary: available *in vivo* results suggest a lack of durability for p-aramid RFP's in the lung - probably due to enzymatic proteolytic attack.

p-Aramid RFP's are of low acute toxicity by oral, inhalation, dermal and intratracheal routes. In rats, no deaths were observed following inhalation exposure at 150 mg/m^3 for 4 hrs. and oral ingestion at doses up to 7,500 mg/kg.

It has also been reported that skin contact tests in experimental animals did not cause "toxic reactions". p-Aramid fibre as such has only slight potential for skin irritation. Skin sensitisation has not been observed.

No data are available concerning the effects of p-aramid fibres on reproduction. However, the lack of potential for systemic absorption of the fibre precludes concern about this endpoint.

Apart from the effects of p-aramid-derived RFP's on the lung, no other toxic systemic effects have been described.

9.3.1 Lung toxicity

In rats exposed to 289 p-aramid RFP/ml for 6 hrs./day, 5 days/week for 2 weeks some pulmonary changes (mainly macrophage accumulation) were observed, but there was no collagen fibre deposition by 6 months post-exposure. In rats similarly exposed to levels of 419 and 772 RFP/ml only minimal to mild pulmonary inflammation and fibrosis (with lesions being most prominent 1 month after the end of the exposure, and therefore showing a reduction in severity) were observed. By 12 months after ending the exposure, no differences were observed between the lung of p-aramid RFP-exposed and control rats.

In rats exposed to p-aramid RFP's at concentrations up to 100 RFP/ml (6 hrs./day, 5 days/week for 2 years), no clinical signs of toxicity, body weight changes, or excess mortality were observed. The lowest level (2.5 RFP/ml) exposure caused no pathological changes in the alveolar architecture of the lungs, except for a few scattered RFP laden alveolar macrophages (effects on alveolar macrophages. This was considered to be the no-observed-adverse-effect level, (NOAEL). In the 25 and 100 RFP/ml group, a dose-related increase in lung weight, and significant particle accumulations in the respiratory bronchioles of the alveolar ducts were observed. Slight type-II pneumocyte hyperplasia, alveolar bronchiolarization, and mild alveolar collagenous fibrosis were also observed. Other rats were exposed to 0 or 400 RFP/ml for 1 year and allowed to recover for another year. Twenty nine males and 14 females of the exposed group died from obliterative bronchiolitis. After 1 year of recovery, no signs of toxicity, body weight changes, or excess mortality were seen in surviving 400 RFP/ml rats. Exposures at 100 and 400 RFP/ml both caused significant increases in lung weight. Exposure-related pathological changes were confined to the lungs (Lee et al., 1988).

From this study, a NOAEL could be concluded at an exposure level of 2.5 RFP/ml [Crl:CD(SD)BR-rats, inhalation, 6 hrs./day, 5 days/week for 2 years, lung histopathology]

The immediate next tested concentration of 25 RFP/ml could be considered as the LOAEL in this study [Crl:CD(SD)BR-rats, inhalation, 6 hrs./day, 5 days/week for 2 years, lung pathology (increase in lung weight; significant RFP accumulation in the respiratory bronchioles of the alveolar ducts; slight type-II pneumocyte hyperplasia; alveolar bronchiolarization; mild alveolar collagenous fibrosis)]

9.3.2 Carcinogenicity studies

Direct injection of p-aramid RFP's into mesothelial tissue failed to show any potential for p-aramid RFP's to produce mesotheliomas, even though it must be mentioned in this connection that, due to the physical properties of this material, it was impossible to inject a stable homogenous suspension.

p-Aramid RFP extracts were not mutagenic to *Salmonella typhimurium* or to Chinese hamster V79 fibroblasts.

The investigation by Lee et al. (1988) represents the key study for the assessment of the carcinogenic potential of p-aramid RFP's by inhalation exposure. At 100 RFP/ml, pulmonary lesions initially described as "cystic keratinising squamous cell carcinomas" (CKSCC) were seen in 4 female rats (6%). CKSCC is a tumour type not observed spontaneously in this strain or in humans. More prominent foamy alveolar macrophages, cholesterol granulomas and alveolar bronchiolarization were reported in female rats.

These were related to the development of CKSCC. CKSCC was also observed in 1 male (1/36, 3%) and 6 females (6/56, 11%) rats of the 400 RFP/ml group. The lesions progressively advanced and often occupied a large portion of the lung. Since biological behaviour and morphological characteristics provided no evidence of malignancy, the authors concluded that CKSCC is probably a benign neoplastic lesions. The lung tumours observed in this study were classified as CKSCC because there was no benign type of squamous cell tumour widely recognised in human or animal tumour classification (Lee et al., 1988). CKSCC's have been classified as metaplastic or dysplastic rather than neoplastic lesions: the authors stated that "it appears appropriate to designate new diagnostic nomenclature indicating a benign type of squamous cell tumour such as keratinising squamous epithelioma in order to distinguish it from squamous cell carcinoma". In fact, Lee (1989) changed his initial diagnostic terminology to "cystic keratinising squamous cell tumour" (CKSCT). Due to some specific properties, i.e. species- and gender specificity, histopathological characteristics and mechanism of tumorigenesis, it has been speculated that these lesions have little or no relevance to humans (Lee et al., 1988; Lee, 1989). No mesotheliomas were seen. More recently, this keratinising lesion has been observed in the lungs of rats with great regularity in several chronic inhalation studies with a variety of dusts.

In an attempt to reach a consensus on an appropriate diagnosis for this lesion, an international panel of pathologists was convened. The panel considered that the most appropriate diagnosis for this lesion was "proliferative keratin cyst" (PKC). The biological potential of PKC lesions remains controversial, but they appear to be unique to the rat with little relevance for humans (Boorman et al., 1996; Frame et al., 1997). They are not considered malignant neoplasms and they seem to occur only in female rats. In male rats, a single squamous cell carcinoma, that was not PKC-related, developed against a background of non-neoplastic lung damage: a NOEL of 2.5 RFP/ml was found. PKC's are considered benign lesions of no relevance to malignant tumour development and the result of a common, non-specific reaction of the rat lung to particle overload. Moreover, the one lung carcinoma that occurred (not statistically significant, 1/36) appears more likely to have occurred spontaneously.

Given the differences in the spectrum of pulmonary toxicity produced by p-aramid RFP's and chrysotile, it is doubtful that an overall comparison of pulmonary/pleural toxicity on a fibre-for fibre basis can be made. It would seem more appropriate to make comparisons with respect to specific end-points. As for mesothelioma occurrence, it would appear that the degree of hazard must be very low for p-aramid RFP's - likely lower than for chrysotile RFP's. A low potential for p-aramid RFP's to produce mesotheliomas is also suggested by inconclusive evidence for penetration of the pleura (Lee et al., 1988). In a recent rat study, Warheit et al. (1995) also reported that increases in cell proliferation in the subpleural and mesothelial tissues following short-time high-level inhalation exposure were not observed - this in contrast with reports relating to chrysotile tested under the same conditions. Finally, the lack of durability of p-aramid RFP's in the lung, as reported by various investigators, further suggests that the degree of mesothelioma hazard induced by p-aramid RFP's is very low - considering the importance of inhaled particle durability for mesothelioma development.

In its evaluation of available data, IARC (1997) concluded that there was inadequate evidence in both humans and experimental animals for the carcinogenicity of p-aramid RFP's and that p-aramid RFP's cannot be classified as to their carcinogenicity (Group 3).

9.4 Assessment of the Occupational Health Hazards and Risks Related to Respirable Fibre-Shaped Particulates (RFP's) derived from p-Aramid

As stated, no human data are available on the disposition and clearance of inhaled p-aramid RFP's. As far as the toxicity to humans is concerned: the only available study dealing with workers exposed to this material did not permit any conclusions on the effects of p-aramid RFP's on human lung function - due to significant sulphur dioxide co-exposure. However, lung effects are unlikely to occur at current occupational exposure levels - see table 3.A.

As for irritation effects: some minimal skin irritation can occur following dermal contact with fabrics made of Kevlar® or Nomex®. It was reported that because these fibres (and particularly Kevlar®) are quite stiff, there is a potential for causing abrasive skin irritation under restrictive contact (i.e. during the wearing of tight clothes). Skin contact tests on humans as well as on experimental animals, failed to show toxic reactions - although some mechanical skin irritation by short fibres at clothing chafing points has occasionally been observed. Airborne fibres can also cause mild irritation of the eyes and mucosa.

9.5 Derivation of a Health-Based Occupational Exposure Limit (OEL)

No human studies providing useful data to derive an OEL are available. We concur with the recent proposal of HSE (1995), that the study by Lee et al. (1988) be used for deriving an OEL. An exposure level of 2.5 RFP/ml was a NOAEL for pulmonary toxicity in this 2-year rat inhalation study; the next higher exposure level of 25 RFP/ml produced generally minimal or slight pulmonary changes, and can be considered a LOAEL for pulmonary toxicity. Applying an uncertainty factor of five to the NOAEL (i.e. in the same range as used under similar circumstances by the European Scientific Committee on Occupational Exposure Limits) a figure of 0.5 RFP/ml (" respirable dust") is recommended as a 8-hr. TWA occupational exposure limit (OEL).

Finally, it seems unnecessary to specify a limit for short-term exposure (STEL).

9.6 Key References

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Table 9.A Summary of lung effects observed in experimental animals exposed by inhalation to p-aramid RFP's (fully described in the text)

animals	exposure time	exposure level	observations	ref.
Crl:CD(SD) BR rats	6 hrs./day, 5 days/ week for 2 years	2.5 RFP/ml	NOAEL for lung histopathology	1
		25 RFP/ml	LOAEL for lung histopathology, increase in lung weight, significant RFP accumulation, slight Type II epithelial cells, alveolar bronchiolarization	1
		100 RFP/ml	increase in lung weight significant fibril accumulation, slight epithelial hyperplasia, alveolar bronchiolarization, CKSCC in 4 females (6%)	1
	6 hrs./day, 5 days/ week 1 year exposure + 1 year recovery	400 RFP/ml	slight centriacinar emphysema, epithelial hyperplasia, alveolar bronchiolarization, CKSCC's in 6/56 females (11%) and 1/36 males (3%)	1
Crl:CD(SD) BR rats	6 hrs./day, 3 or 5 days	600-1000 RFP/ml	transient pulmonary inflammatory response, transient increase in LDH level and BAL fluid at 3 and 5 days post exposure	2
Crl:CDBR rats	6 hrs./day, for 2 weeks	772 or 419 RFP/ml	minimal to mild centriacinar inflammation and fibrosis up to 1 month post exposure, decrease of inflammation by 6 months post exposure, no difference between exposed and control animals at 12 months post exposure	3

Note: CKSCC: cystic keratinising squamous cell carcinoma - more recently diagnosed as PKC (proliferative keratin cyst)

Refs.: 1] Lee et al. (1988), 2] Warheit et al. (1992) and 3] Warheit et al. (1995)

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NIOSHTIC, Occupational Safety and Health Database.

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RTECS, Registry of Toxic Effects of Chemical Substances.

U.S. National Institute of Occupational Safety and Health

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Note: Database consultations have been performed using the following search terms: asbestos toxicity, Kevlar®, aramid fibres, and CAS Registry Number 26125-61-1