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Criteria Documents from the Nordic Expert Group 1993

Brita Beije and Per Lundberg (Eds)

ARBETS MILJÖ INSTITUTET
NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH

The National Institute of Occupational Health employs over 300 scientists in research on the work environment. The research is led by 30 professors. The Institute does mostly applied research, but some questions also require focused basic research.

The scientific competence of the Institute is concentrated in six areas: Physiology, Chemistry, Medicine, Psychology, Technology and Toxicology. This broad base of expertise provides solid support for the Institute's cross-disciplinary approach.

The Institute is responsible for training and educating personnel working within the occupational health services as physicians, nurses, physiotherapists, psychologists and safety and hygiene engineers.

Another of the Institute's responsibilities is disseminating information on occupational health research.

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PREFACE

The Nordic Council is an international body for the governments in the five countries, Denmark, Finland, Iceland, Norway and Sweden. One of the committees within the Nordic Council, the Nordic Senior Executive Committee for Occupational Environment Matters, initiated a project with a view to compiling and evaluating scientific information on chemical agents relevant to health and safety at work and the production of criteria documents. The documents are meant to be used by the regulatory authorities in the Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The management of the project is given to a group of scientists: The Nordic Expert Group for Documentation of Occupational Exposure Limits. At present the Expert Group consists of the following members:

Helgi Gudbergsson	Municipal Institute of Public Health, Iceland
Per Lundberg (Chairman)	National Institute of Occupational Health, Sweden
Petter Kristensen	National Institute of Occupational Health, Norway
Vesa Riihimäki	Institute of Occupational Health, Finland
Adolf Schaich Fries	National Institute of Occupational Health, Denmark

The secretariat is located at the National Institute of Occupational Health, S-171 84 Solna, Sweden.

The criteria documents aim at establishing a dose-response/dose-effect relationship and a critical effect, based on published scientific literature. The task is not to give a proposal for a numerical exposure limit value.

The literature is evaluated and a draft is written by a scientist appointed by the Expert Group with the support and guidance of one member of the group. The draft is then sent for a peer review to experts by the secretariat. Ultimately the draft is discussed and revised at the Expert Group Meeting before it is accepted as their document.

Only studies considered to be valid and reliable as well as significant for the discussion have been referred to. Concentrations in air are given in mg/m^3 and in biological media in mol/l or mg/kg. In case they are given otherwise in the original articles they are, if possible, recalculated and the original values are given within brackets.

This volume consists of English translations of the criteria documents which have been published in a Scandinavian language during 1993. The names of the scientists who have written the separate documents are given in the list of contents, where aslo the dates of acceptance by the Expert Group are given.

Solna in December 1993

Brita Beije
Secretary

Per Lundberg
Chairman

CONTENTS

CRYSTALLINE SILICA

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FOREWORD

There is an almost immense amount of literature on crystalline silica exposure and silica-induced health effects. Any attempt to make a comprehensive review of the literature is therefore condemned to failure.

In this criteria document on the health effects of silica exposure, the "classical" diseases which can result from exposure to naturally occurring crystalline silica have been discussed relatively briefly. More attention has been paid to the possible carcinogenic effects, to the exposure levels to which workers have been exposed and possible exposure-response relationships, and to the current hypotheses on the pathogenesis of silica-induced lung injuries.

In the present document I have tried to highlight relevant literature on the subject from the last one to two decades, but it was also necessary to include some papers of more historical interest. The systematic search for relevant literature was continued until May 1992.

CONTENT

1. PHYSICAL AND CHEMICAL PROPERTIES

CRYSTALLINE SILICA

Chemical formula: SiO_2

Minerals: Quartz (Cas.no. 14808-60-7),
Tridymite (Cas.no. 15468-32-3),
Cristobalite (Cas.no.14464-46-1).

Synonyms: Chalcedony, chert, coesite, cryptocrystalline silica, flint, jasper, microcrystalline silica, novaculite, quartzite, sandstone, silica sand, stishovite, tripoli, keatite.

Trade names: BRGM, D&D, DQ12, Min-U-Sil, Sil-Co-Sil, Snowit, Finmix, Silicron G 910.

Apart from oxygen (O_2), silicon (Si) is the most abundant element in the earth's crust. Together these two elements form the mineral silica (SiO_2) which is present in most rocks and sands in the earth's crust and accounts for more than 28% of its total content. Table 1 shows the approximate content of free silica in some common rocks.

By far the most common form of crystalline silica is α-quartz which is stable at temperatures from 570 to 870°C. Above 870°C it transforms to tridymite, which again transforms to cristobalite at approximately 1,470°C. Both α-quartz, tridymite, and cristobalite occur in semistable forms below the

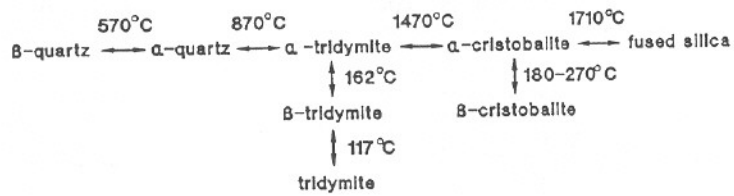

Figure 1. Float sheet of the main types of naturally occurring crystalline silica, and the temperatures at which they transform.

Table 1. Content (in %) of crystalline silica in some common rocks* (Source: 365).

Plutonic rocks	
Granite:	> 20 %
Granodiorite:	"
Tonalite (Trondhjemite):	"
Quartz syenite:	5 - 20 %
Quartz monzonite:	"
Quartz diorite:	"
Quartz gabbro:	"
Syenite:	0 - 5 %
Monzonite:	"
Diorite:	"
Gabbro:	"
Peridotite:	negligible
Serpentinite:	"
Volcanic rocks	
Rhyolite:	> 20 %
Dacite:	"
Quartz trachyte:	5 - 20 %
Quartz latite:	"
Andesite:	0 - 20 %
Basalt:	"
Trachyte:	0 - 5 %
Latite:	"
Sedimentary rocks	
Quartzite:	> 90 %
Sandstones, wide range, mostly:	> 60 %
Mudstone:	10 - 30 %
Limestone:	negligible
Dolomite:	"

* In the plutonic and volcanic rocks the quartz content is calculated as the percentage of the *light-coloured* minerals (in practice quartz, feldspar, and feldspatoids) present in the rock. In the sedimentary rocks the quartz content is calculated as a percentage of *all* the minerals present in the rock.

temperatures at which they transform. When cristobalite is heated beyond 1,710°C it transforms into a viscous glass which, upon cooling, forms a clear glass with an amorphous structure which is stable at all temperatures (96). The different forms of crystalline silica are named with the prefixes α and ß depending on structural and optical properties. Figure 1 gives a float sheet of the most common forms of crystalline silica and the approximate temperatures at which they transform.

Table 2. Some physical and geometrical properties of crystalline silica

Type of silica	Density	Colour	Geometrical form
Quartz	2.65	Colourless, white or variable	α-quartz: trigonal ß-quartz: hexagonal
Tridymite	2.26	Colourless or white	α-tridymite: ortorhombic ß-tridymite: hexagonal
Cristobalite	2.33	Colourless, white or yellowish	α-cristobalite: tetragonal ß-cristobalite: cubic

The basic structure of all crystalline silica is a SiO_4 tetrahedron where the molecules are linked together by sharing each of their corners with another tetrahedron, thus forming three-dimensional and regular frameworks where every silicon has four oxygens and every oxygen has two silicons as its nearest neighbours (96). Table 2 shows the geometrical forms, densities and colours of the main forms of crystalline silica.

2. USES AND EXPOSURE

Man has been exposed to crystalline silica for as long as he has been digging in and excavating the earth's surface. Sand, clay, and rocks containing silica have been used as building materials in most cultures at all times.

2.1. USES OF CRYSTALLINE SILICA

2.1.1. Uses of silica sand and gravel

The quantitatively most important use of silica today is of silica sand for hydraulic fracturing in oil and gas production, sand and gravel in concrete in the building and construction industry, and sand in the production of glass, ceramics, porcelain and abrasives, and as foundry castings.

In 1984 the world production of silica sand was about 182 million metric tons with the USA as the greatest producer with 31.8 million metric tons alone

(95) while Canada produced about 2.4 million metric tons (270). The leading exporter of silica sand in Europe is Belgium with 2.15 million metric tons in 1982 (11).

The consumption figures for sand, gravel and aggregate stone products containing silica in the Scandinavian countries in 1984 are given in Table 3 (275).

Table 3. Consumption (in thousand tonnes) of sand, gravel and hard rock aggregates in the Scandinavian countries in 1984 (Source: 275).

Country	Sand and gravel	Hard rock aggregates	Total
Denmark	38,000	-	38,000
Finland	58,000	7,000	65,000
Iceland	8,000	100	8,100
Norway	30,000	16,000	46,000
Sweden	75,000	11,000	86,000

Iceland has by far the highest consumption of sand (basalt) and gravel with 34 metric tons per inhabitant per year, while Norway has the highest yearly consumption of hard rock aggregates with 4 metric tons per inhabitant. The main uses of the total of the 243 million metric tons of potentially silica-containing materials in Scandinavia were 50% for road materials, 12% for concrete, 18% in construction, and 9% as a fill material.

2.1.2. Uses of silica stones

Silica-containing rocks and stones are still widely used as a building material, as semiprecious stones, and for artistic and sculptural works.

2.1.3. Uses of pure silica crystals

Since the discovery of the piezoelectric properties of quartz crystals in the first half of this century, there has been an increasing demand for pure silica crystals. The leading producer of high-purity quartz is Brazil where the mineral is found in sedimentary strata of sandstone and quartzite. The total export of pure silica crystals from Brazil in the period 1981-83 was 12 metric tons, mainly for the electronic and optical industries in Europe (60%), Japan (17%) and USA (13%) (191,374).

It is also possible to produce synthetic pure silica crystals. The health impact which can result from exposure to dust from such crystals do probably not differ from that which can result from naturally occurring silica.

2.2. EXPOSURE

2.2.1. Environmental exposure

Airborne environmental exposure to free silica is dependent on the local content of free silica in the earth's surface, on the degree of eruption of the surface, on climatic conditions, volcanic activity, and pollution from silica industries or deposits.

Road traffic is a major contributor to environmental silica exposure. Dust concentrations of up to 20 mg/m^3 with up to 15% silica with particles size smaller than 36μm have been measured in airborne city dusts (273).

Concentrations of free silica in the air in desert regions may be considerable. It has been estimated that 4.5 x 10^8 tonnes of sand are shifted annually in the world's deserts (191). The health implications of silica exposure to the inhabitants is not known, but environmental silicosis has been described among bedouins (22).

In high altitude villages in central Ladakh in India, where the inhabitants are exposed to sand storms containing 60-70% free silica every spring, prevalence rates of pneumoconiosis of up to 45,3% have been observed (346).

One case of acute silicosis has also been reported after inhalation of silica-containing domestic cleaning powder (103,104). Pneumoconiosis occurring in rural native women from certain regions in South-Africa was termed "Transkei silicosis" when first described (297). A later investigation of the cases has shown that the condition is more probably due to non-quartz containing dust and smoke from biomass fuelled fires (139).

Airborne dust levels of up to 1.2 mg/m^3, with a free silica content of 3-7% have been registered subsequent to volcanic activity (254). In the nearest neighbourhood to a ferrosilisium plant in Norway, considerable amounts of cristobalite were found in the general dust (Unpublished).

With silica being one of the most stable minerals in the geochemical environment, the content of silica in water is dependent on the types of minerals and rocks in the catchment area for water sources and deposits. Silica may also be present in grain and other foodstuffs depending on the composition of the soil (31). The health implications of the intake of naturally occurring silica in water and food are unsure.

2.2.2. Occupational exposure

Crystalline silica has a vast number of applications in industrial products, in oil and gas extraction, and in the construction and building industry. In the

Table 4. Dust levels in Swedish industries, 1968-71 (Source:126)

Industry or operation	Number of workers studied	Mean total dust concentration (mg/m^3)	Respirable dust concentration (mg/m^3)	Free silica in respirable dust (%)
Mining				
surface	124	8.4	1.5	7
underground	249	5.9	1.2	7
Quartz industry	65	4.5	1.7	46
Stone industry	381	18.9	5.1	18
Stone quarries	226	24.1	5.3	21
Gravel quarries	402	9.3	1.7	24
Construction work	70	11.8	2.0	17
Steel production	195	17.3	4.2	9
Steel foundries				
quartz sand moulding	54	8.8	2.3	12
olivine sand moulding	38	12.4	2.6	5
Iron foundries	821	19.5	5.3	12
Other metal foundries	185	12.1	3.3	13
Ceramics				
porcelain	46	7.1	2.8	9
other ceramics	58	8.2	1.3	11
Glass	52	13.3	3.9	8
Abrasives				
grinding wheels	31	5.5	1.5	5
grinding paper and cloth	9	8.0	3.0	42
polishing materials	5	2.8	0.8	46
Lime and dolomite production	11	51.2	12.8	2
Production of bricks	26	5.8	1.1	17
Cement production	32	61.2	11.0	4
Production of concrete	34	5.9	1.2	8
Production of roofing felt	14	71.6	15.0	4
Production of asphalt	65	9.7	2.5	16
Rubber production	24	35.1	12.3	1
Production of paint	20	14.1	5.6	13
Scouring powder production	9	18.7	7.7	47
Sandblasting	67	39.2	4.7	44
Welding electrode production	8	6.3	1.5	12
Sorting of potatoes	20	6.8	3.8	13
Road sweeping machine	7	12.8	4.9	13

USA it is estimated that up to 3 million workers have significant occupational exposures to silica dust (19,120). It was found in two Norwegian counties studied during the nineteen-eighties, that 8% and 13.6% respectively of males reported occupational exposures to silica dust (21,173). Due to a rapid

Table 5. Respirable silica exposure in US industries, 1972-82 (Source: 274)

Industry or standard industrial classification	Number of samples	% of samples > 2 x PEL*
Agriculture, forestry, and fishing	43	63
Mining	43	57
Construction		
Building construction - general contractors	45	29
Construction other than building	424	30
Construction - special trade contractors	289	10
Manufacturing		
Food and allied products	187	52
Textile mill products	52	27
Apparel and other finished products	16	0
Lumber and wood products, except furniture	13	8
Furniture and fixture	31	0
Paper and allied products	82	13
Printing, publishing and allied industries	31	0
Chemicals and allied products	640	13
Petroleum refining and related industries	214	11
Rubber and miscellaneous plastic products	269	9
Leather and leather products	14	0
Flat glass	82	9
Glassware, pressed or blown	229	11
Glass products from purchased glass	37	11
Hydraulic cement	65	0
Structured clay products	635	26
Pottery and related products	945	23
Concrete, gypsum and plaster products	347	12
Cut stone and stone products	270	27
Abrasive, asbestos and nonmetallic mineral products	558	16
Primary metal industies		
Blast furn., steel works, rolling and finishing mills	639	32
Iron and steel foundries	10,850	23
Primary smelting and refining of nonferrous metals	146	9
Secondary smelting and refining of nonferrous metals	39	0
Rolling, drawing, and extruding of nonferrous metals	23	22
Nonferrous foundries (castings)	2,170	9
Miscellaneous primary metal products	68	46
Fabr. metal prod., ex. machinery and transp. equipment	1,265	22
Machinery except electrical	1,377	13
Electrical and electrical machinery equipment and suppl.	474	23
Transportation equipment	600	20
Measuring, analyzing, and contr. instr., photogr., medical	137	36
Miscellaneous manufacturing industries	211	9
All other industries	460	15

* PEL = OSHA permissible exposure levels at the time of the study based on the formula: $10mg/m^3/(\%$ free silica + 2).

Table 6. Exposure to silica-containing dusts as measured in different studies.

Type of work/industry	Level of exp. mg/m³ Total	Respirable	% content of free silica	Ref.
Mining				
Iron ore mines	0.5-36		23-32	147
Metal mines		0.7-1.7(M)*	2.8-4.0	63
Coal mines	5.6-23		1.5-3.7	327
Graphite mines	17-57		4-7.7	150
Tungsten mine:		0.5-2.4	100	299
Diatomite factory	0.1-2		2.3-19	326
Sandblasting		2.0-37.2	30.8-83.6	431
	0.02-0.1	7.3		272
	0.12-4.77			349
Stone and gravel industry				
Stone quarries		0.5->30	0->30	237
"		17-33	17-24.5	398
Granite sheds		0.2-0.7	100	392
Gravel quarries		1.1 (Md)	3-35	143
Sculptural work		0.02-0.28	10,5-12.2	272
Cutting, sawing		0.02-0.26	4.8-9.5	272
and drilling		1.0-1.3 (Md)	3-35	143
Slate factories	11-177	4-18	48-61	345
Jade workers	1.0-5.6	0.34-0.72	89-97	280
Building and construction				
Drilling		0.03-2.26	15-25	149
Drilling, chiselling		0.5-16.3	7-27	180
and grinding				
Building	0.3-117		1-3	329
	4.2-226	0.27-7.9		14
		23.8-25.5	3-4	40
Sweeping		8.2-11.2	12-23	
Chiselling		7.0-10.9	15-29	
Cutting of concrete		3 (Md)	<1 (Md)	311
Cement industry		0.80 (M)	15.1 (M)	172
Pottery work		1.4 (Md)		8-33 321
"	(0.2-6.6)			
		0.25-1.65	6-30	191
Refractory brick plant		0.2-0.56	6.2-20.9	316
"		1.1-4.3	7.5-21	320
Other ceramic production			11.4	293
Steel and iron foundries	1.32 (M)		10-22	318
Nonferrous foundry	1.01-27.9	0.1-1.23	<2	211
Ferro-alloy industries	2 - 64			80
	0.6-66			314
	2.1-26	5-21		368
	4.6-34 (M)	2.1-19.5		234
Artistic work (sandblasting)	12.1-30.7		26.1-30.8	
Farming				
ploughing and harvesting	7-40		<1-25	23

* M = mean, Md = median

expansion of mining, mineral extraction and construction industries in developing countries, the population at risk and the prevalence of silicosis seem to be increasing (404).

The following tables (4-6) show the variety in occupations and working places entailing silica exposure, and present quantitative occupational exposure data for crystalline silica.

During the early seventies exposure to crystalline silica was surveyed in 1,059 Swedish working places with a total of 3,715 measurements (126). The results of that effort are presented in Table 4.

Table 5 presents the rate of samples exceeding twice the US Occupational Safety and Health Administration (OSHA) Permissible Exposure Level (PEL) (based on the formula 10 mg/m³/(% free silica + 2)) of respirable silica dust as reported in a total of more than 24,000 measurements in different working places (274).

Other reported measurements of silica dust in the working environment from different settings involving silica exposure, and in different countries, are summarized in table 6.

Evidently, sandblasting is the type of job entailing the highest levels of airborne silica dust, with up to 37.5 mg/m³ of respirable dust with a 83.6% content of free silica (431). In the slate industry, levels of 177 mg/m³ with a 61% content of free silica have been measured (345). It is presumed that the workers who are required to work under such conditions have used adequate airway protection.

In diatomite industry surveys in Iceland, both quartz and cristobalite were found in addition to the amorphous silica forms (326).

High levels of total dust were also observed in the building trades, but the content of free silica has mostly been relatively low (329), whereas the silica content in the construction industry may be higher (40,149). On the other hand, dust exposure in the building and construction trade does not seem to contribute much to the risk of silicosis (396).

The high levels of silica dust exposure during artistic work (table 6) were short time measurements performed on different occasions in an art school (234).

Further exposure measurements, mainly from the eastern European countries have been cited in a previous survey (191) but are not repeated here.

2.3. ANALYSIS OF SILICA DUST

During the first two thirds of this century it was customary to sample silica dust by the Impinger method, to analyze it microscopically, and to give the results as the number of particles per cubic foot (ppcf)(18).

Since the early seventies the recommended method for dust sampling has been the gravimetric method where the results are given in mg/m³. For the analysis, the method of choice has been the infrared spectrometry which determines quartz at the absorption peaks of 695, 780 and 799 cm^{-1} with a

detection limit of 50 μg (191). As the Impinger method determines lower dust concentrations quite well, and the gravimetric method has proven more reliable for higher concentrations, the conversion of measurements from ppcf to mg/m^3 has been a quite troublesome task. However, for intermediate exposures in the range from 500 to 1,200 ppcf, or 0.1 to 6-8 mg/m^3 there seems to be a linear relationship between the two methods (94,407).

In order to properly separate crystalline silica from amorphous silica, X-ray diffraction has become established as the method of choice. It has detection limits of 5 μg for quartz and 10 μg for cristobalite (50,51).

Sedimentation analysis has been the traditional method for determining the particle size distribution of dust samples (195). During the last decades, it has also become customary to use a cyclone elutriator to separate the respirable dust fraction. It is also possible to analyze the particle size distribution in the range from 0.6 to 100 μm diameter by a combination of microsieving and the Coulter Counter technique (409).

3. KINETICS

3.1. DEPOSITION

Silica dust follows the same aerodynamic rules as other non-fibrous particles when inhaled (233,391). Silica dust grains with a mass median aerodynamic diameter (MMAD) < 5 μm are able to reach the most distal parts of the airways, and to be deposited there. Due to aerodynamic conditions in the lungs, inhaled particles of about 0.2-1 μm MMAD have the ability of staying airborne and of following the expired air even from the most distal parts of the lungs. Consequently, a smaller proportion of such particles are deposited in the airways (391).

Irrespective of the type of dust inhaled, it seems that particles and fibres small enough to reach the alveolar region are first deposited at the alveolar duct bifurcations which intersect the air flow (42,415). In normal ventilation, particles with MMAD < 2.4 μm are almost entirely deposited owing to gravitational settling, and not by impaction or interception (378). For particles < 0.5 μm, deposition is mainly dependent on the electrical charges of the particles which may be higher in freshly crushed minerals (19,233).

3.2. CLEARANCE

3.2.1. General

Deposited particles are cleared from the airways in healthy persons in three principal ways, namely by the mucous escalator, by macrophage endocytosis, and by dissolution/absorption (391).

Particle clearance from the airways is dependent on particle size, and is usually two-phased with a rapid and slow phase, reflecting clearance from the conducting airways and from the respiratory parts of the airways respectively (20,33). In volunteers who breathed monodisperse 1.9 and 6.1 μm MMAD of fused aluminosilicate particles labelled with ^{85}Sr and ^{88}Y respectively, approximately 8% of the 1.9 μm particles and 40% of the 6.1 μm particles were cleared within 6 days. The remaining dust showed half times in the lungs, averaging 320 days with large intersubject variations, but with little variation as a function of particle size (20).

Even though short term clearance may be increased by acute inhalation of tobacco smoke (233), both the rapid and slow clearance phases have been shown to be slowed down in chronic smokers (33), and in subjects with other significant exposures to airway irritants, such as sulphur dioxide or acid mist (233).

3.2.2. Clearance of silica from human airways

Subjects with lung diseases affecting the conducting airways may have a disturbed rapid phase clearance, while subjects with chronic diseases at the bronchioli level, such as chronic obstructive lung disease or pneumoconiosis, have mostly a delayed slow phase clearance (33,233,338). As an expression of very long term clearance, subjects with silicosis have an increased content of silica in their sputum for years after exposure has ceased when compared to controls (350).

Macrophages containing quartz particles are found in broncheoalveolar lavage fluids in subjects with silicosis (358). The proportion of macrophages with quartz has been found to increase with the duration of silica exposure (73).

In a recent study with bronchoalveolar lavage in silica exposed workers, the amount of silica particles in the lavage fluid was related to both the duration of exposure and the amount of silica to which workers were currently exposed. Retirement led to a decrease in the mineral content, but substantial amounts of dust remained for many years (74).

In coalminers, the content of silica particles in lymph nodes is higher than in the whole lung (60,65). It is uncertain whether delayed lymphatic clearance of quartz is related to the chemistry or the size of particles, but the dust in the lymph nodes is generally found to be finer than the dust found in the lung parenchyma (400).

3.2.3. Animal experiments with silica clearance

In an *in vitro* experiment with quartz particles 1-3μm in diameter, endocytosis by alveolar macrophages in culture occurred very rapidly (260).

In rats exposed by inhalation to 109 mg/m^3 α-quartz for 3 hours, particles were primarily deposited on the alveolar duct surfaces closest to the terminal bronchioles. Within 24 hours, 82% of the dust was cleared from the mucosa. Within 12 hours, 72% of the alveolar macrophages in bronchoalveolar lavage fluid contained quartz particles, and such a proportion was maintained for 24 days after exposure. In the same experiment, quartz particles were found almost immediately in type I pneumocytes; and after three days, particles were also found in the parenchymal interstitium (41,43).

In a comparative study with Fisher 344 rats, the amount of cristobalite 0.9 - 1.1 MMAD given by inhalation in doses of 2.3 - 39 mg/m^3 clearance from the lungs was considerably less than for two quartz materials with approximately the same MMAD, and given in approximately the same doses (61).

3.3. RETENTION

Retained particles are particles which have been deposited in the airway mucosa, but not yet cleared from the lungs. Retention can be expressed as a proportion or as the total amount of retained dust.

The quartz content of the lungs of persons with silicosis has never been found to exceed a total of 5 g (191,406). In a US study, patients with lung cancer were found to have more retained particles, including crystalline silica, than controls without lung cancer (75). The same study showed that prolonged smoking also had a deteriorative effect on particle retention. In another study however, a considerably higher retention of silica was found in non-smokers than in smokers. A suggested explanation was the short term stimulation of clearance by smoking (199).

In an experimental study with guinea pigs breathing cristobalite and two sorts of amorphous silica, the retention of the amorphous forms was about twice and seven times as high as for the crystalline forms, suggesting a more efficient clearance from the lungs of the more reactive cristobalite (312).

In rats inhaling 12-24 mg/m^3 of a mixture of respirable quartz and coal dust for ten months, a retention of 17-38 mg of dust was found in their lungs (334). However, in a similar experiment with rats inhaling 18 mg/m^3 for 9 and 18 months, only 3.6 and 6.7 mg of the dust was found to be retained (417). In another study, inhalation of 1 mg/m^3 for 12 and 24 months gave a retention of 0.07 and 0.10 mg of dust, while inhalation of 20 mg/m^3 for 16 months led to a retention of 3.8 mg, indicating that the retention of quartz dust seems to reach a plateau (387).

For more details on the general principles of inhalation toxicology the reader is referred to an abundant literature on the subject (212,233,302, 348,391).

3.4. ABSORPTION

As silica is slightly soluble in body fluids, dissolved silica may be absorbed into the body as silicic acid (H_2SiO_3), either through the alveolar epithelium into the blood stream, through the lymphatic system, or through the gastro-intestinal tract (206,207). There are no reports on other routes of absorption of silica.

3.5. DISTRIBUTION

The most important deposits of absorbed silica are the liver, spleen and regional lymph nodes (107,263).

3.6. BIOTRANSFORMATION

Silica absorbed in the body as silicic acid does not undergo metabolic changes to other biologically active substances (206,207,263,383).

3.7. EXCRETION

Silicic acid absorbed into the blood stream is excreted through the kidneys. Absorbed silica also increases the level of silica in urine (134,206,207,209). However, in subjects exposed to silica dust, decreasing levels of silica have been observed in blood and urine with advancing stages of silicosis (384). Besides occupational and environmental dust exposure, diet also seems to have an influence on the urinary excretion of silica (134,209).

4. GENERAL TOXICOLOGY

4.1. ACUTE TOXICITY

The first experimental studies of silica investigated the acute toxicity of parenteral administered silicic acid (146). When injected subcutaneously and into the peritoneum, a local inflammatory reaction occurred. Intravenous

administration caused death due to intravasal clotting of the blood a few minutes after the injection of a dose of 100 mg/kg in rodents. At daily doses of 30-72 mg/kg death occurred within a few days with petechial haemorrhages and profound degeneration of the liver and kidneys.

With intravenous injection of colloidal silica in cats, lethal doses varied from 15 to 193 mg/kg. It was concluded that death was more probably due to the destruction of the capillary endothelium than due to intravascular clotting (262).

More recent studies on the acute toxicity of silica have focused on effects of the mineral on specific cell systems. Some of these studies are referred to in chapters 10 and 11.

4.2. FACTORS INFLUENCING TOXICITY

Following intratracheal instillation of 50 mg of different sorts of silica, it has been shown that tridymite has the strongest fibrogenic potential, followed by cristobalite and quartz (209).

Freshly crushed silica particles have been shown to be 4.2-fold more potent in decreasing the membrane integrity of macrophages, 50% more potent in activating hydrogen peroxide secretion by macrophages, and 4.6-fold more potent in stimulating cellular chemiluminescence compared to aged silica (403).

The surface area of the silica particle also influences the toxicity. Dust with smaller particle size provides relatively more surface area with which to interact with target cells. Laboratory measurements have shown that quartz particles with 1 μm diameter have a surface area of 3.5 m^2/g (184).

It has been known for a long time that aluminum salts coated on silica particles inhibit the toxic and fibrogenic action (208,229,242,399)(see also chapters 5.8, 10, and 11).

Other substances which have also been reported to influence the toxicity of silica when given concomitantly, or after pretreatment of the dust, are iron oxides (325), selenium (122), prosil (403), L-αdipalmitoyl-lecithin (352), polyvinylpyridin-N-oxide, polyvinylpyrrolidone (263,287), glutamate given by mouth (264), and inhaled modified superoxide dismutase (239).

Both *in vitro* and *in vivo* the alkaloid Tetrandrine, obtained from the root of a medicinal herb which is employed in China as a treatment for silicosis, has been shown to inhibit the silica-induced activation of alveolar macrophages and polymorphonuclear leukocytes (62).

When native quartz was pretreated with various organosilanes (-CN,-CH_3,-NH_2,-$(N(CH_3)_3)$ influencing the electrostatic charges on the particle surface, the ability to induce inflammation and fibrosis was reduced (403,421). Another study with pulmonary macrophages incubated with silica showed that the toxicity was related to positive charges adherent on the particle surface (19).

Surface infrared modes of silica particles have shown that cell lysis induced by silica *in vitro* is strongly dependent on the presence of silanol groups on the particle surface (298).

In a recent review of the experimental evidence and the pathogenesis of fibrosis caused by mineral dusts, it was concluded that the quantity of retained dust, particle size, and surface area, together with physical forms and reactive surface groups, all have to be seen as determinants of pulmonary fibrosis (165).

5. ORGAN EFFECTS

In this chapter reported non-malignant specific effects on different organ systems from exposure to crystalline silica are described.

5.1. SKIN

5.1.1. Silica granulomas

Almost any substance present within the dermis may result in a foreign body giant cell reaction with the development of a granuloma. Silica is the most frequently found substance in dermal granulomas in man. It is usually introduced to the dermis as a result of an injury. These granulomas may appear as solitary or multiple papillae and nodulae (151). Clinically and histologically the condition has been described as indistinguishable from skin lesions found in sarcoidosis (106,266,337). Localized nodules develop after asymptomatic latent periods lasting from some weeks to up to more than 50 years, with a mean interval of about 10 years (36,266).

5.1.2. Scleroderma

The possible association of silica exposure with scleroderma is dealt with in chapter 5.7.

5.2. RESPIRATORY ORGANS

Inhalation of crystalline silica causes an immediate inflammatory response reflected by an increased number of inflammatory cells and other inflammatory reactions in broncheoalveolar lavage fluids of both humans and animals (25,101,108,415). It has been shown that in subjects with silicosis there is a positive relation between disease severity in terms of restrictive lung function

impairment and the occurrence of inflammatory cell cytokines in bronchoalveolar lavage fluid (332).

After long term exposure, crystalline silica causes three main types of specific non-malignant pulmonary effects, namely silicosis, progressive massive fibrosis and alveolar proteinosis. Historical, legal, clinical, radiological, histological and diagnostic features of these diseases have been reviewed extensively (69,113,324,225,227,274,366). These aspects will only be mentioned briefly here.

Exposure-response relationships for silicosis are dealt with in chapter 12.1.

5.2.1. Silicosis

The term silicosis was first used in 1870 by the Italian pathologist Visconti to describe fibrotic lung lesions from silica exposure (323). During this century the disease has become well known as a nodular fibrosis occurring in miners, stone workers, sandblasters, ceramic workers and many other occupational groups with significant exposure to silica dust. With the inhalation of low concentrations of silica, retained particles accumulate in lymph vessels and regional lymph nodes, where scattered nodular lesions may develop with time. With heavier exposure, nodular lesions are found scattered in the lungs and in the interstitium of individual respiratory units. In most cases of simple silicosis the lesions appear as single nodules.

Radiographically, simple silicosis appears as small and rounded opacities of less than 1 cm in diameter. In early silicosis, lesions are mostly seen in the upper lung fields, whereas all lung fields may be involved in more advanced stages where the opacities usually become larger with an occasional central calcification. CT-scan, in particular high resolution CT-scan, seems to have considerable higher sensitivity for the detection of early silicosis than customary chest X-rays (28,29).

In general the occurrence of lung fibrosis has been associated with cigarette smoking. However, in one recent autopsy study of South African gold miners, there was an inverse relation between smoking and the prevalence of silicosis (168).

As a result of the inflammatory response, workers exposed to silica dust commonly have a productive cough irrespective of the presence of radiographic signs of silicosis. The cardinal symptom in silicosis is dyspnoea, which increases with the severity of the radiographic changes.

The reduction of lung function in subjects with silicosis seems to closely follow the degree of radiographic changes as observed by means of a chest radiograph or a CT-scan (26). In that context, tobacco smoke has been found to potentiate the effect of silica dust on the respiratory impairment (176).

In a recent study the excess loss of lung function for a 50 year old South African gold miner, associated with 24 years of underground dust exposure of an average respirable dust concentration of 0.3 mg/m^3 was estimated to be

236 ml of the forced expiratory volume in one second (FEV_1) (95% confidence interval (CI) 134-337), and 217 ml of the forced vital capacity (FVC) (95% CI 110-324). By comparision, the effect of smoking one packet of cigarettes a day over 30 years was associated with an estimated loss of 552 ml of FEV_1 (95% CI 461-644), and 335 ml of FVC (95% CI 170-500) (179).

Pulmonary hypertension is not uncommon in the advanced stages. In previous times, right heart failure or silicotuberculosis in severe silicosis used to be the main causes of death (361). Recently a case of tracheobronchial obstruction due to broncholithiasis in a patient with silicosis was described (52).

An increased amount of serum angiotensin converting enzyme (ACE) has been observed in patients with silicosis, possibly reflecting a functional enhancement of macrophages producing ACE (53).

In a recent autopsy study of 238 cases of silicosis, fibrosed hilar lymph glands were found in 88% with increasing prevalence rates with an increasing duration of exposure. It was suggested that fibrosis of the hilar lymph glands may predispose to the development of lung parenchymal silicosis (271). In an autopsy study of 1,553 white gold miners from South Africa it was found that the presence of both panacinar and centriacinar emphysema were positively related to silica dust exposure, and that the centriacinar emphysema was also related to the presence of silicosis of the lungs (177).

Life expectancy does not seem to be reduced in mild and simple silicosis. However, in a study of 1,165 silicotics from Quebec who recieved compensation, it was found that factors like age at compensation, smoking, presence of dyspnoea, expectoration, abnormal breathing sounds, radiographic appearance, and vital capacity, all made independent contributions to survival (190).

5.2.2. Progressive massive fibrosis (PMF)

PMF is an uncommon form of silicosis with larger lesions composed of confluent silicotic nodules. By definition, any form of silicosis with radiographic opacities exceeding 1 cm is termed PMF (366). PMF is usually found in the upper lobes and may lead to the contraction of the lobes with a secondary expansion of the lower lobes. Infection by mycobacteria and fungi may also result when the fibrotic contraction of lung tissue leads to reduced vascularization and in some cases to secondary necrosis.

5.2.3. Alveolar proteinosis

This condition, also termed silicotic alveolar proteinosis, or acute silicosis, is a reaction of the lung tissues (mainly type II alveolar cells) producing excess amounts of PAS positive phospholipides which fill the air spaces of the lung (333,428). The condition is commonly seen in laboratory animals, but is rare

in humans. It occurs occasionally, however, after heavy exposure, which may have been of short duration. Radiologically the lungs appear as in pulmonary oedema, and clinically the condition is characterized by a relatively rapid progression of dyspnoea without fever or leucocytosis. In most cases the disease has been lethal within months or at the most 1-2 years.

5.2.4. Pleural reactions in silicosis

In silicosis, focal fibrosis of the visceral pleura is seen in connection with silicotic nodules (366). These so-called "candle wax lesions" range from 3 to 10 mm in diameter and exhibit a waxy, round, grey appearance. Microscopically they have the feature of silicotic nodules and contain variable amounts of silica.

In one patient with silicosis an idiopathic pleural effusion has been described (4). In another patient a subpleural silicotic nodule was described as radiologically resembling a peripheral brochiogenic carcinoma (388).

5.2.5. Rheumatoid silicosis

Rheumatoid silicosis is a special form of relatively rapid progressive nodular fibrosis associated with rheumatoid arthritis. The condition is also termed Caplan's lesions (56,58,59). Besides silica dust exposure, host specific immunological features probably play a major role in the causation of the condition (See also chapter 5.7.).

5.3. LIVER

From early laboratory experiments and case descriptions it has been known that absorbed silica is able to produce structural changes in the liver parenchyma. In experimental animals the lesions have been described as a type of periportal cirrhosis, while changes in man were described predominantly as of central lobar location, predominantly associated with Kupffer cells in the sinusoids and fibrotic degeneration of liver cells (125,146,235,294).

5.4. UROGENITAL SYSTEM

Several reports have described the sporadic occurrence of renal diseases in subjects exposed to silica. Mild focal, segmental, proliferative glomerulonephritis and tubular lesions have been seen in subjects with silica exposure (280,309,310,360). Evidence of renal disease has been found in 40-50% in a series of patients with silicosis (57,344), and several case reports have highlighted glomerular and tubular morphological changes, including the deposition of IgM and Complement C and a raised content of silicon in the kidneys of workers with high exposure to silica (34,128,153,347,360).

In a recent controlled study, it was found that workers with and without silicosis who had previously been exposed to silica had significantly higher urinary excretion of albumin, α-1 microglobulin (AMG) and β-N-acetyl-glucoseaminidase (NAG). Based on these findings, the authors suggested that prolonged exposure to silica is associated with chronic irreversible nephrotoxicity (282).

However, the limited body of laboratory animal evidence connected with this subject (241,279) still makes it difficult to conclude with sufficient certainty that silica exposure as such, results in renal disease in man.

5.5. GASTROINTESTINAL TRACTUS

Apart from a possible carcinogenic effect (chapter 8.1.2.), there is no known evidence of a specific deleterious effect of crystalline silica on the gastrointestinal tract.

5.6. BLOOD AND BLOOD-FORMING ORGANS

Silicotic granulomas have been observed in examinations of the spleen of subjects with silicosis. The granulomas start as simple, periarteriolar accumulations of histocytes, and develop through intermediate stages into fibrohyaline nodules (8). In an autopsy study of 93 subjects with silicosis, such changes were found in 17,2% (76).

There have also been two reports on bone marrow changes in patients with silicosis. In an early study of 7 silica-exposed workers from Egypt, generalized bone marrow hyperplasia was found, in particular with high figures for the myeloid series, reticulum cells and plasma cells, and to some extent also for eosinophils (416).

In a more recent case description silica deposition and characteristic nodular silicotic lesions of the bone marrow were described in a patient with severe lung silicosis, who also had silicotic granulomas of the liver and spleen (107).

5.7. IMMUNOLOGICAL DISEASES

In slate workers exposed to high concentrations of silica dust, high levels of immunoglobulins IgG, IgM and IgA have been observed as compared to nonexposed controls (201). However, there was no association between the observed levels of immunoglobulins and the severity of silicosis. Other studies

of patients with silicosis has also shown increased amounts of immunoglobulins (25,54,92,284).

An association between the autoimmune disease scleroderma and silicosis has been claimed for a long time (38,112,148,154,155,303,331,367). Silica particles from 1-90 μm in diameter were found within the skin of patients with scleroderma in a German study (250).

Two case control studies of South-African gold miners with scleroderma showed no association with the occurrence of silicosis, but a consistent relationship with heavy dust exposure. It was claimed that the intensity of exposure was of greater significance for the risk of scleroderma than the duration of exposure (84,85,372).

A relationship between rheumatoid arthritis and silicosis has also been suggested, at least that subjects with concurrent arthritis have larger silicotic lesions, and in some cases a more rapid progression of their disease (373). On the other hand, silica exposure has not been shown to predispose to rheumatoid arthritis. A common immunological pathogenesis of both diseases is perhaps a more probable explanation of the observed association (92,213,286).

For more details on both humoral and cellular immunologic responses to silica dust, the reader is referred to a recent review of the subject (351).

5.8. ANIMAL EXPERIMENTS ON FIBROGENICITY

The first animal experiments on the fibrogenic properties of crystalline silica were carried out during the twenties and thirties (323). Later the fibrogenic properties of the mineral have been studied extensively (45,91,133,182,209, 265,328,334,341,422).

From these and many other studies, there is firm evidence that both instillation and inhalation of silica dust by different species of animals are able to cause a dose-dependent response in terms of nodular intestinal fibrosis, or in terms of an alveolar proteinosis (165,274,366,420). In this respect there also seems to be a correlation between the cytotoxicity and fibrogenicity of silica dust (203).

In addition to nodular interstitial fibrosis and alveolar proteinosis, recent animal experiments have shown a marked thickening of the walls of the small airways in rats 30 days subsequent to intratracheal instillation of 10 and 30 mg of quartz (425). This may be seen in connection with the small airway obstruction observed in experimental animals and in workers exposed to mineral dust (79,86,370,424,425).

An exposure time of at least 1 year was common in previous inhalation studies aimed at assessing the fibrogenic potential of mineral dusts. Recently, a far less time consuming protocol with 28 days' exposure time has been suggested (30).

More details of the cellular effects of crystalline silica can be found in chapters 10 and 11.

6. IMMUNOTOXICITY AND ALLERGIES

Apart from the effects mentioned in chapters 5.1.2, 5.2.5, 5.7, and 11, no specific immunotoxic or allergenic properties of silica are known.

7. MUTAGENICITY AND GENOTOXICITY

The only investigation using cytogenetic monitoring of stone crushing workers unveiled a significant increase in chromosomal aberrations (gaps) and sister chromatid exchanges in a group of 50 exposed workers as compared to 25 unexposed controls (375). The results were consistent when the effect of smoking and alcohol consumption was coupled with dust exposure, and it was concluded that the observed mutagenic risk was probably associated with the silica dust in the area.

Apart from this, and from the effects mentioned in chapter 10.2., no specific mutagenic or genotoxic effects of crystalline silica are known.

8. CARCINOGENIC EFFECTS

During the last decade the question of a possible carcinogenic effects of exposure to crystalline silica has been reviewed extensively by various authors (2,87,121,132,138,164,184,191,238,246,296,322).

8.1. HUMAN STUDIES

8.1.1. Cancer of the trachea, bronchus and lung

8.1.1.1. Early studies

In 1879 the first occupational lung cancers were recognized as such in silver miners from the Erz mountains on the border between Saxony in Germany and Bohemia in Czechoslovakia (186,187,188).

Later investigations on the Saxon side of the border considered the concomitantly occurring silicosis observed among the miners as the primary aetiologic factor for the malignant degeneration (336). In a similar investigation conducted in Joachimsthal on the Bohemian side of the mountains, however, excess lung cancer rates were also found, but no notable degree of silicosis was found among the miners. Thus, on the Bohemian side,

exposure to radon daughters was considered to be the most probable cause of malignancy (308).

Following these early observations, several autopsy materials were published, some of them indicating a relation between silicosis and lung cancer (35,66,99,130,215,390), and some of them not (47). In one of the studies (130) there was some evidence of an increased occurrence of lung cancer even in silica-exposed subjects without silicosis. One early study of patients with lung cancer also indicated an increased silica content in lung tissue from autopsies as compared to control autopsies of patients without lung cancer (9).

In spite of these early communications indicating a possible relationship between silicosis and lung cancer, relatively little light was thrown on the matter during the subsequent decades. In a comprehensive review of the literature on silica-induced neoplasms published in 1967 (47), there was no mention of any epidemiological studies on the subject. In a Swiss autopsy study published in 1969, lung cancer was less prevalent among subjects with silicosis than among subjects without (340).

8.1.1.2. Cohort studies of silica-exposed groups

Since 1976, when the first epidemiological study unveiled an excess lung cancer mortality among South Dakota gold miners (129), there have been several papers showing a possible connection between silica exposure in different trades and an increased risk of lung cancer. Table 7 summarizes the main results regarding lung cancer from cohort studies of silica-exposed groups cited in the 1987 IARC monograph on the evaluation of the carcinogenic risk of crystalline silica (191).

While the first study conducted among South Dakota gold miners showed indications of an increased lung cancer risk (129), the two following studies from the same study base, but with a higher number of men, were not able to confirm this finding (44,244).

Adjustment for the effect of cigarette smoking was not made in any of the studies cited, and confounding from other carcinogenic exposures such as radon daughters, polyaromatic hydrocarbons, arsenic and asbestos may well have acted as possible competitive causes of increased risks in nearly all of the studies. In only one of the studies, namely that among UK foundry workers (117), was there a positive relation between the length of employment involving silica exposure and lung cancer risk. In one of the studies conducted among Vermont granite workers, an estimate of each worker's cumulative exposure to silica dust was made, but no relation was found between that variable and SMR (94). No other study gave any information about any exposure response relationships.

Excess mortality from lung cancer has also been reported in several other metal mining groups, in all of which radon daughters was a probable confounding factor, e.g. British hematite miners (37), Canadian flour-spar miners (98), Swedish iron and sulphide ore miners (105,196,228,309,377), Swedish zinc and lead miners (16,17).

Table 7. Cohort studies on silica exposure and lung cancer risk 1976-86.

Cohort	Number of cohort members	Observ. period	Exposure time/place	Relative risk [1]	Comments	Ref.
US gold miners	440	1960-73	>5	370[b]*	Highest SMR within 20 years since first employment.	129
US gold miners	1,321	1937-73: 1937-55	>21 yrs: >21 yrs:	103[b] 176	No consistent exposure-response relationship.	244
US gold miners	3,328	1940-77	>1 yr	100	No relation with exp. time, latency or estimated dose	44
USSR gold miners	?	1948-74		7.9[2]*	Observed among underground miners.	202
USSR fire-clay plant	?	1948-74		2.8-4.5[2]*	Both men and women with heavy exposure.	202
Australian gold miners	1,974	-75		140[b]*	Concomitant exposure to radon and arsen.	12
Canadian miners	50,201	1955-77		145*	Several subgroups studied	191
US miners	12,258			126.6*	Several subgroups showed a raised risk	191
US taconite miners	?	1952-76		84[b]	Generally low silica exposure	171
US haematite miners Underground: Above-ground:	10,403	1937-78		100 88	Underground miners had raised risk of Hodgkin's disease.	191

cont.

table 7 cont.

French iron miners	1,173	1975-80	mean 16.7 yrs	350[3]*	Radon exposure not considered	306 as a sufficient explanation.
New York City tunnel workers	932	1955-72	All: <10 yrs: >29 yrs:	160 125 444*	No data on silica levels or exposure to radon daughters available.	191
Italian ship yard sandblasters	190	1960-75		376*	Concomitant exposure to asbestos and mineral oil.	315
Vermont granite workers	969	1952-78	Employed <1940: >1940:	1.3 (PMR) 1.1 " 1.4 "	No clear exposure-response relationship observed.	94
Vermont granite workers	5,414	1950-82	All Shed & quarry	116 127*	No increased risk for other silica-exposed workers.	83
Finnish granite workers	1,087	1940-75	>3 month	129	Excess risk of cancer of the stomach observed.	221 218
US granite cutters	1,905			1.2 (PMR)	No relation between cancer risk and duration of exposure	380
US pottery workers	2,055	1955-77		1.2 (PMR)	Excess risk observed in sanitary division, exposure to asbestos possible.	394
Italian refractory brick plant	231	1960-79		183	Striking excess of non malignant respiratory diseases.	191 316

cont.

table 7 cont.

Danish foundry workers	5,579		All: <25 yrs: >25 yrs:	115 96 159*	Concomitant exposure to PAH, mixed dust, and carbon monoxide reported.	359
UK foundry workers	8,596	-78	>1 yr:	149*	Relationship between lung cancer risk and length of employment.	117
Deceased UK foundry workers	1,540	1978-82	 Moulders: Dressers: Labourers:	1.2 (PMR) 1.3 " * 0.8 " 1.1 "	Among moulders there was a positive relation between length of union membership and PMR	117
US iron foundry workers	221		>10 yrs:	146 (SPMR)*	Level of exposure: appr. 0.1-0.2 mg/m^3	369
Norwegian ferroalloy industry	2,533	1953-82	>18 months	0.8-1.0	Concomitant exposure to other carcinogens probable	211

1) When not otherwise indicated the figures are based on the observed-expected ratio multiplied with 100.
2) Relative risk based on comparison of age-adjusted death rates.
3) All respiratory cancers.
* $p < 0.05$
PMR = Proportional mortality ratio
SPMR = Standardized proportional mortality ratio

The levels of free silica in the dust generated in coal mining is normally relatively low. No elevated risk of lung cancer has been seen in studies of British coal miners (77,131,193,204,232,259,382). In some studies among U.S.coal workers, an elevated lung cancer risk has been observed, but in these studies, the excess risk may also be explained by cigarette smoking (109,110,330). Other U.S. studies of coal miners have not shown an increased risk (81,291).

8.1.1.3. Cancer studies among subjects with silicosis

Table 8 summarizes the main results of cohort studies on lung cancer mortality among workers with silicosis. Most of these studies show an increased lung cancer mortality with overall relative risks between 2.0 and 6.5. In a recent summary of most of these studies, it was pointed out that also non-mining occupational groups with silicosis have been shown to have an increased lung cancer risk in some studies (7). However, as there are several problems which can lead to selection bias when using subjects with silicosis to define a cohort, these studies cannot be taken as any conclusive evidence for a causal relationship.

There is only one study on the relationship between anthracosis and lung cancer risk which has shown an increased relative risk (401). All other studies on the subject have been negative (77,232,259).

8.1.1.4. Case-control studies

Since 1983 there have been several case-control studies using different study bases in the elucidation of silica exposure or the occurrence of silicosis among subjects with lung cancer. Table 9 summarizes the main results of these studies, showing odds ratios between 0.8 and 3.16. In some of the studies (380,410) the comparability of the control groups is open to question. As in cohort studies based on subjects with silicosis, there may be confounding from smoking and other selection biases when using the presence of silicosis as the characterization of exposure to silica. None of these case control studies gave any quantitative data on silica exposure.

8.1.1.5. Recent epidemiological studies

Since 1987, when the IARC evaluation of the carcinogenic risk of silica was issued, several new and extended epidemiological studies from workplaces and occupations with more or less "pure" exposure to crystalline silica have been published.

The observation period of the study first published in 1982 (221) on 1,026 Finnish granite workers exposed to "pure" silica without significant exposures to radon daughters, asbestos or polycyclic aromatic hydrocarbons, has later been extended to 1985 (216,217,218). The number of person years in the latest extension was 23,434 and the total number of deaths 296. The observed number of lung cancer was 31, while 19.1 were expected when compared to the national rates (SMR 156, $p<0.05$). Exposure levels to quartz were between 1.0 and 1.5 mg/m^3, but the study did not provide any further

Table 8. Follow-up studies of lung cancer in subjects with silicosis.

Source of silicosis records	Number of cases	Year of diagnosis	Follow-up until	Lung cancer SMR by industry						Total	Ref.
				Mining only	Mining, quarry, tunnel or stone	Foundry	Ceramic	Other			
Swedish pneumoconiosis registry	3,610	1931-48	1969	590*		80	130	330		270*	418
		1949-69	1969	380*		220*	290	100		280*	
Finnish register of occupational diseases	331	1964-74	1975							300*	142
Hospital records in Japan (person years)	4,413	1971-81	1981							650*	71
Swiss accident insurance fund	2,399	1960-78	1978	250*	120	390	190			240*	356
Cases compensated for silicosis in Austria	2,212	1950-60	1979							140*	276
Swedish pneumoconiosis registry	712	1959-77	1979		540*	390*				440*	419
Finnish register of occupational diseases	961	1935-77	1982	440*	270*	210*		340*		310*	222
Cases compensated for silicosis in Italy	1,313	1959-63	1984	140*	180*			220		190*	430
Cases compensated for silicosis in Ontario	1,479	1940-85	1985	230*	360		290	270		300*	114
Italian refractory brick workers	136	-60	1979				167			167	316
Cases compensated for silicosis in Italy	952	1946-84	1984	250*	140	160	210*			150*	119
Cases compensated for silicosis in Quebec	1,165	1938-85	1986	380*	200*	300*	500*	690*		350*	189
Cases compensated for silicosis in Hong Kong	1,419	-1980	1986							200*	281
North Carolina silicosis registry	369	1959-61	1975	200*						200*	6,7
Medical records of silicosis in Genoa	520	1961-80	1982							685*	256
Medical records of silicosis in Sardinia	724	1964-70	1987							129*	61
Silicosis registry in Singapore	184	1970-84	?							201	70

* $p < 0.05$

Table 9. Case-control studies of the occurrence of lung cancer and exposure to crystalline silica (95% confidence interval in parenthesis).

Cases	Controls	Characterisation of exposure to silica	Odds ratio	Comments	Ref.
1,580 Austrian male lung cancer cases	3,160 males from a nationwide study	Occupational group: Mining and processing of stone	2.0 $p<0.01$	Result could be due to differences in social class	410
72 Italian male lung cancer cases from Civitacastellona 1968-84	319 matched males from the same municipality without lung cancer	Inteview based: - Employed in ceramic industry - Ceramic workers with silicosis - Ceramic workers without silicosis	2.0 (1.1-3.5) 3.9 (1.8-8.3) 1.4 (0.7-2.8)	Smoking habits accounted for. Increasing risk with increasing length of employment.	118 224
133 white South African gold miners with lung cancer. 1979-83	266 white males matched for age and smoking.	Subjects with silicosis: Subjects with silicosis at autopsy:	1.08 1.49	Decreasing odds ratio with degree of exp. Misclassification not ruled out.	166
97 U.S. granite workers with lung cancer	135 granite workers with other cancers	Silicosis mentioned on benefit records.	3.16 $p<0.001$	Nested case-control analysis in a cohort study.	380
333 male lung cancer cases from New Mexico	499 matched males from the same area.	Uranium mining industry. Underground mining.	1.9 (0.8-4.9) 2.1 (1.1-3.7)	Misclassification of exposure not ruled out.	230

cont.

table 9. cont.

309 lung cancer patients in a chest hospital in Balluro, Italy. 1973-80.	309 matched at the same hospital without lung cancer.	Clinical records: - silica exposed without silicosis - Silica exposed with silicosis	1.3 (0.0-4.9) 5.3 (0.5-43.5)	Other lung diseases among controls may be associated with silica exposure as well.	243
Autopsies of 381 lung cancer patients in the Netherlands. 1972-88	381 matched autopsied patients without lung cancer.	Judgement of occup. hygienist. Employment in Ceramic industry	1.11 (0.77-1.61)	Positive relation between exposure index and odds ratio	252 253
231 autopsies of lung cancer cases from South-Africa from 1975 minus 1979-83	318 matched controls from necropsy records at Centre of Occup. Health	Presence of silicosis or silica exposure on personnel records	0.8-1.84	No pos.relation between degree of silicosis or degree or duration of silica exposure observed.	167
5,935 lung cancer cases in Detroit.	3,956 colon and rectum cancer cases	Telephone interview: - Work in excavating and mining. - Masons	4.01 (1.3-12.1) 1.79 (0.9-3.5)	Characterisation of exposure unspecific regarding silica exposure.	49
73 hospital-based Japanese patients with lung cancer	72 pneumoconiosis patients from the same hospital.		12.0	No relation observed between duration of exposure or degree of silicosis and OR.	72
316 male lung cancer cases among Chinese pottery workers and miners.	1,352 workers without lung cancer.	Employment records from mine or factory files.	1.1 - 3.1	OR adjusted for smoking habits. OR significantly increased in subjects with silicosis.	248

information for an exposure-response analysis. There was a positive relationship between the time since entry into granite work and SMR.

Among 6,187 British pottery workers at different workplaces followed from 1970 to 1985 there were no particular excesses of lung cancer with any product or job group. However, for men who were under 60 years of age at the start of the follow-up, the SMR for lung cancer was 140 when compared with the national rates, and 132 when compared with locally adjusted rates ($p < 0.05$ for both) (423). The same study showed an increasing SMR of lung cancer with the number of years since entering employment with silica exposure. The amount of respirable free silica was measured at the start of the follow-up in 1970-71. Table 10 shows both crude and smoking-adjusted relative risks of lung cancer up to 1985 in relation to the dust concentrations measured, and the estimated cumulative exposure prior to the start of follow-up. Except for those with the lowest exposure (who may have been subject to health-related selection), there are indications of an exposure-response relationship both for the levels of exposure measured at the start of the follow-up, and the estimated cumulative exposures.

Mortality was followed from 1960 to 1979 among 231 Italian refractory brick workers with exposures in 1975 of between 0.09 and 13.0 mg/m^3 of dust containing 5.9 to 20.9 % of respirable silica. The number of person years under observation was 4,023. The SMR for lung cancer was 183 (95% confidence interval (CI) 91-327)(316). In spite of the given levels of exposure, the study did not provide figures making any analysis of an exposure-response relationship possible. In a recent extension of the cohort to 1,022 workers with a total of 17,508 person years at observation, total SMR for lung cancer was 151 (95% CI 100-218). For workers first employed before 1958, who were likely to have shared the highest exposure to crystalline silica, lung cancer SMR was 177 (95% CI 103-284). Workers with at least 19 years employment at the plant had an SMR of 201 (95% CI 107-344) when a 19 years' latency time was also acounted for (257).

The mortality from lung cancer was followed from 1940 to 1980 in 2,055 U.S.pottery workers, who in the period from 1939 to 1966 had been employed for at least one year in the production of ceramic plumbing fixtures. During their work they were exposed to silica dust and non-fibrous talc. Apart from an assessment of "high" and "low" exposures, there were no quantitative data on silica exposure. The SMR for lung cancer of 143 was statistically significantly raised. The highest SMR, 193, was for men who started work in the industry between 1940 and 1949, and there was a higher SMR for men with "high" exposure to silica than for those with "low" exposure (395).

In Austria, occupational and smoking histories were collected from 247,064 workers in Vienna between 1950 and 1960. Of these, 1,630 male workers aged 40+ years were selected because of their occupational exposure to silica. The same number of unexposed controls from the same study base were matched for age and smoking habits. The mortality of the two groups was followed from 1980 to 1985, giving a total of 20,172 person years under

Table 10. Relative risk of dying from lung cancer 1970-85 among 6,187 British ceramic industry workers in relation to respirable quartz exposure (mg/m^3) in 1970-71 and cumulative exposures (mg/m^3 x years) up to 1970-71. (Source: 423).

	Respirable quartz concentration measured in 1970-71			
	0.0-0.02	0.03-0.04	0.05-0.09	0.1+
National rates	1.67	1.13	1.53	1.73*
Smoking adjusted rates	1.68	1.06	1.43	1.63
	Cumulative exposure to respirable quartz up to 1970-71			
	0.0-0.14	0.15-0.49	0.5-1.49	1.5+
National rates	1.18	1.01	1.68*	1.58
Smoking adjusted rates	1.08	0.99	1.62*	1.51

* $p < 0.05$

observation. The rate ratio of lung cancer between the two groups was 1.27. The SMR for lung cancer based on the Vienna population was 169 for the silica-exposed group, and 118 for the non-exposed group. When analyzed by the type of work or industry in which silica exposure had occurred, lung cancer SMR's were as follows: foundries: 164, other metal industries: 133,ceramics and glass industries: 237, stone and construction work: 294, and "others": 149 (277,278). No quantitative exposure data were given.

In a study of 2,071 Danish stone workers who had been followed for 42 years, the observed and expected cancer figures were based on figures from the national cancer registry and adjusted according to regional variations (144). The standardized incidence ratio (SIR) for lung cancer was 200 (95% confidence interval (CI) 149-269) for all cohort members, 808 (CI 323-1,657) for skilled sandstone workers in Copenhagen, 404 (CI 202-723) for skilled granite workers in Copenhagen, 119 (CI 51-235) for skilled granite cutters in Bornholm, 181 (CI 116-270) for all stone workers in Bornholm, 246 (CI 143-394) for unskilled workers in the road and building material industry, and 111 (CI 45-229) for unskilled workers in the stonecutting industry. In 1970 the median exposure level to respirable silica in the road and building material industry was 0.16 (range 0.02-12.7) mg/m^3. In the stone cutting industry it was 0.05 (0.02-0.57)mg/m^3 (143). As smoking data for the cohort members could not be obtained, the occurrence of bladder cancer was studied concomitantly among the cohort members. No significant excess incidence of bladder cancer was observed, leading the investigators to conclude that smoking habits among the stone workers were not entirely different from the general male population from which the expected number of lung cancers was calculated.

Table 11. Relative risk of lung cancer (ICD 162) in some selected industries/-occupations in the Scandinavian countries. (95% confidence interval in parenthesis) (Source: 236)

	Norway	Sweden	Finland	Denmark
Foundry workers	1.73 (1.12-2.25)	1.38 (1.16-1.64)	1.56* (1.31-1.86)	1.6 (0.85-2.74)
Iron ore mining	1.36 (** - 3.17)	3.19 (2.92-3.49)	1.78 (0.22-6.45)	
Other metal ore mining	1.0 (0.33-2.34)	3.71 (3.1-4.4)	5.02 (3.11-7.68)	
Construction work with mining and quarrying	1.39 (0.69-2.49)	1.01 (0.87-1.18)	1.97 (1.32-2.83)	
Stone cutting	0.83 (0.17-2.44)	0.98 (0.83-1.16)	1.75 (0.98-2.89)	2.1 (1.77-4.57)
Self employed stone cutters				2.9 (1.17-5.98)

* Iron and steel basic industry.
** The figure given by the authors was 1.44 which is obviously incorrect.

Cause specific mortality from the previously published study of 5,414 Vermont granite workers (94) has now been followed from 1950 through 1982 (82). For all shed workers there was a statistically significant excess mortality from cancer of the respiratory system with an SMR of 127, based on 104 cases. The highest excess (SMR 146) was found for shed workers who had been hired before 1930 when dust levels were probably about 0.5 mg/m^3. The average dust exposure levels for cutters were about 0.37 mg/m^3 before 1940,and about 0.07 mg/m^3 thereafter. No clear exposure response relationship was shown, but the SMR for lung cancer showed an increase with increasing latency, and with duration of employment.

In the Scandinavian countries, census data were used to identify occupational groups potentially exposed to silica dust. These groups were followed by linkage with the national cancer registries and mortality data. The relative risks of lung cancer by country and type of industry or occupation are shown in table 11. No excess lung cancer risk was found for other workplaces not shown in the table (glass, porcelain, ceramics and tile industries, stone quarries, or sand and gravel pits) (236).

8.1.1.6. Other epidemiological studies

There have also been several studies during the last years on cancer in the iron and steel industries (10,115,116,261,362) and in the mining industry (3,68,181,223,251,427,429). However, as none of these studies provides

quantitative data on silica exposure, and they are probably more or less confounded by other carcinogenic exposures, they do not contribute any solutions to the question of a causal relationship between exposure to crystalline silica and lung cancer.

However, in a recent review of the literature on lung cancer risk in iron and steel foundries, polynuclear aromatic compounds and silica are considered as the most probable causes of excess lung cancer risk in iron foundries, along with chromium and nickel fumes in steel foundries (397).

8.1.2. Cancer of the gastrointestinal system

The first epidemiological indications of a possible association between silica exposure and stomach cancer probably came from a mortality study among 472,062 British coal miners, who were followed from 1949 to 1959. An SMR for stomach cancer of 149 was found, based on 969 cases (382). The results were later supported by another study of 25,000 British coal miners followed from 1958 to 1980, which also showed some evidence for a dust-related increase in deaths from cancer of the digestive system (259). However, other studies of coal miners and other mining groups have not been able to show any excess of gastrointestinal cancer (15,247,389).

In a Finnish study of 1,087 granite workers who had been followed from 1940 till 1975, and who had no known exposure to radon daughters, poly-aromatic hydrocarbons or asbestos, there were 15 deaths from gastrointestinal cancer against 7.4 expected (SMR 203, $p < 0.02$) (221). In a further follow-up of the same cohort through 1985 there were 18 gastrointestinal cancers against 11.6 expected, giving a statistically nonsignificant SMR of 155 (218).

In the follow-up study of 1,626 Austrian men who had been exposed to silica dust between 1950 and 1960, and who had been followed up to 1985, there was a statistically significant raised SMR of 166, based on 77 cases of stomach cancer (277,278).

In the Canadian study of 50,021 Ontario miners there was also a statistically significant increased risk of stomach cancer with 60 cases observed and 40.4 expected (191).

On the other hand, the other recent studies of workers with a well defined, more or less "pure" exposure to crystalline silica have not shown any excesses of gastrointestinal cancers (82,144,316,395,423).

It should also be mentioned that the large Montreal multi-center case control study of cancer at multiple sites, in an "in-depth" analysis, gave an odds ratio of 1.2 for silica and stomach cancer (364). In a recent case-control study from Spain, the risk of gastric cancer was positively associated with coal mining (OR 11.8, 95% CI 1.36-103), and with occupations entailing exposure to silica and other mineral dusts (OR 1.8, 95% CI 0.9-3.59) (135). In an American study of 1,342 cases of adenocarcinoma of the stomach, proportional incidence ratio for men who worked in dusty jobs was 1.3 times

(95% CI 1.2 - 1.4) that of unexposed controls. The association increased with increasing levels of dust exposure (425).

There have also been some reports suggesting a connection between an increased incidence of oesophagus cancer in certain regions in China and the content of silica fibres in plants used as human food (31,289).

8.1.3. Other cancer forms

In studies of silica-exposed groups there have been single indications of an increased risks of cancer of the haematopoietic system with SMR 161, 95% CI 42-422 (222), cancer of the bone and connective tissue with SMR 410, 95% CI 130-950 (119), and larynx with SMR 504, 95% CI 105-1,485 (315) and SMR 405, 95% CI 83-1,182 (316). An increased risk of leukaemia has been observed in one of the studies of US gold miners with SMR 169, 95% CI 81-312 (44), and in the study of US iron foundry workers with SPMR 284, 95% CI 123-655 (369). In a German case-control study of 1,119 patients with larynx cancer compared to patients with stomach cancer there was an odds ratio for larynx cancer of 1.6 among workers in the glass, porcelain, clay and quartz industries, and 2.3 among steel and foundry workers (339).

8.2. EXPERIMENTAL CANCER STUDIES

What is probably the first experimental study indicating an association between silica and lung cancer was published in 1940 (55). In an inhalation study, mice of both sexes were exposed to respirable dust from silica, iron oxide and natural dust containing 57% free silica from the Joachimsthal mines. Lung cancer incidence rates for the exposed animals living 10 months or longer compared with controls were 21.3% versus 7.9% for silica, 32.7% versus 9.6% for iron oxide and 11.6% versus 1.6% for the Joachimsthal dust. Even though it should be noticed that the number of animals surviving for a sufficient length of time was rather limited, these early and carefully conducted experiments with a long observation period showed some remarkable results which had probably deserved more attention than they actually received from the contemporary scientific community.

In a later study with a surgical intrapulmonary implantation of quartz dust of about 2 μm diameter into rabbits, tumours were found in both lungs of 4 out of 5 animals surviving 5-6 years (198). The early experimental studies with an intrapleural injection of mineral dust, including asbestos and quartz, also showed some evidence of a carcinogenic effect of silica (411).

The main results of some experimental studies with the inhalation of silica dust allowing for observation periods of sufficient length are summarized in table 12, while tables 13 and 14 (pages 46-49) summarize studies with intratracheal instillation, and with the intrapleural, intrathoracic and intraperitoneal administration of different types of crystalline silica in animals.

In addition to the studies cited in the tables, studies with the administration of crystalline silica along with benzo-a-pyrene (283,317,381) and with radon daughters (32) have shown an increased response which has been supposed to result from the combined exposure. In an inhalation study with rats, the rate of both lung tumours and mesotheliomas was greater, and the tumours occurred earlier when chrysotile asbestos was given concomitantly with quartz (Silicron S 600; Eurostandard A 9950) with a mean particle size of 2,5 μm, compared with the inhalation of asbestos alone (93).

The only animal species in the studies cited in tables 12-14, in which any clear neoplastic effect has been shown so far, is the rat. Neither the Syrian hamster, nor the mouse has responded in a similar fashion.

No single study has shown any clear dose-response relationship. Considering three of the most recent animal studies, there seems to be nearly the same tumour response rate over a wide range of exposure. An 18% incidence rate of pulmonary tumours after inhalation of 0.73 mg/m^3 over 24 months (267) was not very different from that found in animals breathing 12 mg/m^3 for about 20 months (30%) (183), or in animals breathing 50 mg/m^3 for 24 months (21%) (89).

In spite of these reservations regarding the causal inference which can be drawn from the animal studies, the results from the repeated studies in rats published up to 1986 were sufficient for the IARC group of experts, who finished their work in 1987, to consider the evidence for the carcinogenicity of crystalline silica in experimental animals as sufficient (192).

9. REPRODUCTIVE HAZARDS

Apart from the effects mentioned in chapter 10.2, no specific reproductive hazards are known to be caused by exposure to crystalline silica.

10. IN VITRO STUDIES OF CYTOTOXIC AND CYTOGENETIC EFFECTS

10.1. STUDIES OF CYTOTOXICITY

A number of *in vitro* studies have shown that crystalline silica is cytotoxic to both macrophages and polymorphonuclear leucocytes (242,352,363), and probably to a number of other cell types as well (342). The red blood cell haemolysis system has been used to study the cytotoxicity of quartz, and to

Table 12. Animal carcinogenicity studies with inhalation of crystalline silica.

Species	Number of animals	Exposure	Observation time (days)	Effects and rate	Comments	Ref.
Female BALB/cBYJ mice 6 weeks old	60	1.47, 1.8, and 1.95 mg/m^3 of Min-U-Sil <1.2 μm diam. 8 h/day, 5 d/week for:	0 - 150	Pulmonary adenomas	Unexposed controls sacrificed by the same schedule as exposed animals.	422
		150 days:		0/22		
		300 "		5/22		
		570 "		4/16		
		Unexposed controls:		7/59		
Charles River Fischer 344 rats. 3 months old.	144	0 - 51.6 mg/m^3 Min-U-Sil 1.7-2.5 μm MMAD 6 h/day 4 d/week for 24 months	Survived >494	Epidermoid carcinomas	Additional lesions in quartz-treated rats where pulmonary adenomatosis, cuboidal metaplasia of alveolar epithelium, alveolar proteinosis, lymphoreticular hyperplasia, and nodular fibrosis were found.	89
Males:	72			1/47		
Females:	72			10/53		
		Females with 4 months exp:		3/5		
		Unexposed controls:		0/89		
Charles River Fischer 344 rats	3 x 62	Nose only inhal. of 12±5 mg/m^3 Min-U-Sil < 5 μm diam., 6 h/day, 4 d/week for 83 weeks:	mean 683	Lung tumours	Tumours in exposed rats: 3 squam. cell, 11 adenocarc. 6 adenomas. Pronounced fibrosis observed in most animals.	182 183
		Sham exposed controls:		18/60		
		Unexposed controls:		0/54		
				1/15		
Charles River Fischer 344 rats (VAF-SPF) 8 weeks of age	3 x 100	DQ 12 (87% quartz), 1.3 μm MMAD with resp. fraction 74% 6 h/day, 5 d/week, 24 months:	median 750	Malignant lung tumours 12/100	Malignant tumours were 10 adenocarcinomas, 1 squamous cell carcinoma, and 1 mixed type. Lipoproteinosis, fibrotic foci, and peribronchial granulomatous foci were also found more frequently in silica-exposed animals.	267
		TiO_2 exposed controls:		1/100		
		Sham exposed controls:		0/100		

Table 13. Animal carcinogenicity studies with intratracheal instillation of crystalline silica.

Species	Exposure	Number of animals	time	Obs. tumour rate	Lung Comments	Ref.
Spraque-Dawley rats	7 mg Min-U-Sil < 5μm diam. in 0.2 ml saline weekly for 10 weeks.:	3 x 40	lifespan	6/36	In quartz-treated rats there was 1 adenoma and 5 carcinomas. Fibrotic lesions also observed in quartz-treated animals.	182
	Saline only controls:			0/40		
	Untreated controls:			0/18		
Male Charles River Fischer 344 rats	Into left lung 20 mg of Min-U-Sil (0.1% > 5μm diam):	3 x 85	3,12,18,22 months	30/67	Mostly adenocarcinomas in both treated groups. Min-U-Sil group had larger lesions.	141
	Novaculite (2.2% > 5 μm diam):			21/72		
	Deionized water:			1/75		
Syrian golden hamsters	Min-U-Sil with mean 1.7 μm diam. once a week for 10 weeks.		lifespan		Survival rates not mentioned.	182
	- 3 mg in 0.2 ml saline:	48		0/31		
	- 7 mg in 0.2 ml saline:	48		0/41		
	- 0.2 ml saline only:	68		0/58		
	- Untreated controls:	72		0/36		
Male Syrian golden hamsters, 7-9 weeks old	Weekly for 15 weeks in 0.2 ml saline:		92 weeks		One tumour of the larynx observed in the ferric oxide treated group. The only tumour in the Min-U-Sil group was an adenosquamous carcinoma.	183
	- 0.7 mg respirable Min-U-Sil:	50	"	1/35		
	- 1.1 mg respirable Sil-Co-Sil:	50	"	0/50		
	- Saline only controls:	50	"	0/48		
	- 3 mg ferric oxide:	50	62 weeks	0/34		
	- 3 mg Min-U-Sil + ferric oxide:	50	"	0/49		
Male strain A/J mice 11-13 weeks old.	Weekly for 15 weeks: 9.75 mg/kg Min-U-Sil		sacrified at 20 weeks		Lung adenomas. Single dose of quartz was mg/mouse. Observation time may have been of insufficient length.	249
	(1-5μm) in saline:	20		20%		
	Saline only controls:	30		31%		
	64.1 mg/kg Urethane controls:	30		60%		

Table 14. Animal carcinogenicity studies with intrapleural (IP), intrathoracic (IT) and intraperitoneal (IPe) admnistration of crystalline silica.

Species	Exposure	Number of animals	Obs. time	Effects rate	Comments	Ref.
Male Marsk mice, 3 months old	IT 10 mg tridymite with 20% < 3.3 μm and 40% 3.3-6.6 μm:	32	19 months	Tum L&P* 4/32	Lymph node hyperplasia: 19/32	48
	IT 5 mg chrysotile:	32	"	8/32	1/32	
	Saline only control:	34	"	0/34	1/34	
SPF Wistar rats, 48 of each males and females	IP 20 mg quartz (<5μm diam) in 0.4 ml saline:	96	lifespan "	MTRS** 39/95	Tumours found in mediastinum, pericardium, diaphragm, liver, and spleen. Distribution of malignant tissue corresponded to that of silicotic nodules.	412
	IP 0.4 ml saline controls:	96	"	8/96		
Standard Wistar rats,	IP Quartz in 0.4 mg saline:	96	"	31/94		
	IP 0.4 ml saline controls:	96	"	7/85		
SPF inbred Wistar rats, 6 weeks old, 80 of each males and females.	IP 20 mg quartz (<5μm diam) in 0.4 ml saline:	160	Up to 120 weeks	MTRS 23/160	One thymoma/lymphosarcoma in the control group.	413
	IP 0.4 ml saline controls:	160		0/160		
SPF inbred Wistar rats 6 weeks old, 16 of each males and females	IP 20 mg Min-U-Sil in 0.4 ml saline:	32	Mean: 678 days	MLHT*** 8/32	In the quartz-treated group and in the coal-treated group there were 3 and 1 thymomas/ lymphomas respectively.	413
	IP 0.4 ml saline control:	15	720 "	0/15		
	IP respirable coal dust in 0.4 ml saline:	16	618 "	0/16		
Wistar derived ICI rats, 16 of each males males and females.	IP 20 mg Min-U-Sil (<5μm diam) in 0.4 ml saline:	32	Mean survival 545 days	MLHT 11/32	Tumour morphology was similar in all strains.	414
	IP 0.4 ml saline controls:	32	"	0/32		
PGV rats, 12 of each males and females.	IP 20 mg Min-U-Sil (<5μm diam) in 0.4 ml saline:	24	666 days	2/24		
	IP 0.4 ml saline controls:	12	"	0/12		
Agus rats, 20 of each males and females (all groups 5-6 weeks old)	IP 20 mg Min-U-Sil (<5μm diam) in 0.4 ml saline:	40	647 days	2/40		
	IP 0.4 ml saline controls:	24	"	0/24		

cont.

Table 14 cont.

Species	Exposure	Number of animals	Obs. time	Effects rate	Comments	Ref.
SPF inbred Wistar rats, 6 weeks old, 16 of each males and females.	IP 20 mg cristoballite (<5μm diam) in 0.4 ml saline:	32	Mean survival 714 days	MLHT 13/32	Thymoma/lymphosarcoma was found in 5 of the quartz-treated animals, and in 1 of each of the saline and coal-treated groups.	413
	IP 0.4 ml saline controls:	15	720 "	0/15		
	IP respirable coal dust in 0.4 ml saline:	16	618 "	0/16		
Wistar derived ICI Alderley-Park rats, 5-6 weeks old, 16 of each males and females.	IP 20 mg cristoballite (<5μm diam) in 0.4 ml saline:	32	Mean survival 597 days	4/32	The tridymite was obtained by dissolving impurities from silica cement which had had long service at approx. 1,380°C in a gas-retort house.	414
	IP 20 mg tridymite (<5μm diam) in 0.4 ml saline:	32	525 "	16/32		
	IP 0.4 ml saline controls:	32	717 "	0/32		
SPF inbred Wistar rats, Two groups with 16 of each males and females. 6-8 and 8-12 months old.	IPe 20 mg Min-U-Sil in 0.4 ml saline:	64	Mean survival 462 days	ML**** 9/64	Tumours were 2 of the histiocytic type and 7 of the thymoma/lymph-sarcoma type. One of the controls developed thymoma/lymphosarcoma.	413
	IPe 0.4 ml saline controls:	20	332 "	0/20		

* Tum L&P = Tumours of lung and pleura.
** MTRS = Malignant tumours of the reticuloendothelial system.
*** MLHT = Malignant lymphomas of the histiocytic type.
**** ML = Malignant lymphomas.

compare the effect of different types of silica dust. It has been shown that the cytotoxic effect depends on the "freshness" of the particle surface (226,403). The levels of haemolysis obtained with several silica varieties occurred in increasing order with tridymite, quartz, crystoballite, vitreous silica, and coesite (379).

In a study using the spin labelling technique to determine the cytotoxic effects on bovine red cells, both chrysotile asbestos and titanium dioxide induced modifications in membrane proteins, whereas quartz did not (231).

When assessing the ability of quartz and asbestos to induce the production of reactive oxygen metabolites in human polymorphonuclear leucocytes, quartz was the most potent, and there was a positive correlation between the induction of such reactions and red cell haemolysis (157).

In a study using three *in vitro* mammalian cytotoxicity assays, quartz proved to be highly toxic in the erythrocyte haemolysis assay, but far less toxic in the Chinese hamster ovary cell clonal cytotoxicity assay, and the V79 cell clonal cytotoxicity assay (78).

The presence of other minerals or coal in the tested dust, and in particular that of soluble aluminium seems to strongly subdue the toxic action (122,163,242). One recent study also showed a reduction in the cytotoxicity of quartz when given simultaneously with L-α-dipalmitoyl lecithin (352).

10.2. CYTOGENETIC STUDIES

Studies with silica (type not specified) have been negative when using the *Bacillus subtilis* rec. assay, commonly used to indicate the ability of a substance to induce DNA-damage, (197,200).

Results were also negative when applying silica (silicron G-910, physical form not specified) in mutation tests with different strains of *Salmonella typhimurium* or *Escherichia coli*, both with and without a metabolic activation system (191).

Min-U-Sil (Pennsylvania Glass Sand Co) at doses of 20 $\mu g/cm^2$ has been shown to induce the formation of micronuclei in hamster embryo cells (170). Another study with DQ 12 in doses of 500 mg/kg body weight did not show a similar effect in bone marrow cells from mice (405).

Using Chinese hamster V79 cells, Min-U-Sil at concentrations of 1-15 $\mu g/ml$ was not able to induce sister chromatid exchange (313), and α-quartz at a dose of $2 \mu g/cm^2$ did not induce chromosomal aberrations or aneuploidi in hamster embryo cells (292). On the other hand, morphological transformation has been shown to occur in hamster embryo cells both after exposure to Min-U-Sil and α-quartz at doses greater than 2 $\mu g/cm^2$ (169). In a study with several types of crystalline silica, the occurrence of DNA double strand breaks was high for F600 quartz, cristobalite and Min-U-Sil, intermediate for tridymite and HF-etched MQZ, and low for Chinese standard quartz and DQ12 (More details on the applied essay and types of dust not given) (343).

When studying intercellular communication, quartz has been shown to influence metabolic cooperation with Chinese hamster cells (64).

In a recent study assessing the ability of Min-U-Sil (quartz) and tridymite to induce sister chromatid exchanges (SCE) in cultures of human lymphocytes and monocytes, the level of SCE was significantly enhanced after treatment with 50 $\mu g/cm^2$ of tridymite, whereas the quartz-treated cells did not show such clear cut effects. Complementary experiments showed that phagocytosis of tridymite particles by monocytes was a prerequisite for the induction of SCE in lymphocytes (295).

11. PATHOGENESIS OF LUNG INJURY

Upon contact with inhaled silica particles, various types of pulmonary cells react, and subsequently induce the mechanisms which both in the short and long term are responsible for the different lung injuries which may occur. In bronchoalveolar lavage studies in rats, it has been shown that a single instillation of silica caused a progressive reaction which lasted for months and involved both alveolar and interstitial tissues (108). It also seems to be important to study lung wall tissue cells in order to further elucidate the mechanisms involved in silica-induced lung damage (371).

Despite the huge amount of research carried out in order to elucidate the various health effects of silica exposure, the mechanisms by which silica particles deposited in the lungs exert their effects are still not fully understood. The recent concept of which pathogenetic mechanisms are involved has been reviewed from various angles by different authors (27,92,160,163,165,363).

11.1. INTERACTION WITH PULMONARY MACROPHAGES

The primary key to the understanding of the effects of silica dust seems to be the phagocytosis of silica particles by alveolar and interstitial macrophages (1). Some authors claim that the process of phagocytosis is dependent on, or at least stimulated by, the coating of the silica particles with host-derived proteins, so-called opsonins, which are thought to be members of the immunoglobulin and its complement system, and acts chemotactically to specific receptors on the macrophage surface (42,363). Induction of C5a complement by the deposition of silica particles is also believed to exert a chemotactic effect in attracting alveolar macrophages to the site of deposition (27).

Based on *in vitro* studies, it was earlier thought that the events following phagocytosis were a result of the cytotoxic potential of the quartz particle leading to the death of the macrophage and the subsequent release of lysosomal enzymes (5). More recent studies have shown that silica particles *in vivo* do

not influence the viability of the macrophages to the same extent as *in vitro*, but that they influence their function more (162,305). One early event in the interaction of silica with alveolar macrophages may involve perturbation of the intracellular calcium homeostasis (67).

Stimulated macrophages release different cytokines, which are able to exert an effect on other cell systems, e.g.lymphocytes (27,290,305,380), fibroblasts (92,305), and polymorphonuclear neutrophilic leucocytes (92,160). The most important type of macrophage-derived cytokine is now believed to be interleukin-1 (IL-1), which is able to stimulate T-helper lymphocytes unspecifically and to stimulate fibroblasts both to proliferate and to enhance the production of collagen (27,92,305,353).

Other suggested cytokines which are supposed to stimulate the fibroblast are macrophage-derived growth factor (MDGF) (92), fibroblast growth factor (FGF) (1), macrophage fibrogenic factor (MFF) (165) and fibronectin (27,101,220).

On the other hand, prostaglandin E2 (PGE2) is supposed to suppress the action of IL-1 on fibroblasts (92). In an *in vitro* study it was suggested that the fibrogenicity of silica could be based on an inhibition of PGE2, since silica dust caused macrophages to release growth factor for fibroblasts without triggering the release of PGE2, which would again have inhibited the subsequent fibroblast proliferation (46).
Tumour necrosis factor (TNF) and leukotrine B_4 (LTB_4), also derived from macrophages, are both supposed to act fibrogenically and to induce an inflammatory response (102,123,136,156,307).

11.2. EFFECTS ON NEUTROPHILIC CELLS

LTB_4 produced by the macrophage acts chemotactically to attract neutrophilic cells, which again induces an inflammatory reaction (123,156,160). An increased number of neutrophils has been observed in bronchoalveolar lavage from patients with silicosis (25), and in experimental studies (108,415).

11.3. EFFECTS ON LYMPHOCYTES

IL-1 released by the silica-stimulated macrophage induces the growth of T-helper lymphocytes, probably making the immune system more prone to respond to antigen stimulation with the production of IL-2-5 or gamma-interferon, and to react with a hypersensitive, inflammatory or specific allergic response.

IL-4 produced by an increased number of stimulated T-helper cells may induce the maturation of B-lymphocytes to plasma cells, which again are responsible for the increased amounts of immunoglobulins, IgA, IgG and IgM which have been observed in patients with silicosis (25,54,92,284).

Stimulated T-helper lymphocytes also produce an increased amount of soluble products called macrophage Ia recruitment factor (MIRF), inducing the expression of Ia antigens on the macrophage surface, which in turn increases its immunological potencies, such as, for example, as an antigen presenting cell (92,386,351).

Expansion of the lymphocyte population has been observed in lung lavage fluids from both humans and animals with silicosis (25,74,90).

11.4. EFFECTS ON OTHER PULMONARY CELLS

Deposited silica reacts not only with the alveolar macrophages, but also with alveolar epithelium, especially the alveolar type II cell responsible for the production of alveolar surfactant and for the regeneration of alveolar epithelium (163).

Alveolar type II cell hyperplasia has been noted in lung tissue and in lavage fluid in cases of silicosis (27,355). These cells are capable of producing a different composition of, and increased amounts of, phospholipids which probably also play a role in the fibroblast-derived fibrogenesis as well as in the alveolar proteinosis which can be induced by massive exposure (25,88).

In studies of type II pneumocytes from rats exposed to silica it has been found that part of the cells were hypertrophic and contained larger lamellar bodies and elevated amounts of protein and total RNA than did normal type II cells (258,415). In an experimental study where rats were exposed to quartz by inhalation, it was observed by electron microscopy that the lung cancers were mainly alveolar type II cell tumours (194).

Electron microscopic studies have shown that after 24 hours, inhaled silica particles may also be found within the type I pneumocytes (41). In an other study it was found that dust penetration into the interstitium could occur by the migration of dust-loaded macrophages through alveolar intracellular spaces, or by dust-induced necrosis of alveolar cells with reepithelization engulfing the dust particle (210). Experimentally it has been found that silica increases the alveolar-capillary membrane permeability in a dose dependent way (255).

Enhanced peroxidase activity, probably from neutrophilic cells, eosinophils and mast cells has also been observed in bronchoalveolar lavage fluids of hamsters exposed to silica. This indicates a silica induced stimulation of the non-immune immediate pulmonary defence system (97).

11.5. FORMATION OF FREE RADICALS

For more than thirty years it has been known that the oxidative and hydroxylative actions of quartz might play a role in the development of silicosis (240). In a number of recent experimental studies it has been shown that human neutrophils (100,140,160), and human and animal macrophages (152,286,408)

produce highly reactive and cytotoxic oxygen and hydroxyl radicals when exposed to fibrogenic silica (205,376).

Upon contact with water, silanol (SiOH) groups are formed on the surface of the silica particle. These groups have a high affinity to iron, which again acts as a catalyst enhancing the reaction leading to the production of free radicals (127,363). Freshly crushed silica has been shown to have a particular potential for forming free radicals, which might also explain the observation of an increased pathogenicity of such silica particles (402).

A positive relation has also been observed between the pathogenicity of dusts and their ability to form oxygen and hydroxyl radicals (157,158,205). It has also been shown that the formation of free radicals is restrained by the presence of polyvinyl-n-oxide (PVPNO) which binds to the particle surface (145,159), and polyvinylpyridin-n-oxide (287). The production of free radicals is strongly enhanced in the presence of immunoglobulins and Interferon-γ (214,286,288,304). Together with the genetically induced expression of histocompatibility antigens (219), this phenomenon might to some extent explain some of the individual susceptibility to lung injuries from silica exposure.

The ability of silica dust to induce the formation of free radicals in macrophages and other pulmonary cells, may also be of crucial importance when discussing how the substance might exercise a carcinogenic effect (111).

11.6. EXPRESSION OF SERUM ONCOPROTEINS AND GROWTH FACTORS IN SILICOSIS.

As opposed to what was found to be the case in a study of serum ras-oncogens in patients with asbestosis, patients with silicosis in the same study did not show increased level of serum oncoproteins whether they later developed cancer or not. However, patients with silicosis had a stronger expression of growth factor PDGF which seemed to increase with increasing degree of lung fibrosis. This finding could suggest that elevated serum PDGF levels might be a marker for the development of severe and progressive pneumoconiosis (39).

12. EXPOSURE-RESPONSE RELATIONSHIPS

12.1. EXPOSURE-RESPONSE FOR SILICOSIS

There is a positive relation between the quantitative measures of silica dust exposure and the occurrence of silicosis. A positive relation between the degree of exposure and the time lag for the disease to develop has also been observed (300).

There have been many previous studies showing a clear relationship between dust content in the lungs of silica-exposed workers and radiographic signs of pneumoconiosis (335).

In 1986 a WHO study group aimed to recommend *health-based* limits in occupational exposure to crystalline silica and coal dust (420). When applying strict criteria to epidemiological studies requiring that (i) both dust concentration and exposure time were given, and (ii) that the effect was given in terms of radiographic changes measured on a progressive scale, only the four following studies were found to fulfill the criteria:

1) In a British study of 623 coal miners, increased risk of coal-worker's pneumoconiosis was found in workers with an estimated exposure to free silica at concentrations of 0.1 mg/m^3 (357).

2) In 1,115 Japanese silica-exposed stone workers, ceramic workers, and welders, it was found that the limits for a 5% prevalence rate of silicosis after 25 years of exposure, were 0.47 mg/m^3 of dust containing 30% of free silica, 0.54 mg/m^3 of dust with 16 % of free silica, and 0.96 mg/m^3 of dust containing 7% of free silica (420).

3) In a study of 241 British gypsum miners with exposures to respirable quartz between 0.07 and 0.12 mg/m^3, 16 of 64 miners with at least 20 years' service showed evidence of silicosis grade 1/0 or more (ILO-classification). There was a linear relationship between the prevalence of radiographic abnormalities and exposure, and the cases of pneumoconiosis were observed in miners exposed to dust concentrations of not more than 0.05 mg/m^3 (245).

4) In one of the studies of Vermont granite workers published in 1974, no case of silicosis was observed among workers with exposures < 5 million particles per cubic foot (mppcf) (approximately 0.03 mg/m^3) for up to 20 years (13,392,393,420).

In two studies of *total dust* exposure, a 1% prevalence rate of silicosis was observed in Peruvian miners at exposure levels of 8 mppcf of dust with 30% of quartz and exposure times of up to 12.7 years (301), and no case of silicosis was found among miners exposed to 5-10 mg/m^3 with a quartz content of 14-35% (91).

Based on these studies the WHO study group recommended a *health-based* exposure limit for respirable crystalline silica of 0.04 mg/m^3 in order to avoid the occurrence of silicosis (420).

In a proportional mortality study among the Vermont granite workers the relative risk of silicosis was 1 for lifetime exposure levels < 200 mppcf x years (94).

A more recent follow-up and reanalysis of the Vermont data included an exposure survey and a method for the conversion from konimetric exposure

measurements to gravimetric values (268,269,407). Demanding that three or more of five radiographic readers had classified the chest radiographs of the study participants as with small opacities of profusion 1/1 or more (ILO-classification), the risk of silicosis based on 40 years' exposure was 0.4% (95% confidence limits 0.2-0.8%) at mean exposure levels to respirable silica at 0.05 mg/m^3, 1.3% (0.7-2.1%) at 0.1 mg/m^3, 2.4% (1.4-3.9%) at 0.15 mg/m^3, and 3.8% (2.2-6.5%) at 0.2 mg/m^3. Based on the same radiographic criterion, the estimated cumulative exposure needed for a 1% risk of developing silicosis was 3.5 (2.5-4.9) mg/m^3 x years, for a 2% risk 5.4 (4.0-7.3) mg/m^3 x years, for a 5% risk 9.5 (6.6-13.6) mg/m^3 x years, and for a 10% risk 14.6 (9.3-23.7) mg/m^3 x years (269).

In another recent publication regarding the Vermont granite workers who had been exposed to an average of 0.06 mg/m^3 of silica, 0.7% of the group showed nodular or rounded opacities of the type usually seen in uncomplicated silicosis. It was claimed that the low exposure level had been sufficient to essentially eliminate radiographic changes indicating silicosis (137).

Following relatively high exposures to silica dust among Indian slate pencil workers with respirable fractions between 3.7 and 18.4% with a content of free silica between 47 and 62%, a prevalence rate of silicosis of 54.6% was found despite the fact that no worker had more than 20 years of exposure (345). In a recent study from China, a safe level of exposure to respirable silica was estimated to be 0.25 mg/m^3, based on individual exposure characteristics and the prevalence of silicosis (299).

12.2. EXPOSURE-RESPONSE FOR LUNG CANCER

As mentioned in the commentaries to the tables 7-9 (chapter 8.1.), only one of the early epidemiological studies of silica exposure and lung cancer gave any information that could be used to assess any relation between exposure and a carcinogenic response. In this one study, no positive relation between the estimated cumulative degree of exposure and response was found (94). Compared to the early epidemiological studies, more recent studies have had two great advantages, namely that they have dealt with groups with a "pure" exposure to silica, and that they have given more reliable information about the exposure levels of the workers studied.

Table 15 shows a brief summary of the exposure levels and the relative risks of lung cancer in some of the studies cited in chapter 8.1.

As the lower limits in the given ranges of exposure are rather small, it is difficult to obtain any firm limit of no effect from these studies. In the study of British pottery workers, no excess risk of lung cancer was observed at exposure levels in 1972 < 0.05 mg/m^3, or at cumulative exposure levels < 0.5 mg/m^3 x years (423). Among the Vermont granite workers no excess risk of lung cancer has been shown in workers hired after 1940-50. At that time a new ventilation system was installed, and after that, exposure levels only very seldom exceeded 0.075 mg/m^3 (82). In the Danish study an estimated median

Table 15. Exposure levels to crystalline silica and relative risk of lung cancer.

Study population	Exposure levels mg/m^3	SMR (95% CI)	Comment	Ref.
Finnish granite workers	1.0-1.5	156	No information for an exposure-response relationship	217
British pottery workers	1972: 0.1 Cumulative 0.5-1.49	173 (101-278) 168 (100-249)	No excess risk < 0.05 mg/m^3 or 0.5 mg/m^3 x years cumulative exp. See also table 11.	423
Italian refractory brick factory	0.09-13.0	183 (91-327)	No information for an exposure-response relationship.	316
Danish stone-cutting industry	0.02-0.81 (respirable quartz)	111 (45-229)	Locally adjusted SIR. No information for an exposure-response relationship	143 144
Danish road material industry	0.003-13.9 (respirable quartz)	246 (143-394)		
Vermont granite shed workers	Hired before 1930: 40 mppcf* Hired after 1950: 10 mppcf	143.5 ($p<0.05$) 81.1	Exposure values estimated conversion from konimetric measures.	82
US iron foundry workers	cumulative <1.45 mg/m^3	148 (PMR)	At least 10 years exposure	369

* mppcf = million particles per cubic foot.

exposure level of 0.16 mg/m^3 was associated with a slightly increased risk oflung cancer, whereas a median exposure of 0.05 mg/m^3 was not (144).

Table 16 shows relative risks of lung cancer in relation to the reported duration of exposure and in relation to the reported time since first exposure in some of the cohort and case-control studies cited. As the relative risk is probably also dependent on the degree of exposure, these figures are of

In another recent publication regarding Vermont granite workers exposed to an average of 0,06 mg/m^3 of silica, 0,7% of the cohort showed nodular or rounded opacities of the type usually seen in uncomplicated limited value. However, it seems from some of the studies that an increased risk of cancer has been observed even after relatively short exposure times. Regarding the time since first exposure, the Finnish study (218) showed an increased risk even after a very short latency period, while a considerable time between first exposure and an increase in

Table 16. Relation between length of exposure and time since first exposure in studies of lung cancer and exposure to crystalline silica.

Study group	Years of exposure and relative risk	Years since first exposure and relative risk	Ref.
Cohort studies:			
Vermont granite shed workers hired before 1940	<10: - 10-29: 104.6 30+: 154.8	15-24: 95.2 25-39: 96.3 40+: 164.9	82
Vermont granite shed workers hired after 1940	<10: 65.0 10-29: 136.4 30+: 117.8	15-24: 84.4 25-39: 129.3 40+: -	
Finnish granite workers		<5: 156 5-9: 168 10-19: 205 20-29: 247 30+: 226	218
UK pottery workers		0-19: 110 20-39: 130 40+: 140	423
UK foundry workers	Significantly increased SMR by 5-9 years of exp.		117
US pottery workers	<15: 162 15-29: 168 30+: 112	<15: (146) 15-29: 132 30+: 156	395
Case-control studies:			
Austrian stone workers	<10: 220 10-20: 270 21-30: 260 31-40: 160 40+: 180		410
Italian lung cancer patients	1-4: 100-110 10-14: 140		243

lung cancer risk was found in the other studies. In a study of proportionate mortality among US foundry workers, an increased SPMR for lung cancer of

148 ($p < 0.05$) was found at cumulative mean exposure levels averaging 10 mppcf over 30 years. This is roughly equivalent to an exposure of 0.145 mg/m^3 of respirable crystalline silica (261,369).

In a recent study of 2,209 white South African gold miners who started work in 1936-43, lung cancer mortality was followed from 1968 to 1986. The workers had been exposed to 0.05-0.84 mg/m^3, and the average exposure time had been 23.5 years. Based on 39 deaths due to lung cancer, the SMR was 161 (95% conf.int.114-220). By means of logistic regression analysis it was estimated that the relative risk of lung cancer associated with a cumulative exposure unit of 1,000 x years (further details not given) increased with 0.023 (95% conf.int.1.005-1.042) (178). In the same study, and in a study of Chinese tin miners exposed to silica (429), a multiplicative interaction between silica exposure and tobacco smoke for the risk of lung cancer was suggested.

13. RESEARCH NEEDS

Based on the available data and the remaining uncertainty regarding a possible carcinogenic effect from crystalline silica, the following main focuses for further research are proposed:

- More groups of workers with "pure" exposure to crystalline silica dust have to be defined in epidemiological studies, and the past and present levels of exposure have to be assessed more accurately than in most previous studies.

- Another challenge in epidemiology is to further study the impact of combined exposures in different settings, and the prevention which can be achieved by different modes of intervention in the most important causative factors.

- There also seems to be a need for more properly designed epidemiological studies regarding other non-malignant effects of the exposure to crystalline silica.

- Animal experiments into the effects of crystalline silica have been surprisingly limited during the last years. It is evident that what is particularly needed is more inhalation experiments with different species and different doses.

- There is also a need for further studies to throw light on the mechanism of the effect of crystalline silica on different cell systems. This involves both *in vitro* studies, animal experiments and clinical investigations. Among other crucial questions to be solved is that of the individual susceptibility to noxious agents, both short and long term.

14. DISCUSSION

As silica is the most abundant mineral in the earth's crust, and low-grade occupational exposures are rather common, the implications of any adverse health effects from low grade environmental and occupational exposures may become immense.

It has been shown, beyond any doubt, in a large number of case series, epidemiological studies, and animal experiments that inhaled crystalline silica causes various types of fibrotic lesions in lung tissue, and that this effect is dose-dependent. Silicosis, progressive massive fibrosis, and alveolar proteinosis are serious diseases which may be life threatening, in particular when exposure to silica is high.

Cases of fibrotic lesions have been reported at exposure levels down to 0.05 mg/m^3 of respirable silica. It has been estimated that the risk of developing lung fibrosis from an exposure of 0.1 mg/m^3 is between 0.7 and 2.1%, based on long term exposure measurements and long term follow-up.

Reports of other non-malignant diseases which have been shown to be associated with silica exposure are mainly based on case series and autopsy materials. The evidence for a causal relationship from epidemiological studies and animal experiments is rather scarce, and no exposure-response relationships have been demonstrated. Based on today's available data, it seems fair to assume that if exposure to respirable free silica is low enough to prevent silicosis (0.04 mg/m^3)(420), it will also be low enough to prevent the occurrence of other non-malignant adverse health effects.

In 1987 the IARC expert group considered the evidence from animal studies for a causal relationship between silica exposure and cancer as sufficient, and the evidence from human studies as limited (191). Crystalline silica was thus classified as a group 2 A carcinogen (192).

The evidence of a causal relationship between exposure to crystalline silica and cancer of the respiratory organs in man is still limited. Recent epidemiological studies of groups with more or less "pure" exposure to crystalline silica, some of them indicating the presence of an exposure-response relationship, have contributed significantly to the evidence of a possible causal relation. However, as the results of these studies, like the results of most previous epidemiological studies, may, at least to some extent, have been influenced by selection bias and confounding, the evidence is still too scarce to conclude from the epidemiological data, with enough confidence, that there is a causal relationship between silica exposure and lung cancer.

It is remarkable that the animal studies have only been able to show a carcinogenic effect in rats, and not in hamsters and mice, which have also been investigated thoroughly. In experimental studies there is also a remarkable lack of a dose-response relationship. On the other hand, carcinogenicity has been shown in rats in repeated studies and by different routes of administration. A clear carcinogenic effect has recently been demonstrated in inhalation experiments with doses almost similar to that which can be found in a workplace situation.

Another serious problem with the experimental data is the question of the relevance of rodent histological tumour types to human diseases, which is still open to debate. A sex difference in both effect and response rates has also been suggested, but the data are insufficient for any conclusions to be drawn.

In vitro studies have shown a clear cytotoxic effect of crystalline silica where effects on the genetic material in some cell types may also be involved. However, the data are far too scarce to conclude that crystalline silica is in itself able to exert a genotoxic effect.

It is also interesting that recent studies into the pathogenetic mechanisms of the lung tissue reaction by exposure to crystalline silica have unveiled pathways, which include, among others, the stimulation of free oxygen and hydroxyl radicals, which may be common for both the fibrogenic effect and perhaps also for a carcinogenic effect.

Nevertheless, it still seems fair to conclude from the experimental data, and partly from the epidemiological data, with some uncertainty remaining, that crystalline silica probably plays a role in neoplastic development subsequent to exposure.

From these considerations it may seem difficult to decide whether only silicosis or both silicosis and lung cancer should be regarded as the *critical effects* following exposure to crystalline silica. In any case, it seems fair to suggest from the available epidemiological data, that an exposure level which is regarded as low enough to prevent silicosis (420) will also be sufficiently low for the prevention of any observable increased risk of lung cancer in humans.

If crystalline silica were to be introduced today as a new substance, the uncertainty regarding its possible carcinogenic effect would probably have justified strict precautions for avoiding any exposure. As man has been exposed to the substance to some degree during his entire stay on this planet, and lung cancer incidence has shown its dramatic increase first during the last 40-50 years, however, it seems to be little reason to fear serious adverse health effects of everyday dust exposure to the general population.

The important point must be to protect exposed workers from such lifelong exposures that can cause lung fibrosis, and which may possibly, alone or in combination with other exposures, also contribute to the development of lung cancer.

15 SUMMARY

Hilt B. Crystalline silica. Nordic expert group for documentation of occupational exposure limits. Arbete och Hälsa 1993:35, pp 1-82.

Crystalline silica (SiO_2) is the most abundant mineral in the earth's crust. This criteria document reviews environmental and occupational exposure to silica-containing dust and various health effects which may result from such exposure. The epidemiological and experimental evidence for a possible carcinogenic effect of silica dust is reviewed and discussed. From the available data it is concluded that working conditions with exposures which are today regarded as low enough to prevent the occurrence of silicosis (< 0.04 mg/m^3 of respirable crystalline silica), will probably also be sufficiently low to prevent any possible observable increased risk of lung cancer in humans.

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Appendix

List of permitted or recommended maximum concentrations of crystalline silica in air

Country	mg/m^3	ppm	Comments	Year	Ref
Denmark				1988	2
Cristobalite	0.15	-	total dust		
	0.05	-	respirable dust		
Quartz	0.3	-	total dust		
	0.1	-	respirable dust		
Tridymite	0.15	-	total dust		
	0.05	-	respirable dust		
Finland				1987	8
Cristobalite	0.01	-	fine-grained		
Quartz	0.2	-	fine-grained		
Tridymite	0.1	-			
Iceland				1989	6
Quartz	0.3	-	total dust		
	0.1	-	respirable dust		
The Netherlands				1989	4
Cristobalite	0.075	-	respirable dust		
Quartz	0.15	-	respirable dust		
Tridymite	0.075		respirable dust		
Norway*				1989	5
Cristobalite	0.05	-	total dust		
	0.1	-	particle sice<5µm		
Sweden				1990	3
Cristobalite	0.05	-	respirable dust		
Quartz	0.1	-	respirable dust		
Tridymite	0.05	-	respirable dust		
USA (ACGIH)				1990	1
Cristobalite	0.05	-	respirable dust		
Quartz	0.1	-	respirable dust		
Tridymite	0.05	-	respirable dust		
Tripoli	0.1	-	respirable dust		
USA (NIOSH)				1989	7
Cristobalite	0.05	-	respirable dust		
Quartz	0.05	-	respirable dust		
Tridymite	0.05	-	respirable dust		
Tripoli	0.05	-	respirable dust		

*The additive effct of the three types of silica, when present together, is taken into account: $\frac{C_1}{N_1} + \frac{C_2}{N_2} + \frac{C_3}{N_3} < 1$, where C = concentration and N = exposure limit for each type of silica

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DIESEL EXHAUST

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Contents

Abbreviations

AHH	Arylhydrocarbon hydroxylase
AQI	Air Quality Index
ARP	Respirable particulate adjusted for the contribution of tobacco smoke
BALF	Bronchoalveolar lavage fluid
BaP	Benzo(a)pyrene
CA	Chromosomal aberrations
CD	Coal dust
CHD	Coronary heart disease
CI	Confidence interval
CV	Closing volume
DE	Diesel exhaust
DEP	Diesel exhaust particles
DF	Diesel fuel
DNA	Deoxyribonucleic acid
EPA	Environmental Protection Agency
FEV%	Forced expiratory volume in % of FVC
$FEV_{1.0}$	Forced expiratory volume in 1 sec
FVC	Forced vital capacity
GI	Gastrointestinal
GSH	Glutathione
HC	Hydrocarbon
HDD	Heavy duty load diesel
IARC	International Agency for Research on Cancer
LDD	Light duty load diesel
MN	Micronucleus
MSHA	Mine Safety and Health Administration (USA)
MTU	Michigan Technologic University (USA)
NIOSH	National Institute for Occupational Safety and Health (USA)
NP	Nitropyrene
OEL	Occupational Exposure Limit
OR	Odds ratio
PAH	Polycyclic aromatic hydrocarbons
PAM	Pulmonary alveolar macrophages
Phe	Phenantrene
PMR	Proportionate mortality ratio
RCD	Respirable combustible dust
RR	Relative risk
SCE	Sister chromatid exchange
SMR	Standardized mortality ratio
TL_{CO}	Transfer factor for CO
USBM	United States Bureau of Mines
USEPA	United States Enviornmental Protection Agency
VC	Vital capacity

Introduction

In occupational settings it is usually impossible to separate between exposure to diesel exhaust (DE) and diesel vapours. The occupational groups that are more directly exposed to primarily exhaust (and not diesel fumes), such as car-park attendants and toll-booth workers, are too small and, furthermore, they are also exposed to petrol exhaust. Another problem when estimating the hazards of DE is the fact that the composition of DE may vary considerably due to the type of diesel fuel used, as well as the type of engine used, the driving conditions, such as heavy load/ light load, speed etc. In the animal studies, the interest has, predominantly, been focused on the particle fraction of DE as it contains a large number of polyaromatic hydrocarbons (PAH), known to be mutagenic and carcinogenic. Furthermore, only the particle phase is necessary to induce lung tumours in rats.The major concern regarding the gas phase has been directed towards the sulfur content of diesel fuel, which has been markedly lower over the years. However, the gas phase also contains compounds with known carcinogenic potential (such as formaldehyde) or with a cocarcinogenic effect. The literature on DE is ginourmous, and thus the present document has focused mainly on literature published 1989 and later, and in particular literature dealing with effects in humans, when such is available. Reference has also been made to important earlier studies, especially regarding carcinogenicity. For detailed information on pre-1989 studies, the reader is referred to reviews published by IARC (99), NIOSH (152), and USEPA (221).

1. Physico-Chemical Composition

No explicit physico-chemical data are available for DE.

Diesel-engine exhaust (DE; Table 1) is produced during combustion of diesel fuel and it includes *gases* such as carbon monoxide, sulfur oxides, nitrogen oxides, aldehydes (including acetaldehyde, acrolein, benzaldehyde, and formaldehyde), and other compounds, i.e. PAHs, such as benzene and nitrated PAHs etc, and a *particulate fraction*. (DEP). Generally the particle concentration in the DE goes down with increasing cetane number (see below). The particulate fraction contains carbon cores that tend to form chains or other aggregates following the combustion process; more than 95% of these particle aggregates are less than 1 micrometer in diameter (36, 41, 44, 55, 56, 72,190, 197, 215, 224, 233). Organics, such as PAHs, as well as sulfates and nitrates, are associated to the carbon cores. A large number of the PAHs have been identified and quantitated (Table 2; 69, 70, 166, 176, 189, 214, 233, 234). However, diesel exhaust is a mixture of hundreds of compounds, which may vary in composition due to several factors; the source of diesel, engine design, operating conditions, i.e. heavy duty load diesel (HDD), light duty load diesel (LDD) etc (232). One of the compounds identified in DE, the highly mutagenic dinitropyrene, has been suggested to be a collection artifact (120).

Diesel fuel (Table 2) is a gas oil fraction obtained from the middle distillate in petroleum separation. It is available in various grades (e.g. D1-D9, with different

Table 1. One example of the variability in diesel exhaust emission due to the type of diesel fuel (D1-D9) used. The numbers have been estimated from diagrams presented by Westerholm and Egebäck (235).

	D1[a]	D2[a]	D4[a]	D5[a]	D6[a]	D7[a]	D8[a]	D9[a]
Particulate (g/km)	0.4[b]/0.3[c]	0.4[b]/0.32[c]	0.45[b]/0.37[c]	0.46[b]/0.34[c]	0.5[b]/0.37[c]	0.45[b]/0.3[c]	0.46[b]/0.34[c]	0.45[b]/0.2[c]
Particulate carbon (g/km)	0.17[b]	0.18[b]	0.18[b]	0.24[b]	0.25[b]	0.23[b]	0.26[b]	0.22[b]
Soluble org. fraction (mg/km)	10[b]	8[b]	20[b]	40[b]	70[b]	28[b]	25[b]	30[b]
Hydrocarbons (HC) (g/km)	1.2[b]/0.8[c]	1.3[b]/0.8[c]	1.35[b]/0.8[c]	1.2[b]/0.9[c]	1.3[b]/0.8[c]	1.4[b]/1.0[c]	1.5[b]/1.1[c]	1.35[b]/0.8[c]
CO (g/km)	2.4[b]/2.1[c]	3.5[b]/2.5[c]	3.4[b]/2.3[c]	2.9[b]/1.9[c]	3.1[b]/1.8[c]	3.3[b]/2.2[c]	2.8[b]/1.9[c]	2.5[b]/2.0[c]
NO_x (g/km)	11.8[b]/9.5[c]	12.5[b]/10[c]	12.5[b]/10[c]	12.8[b]/10[c]	13[b]/10[c]	12.5[b]/9.9[c]	11.8[b]/9.8[c]	12[b]/9.8[c]
Nitrate (mg/km)	1.0[b]	2.0[b]	3.1[b]	0.5[b]	1.8[b]	2.6[b]	n a	0.5[b]
Sulfate bound to water (mg/km)	n a	n a	36[b]	3[b]	18[b]	5[b]	n a	n a
Soluble sulfates (mg/km)	n a	n a	28[b]	2[b]	14[b]	4[b]	n a	n a
Formaldehyde (g/km)	0.12[b]	0.14[b]	0.08[b]	0.08[b]	0.06[b]	0.26[b]	0.12[b]	0.06[b]
Acetaldehyde (g/km)	0.05[b]	0.04[b]	0.02[b]	0.06[b]	0.03[b]	0.05[b]	0.07[b]	0.06[b]
Ethylene (mg/km)	n a	n a	31[b]	40[b]	33[b]	38[b]	40[b]	33[b]
Propylene (mg/km)	n a	n a	9.5[b]	11[b]	8[b]	12[b]	7[b]	7.5[b]

Table 1 cont.

	D1	D2	D4	D5	D6	D7	D8	D9
Oxygenated and light aromatic compounds								
Acrolein (mg/km)	18.5[b]	15[b]	8[b]	16[b]	14.5[b]	12[b]	13[b]	15[b]
Methacrolein (mg/km)	3.2[b]	2.2[b]	1.8[b]	2.4[b]	2.3[b]	1.8[b]	1.8[b]	2.2[b]
Benzene (mg/km)	8[b]	5[b]	5.5[b]	6[b]	7[b]	4[b]	4.5[b]	5[b]
Toluene (mg/km)	9[b]	4.5[b]	3[b]	6[b]	5[b]	2.5[b]	2[b]	2.5[b]
Polycyclic aromatic hydrocarbons								
The org. soluble fract (g/km)	0.18[b]	0.21[b]	0.18[b]	0.18[b]	0.2[b]	0.18[b]	0.16[b]	0.16[b]
The particulate phase associated PAHs (sum of 29; µg/km)	40[b]	30[b]	20[b]	110[b]	220[b]	100[b]	70[b]	120[b]
The semi-volatile phase associated PAHs (sum of 29); µg/km)	110[b]	<5[b]	10[b]	100[b]	220[b]	30[b]	50[b]	140[b]
The particulate phase associated 1-nitropyrene (µg/km)	0.6[b]	0.1[b]	0.85[b]	0.25[b]	1.1[b]	0.15[b]	0.15[b]	0.1[b]

a D1-D9 indicates DE emission from the various diesel fuels (D1-D9) tested. For further information on the fuels see Table 2.
b Bus cycle
c US transient cycle
d The 29 PAHs are listed in Table 2[e]
n a = not analysed

Table 2. Diesel fuel characteristics from Westerholm and Egebäck (235).

Type of fuel	Cetan no	Density (g/L)	FBP (°C)	Aromatics (vol%) Total	Mono-	Di-	Tri	Olefins (vol%)	Nitrogen (wt%)	Sulfur (wt%)	EHN (wt%)	Sum of 29 PAH (mg/l)
D1	52.8	811.7	261	1.8	1.8	<0.05	<0.05	1.4	0.212	<0.01	-	1.6±0.9
D2	50.0	821.3	260	16.6	16.2	0.4	<0.05	2.0	0.34	<0.01	-	4.1±0.4
D4	47.2	832.0	261	23.0	18.1	4.9	<0.05	2.2	3.9	0.29	-	1.39
D5	47.0	831.3	323	25.1	21.1	3.8	0.2	1.6	29.2	0.02	-	340±17
D6	48.3	836.8	364	26.1	20.2	4.8	1.1	1.0	110	0.16	-	1100±270
D7	44.7	808.3	300	20.0	17.2	2.7	0.1	0.9	14.3	0.02	0.0	230±40
D8	55.7	808.7	299	20.5	17.2	2.7	0.6	0.2	207	0.01	0.2	180±21
D9	52.8	813.2	301	17.3	14.5	2.2	0.6	0.7	11.0	<0.01	-	310±25

EHN = ethyl hexyl nitrate

FBP = final boiling point

PAH = polyaromatic hydrocarbons

Fuels D5, D6, D7, and D8 are commercially available on the Swedish market.

Fuels D5, D7, and D8 are commonly used for city buses.

Fuel D8 is fuel D7 with added EHN (2000 ppm) as ignition improver.

Fuel D9 is a blend of cracked petroloils with a low content of sulfur.

The 29 PAHs:

2-Me-9H-fluorene, Dibenzothiophene, 4-Me-dibenzothiophene, 3-Me-dibenzothiophene, Benzo(ghi)-fluoranthene, Cyclopenta(cd)pyrene, Benzo(a)anthracene, Chrysene/triphenylene, Benzo(b&k)fluoranthene, Benzo(e)pyrene, Benzo(a)pyrene, Perylene, Indeno (1,2,3-cd) fluoranthene, Indeno(1,2,3-cd) pyrene, Picene, Benzo(ghi)perylene, Coronene, Phenanthrene, Anthracene, 3-Methyl-phenanthrene, 2-Methyl-anthracene, 4&9-Methyl-phenanthrene, 1-Methyl-phenanthrene, Fluoranthene, Pyrene, 1-Me-7-Isopropyl-phenanthrene, Benzo(a) fluorene, 2-Me-Pyrene, 1-Me-Pyrene

boiling points) as required by different engine types, one of them being synonymous with fuel oil No.2. Their compositions vary in mixtures of predominantly aliphatic, olefinic, cycloparaffinic, and aromatic hydrocarbons, and also appropriate additives, such as cold flow improvers (olefin-ester copolymers), antistatics, anti-corrosion chemicals- (alkenyl succinic acids, esters, dimer acids, amine salts), antioxidants, antifoam additives (silicones), biocides (imines, amines etc). The slightly viscous, brown fluids are flammable. Organometallic compounds, e.g. barium, calcium, manganese and iron, have been used to reduce diesel smoke (77). The Association of Swedish Automobile Manufacturers and Wholesalers (BIL) proposed, in 1989, an environmental orientated diesel fuel with the following specification of some main properties; sulfur 0.001 wt-% (max), aromatics 5 vol-% (max), olefins 1 vol-% (max), initial boiling point 180 (min), final boiling point 300 (max), density 830+30 kg/m, and cetane number of at least 50. In 1993, about 1/3 of the diesel produced in Sweden has a sulfur content of < 0.001 %, and a PAH content of < 5 %. The State of California has decided that low sulfur, 500 ppm by weight, and low aromatics, 10 % by volume or less are required as from October 1, 1993.

The cetane number indicates the ability to ignite quickly. Usually, the higher the cetane number, the more easily is the fuel ignited. However, other properties such as premixed combustion fraction, premixed combustion index and diffusion combustion index must be added for an accurate description of combustion and emission characteristics.

2. Occurrence and Exposure

Diesel fuel is used for diesel or semidiesel, high-speed engines requiring a type of fuel with low viscosity and moderate volatility. The heavier grades are used for railroad and marine diesel engines. In 1990, 52 % of the domestic transport work within Sweden was done on diesel powered lorries. Within EC (European Community) lorries were utilized to 59 %, whereas in the US only to 25 %. Another big source of DE exposure is in the mining industry.

In Stockholm, Sweden, diesel powered buses were first introduced in the beginning of the 1930´s, and since 1945 all buses equipped with combustion engines have been diesel fuelled. However, during latter years, ethanol has been introduced as a potential alternative to diesel. Some buses in Stocholm are run on ethanol since the end of the 1980's. In Copenhagen, Denmark, another alternative to diesel has been chosen; today's buses are run on rape-oil. On the whole, the Nordic countries are reducing the diesel driven vehicles wherever possible, due to the potential hazard to humans.

In 1992, the refinaries in Sweden produced/sold 2 994 000 m^3 diesel , which was an increase compared to 1991 of about 400 000 m^3. During the first six months of 1993, 1 335 000 m^3 of diesel oil was used, which is somewhat less than during the same period in 1992. Of the diesel volume used in Sweden, 75-80 % is classified as mileau diesel, class I and II (class I being the top quality). The work places in Sweden with DE exposure are garages and terminals for buses and lorries, magazines where trucks are used, Roll-on Roll-off (Ro-Ro) ships, and

mines. However, in the mines, there is a continuous change from diesel to elctricity, wherever possible. Fire men and entrepreneurs are also exposed to DE.

In 1992, Denmark used 1 478 000 m^3 ordinary diesel and 727 000 m^3 milieau diesel, the latter being low in sulfur and PAH (183).

Norway used, in 1992, 1 398 000 m^3 diesel, including mileau diesel. The latter represents, however, only a small portion of the total diesel consumption (118).

The estimated use of diesel in Finland is, for 1993, 1 459 000 metric tons, of which about 370 000 metric tons are mileau diesel (Ditydiesel, mileau class II). During 1994, 70-80 % of the total diesel consumption is estimated to be mileau diesel (172).

On Iceland about 320 000 metric tons of diesel is used per year (74).

In a study by Westerholm & Egebäck (235), eight different diesel fuels were tested in two vehicles, one Scania-bus and one Volvo-lorry. The DE emissions were analysed (Table 1), regarding CO, NOx, HC (hydrocarbons) and particles, which are regulated in Sweden, as well as the unregulated contaminants aldehydes, PAHs, light aromates, olefines and a few others. The results indicated that there is a clear correlation between the density of the diesel fuel and the cetane number, respectively, and the emissions of contaminants. There was also a correlation between PAH-level in the fuel and the emission of PAHs in the exhausts.

In London, UK, diesel buses were first introduced in the 1930's and in the 1950's they had replaced all trams and trolley-buses.

Robertson and co-workers (174) at the Institute of Occupational Medicine in Edinburgh studied the consequences of using diesel FSVs (free steer vehicles) in British coalmines. In all three colliers studied, levels of noxious emissions were low. The highest levels measured for NO_2, CO and formaldehyde were 0.3 ppm, 15 ppm and 0.5 ppm, respectively, well below the current UK OELs (3 ppm, 50 ppm and 2 ppm, respectively). Only traces of PAHs (including pyrene, benzo(a)anthracene, chrysene, benzo(a)pyrene and benzofluoranthene) were detected, all at concentrations below 80 ng/m^3. No measurements of DE particulate concentrations were done. The authors explained the low values with good ventilation, care taken in routine vehicle maintenance and good working practices.

In the US, diesel locomotives were introduced in 1928, and in 1959, 95% of the locomotives were dieselized. Most lorries did not have diesel engines until the late 1950s or early 1960s, and most smaller lorries are still powered by petrol engines. However, estimates for the US were, in 1988, 1.35 million workers in 80 000 workplaces exposed to the combustion products of diesel engines. Some of the occupations were mining, agriculture, motor vehicle maintenance, fork lift truck driving, lorry driving, and tunnel and bridge operation (152). In a study of job categories most frequently exposed to DE, the percentage within a job category that was exposed to DE was, for railroad workers 49.7%, for lorry drivers 47.0%, for heavy equipment operators 46.5%, and for farmers 45.7%.

In another study of DE exposure of 651 men involved in motor transport operations (30.6%), the percentage was; mechanics and repair men except electrical (13.2%), excavating, grading, paving (9.1%), mining and quarring (8.6%) (194). See also Tables 3 and 4.

Table 3. Some work places with measured DE exposure.

Work place	Country	Particle conc. (mg/m^3)	Comments	Reference
Bus garage location A	USA			229
		0.138	outside (roof)	
		0.137	inside (no busdriving)	
		0.584-1.143	inside (busdriving)	
Bus garage location B	USA			229
		0.033	outside (roof)	
		0.047	inside (no busdriving)	
		0.105-0.346	inside (busdriving)	
Bus garage	Sweden	0.46[a]		219
Car ferry	Sweden	0.1-0.3	(while loading ~20 min)	219
RoRo ship	Sweden	0.13-0.59	loading (winter)	219
		0.3-1.0	loading (summer)	
Underground mines		USA	0.2	estimated
average	230			
		1.7	estimated unfavourable situation	
Round-houses	Finland	1.99		145
Locomotive cabs	Finland	0.38		145

a) Exposure to mixed gasoline and diesel exhaust

Two mines, one in Illinois and the other in Utah, have been compared regarding the contaminant levels due to DE exposure (136). Despite a big difference in ventilation, the Illinois mine supplying over 1.5 times the amount of air in the Utah mine, there was no difference in the overall contaminant levels. Using the inertial separation technique, the particulate level was between 0.31 and 0.77 mg/m^3 for particulate size <1 μm, at different locations in the mine. The intake air had a particulate level of 0.10 mg/m^3 (Utah) and 0.22 mg/m^3 (Illinois). The NO_2 level varied between 0.26 and 0.53 ppm (intake air 0.17 ppm in Utah and 0.14 ppm in Illinois), and the NO level between 1.10 and 4.38 ppm (intake air 0.11 ppm, Utah and 0.51 ppm, Illinois).

Levels of diesel exhaust particulate (DEP) exposures for coal miners have been estimated by McCawley et al (137) to range from approximately 0.3-0.7 mg/m^3, and Haney (79) has reported measurements ranging from 0.2-1.0 mg/m^3 in coal mines, and 0.3-1.5 mg/m^3 in metal and non metal mines.

The Canadian mining industry is also heavily dieselized with 3 000-4 000 units underground, as compared to 5000 units in the USA.

Table 4. Some examples of workers in the USA exposed to diesel exhaust.

Job description		Soot μg/m^3	Comments	Ref
Railroad workers		17-134	Respirable particulates	247, 248
Lorry drivers		~20		254
Dockworkers	cold*	18.5	Submicrometer elemental carbon	254
	warm*	27.3	obtained by full-shift (8 h) personal samples with a modified dichotomous sampling cassette (ref. McCawley & Cocalis)	
Mechanics	cold	44.4		254
	warm	7.1		
Local drivers	cold	3.1		254
	warm	8.3		
Highway background	cold	1.9		254
	warm	5.3		
Residential background	cold	1.7		254
	warm	1.1		
Road drivers	cold	2.4		254
	warm	7.6		

* Cold (<50° F = <10°C) or warm (>50° F = >10°C) weather

Table 5. Average exposure of LHD operators from three metal mines in Canada with no emission control device on the machine and using diesel containing 0.2% sulphur, from Grenier & Hardcastle (72).

Contaminant	Avg. conc.	AQI	Danger
RCD/soot	0.85 mg/m^3	57%	Carcinogenic
SO_2	1.19 ppm	22%	Irritant & asphyxiant
NO_2	0.80 ppm	11%	Acidic reaction in lung & edema
NO	5.68 ppm	8%	-"-
CO	2.65 ppm	2%	Affinity to hemoglobin

AQI (Air quality index) = 2.80

For the reduction of DEP, the manufacturers of vehicles have produced various exhaust traps. Rasmussen et al (171) have shown that in diesel automobiles equipped with such exhaust traps produced 87-92 % of the total amount of particulate material was retained by the filter (collected by 20" x 20" Teflon-coated fiber glass filters). The US Bureau of Mines (USBM) and the Ministry of Labour of Ontario (72) have sponsored the adaption of an exhaust trap, a ceramic filter element, to mining diesel engines. The standard ceramic filter performance data, assuming the diesel engine not to be working sufficiently hard to cause

combustion of trapped soot and on-going regeneration of the filter, are; 90% soot retention, slight increase in CO, and negligible changes in other Air Quality Index (AQI, see Chapter 6 & Table 5) gases. Maximum filter life is more than 4 000 hrs. The use of ceramic filter increases the air quality underground, removes engine smoke, and thereby improves visibility and safety, eliminates nuisance maintenance, and reduces diesel smell and noise. Already in 1991, there were a number of mines with such diesel particulate filter installations:

Vehicle type	Canadian mines	"International" (non-Canadian) mines
LHD	14	11
Teletrams	6	20
Dozers & tractors	3	11
Loaders	3	
Bolters	2	1
Boom Trucks	24	47

Ulfvarson et al (220) have studied the pulmonary function in 15 workers (median age 39, median time of employment 9 years) exposed to DE at a tunnel (3 km long) construction site. Lorry and loading machine drivers, rock workers and others were studied. The diesel engines were run on so-called "light" diesel 'fuel, with a lower content of sulfur than "heavy" diesel fuel. Dust, respirable dust, CO, NO, and NO_2 were measured using man-carried sampling equipment as described by Ulfvarson and Alexandersson (218). The exhaust pipe filters were of a ceramic type, which decreased the particle emission by 85%. No oxidation catalyst for the gaseous components was used. The respirators were either "air stream helmets" fitted with a coarse dust filter and a fine dust filter, or half-face masks with dust filter. Official leakage value was less than 0.1%. The dust level was significantly reduced from 2.61±1.32 mg/m^3 (n=35) without filter to 1.80±0.728 mg/m^3 (n=16) with exhaust pipe filter. However, gaseous substances were not retained to any appreciable extent.

3. Methods of Analysis

There are problems attached to measurements of solvent extractable matter from particulates, and substituted PAHs, or common combustion gases, as they are frequently ubiquitously present in the occupational environment at levels above normal back ground pollution from sources such as tobacco smoke and work with solvents, fuels, oils, and gases. Furthermore, Zaebst et al (254) showed that the outdoor temperature may influence the exposure level of DE. There is no consensus regarding which compound/s might give an accurate estimate of the DE exposure if analysed separately. Benzo(a)Pyrene (BaP) were used as an indicator of DE exposure in many earlier studies. When comparing the BaP-containing diesel emissions from different engines, the observed tumour levels were related to the measured BaP levels. However, it was not feasible for comparison between complex mixtures from different mixture classes, such as diesel, coke oven,

roofing tar etc (149, 150). Particle analysis, using gravimetric methods or thermal-optical analysis, are more commonly used today.

Gravimetric methods have been used in many studies of the exposure of workers to DE. Unfortunately, the gravimetric method is rather insenstive, with a limit of detection of 200 µg/filter, and it is lacking in specificity as other particles may cause a variable and potentially large positive bias.

Gravimetric determination of submicrometer - sized particulates with a custom - designed "dichotomous" sampling cassette, designed to accept and collect airborne particles with a mass median aerodynamic diameter of <1µm was used by the Mine Safety and Health Administration (MSHA), USBM, and NIOSH when measuring diesel aerosol exposure in coal mines (136). In 1986, NIOSH and USBM, in collaboration, showed that *inertial separation techniques* can be used to separate diesel particulate from coal mine dust (136), the former has a particulate size of <1 µm.

Thermal-optical analysis quantitates, separately, elemental carbon and organic (volatile and semivolatile) carbon species in particulate matter collected on a filter (32, 108, 254). This method is 100 times more sensitive than the gravimetric method (limit of detection ~2 µg/filter). About 60-80% of diesel particulate carbon is elemental carbon, and tobacco smoke is thought to be almost entirely organic carbon (only ≤ 2% elemental carbon, and thus no significant positive bias). Furthermore, almost all particulate carbon associated with vehicular traffic is due to DE, the contribution from other sources, i.e. petrol exhaust and tire debris, appears to be minmal (164).

Measurement of ARP (respirable particulate adjusted for the contribution of tobacco smoke by quantitation of nicotine, extracted from the same filter) has been used by Woskie et al (247, 248) in a study on DE exposures of railroad workers in thirteen job groups from four railroads in the US. The national average exposures were; 31-35 µg/m^3 for clerks/dispatchers/station agents; 50-66 µg/m^3 for signal maintainers; 65-77 µg/m^3 for engineers/firers; 83-95 µg/m^3 for brakers/conducters; and 125-157 µg/m^3 for locomotive shop workers. Using the limited historical records available, they also showed the past DE exposure to be approximately constant from the 1950's to 1983.

Recently, Cantrell & Rubow (33) have, in collaboration with Marple (129), developed a personal diesel aerosol sampler for USBM. The design criteria for the sampler is based on *size-selective sampling*, with a size separation of 0.8 ± 0.1 µm and a sample flow rate of 2 l/min. Coal dust and DE aerosol can be separated and measured on the basis of size.

At the Michigan Technological University (MTU) it has been demonstrated that each of the major pollutants (CO, NO, NO_2, SO_2, and RCD) and the AQI in dieselized mines are related functions of the CO_2 concentration for the operation of a single machine (72). MTU suggests that CO_2 might be used as surrogate for DE measurements, which gives a cheap and reliable measure also for required monitoring of a vehicle´s compliance with the regulations. The possibilities of extending CO_2 as a surrogate for DE measurements in automated mine ventilation monitoring and control is under investigation (72).

For laboratory analyses of DE components, there are basically three different driving cycles; a bus cycle, the US transient cycle for heavy-duty vehicles, and the 13 mode test, ECE R49 (237).

The *bus cycle* has been developed at the University of Braunschweig, Germany. It simulates the driving condition of a bus in city traffic. The driving distance is 11 km and the duration 29 minutes. The top speed is 58.2 km/h and the average speed 22.9 km/h.

The *US Federal cycle* for heavy-duty vehicles is a transient cycle defined by a speed versus time schedule. It simulates heavy-duty vehicle driving in city areas and on a free-way. Its duration is about 2x18 minutes, the top speed is 93.3 km/h, and the average speed is 30.4 km/h.

The *European ECE R49 cycle* is a 13 mode, steady state driving cycle. The engine is driving at a constant speed with 10 loaded modes and 3 idle modes, and it was constructed for driving the engine in a motor test bench.

4. Deposition and Clearance

Apart from the complexity of DE emission, another major problem, when estimating the human hazard due to DE exposure, is associated with the deposition of DE particles (DEP) in the respiratory tract and the clearance of the particles and/or the PAHs associated with the particles (12, 35, 37, 64- 68, 162, 210, 251). The difficulties associated with realistic studies regarding the bioavailability of PAHs, i.e. the amount of PAHs eluted from the particles in the respiratory tract and/or the lungs as well as the speed with which the PAHs might be eluted, are ginormous.

Already in the 1970s and 1980s, the importance of the bioavailability of PAHs was discussed, and it was shown, in vitro, that they could be extracted from the DEPs by biological fluids. In a later study, Bevan and Ruggio (12) have used phospholipid vesicles composed of dimyrostoylphosphatidylcholine (DMPC) to elute PAH from DEP.

The lung retention of the radiolabeled model PAH, (^{3}H)benzo(a)pyrene (3H-BaP), has been studied by Sun et al (210). Male and female Fischer 344 rats were exposed for 30 min to DEP-associated 3H-BaP by nose-only inhalation. The total mass of the aerosol, calculated with a rat minute volume of 270 ml/min, was 31.6 x 10^3 ng, and the total 3H-BaP inhaled was 45 ng. Based on a lung deposition efficiency of 16% (35), the initial lung deposition of the aerosol was calculated to 5.1 x 10^3 ng and of the 3H-BaP, 7.1 ng. The lung clearance of inhaled 3H-BaP occurred in two phases. The initial phase was very rapid with a biological half-life of <1hr, followed by a long-term rate component with a biological half-life of 18+2 days, and representing 50+2% of the total calculated amount of 3H-BaP that was initially deposited in the lungs.

A number of models have been developed, primarily to predict the deposition of DEP in the respiratory organ of humans, but also the release of PAHs from deposited DEPs. Yu and Xu (251) developed one such model for predicting deposition of DEPs; the deposition fraction for humans (0.23) agreed with experimental results from studies with human volunteers (37). However the respiratory deposition is influenced by changes in breathing rate and particle size

Table 6. Lung burden of "soot" in rats, after exposure to DEP.

Strain	Dose	Exposure	Lung burden (mg)	Post exposure (weeks)	Reference
F 344 rats	50 µg/m3*	20h/d, 7d/w	0.047±0.003	0	206
		for 4 w	0.019±0.003	6	
			0.000±0.001	26	
		for 13 w	0.313±0.041	0	
			0.030±0.019	52	
		for 26 w	0.323±0.024	0	
			0.049±0.018	13	
			0.123±0.011	26	
			0.023±0.023	52	
		for 52 w	0.557±0.033	0	
			0.236±0.013	13	
			0.201±0.035	26	
			0.100±0.039	52	
F 344 rats	0 mg/m^3	7h/d, 5d/w	0		88, 89
	0.35		0.6		
	3.3	for 30 mo	12.0		
	7.0		20.0		

* The measurement has a potential for serious error due to the background chamber aerosol, which varied between 6-20 µg/m^3 depending on the number of animals present. However, the authors used a high-velocity sample probe which effectively reduced the contribution of the background aerosol to a maximum of 2-4% of the DEP concentration.

Table 7. Lung clearance in rats exposed to DEP and ^{3}H-BaP.

Species	Dose	Totally inhaled	Initial lung deposition +	Exposure time	Comments	Reference
F 344 rats (26 of both sexes)	3900/5.5* ng/l (nose only - inhalation)	31600/45 ng	5100/7.1 ng	30 min - 26 d	Lung clearance t1/2 <1h (0-30) t1/2=18±2 d (30'-26h) at 26 d:~20% left Max levels of ^{3}H-radioact in other organs: 0-30; esophagus, small intestine, liver, kidneys and blood 2-6h; stomach, cecum, & large intestine at 5 d; ^{3}H-radioact undetectable Excretion: total amount ^{3}H-radioact: 7.5±1.2 ng equi ^{3}H-BaP In urine; 17± 3% until day 8 In feces; 83+6% until day 12	210

* DEP-associated ^{3}H-BaP

\+ Based on a rat minute volume of 270 ml/min, and a lung deposition efficiency of 16%

distribution, and there seems to be a large inter-individual variability in deposition rates. These factors have been taken into account in the further development of this model (252, 253).

A more recent effort to solve the problems of DEP retention as well as the release of PAHs from DEPs and their retention, have been done by Gerde and coworkers (64-68). They developed a theoretical model for the retention of PAHs in the bronchial airways and in the alveolar region. According to their model, the two major determinants of the retention time for an unmetabolised lipophilic substance in a specific cell are the lipid-aqueous partition coefficient and the distance to the nearest blood capillary. The larger the partition coefficient and the longer the distance of diffusion, the longer the retention time. Thus, the retention of PAHs should be fundamentally different between the bronchi, where the distance between the air interface and the capillary blood probably exceeds 50 µm, and the alveoli with a distance of about 0.5 µm. Using their theoretical model, Gerde et al (64) showed that BaP may be retained in the bronchi for hours, compared to less than 1 min in the alveoli. Furthermore, they were able to demonstrate that, the low-dose exposure conditions that are typical for the human DE exposure lead to a rapid release of PAHs from the DEP (65).

Table 6 and 7 give some examples of lung burden and clearance in rats exposed by inhalation to DEP.

5. Distribution and Biotransformation

Due to the fact that BaP has been used as an indicator substance for DE, several investigators have studied the uptake, distribution and biotransformation of BaP in combination with DE exposure. Two such studies are included here.

BaP metabolism in A/Jax mice exposed to DE, which was diluted with filtered air at a 16:1 to 18:1 dilution ratio (~6 mg/m^{3} DEP) for 8 h/day, 7 days/week for nine months has been investigated (216). The DE-exposed and non-exposed mice were instilled intratracheally with ^{14}C-BaP. Within 2 h after instillation, radioactivity was detected in the whole mouse, with most in the lungs, liver and GI tract. By 24 h, considerable radioactivity had redistributed to the GI tract. At 168 hours, only a trace of label was found in the GI mucosa. Qualitatively there was no obvious difference between the DE- exposed and non-exposed mice.

DE exposed and non-exposed mice were given ^{3}H-BaP, by intratracheal instillation and the presence of ^{3}H- BaP and its metabolites, (i e. nonconjugated primary metabolites, sulfate conjugates, glucuronides, glutathions & other conjugates) was measured (34). By 2 h after instillation, primary metabolites were found in liver and lung, but very little was conjugated. The unconjugated BaP was mainly in the form of free BaP and phenolic metabolites. The lungs of DE-exposed mice had less capacity to dispose of "bound" BaP one week after instillation. The content of the caecum in a 24 h mouse contained some free BaP, a large amount of primary metabolites, and very little conjugates. Either conjugates in the large bowel had been hydrolyzed by the bacterial flora, or the mucosal cells of the small intestine have metabolized BaP and then excreted the primary metabolites back into the lumen. During the first 16 h after instillation, 18

% of the BaP was excreted in the urine of non-exposed mice and 14% of the BaP was excreted in the urine of DE-exposed mice.

DE suppressed the ability to clear the small amount of BaP. The extension time carcinogens spend in the lung will markedly increase the carcinogenic risk.

The distribution and biotransformation of BaP has also been studied after 30 min inhalation of DEP-associated 3H-BaP by F344 rats. Within 30 min postexposure, the maximum levels of radioactivity was obsereved in esophagus, small intestine, liver, kidneys, and blood, whereas the maximum levels in stomach, cecum, and large intestine were detected between 2-6 hr after exposure. After reaching the maximum, the levels decreased exponentially, and after 5 days only the lungs still contained a detectable level of radioactivity (almost 40% down to 20% after 25 days). After day 8, no further radioactivity was excreted in the urine, while substantial levels were measured in the feces until after day 12. The total amount excreted was 7.5+1.2 ng equivalents of 3H-BaP, 17+3 % being excreted in the urine and 83+6 % in the feces. In the lungs, 65 % of the total 3H-radioactivity was found as BaP, 17 % as BaP-phenols (3-OH- and 9-OH-) and 18 % as BaP-quinones (-1,6-quinone and -3,6-quinone) at 30 min postexposure. At 20 days after the exposure, the findings were 76, 13, and 5 %, resp. (209-211).

Gerde et al (66-68) have studied the blood borne clearance of BaP and phenantrene (Phe) in Beagle dogs, by exposing them to a bolus of aerolized crystals of BaP (77 mg ^{14}C-BaP) or Phe (28 mg ^{14}C-Phe) in a single breath, and the blood borne clearance of BaP and Phe was monitored by repeatedly sampling blood. Half of the BaP and Phe cleared from the alveoli within 2.4 and 1 min, respectively. The clearance from the blood proceeded with a half-time (measured over 4 hrs) of 2.5 hrs for Phe and 2.0 hrs for BaP, indicating a mostly perfusion-limited uptake of Phe and a diffusion-limited clearance of BaP. The BaP clearance curve was mono-phasic, whereas the Phe-clearance was multi-phasic (66-68).

Bevan and Ruggio (12) have studied the elution of BaP from DEP after intratracheal instillation of 1 mg DEP with associated ^{3}H-BaP (in 0.3 ml 0.15 M NaCl) to male SpD rats. About 50% of the radioactivity remained in the lungs at 3 days following instillation, 30 % was excreted in the feces, and the remainder was distributed throughout the organs of the rats; about 3 % in the liver and urine, about 2.5 % in the stomach, and about 2 % in the intestine and carcass.

6. General Toxicity

In Canada, medical specialists have used a method to evaluate the over-all toxicity of DE, the Air Quality Index (AQI) (60), which mathematically equates the five toxic exhaust elements (in mg/m^3) with their respective limit value:

$$AQI=\frac{CO}{50}+\frac{NO}{25}+\frac{RCD}{2}+1.5\left(\frac{SO2}{3}+\frac{RCD}{2}\right)+1.2\left(\frac{NO2}{3}+\frac{RCD}{2}\right)$$

RCD = Respirable Combustible Dust

The suggested maximum for the AQI, to avoid tissue damage or diminution of respiratory function, is 3. See also Table 5.

It should be noted that RCD/soot, is heavily weighted in the equation (occuring three times), which reflects the ability of RCD/soot to cause tissue damage when in conjunction with the acid gases as well as its potential carcinogenicity. The second major contributor to the AQI is SO_2, which, however, can be reduced by using low sulphur fuels (i.e. <0.1%).

The phagocytic cells of the lung (alveolar macrophages and neutrophil granulocytes) are of importance when diesel soot particles are inhaled. Neutrophils, harvested from fresh human blood obtained from healthy adult donors, were incubated with DEP, sulfite, and DEP+ sulfite in a chemiluminescence-test. The phagocytosis rate of human neutrophils was clearly increased after incubation with DEP + sulfite (100 to 196 %) whereas the single substances had no effect. Neutrophils isolated from porcine blood were much more affected by the treatments. Diesel soot had a slightly stimulatory effect on the phagocytosis (100 to 154 %) and DEP + sulfite caused a significant increase (100 to 269 %), whereas sulfite alone decreased the phagocytosis (100 to 73 %). Oxygen uptake of activated granulocytes was reduced in all three treatment groups (92).

In order to study the DE effect on some biotransformation enzymes in liver and lung, male Fisher 344 rats were exposed by Chen and Vostal (39) by inhalation to clean air or diluted DE at the particle concentrations of 750 µg/m^3 or 1500 µg/m^3 for 20 h/day, 5.5 days/week for 9 months. No differences were observed in body, liver or lung weights between controls and exposed rats. No statistically significant changes in arylhydrocarbon hydroxylase (AHH) activity in liver microsomes were observed during 9 months, whereas the AHH activity in lung microsomes was reduced after 9 months at 1500 µg/m^3 compared to controls; 8.4+0.6 and 13.0+1.0 picomoles/min/mg protein, respectively. There was no significant effect observed in liver microsomal cytochrome P450 content (39). It was also demonstrated that DEP may function as an inducer of the AHH activity in rat lung microsomes after i.p. injection, daily for 4 days of 25 mg DEP/kg bw/day (213 % induction), and 120 mg DEP/kg bw/day (938 % induction). The AHH activity of liver microsomes of the same rats was only induced at 25 mg DEP/kg bw/d (140%), with no effect at 120 mg DEP/kg bw/d, whereas the hepatic cytochrome P450 activity was inhibited by both 25 (97 % of control) and 120 mg (77 % of control) DEP/kg bw/d. In a second study (40), male F344 rats were exposed by inhalation to diluted DE at 1500 µg/m^3 or 6000 µg/m^3 DEP for 20 h/day, 7 days/week and 2-28 days. Pulmonary alveolar macrophages (PAMs) were obtained by lavage. The number of PAMs recovered per animal increased approx. 1.5 - fold ($P< 0.05$) after 6000 µg/m^3 for 14 days compared to controls. After 28 days of exposure the number of macrophages increased 1.3-fold ($P<0.05$) at 1500 µg/m^3 and 2-fold after 6000 µg/m^3 ($P<0.05$) compared to controls. The AHH activity after 14 days exposure to either dose was not affected, whereas 28 days of DE exposure reduced the AHH activity from 0.179 (control) to 0.130 (1500 µg/m^3) and 0.196 (control) to 0.091 (6000 µg/m^3) picomoles/min/10^6 cells. See also Table 8.

DE exposure of SpD rats, 14.2 ppm (v/v, average of weekly means) for 15, 33 or 42 days increased AHH activity in lung after 33 and 42 days (5.8±0.24 vs. 4.19±0.32 and 5.11±0.24 vs. 3.67±0.28) and in liver after 33 and 42 days

Table 8. Effects of exposure to DE gas phase and/or particulate phase (DEP) on liver and lung in monkeys, rats, mice, and hamster.

Species	n	Gas phase	DEP mg/m^3	Exposure	Effects	Reference
Monkeys	60	+	2.0	7h/d,5d/w	Lung function; no restrictive lung diseaese, but	123
Cynomolgus		+	1.0 + 1.0CD		obstructive responses. CD also lower forced expir. flow	
Fischer 344	6	+	0.75-1.5[a)]	20 h/d, 5.5 d/w	Liver mic. P450; no effect	39
rats, male	6	+	0.75[a)]	1-9 mo	Lung mic. AHHact; no effect at 0.75 mg/m^3	
			1.5[a)]	1-9 mo	Lung mic. AHHact; signif. red. at 6&9 mo (65% of ctrl)	
Fischer 344	40	+	1.5-6.0[a)]	20 h/d, 7 d/w	PAMs increased by 50% ($p \leq 0.05$) at 6.0 mg/m^3 for 14 d	40
rats, male				2-28 d	and ≈20 % ($p = \leq 0.08$) at 15 mg/m^3 for 30 d	
					AHH act red ($p \leq 0.05$) at 29 d	
	25	-	12 mg/kg bw	single[b)]	PAMs increased by 20% at 4 d	40
					AHH act red (ND) at 1.5 d	
SPF Wistar	96	+	-	19 h/d, 5 d/w	Lung clearance; no effect	85
rats, female	96	+	4.24	3 mo	Lung clearance; increased 2.5 - fold	
SPF Wistar	92	+	-	19 h/d, 5 d/w	Lung function; no effect	85
rats, female	95	+	4.24	12 mo	Lung function; compliance signif decreased,	
					resistance significant increased	
Syrian golden	96	+	-	19 h/d, 5 d/w	Lung clearance; no effect	85
hamster	96	+	4.24	12 mo	Lung clearance; insignificant decline	
male & female						
Fisher 344		-	250 µg/m^3	20 h/d, 5.5 d/w	Liver mic. ability to ox B(a)P is signif. red to	145,188
rats		-		6 w	58 % of control	
			1500 µg/m^3		Liver mic. ability to ox B(a)P is signif. red to	
					50 % of control	
		-	250 µg/m^3	20 h/d, 5.5 d/w	Lung mic. ability to ox B(a)P is signif. red. to	
				12 w	55 % of control	
		-	1500 µg/m^3	53 w	Lung mic. ability to ox B(a)P is signif. red. to	
					6 % of control	

a) DE diluted with air; b) intratracheally; PAM = pulmonary alveolar macrophages; CD = coal dust; ND = significance not determined

(150±6.3 vs. 117.5±4.6 and 164±10.9 vs. 113.9±8.9). Epoxidehydratase (EH) activity was not affected in any of the organs studied (119, 163, 167).

7. Acute Toxicity

Kahn et al (109) have reported acute overexposure to diesel exhaust in 13 miners, of whom 12 had symptoms of mucous membrane irritation, headache and light-headedness, 8 reported nausea, 4 had symptoms of a sensation of unreality ("being high") and heartburn, 3 reported weakness, numbness and tingling in extremities and vomiting, and 2 experienced chest tightness. The symptoms caused lost work time, but resolved within 24-48 hours.

Sandström and Rudell (182) studied the bronchoalveolar inflammatory response to inhalation of DE, using bronchoalveolar lavage (BAL). Eight healthy non-smoking individuals were exposed, for one hour, to DE from an idling diesel engine, diluted with air. Median concentrations in the breathing zone were 3.7 ppm NO, 1.6 ppm NO_2, 27 ppm CO, $3x10^6$ particles/cm^3, and 0.3 mg/m^3 formaldehyde. A mild but significant increase in neutrophils was found in the broncioalveolar portion (BAP) of the BAL fluid after DE exposure, but not in the bronchial portion (BP). The total number of mast cells in BP was reduced following DE exposure, but unchanged in BAP. The authors concluded that the exposure to DE causes a different acute inflammatory reaction in the human lung compared to exposure to SO_2 or NO_2 alone, which may be due to DEP, PAHs and other components in the DE.

8. Organ Effects

8.1. Cardiovascular Organs

Edling and Axelson (54) showed that the most heavily exposed group in a bus company, i.e. bus garage workers, had a four-fold increase in risk of dying from cardiovascular disease after correction for smoking and allowing for 10 years of exposure and at least 15 years of induction - latency time. CO exposure was suggested as a possible cause.

In a study by Rosengren and coworkers (175), it was demonstrated that middle-aged bus and tram drivers (n=103) in Gothenburg, Sweden, had an incidence of coronary heart disease (CHD) of 18.4 % compared to 6.4 % among the other occupation groups (n=6596), OR = 3.3. The follow-up was extended through a mean of 11.8 years. There was also an increased risk for taxi drivers, although not statistically significant, whereas no risk was observed for lorry drivers. The increased incidence for CHD could not be explained by other known risk factors (e.g. smoking habits, serum cholesterol, blood pressure, alchohol abuse). However, air pollution due to automobile exhaust fumes could be an important factor, CO being associated with cardiovascular disease.

8.2. Respiratory Organs

8.2.1. Humans

Aqueous suspensions of DEP are able to oxidize biological target molecules. One of the main components of urban air pollution, SO_2, seems to cooperate synergistically with the toxicity of DEP in aqueous solution (91, 223). Thus, DEP + SO_2 might be responsible for certain bronchial diseases and lung dysfunctions, especially in sensitive individuals, i.e. asthmatics.

Wong et al (244, 245) studied 34 156 male members of a heavy construction equipment operators union with a potential exposure to DE. Mortality from emphysema was significantly increased, 116 deaths compared to 70.17 expected. The corresponding SMR of 165.3 was significant at the 0.01 level. However, the contribution of DE could not be estimated.

Ames et al (5) have studied chronic respiratory effects of exposure to DE in coal miners. Changes in respiratory function and development of chronic respiratory symptoms were measured over 5 years in 280 DE exposed and 838 control miners from underground coal mines in the US. No association was found between DE exposure and respiratory effects (such as chronic cough and phlegm, or breathlessness) or pulmonary function decrements (FVC or $FEV_{1.0}$). The authors suggested that the results should be interpreted with caution, despite adjustment for confounders such as age, smoking, and coal dust effects. In another study, Ames and Trent (6) demonstrated that there was no "healthy worker" effect among diesel coal miners, i.e. those with some respiratory impairments or symptoms did not leave their jobs more often than those with no symptoms.

Wolff (239) has reviewed the data on effects of the airborne pollutants SO_2 and NO_2, on mucociliar clearance. SO_2 is highly water soluble and therefore absorbed primarily in the nose and upper airways with a rapid drop off in concentration down the airways. NO_2 tends to produce effects predominantely in the region of the terminal bronchioles. Studies on NO_2 effects are very limited and performed with animals. Exposure of humans to 1-5 ppm SO_2 causes significantly slower nasal mucous clearance, whereas 5 ppm increases the bronchial clearance in both exercising and sedentary humans, with the greater effects in exercising subjects. Other studies have shown very little effect of SO_2. Prolonged exposure to SO_2 at very high doses (≈500 ppm) produces changes characteristic of chronic bronchitis. The effect seems to be increased secretion, as ciliary activity is not altered. Asthmatics may suffer from increased airway resistance at SO_2 levels as low as 0.25 ppm.

Jacobsen et al (105) investigated whether long-term exposure to low concentrations of nitrogen oxides (NO and NO_2) might be associated with increased susceptibility to respiratory infections. Nearly 20 000 miners from nine British coalmines were interviewed and 7 463 of them reported of colds, influenzas or bronchitis. No association was found for long-term exposure to nitrogen oxides in the mines and absence from work due to chest infections. The levels of nitrogen oxides in the British coalmines of the 1970s were similar to the levels recorded in some cities polluted by motor vehicle exhaust emissions.

Bofetta et al (16) analyzed the two-year mortality of 461 981 males in relation to DE exposure and to employment in selected occupations related to DE

exposure (see Chapter 10.1). They found no association for DE exposure and mortality for non-neoplastic pulmonary diseases. However, the statistical power was rather low and a further analysis on a larger cohort or a longer follow-up period would be necessary.

In their study on the pulmonary functions of 15 workers exposed to DE at a tunnel construction site (see Chapter 2), Ulfvarson and coworkers (220) tested the efficiency of different control measures (exhaust pipe filter and respirator) in protecting against lung irritation of DE. The subjects served as their own controls. In the study FVC, $FEV_{1.0}$, FEV %, TL_{CO}, VC, CV, and CV% were measured. All paramters were determined when using the exhaust pipe filter, and VC, CV % and TL_{CO} were determined in the respirator test (218). The conclusion of the authors was that it is possible to evaluate the effects of control measures regarding DE exposure by the evaluation of the acute effects on the pulmonary function of occupationally exposed subjects. Furthermore, the catalytic exhaust pipe filters clearly protected against the effect of the DE exposure on the FVC in the drivers; mean quotient of filter/no filter was 1.044 ($p<0.001$). No detectable effects were observed with the respirator. However, the number of subjects was small, and a certain discomfort in wearing the respirators may have influenced their proper use.

Krause et al (117) evaluated distinct parameters (functional lung diagnostic and laboratory parameters) both in miners exposed to DE gases and control persons. Three groups were studied; the DE exposed group with 380 miners (DE); 381 miners with similar work but without DE exposure (DV), and the third group (187 miners) had some exposure to DE (DZ). No differences in the frequency and severity were found in the respiratory results due to DE-exposure. The only laboratory parameter which was associated with DE-exposure was the mercapturic acid excretion in urine, which, however, also can be influenced by other factors.

8.2.2. Animals

Subchronic studies of DE exposure of rats indicate some transient changes over the first few weeks, which subsequently resolves after about 18 weeks. Despite its irritant gases, DE emissions seem to produce little change in mucociliary clearance (239, 241, 242). However, NO_2, per se, causes impairment of tracheal clearance in rats exposed to 6 ppm for 6 weeks, but not at lower levels, and 15 ppm NO_2 for 2 hr reduced tracheal mucous velocity in sheep while 7.5 ppm had no effect (241). In Table 8, some effects of DE gas phase and DEP on lung and liver are summarized.

In a long-term inhalation study by Lewis et al (123,124) both rats and monkeys were used. The major objective of the rat study was to assess long-term biologic responses including tumour incidences. The monkeys were used to study the effect of long-term exposures on organ systems with major emphasis on the respiratory system. Male and female Fisher-344 weanling rats (864 and 288, resp.) and 60 male Cynomolgus monkeys (Macaca fascicularis) were exposed 7h/day, 5 days/week, for 24 months, 72 rats of each sex per exposure level and 15 monkeys per exposure level. An additional 576 male rats (144 per exposure level) were exposed up until interim sacrifice at 3, 6, 12 and 24 months to assess toxicological responses as a function of duration of exposure. Further short-term

exposures (lasting 1-6 months,)were conducted in rats and mice for purposes of studying particle clearance from the lungs, dominant lethal mutagenicity in rats, and susceptibility to viral infection in mice. The four experimental atmospheres were: filtered, conditioned ambient air, control (FA); 2 mg/m^3 respirable coal dust (particle range <7 μm) (CD); 2 mg/m^3 of DEP with specific limits on gaseous or vapour constituents, such as oxides of carbon, nitrogen and sulfur, ammonia, and hydrocarbon (DE); and 1 mg/m^3 respirable coal dust plus 1 mg/m^3 DEP with the same gaseous and vapour airborne concentrations as in DE, i.e. about 10 ppm CO, about 8 ppm NO, about 1.5 ppm NO_2, about 0.7 ppm SO_2, and about 0.6 ppm NH_3. It was shown that both DE and CD particles were deposited in the lungs and retained in the alveolar tissue. Alveolar type II cell hyperplasia and pulmonary lipidosis was most evident in rats exposed to DE alone. However, there were no evidence of emphysema or chronic bronchitis, and only minimal fibrosis was seen in association with the retained particles. Both DEP and CD particles affected the defense mechanism of the lung. Responses associated with phagocytosis were activated by CD particles but depressed by DEP. Severity of influenza challenge increased concomitantly with decreased interferon production in DE-exposed mice (female CD-1). In monkeys, mild, obstructive airway disease was observed after DE, CD or DECD treatment. No induction of xenobiotic metabolizing enzymes was detected in the lung or liver (rats), and the humoral and cellular immunities were not significantly affected (rats). No evidence of chronic toxicity, such as changes in weight gains, organ-body weight ratios, or clinical parameters was found (rats and monkeys). No synergistic effects between DE and CD could be demonstrated.

Wolff et al (240) determined inflammatory responses due to DE and carbon black by analyzing bronchoalveolar lavage fluid (BALF) from exposed rats and from histopathology. Male F344/N rats were exposed for 7h/day, 5days/week for 12 weeks to DE and carbon black at a particle concentration of 10 mg/m^3. The control group was exposed to filtered air. The lung burdens that resulted from inhaling the two similarly seized particles were also similar. The DEP had 33% of their mass as extractable organic compounds, as compared to 0.04% for carbon black. At the end of a 12-week exposure, rats exposed to DE soot or carbon black showed similar, mild inflammatory responses, with a statistically significant ($p<0.01$) increase in polymorphonuclear neutrophils ($1.5x10^3\pm0.4$ cells for DE, $1.7x10^3\pm0.5$ cells for carbon black, and $0.03x10^3\pm0.03$ cells for filtered air) and acid proteinase (42±2 μg hemoglobin solubilized/4h/ml for DE, 42±3 μg hemoglobin solubilized/4h/ml for carbon black, and 30±2 μg hemoglobin solubilized/4h/ml for filtered air), and also similar histopathological responses. The major histopathological effect was the presence of numerous large alveolar macrophages, engaged with black particles in the airway lumen of distal terminal bronchioles and proximal alveolar ducts. There was mild type II cell hypertrophy and hyperplasia in the lungs of DE exposed rats, and only type II cell hyperplasia in the lungs of carbon black exposed rats. The inflammatory responses produced by carbon black and DE are similar, and these responses may result in conditions that could lead to promotion and progression of lung cancer.

Henderson et al (88,89) have studied biochemical and cytological changes in BALF and in lung tissue from rats and mice. Male and female F344/Crl rats and CD-1 mice were exposed 7 hr/day, 5 days/week for up to 30 months to diluted DE

from light-duty diesel engines containing 0.35, 3.5, or 7 mg particles/m^3. Every six months, analysis were performed on bronchoalveolar lavage fluid (BALF) and on lung tissue. No biochemical or cytological changes were observed in BALF or in lung tissue in either species exposed to the lowest dose. In the two higher dose levels, a chronic inflammatory response was observed in both species by dose-dependent increases in inflammatory cells, cytoplasmic (CDH, GSH-R) and lysosomal enzymes (BG2U, acid proteinase, AcP) and protein in BALF. After one year of exposure, the rats had developed focal areas of fibrosis associated with the presence of soot, whereas the mice showed only a fine fibrillar thickening of an occasional alveolar septa in the high exposure group. In the rats, the BALF ß-glucuronidase activity and hydroxyproline contents were also higher. Levels of glutathione (GSH) and GSH reductase activity were increased in a dose-dependent fashion in BALF (mice >rats). In lung tissue from exposed animals, the increase in both cytoplasmic enzymes (CDH, GSH-R) and lysosomal enzymes (BG2U, acid proteinase, AcP) followed the pattern in BALF, but the degree of increase was less than in BALF. The cytochrome P450 content in lung tissue was decreased in rats at all exposure concentrations. In the mouse lungs no such effect was seen. Like Wolff et al (240), Henderson et al (88) also showed in their study that pure carbon particles cause responses in rat lung similar to those caused by DEP, i.e. the observed effects are due to the particles per se.

Male rats, totally 72 (Charles River), were exposed to DE emissions at concentrations of 0, 250 and 1500 μg/m^3 DEP, for 20 hr/day and 51/2 days per week. After 12, 24 and 36 weeks of exposure, the rats were sacrificed and the lungs analyzed by morphological and biological methods. Lung weight was significantly higher ($p<0.01$) after 12 weeks exposure to 1500 μg/m^3 DEP. Total lung collagen content increased proportionately with the change in lung weight. Cell content in the lung tissue (DNA) was significantly increased at 1500 μg/m^3 after six months. Prolyl hydroxylase, an enzyme intimately associated with collagen synthesis was increased only after 12 weeks exposure and it's activity decreased with the age of the rats. Phospholipids and cholesterol increased significantly in rats exposed for 36 weeks at 1500 mg/m^3. The profile of fatty acids was not significantly changed (13, 38, 142, 221).

Fisher 344 rats were exposed to DE at two concentrations 250 μg/m^3 and 1500 μg/m^3 of DEP. One year of exposure had no effect on the size of the rat or their livers, however, the higher exposure increased lung weight. The ability of lung microsomes to oxidize BaP was impaired at both concentration. Liver microsomes were less able to oxidize (BaP at six and twelve weeks after exposure, but were no different from controls after one year (38).

Pulmonary function was tested in 25 male Fisher 344 rats chronically exposed to diluted DE. The particulate concentration was 1500 μg/m^3 and the exposure time was 20 hours/day, 5.5 days/week for 267 days. When comparing the data regarding transpulmonary pressure, lung air flow and volume, and respiratory rate with those from 25 clean air controls, there were no apparent significant changes in lung functions due to DE (73).

Vostal et al (224) studied the effect of diluted DEP (1500 μg/m^3) and DECD (DE+coal dust) on lymphatic transport of the inhaled DEP. Prebronchial and perivascular aggregates of lymphoid tissue contained DEP even after a short exposure at low dose. After one or two years exposure, ten Fischer 344 rats from

each exposure group were evaluated for responses in functional residual capacity and airway resistance and conductance. Rats exposed for two years were also evaluated for maximum flow volume analysis: No significant differences were observed between the different treatment groups in functional residual capacity, airway resistance or alveolar morphometry. However, significant obstructive impairment was found for rats in the DECD (DE+coal dust) group. Of the ten rats exposed, six showed severe flow impairment (30-40% of controls).

It has been shown in hamsters, mice and rats that the redox balance of the lung can be effected by inhalation of DEP. Furthermore, inflammatory responses may be provoked, and, in parallel, a decrease in lung clearance. Unfiltered diesel soot can also provoke adenocarcinoma in the lung (85,87).

In other studies, with higher levels of DE exposure, morphometric changes were observed in the lung parenchyma of guinea pigs and cats exposed to DE (61). Furthermore pulmonary function impairment has been reported in rats, hamster and cats exposed to DE (57) as well as in rats and monkeys exposed to coal dust (123, 124, 144, 200).

8.3. Central Nervous System

Impaired function in neuropsychological tests and neuroastenic symptoms hasve been demonstrated in a study on bus garage workers after long-term exposure to high DE emissions (114). DE exposure was not measured, however, during winter the engines were often kept running through the whole night. Eleven garage workers, with exposure to great quantities of DE exposure for 2 to 29 years, all presented acute symptoms in the form of headache, vertigo, fatigue, irritation of mucous memebranes, nausea, abdominal discomfort or diarrhoea. Out of seven persons, employed for more than five years, six complained of failure of memory, difficulty in concentration, irritability, increased sleep requirement, psychological changes and reduced libido. Neuropsychological examination was undertaken, and in five of them impaired function, mainly of slight extent, was demonstrated.

Bofetta et al (see chapter 10.1; 16) observed a dose-response effect for duration of DE exposure and cerebrovascular disease; RR for 1-15 years DE exposure was 1.43 (95% CI, 0.89-2.29), and RR for > 15 years of DE exposure was 1.68 (95% CI, 1.06-2.66). High CO level was suggested as an explanation.

8.4. Reproductive Organs

An in vitro test system employing human spermatozoa has been used by Fredricsson et al (59) to study effects of extracts from DEP material. The highest concentration tested corresponded to 0.38 mg particles, equivalent to 0.06 µg PAH/100 µg sperm suspension. Immediate effects were very moderate, but with increasing exposure (≤18h), motility, linearity, straight line velocity and curvilinear velocity were induced at the lower doses (0.38 µg) and thereafter reduced. At the highest dose (0.38 mg) very few sperms remained mobile, which makes the data rather uncertain.

Lewis and coworkers (123) performed semen analysis on monkeys (Cynomolgus) exposed to DE (2.0 mg soot/m^3), coal dust (2.0 mg/m^3),

DEP+coal dust (1.0+1.0 mg/m^3), or filtered air. Sperm motitlity, mean sperm density, and incidence of abnormal sperms were essentially the same in all four groups.

DE exposure, 14.2 ppm (v/v, average of weekly means) for 15, 33 or 42 days increased the arylhydrocarbon hydroxylase (AHH) activity significantly in the prostate gland in SpD-CD rats compared to controls (1.3±0.28 vs. 0.29±0.06; 0.94±0.08 vs. 0.28±0.16; 0.6±0.02 vs. 0.3). However, AHH activity was not affected in testis. Epoxide hydratase (EH) activity was not affected, neither in the prostate gland nor in the testis (119). Effects on reproduction in terms of sperm abnormalities have been shown in Syrian hamsters following inhalative exposure to DE (DEP; 12 mg/m^3, 8h/d, 7d/w for 3.5 mo; Periera ea 1982) and in C57B1/6 x C3H F1-mice following intraperitoneal injections of 50, 100, or 200mg/kg bw DEP for 5 days (163, 167).

9. Genotoxicity

It has been demonstrated that a large number of mutagenic organic compounds are associated with DEP (43, 97, 143, 171, 176, 177), of which the nitro-PAH are some of the most potent ones. These compounds are bioavailable following deposition in the lung, as described in Chapter 4 (25, 113, 241). However, there is also some mutagenic activity associated to the gaseous phase of DE (99, 107, 125-127, 130). See also Table 9.

Fredga et al (58) measured chromosome changes (chromosome aberrations, CAs, and sister chromatid exchanges, SCEs) in lymphocytes from 12 drivers of diesel lorries (6 smokers and 6 non-smokers). No information was given about the DE exposure level. Both chromosome breaks and gaps were increased in non-smoking, diesel lorry drivers (3.6 and 7.7 % CA, resp.) compared to non-smoking controls (1.4 and 4.3 % CA, resp.). However, the increase was not statistically significant. No effect was observed on the frequency of SCEs in DE-exposed compared to non-exposed controls. A study by Nordenson et al (153) of chromosome aberrations in underground miners exposed to DE for 2-9 years did not show an increase in the frequency of CAs (breaks and gaps) in lymphocytes from the DE-exposed miners compared to controls.

Schenker et al (185) measured postshift urinary mutagenicity on 87 railroad workers with a range of DE exposures, using Salmonella TA 98 ± S 9 mix in microsuspension. The DE exposure was measured over the work-shift (7-10 hr) by constant-flow personal sampling pumps. Respirable particle concentrations were adjusted for the contribution of environmental tobacco smoke (estimated from nicotine concentration in treated filters). Respirable particle concentration (RSP) adjusted for environmental tobacco smoke (ETS) gives the DE exposure adjusted respirable particles (ARP). They found no independent association of DE exposure (as ARP) with postshift urinary mutagenicity among smokers or nonsmokers. Mutagenicity in urine and faeces from DE exposed workers has also been studied by Willems et al (237) in a plate-incorporation assay, using Salmonella TA98 (urine & faeces) and TA100 (faeces) as tester strains.

No indication of enhanced incidence and/or degree of either faecal or urinary mutagenicity was observed.

Table 9. Mutagenicity of DE emissions from a Scania 113 bus driven according to the bus cycle described in chapter 3.

	particulate phase				semi-volatile phase			
	TA 98		TA 100		TA 98		TA 100	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
	(revertants/ml)*		(revertants/ml)*		(revertants/ml)*		(revertants/ml)*	
D1	7	8	30	25	-1NS	-3NS	-3NS	7NS
D2	15	12	50	45	6	20	25	25
D4	28	20	90	65	10	40	35	36
D5	25	25	90	90	3NS	17	12	35
D6	48	50	120	155	1.5NS	10	16	23
D7	16	12	60	60	4.5	10	26	35
D8	5	5	10	30	3NS	85	3NS	33
D9	20	18	70	80	1.5NS	35	13NS	38

Mutagenicity tests in a plate-incorporation assay with Salmonella typhimurium strains TA 98 and TA 100

D1-D9 composition, see Table 1

* The values have been estimated from diagrams in Westerholm & Egebäck (235)

NS = not significant

In the long-term inhalation study by Lewis et al (123) with four different exposure conditions as described in Chapter 8.2.2, bone marrow cells were harvested from femurs of male F344 rats exposed for 24 months , and female Swiss-Webster CD-1 mice exposed for 6 months, in order to score for micronuclei (MN). The mean frequencies of micronuclei in polychromatic erythrocytes from the Swiss-Webster CD-1 mice exposed to DE or DECD for 6 months were twice that of the control groups . No difference was observed in the F 344 rats exposed for 24 months (no figures were given). Lymphocyte SCE was studied in ten male F344 rats exposed for three months to DECD. The mean frequencies of the SCE were 0.22 per chromosome for controls, and 0.23 per chromosome for exposed rats. Dominant lethal effects were studied, using male, F 344 rats after 6 months exposure, 15 per treatment, and two naive F344 females. Numbers of live implants, dead implants and preimplantation losses were equivalent across the four treatments. Accordingly, no dominant lethal effects were observed (no figures were given). In Drosophila melanogaster, 8-h exposure to diluted DE (2.2 mg soot/m^3) did not increase the incidence of sex-linked recessive lethal mutations (99, 192).

Several in vitro studies have been performed with cultured mammalian cells, such as Chinese hamster V79 cells (82, 99, 112). Hasegawa et al (82) examined the ability of DEP from light-duty and heavy-duty engines to induce SCE and CA in Chinese hamster V79 cells, and morphological transformations in BALB/c 3T3 cells. The light-duty engine produced DEP which increased the number of cells with CA (mainly chromatid gaps and breaks) in a dose-dependent manner, however, only the highest dose tested (250 µg/ml) caused a significant ($p<0.01$) increase (3% in controls to 17% in treated). The number of cells with SCE also increased in a dose-dependent fashion , up to 1.6-fold at the highest dose tested (200 µg/ml). DEP produced by heavy-duty engine had no effect on CA, but increased the number of cells with SCE (up to 1.3-fold). In both cases was the control value 7.5 SCEs/cell. In the BALB/c 3T3 cells, DEP (30 and 50 µg/ml) from light-duty engine induced a significant number of transformed Type III foci (0.24 and 0.40 foci/dish, respectively, as compared to 0 foci/dish in control). DEP (10-50 µg/ml) from heavy-duty engine caused no significant increase in the number of transformed cells. However, when a number of Type III foci were isolated from cultures treated with DEP from light-duty or heavy-duty engine, and injected subcutaneously into nude mice (BALB/c nu/nu) at a dose of 10^6 cells per animal, the transformed cells, from both sources, produced tumours in the nude mice at the site of injection. Untreated and untransformed cells produced no tumours, even at a dose as high as 10^7cells/animal.

A large number of mutagenicity studies using Salmonella bacterial test system, have been conducted with DE and DEP (8, 99, 112, 171, 189, 191, 226, 227). Soluble or extracted organic matter from the particulate fraction were used in the earlier studies, the results showing an unequivocally positive effect, both with and without exogenous metabolic system from rat liver. By fractionation of the DEP extracts it was demonstrated that the mutagenic activity was associated to the moderately polar and highly polar neutral fractions (99, 189). Further separation of the neutral fraction on the basis of polarity resulted in mutagenic activity in predominantely the aromatic, moderately polar and highly polar oxygenated fraction (97, 99, 189), and chemical characterization showed that nitrated PAHs

contribute to 20-55% of the mutagenicity of DEP (191). Other oxidized PAHs associated with DEP have been shown to be mutagenic to S. typhimurium. Furthermore, Rasmussen et al (171) demonstrated that diesel automobiles equipped with the manufacturer´s exhaust trap, produced a reduction of mutagenicity in the Ames assay which directly followed the 87-92 % reduction of particles. It has also been shown (226, 227) that physiological fluids, such as dipalmitoyl lecithin (present in lung surfactant) are capable of dispersing or solubilizing agents from DEP that are mutagenic in the Salmonella assay. Keane et al (112) have demonstrated that DEP dispersed in aqueous mixtures of dipalmitoyl phosphatidyl choline, which is a major component of pulmonary surfactant, induce mutagenicity in Salmonella TA 98 (2-4 fold) and SCEs in Chinese hamster V79 cells (by approximately 30-65%). The genotoxic activity was associated to the sediment fraction, i.e. the particulate fraction. Filtered DE has also been shown to be mutagenic to S.typhimurium and E. coli, but only in the absence of an exogenous metabolic system (99, 107, 125-127, 130).

9.1. DNA Adducts

Wolff et al (240) exposed male F344/N rats for 7h/day, 5days/week for 12 weeks to DE or carbon black at a particle concentration of 10 mg/m^3. The control group was exposed to filtered air. Genotoxicity was assessed by using a ^{32}P-postlabeling method to measure lung DNA adducts. The lung burdens that resulted from inhaling the two similarly sized particles were also similar. The DEP had 33% of their mass as extractable organic compounds, as compared to 0.04% for carbon black. The mutagenicity in the Salmonella TA 98 test without S9 was 0.4 revertants/µg extract for organic extract from diesel soot and <0.002 revertants/µg for carbon black extract. The level of total DNA adducts was significantly higher ($p<0.05$) in lungs of DE exposed rats (16 adducts per 10^9 bases) than in lungs of rats exposed to filtered air (7.5 adducts per 10^9 bases), whereas there was not a significant difference between carbon black exposed rats (~ 11 adducts per 10^6 bases) and filtered air. The highest level of DNA adducts occured in peripheral lung, where the tumour formation is located.

Bond and coworkers (22-24, 26) have demonstrated that both DE and 1-nitropyrene (NP) induced DNA adducts in lung tissue of male F 344/N rats (11-15 weeks old), exposed for 7 h/day, 5 days/week for up to 12 weeks to diluted DE (0.35-10 mg soot/m^3) or for 4 h/day, 1 day/week for up to 12 weeks to NP (2 mg/m^3) or NP adsorbed on carbon black (NP/CB; 2 mg NP + 98 mg CB/m^3) respectively. Mice were also intratracheally instilled with ^{3}H-NP (4,5,9,10-^{3}H-NP; 1.8 Ci/mmole). The DNA adduct levels were independent of DE exposure concentration; 0.35, 3.5, 7.0, and 10.0 mg DEP/m^3 gave rise to 13.6, 13.1, 14.0, and 14.0 DNA adducts per 10^9 bases, respectively. The control value was 7.3 adducts per 10^9 bases. Furthermore, it should be noted that DNA adducts were elevated (13.6 $\pm$ 1.7 adducts/ 10^9 bases) at an exposure concentration (0.35 mg soot/m^3) that does not significantly increase lung tumour incidence according to Mauderly et al (132,135). This suggests that additional factors probably play a role in DE-induced pulmonary carcinogenicity. The studies with NP, which gave rise to C8-dG-AP DNA adducts, indicate that lung tissue is capable of metabolically activating NP to a reactive metabolite that binds to DNA.

However, the NP induced C8-dG-AP adducts and the DE induced adducts showed different patterns in the post-labelling assay (24).

Törnqvist et al (217) exposed F 344 rats and Syrian Golden hamsters for 16 h/day, 5 days/week for 2 years to DE or filtered DE. The DE gases were diluted with ambient air to 8.2% (high dose), 2.7% (medium) and 0.9% (low) respectively. The observed dose-related incidence of lung cancer in rats exposed to unfiltered DE (27) was correlated/related to the presence of Hb-adducts in the blood. The mean age of adducts in the erythrocytes at steady-state was about 18 days (the life-span of erythrocytes is about 60 days in rats and hamster). No long-term adaptation was observed.

10. Carcinogenicity

Already in 1955, DE extracts were demonstrated to be carcinogenic when applied to mouse skin (116). However, major concern for the potential carcinogenicity of DE did not develop until the late 1970s, when Huisingh et al (98) published results demonstrating that DE extracts were mutagenic in bacterial assays. NIOSH (152) and IARC (99) have published extensive reviews covering the work on DE exposure and cancer up until the late 1980s. However, the more important studies from that period are included below, together with studies published between 1989 and 1993. A summary of the majority of epidemiological studies regarding lung and bladder cancer are presented in tables 10 and 11. A summary of lung tumour incidence in animals after long-term exposure to DE is presented in table 12.

10.1. Humans

In a mortality study among members of a heavy construction equipment operators union, Wong et al (245) showed an overall mortality for the entire cohort (34 156 men and several subgroups) that was significantly lower (SMR = 81.4, p =0.01) than expected, when compared to national rates. The SMR for all cancers was also reduced (SMR = 93.0, p = 0.05). However, the expected deaths due to cancer of the liver (SMR = 166.7, p = 0.05) were increased, as well as deaths by emphysema (SMR = 165.3, p = 0.01) and accidents (SMR = 127.0, p = 0.01). With the help from union personnel, the 200 different job titles were reduced to 20 functional job titles, which were further grouped into three categories of potential exposure to DE emissions; high, low, and unknown exposure. The mortality of all causes, and all cancers were significantly lower for all three groups. There was no significant increase in cancer of liver, and neither in cancer of lung. However, in the group which comprised subjects with no dispatch history (i.e. the individuals performed the same job for the entire period), there was a significant increase in cancer of lung (SMR = 119.3, p = 0.05), as well as stomach (SMR = 199.1, p = 0.01) leukaemia and aleukaemia (SMR = 265.9, p = 0.05), and also an increase in emphysema (SMR = 235.5, p = 0.01). Smoking was not taken into account.

Garshick and coworkers have conducted a case-control study (62) and a retrospective cohort study (63) of lung cancer deaths and DE exposure in US

railroad workers, taking into consideration confounding factors such as asbestos exposure and cigarette smoking. Asbestos exposure in the railroads occurred mainly during the steam engine era, which ended in 1959. The asbestos exposure category was on job held in 1959 (or earlier if available), or on the last job held if the subject had retired before 1959. Smoking history was obtained by next-of-kin. The case-control study was done to test the hypothesis that DE exposure results in an increased risk of lung cancer. For cases ≥ 64 years of age at death (in 1981-82) and their matched controls (randomly selected workers, who did not have cancer on their death certificate or died of suicide or unknown cause) no effect of DE exposure was seen. In the younger group, ≤ 64 years of age at death and 20 years in DE exposed job, there was both an increased prevalence and duration of DE exposure. A significantly elevated odds ratio for lung cancer was observed (OR = 1.41, 95% CI 1.06-1.88). However, as the relative risk increase was low, it may be difficult to rule out remaining confounding factors. In order to confirm these results, Garshick and coworkers (63) conducted a large retrospective cohort study with 55 407 white male railroad workers who were 40-60 years old in 1959 and who had been working for 10-20 years in the railroad service. The cohort was traced until the end of 1980. Also this study supports the hypothesis that occupational exposure to DE results in a small but significantly elevated risk for lung cancer.

Siemiatycki et al (193, 194) have performed a large population-based case-control study in Montreal, which focused on occupational exposure as potential risk factors. One part of the study investigated 10 types of exhaust and combustion products, among them DE and its association with several sites of cancer. Interviews were carried out with 3 726 cancer patients (men, 35-70 years). Through in-depth interviews, detailed information about job history and possible confounders was obtained. Chemists and hygienists translated each job into a list of potential exposure; 651 subjects were exposed to DE at some level. Of 15 types of cancer studied, only colon cancer showed a statistically significant (p=0.05) associaton with DE (OR=1.3, 90% CI 1.1-1.6), and also a dose-response effect, based on short- and long-term exposure (short- and long-term exposures were defined by a 10 year cut point). The association for DE and rectum cancer was not statistically significant, but the data suggested a weak dose-response effect. Both squamous cell lung cancer and prostatic cancer showed an increased OR (1.2), though not statistically significant, suggesting an association to DE exposure. The squamous cell lung cancer risk was higher among subjects with short-term DE exposure than among those with long-term DE exposure. The risk for prostatic cancer due to DE exposure showed a similar dose-response as did colon cancer.

Bofetta et al (16) analyzed the two-year mortality of 461 981 males, with known smoking status and aged 40-79 years, in relation to DE exposure and to employment in selected occupations related to DE exposure. The subjects were selected from 1 200 000 men and women, who were enrolled in 1982 in a long-term cohort study in the US, Cancer Prevention Study II. They filled out a confidential questionaire at the time of enrollment. Among the 461 981 subjects, 92 038 did not give information about DE exposure, 62 800 were exposed to DE and 307 143 were unexposed. The relative risk (RR) for exposed subjects for all mortality causes was 1.05 and for lung cancer mortality 1.18 when adjusted for age, smoking, and other occupational exposures, such as asbestos, coal and stone

dust, coal tar pitch, gasoline exhausts, based on information from the questionaires. DE exposure for 1-15 years showed RR for all causes of 0.94 (95% CI 0.85-1.05), and for lung cancer 1.05 (95% CI 0.94-1.56). The significance for trend ($0.05 < p < 0.10$) suggests a dose-response effect. Furthermore, it is suggested that the interaction between DE and smoking is additive rather than multiplicative. Railroad worker, lorry driver, heavy equipment operator, and farmer were the job categories most frequently exposed to DE. It should be mentioned that DE exposed farmers were excluded from futher studies, only because they showed low mortality risk in lung cancer in the preliminary analysis. Instead miners were investigated further, despite the fact that only 14.4% reported DE exposure, as other studies have indicated high DE exposure among miners. There was no mention of radon exposure, which is a strong confounder in a mine. The RR for lung cancer was elevated for railroad workers (1.59; 95% CI 0.94-2.69), lorry drivers (1.24; 95% CI 0.93-1.66), heavy equipment operators (2.60; 96% CI 1.12-6.06), and miners (2.67; 95% CI, 1.63-4.37). All-cause mortality was also elevated; railroad workers (RR 1.43, 95% CI 1.20-1.72), heavy equipment operators (RR 1.70, 95% CI 1.19-2.44), miners (RR 1.34, 95% CI 1.06-1.68), and lorry drivers (RR 1.19, 95% CI 1.07-1.31). When comparing lorry drivers with reported DE exposure (18 cases) and those without (18 cases), there was no difference in lung cancer risk (RR=1.22 and 1.19 respectively). There was, however, a possible dose-response effect when comparing the duration of DE exposure; lorry drivers with 1-15 years of DE exposure (6 cases) showed RR=0.87 (95% CI 0.33-2.25), and those with 16 years of DE exposure (12 cases) showed RR=1.33 (95% CI, 0.64-2.75). Reference category was unexposed lorry drivers. There were too few cases in the other job categories to do a similar analysis. Mortality for all malignant tumours was nonsignificantly increased and there were no deaths from chronic bronchitis when looking at all DE exposed subjects with no regard to occupation. Mortality from Hodgkin´s disease and lymphoid leukamia was increased among those exposed to DE. No increase in non-Hodgkin's lymphoma or other leukemia than lymphoid. Significant ($p<0.05$) increases in deaths occurred due to cerebrovascular disease, arteriosclerosis, pneumonia and influenza, cirrhosis of the liver, and accidents.No association was found between DE exposure and mortality in bladder cancer.

Boffetta et al (14,15) performed a case-control study on occupational exposure to DE and lung cancer risk. The data used in the study were derived from a large ongoing case-control study of tobacco-related diseases that was also used by Iyer et al (104). The occupations were aggregated according to diesel exposure. The analysis included 2 584 lung cancer cases and 5 099 controls who were interviewed in the hospital at the time of diagnosis by trained interviewers using a structured questionnaire. No increase in risk for occupations with possible exposure and only modest increases in the crude ORs for occupations with probable exposure (OR=1.31, 95% CI 1.03-1.69) and for lorry driving per se (OR=1.31, 95% CI 1.09-1.57) were found. The excess in risk disappeared when controlling for smoking and education. The lung cancer risk according to duration of DE exposure (up to 30 years) showed a trend of increased risk with increasing duration for self-reported DE exposure ($p<0.12$). However,the trend was primarily due to high point estimate in the category aged over 30. A further analysis was performed of lorry-drivers cross-classified by self-reported DE exposure. The

results were, however consistent with those mentioned above (OR=1.25, 95% CI 0.85-2.76).

Gustavsson et al (76) investigated mortality and lung cancer incidence among bus garage workers in Stockholm, Sweden. All 695 men, who had worked as a mechanic, service man or hostler had been employed for at least 6 months between 1945 and 1970. The DE exposure was assessed by an industrial hygienist, the level (exposure intensity) for every work period in the work history was classified on a ratio scale of six degrees. Cohort and case-referent techniques were used for the analysis, and a standardization for occupational activity was performed in order to reduce bias from the "healthy worker effect". Expected numbers of deaths and cancers were computed according to the person-year method (99). Six referents were selected for every case, and individuals with a primary form of lung cancer (20 cases) were selected as cases. The results showed that the overall mortality in the cohort equaled the expected when the local rates, adjusted for occupational activity, were used as the reference. Several histological types of lung cancer were represented, and also two mesotheliomas, but the numbers were too few to permit conclusions regarding excess of individual types. There were 17 lung-cancer deaths, whereas 13.9 would be expected, 4 cases of oesophageal cancer were found against 2.1 expected. In the case-control study, the relative risk (RR) for lung cancer, however, increased with increasing exposure to DE, when estimated with logistic regression. Data on smoking habits were not possible to obtain in this retrospective study. In the case-referent study, however, it is not probable that the smoking habits differed substantially between the workers with high and low exposure, since both groups belonged to the same occupational category. Analysis of the lung cancer mortality by increasing cumulative DE exposure indicated no definitive evidence for a dose-response relationship. However, taking the total dust levels into account, the authors suggest a doubled lung cancer risk after 20 years of work in bus garages with a total dust level of 0.9 mg/m^3.

Steenland et al (201) conducted a case-control study of lung cancer deaths in the Teamster Union based on 996 cases and 1085 controls who had died in 1982-1983 after applying for pensions. The four principal job occupations within the Teamster Union were long-haul drivers, short-haul or city drivers, lorry mechanics, and dock workers. There was, however, no information on whether the men drove diesel or petrol driven lorries. Thus, "next-of-kin" data were compared with the Teamster records in order to get an estimate of exhaust exposure. Of the men identified by next-of-kin as primarily diesel lorry drivers, 90% were long-haul drivers by Teamster Union data, and the corresponding proportions were 82% for mechanics and 81% for dock workers. There were positive trends in lung cancer risk with duration of employment for long-haul lorry drivers after 1959 or 1964 (Teamster work history), and for lorry drivers who drove primarily diesel lorries (next-of-kin work history). For lorry drivers who drove 35 years or longer and primarily diesel lorries, the odds ratio for lung cancer was 1.89 (95% CI: 1.04, 3.42), when adjusted for age, smoking, and asbestos.

Table 10. Epidemiological studies of lung cancer and DE exposure

Study design	Population studied (follow-up years)	Results	Comments	Year (Reference
Cohort	15 995 man years London transport employees (4 yr)	84 deaths. RR=1.42 for garage workers. Signif. N.R.	DE exposure by job records. No control for smoking	1957 (168)
Cohort	6 506 railroad workers Baltimore and Ohio (5 yr)	6506 deaths. RR=0.88 for most likely exposure Signif. N.R.	DE exposure by job record: No control for smoking	1959 (110)
Cohort	3 886 US pot ash miners (27 yr)	433 deaths reported, lack of excess in lung cancer	Diesel use in mines. No control for smoking	1973 (231)
Cohort	179 756 members of US Teamster Union (0.25 yr)	245 deaths. RR=1.21 for entire cohort. RR=1.37 for age 50-59	Exposure by union membership	1975 (122)
Case-control	3 938 cases (lung cancer death) from hospitals in Los Angeles County matched controls (5 yr)	SMR=165 for lorry drivers S.S. (109 cases) SMR=344 for taxi drivers (23 cases)	Exposure assessment by last job. No control for smoking	1976 (140)
Case-control	6 434 cases Buffalo, N.Y. matched controls (10 yr)	RR= 0.92 for lorry, taxi, bus drivers. RR=0.94 for train engineers and firemen N.S.	Exposure by occup. RR for 66 cases. Control for smoking	1977 (51)
Case-control	US 3rd Natl Cancer Survey, 22 cases matched controls (3 yr)	RR=1.52 for male lorry drivers N.S. (22 cases)	Occup by interview. Control for smoking	1977 (238)
Cohort mortality	London transport staff (20 000), incl. bus garage workers, males, employed ≥25 yr	667 lung cancer cases Mortality ratio: Bus drivers 1.34 Bus conducters 1.34 Engineers in garages 1.11 -"- central work 1.50 Motormen and guards 1.15	High concentration diesel smoke in bus garage. No smoking data	1981 (228)
Cohort mortality	34 027 Swedish male lorry drivers (12 yr)	RR=1.33 for entire cohort S.S.	Exposure by job record. No control for smoking	1981 (1)
Cohort mortality	1 558 New Jersey vehicle examinors (29 yr)	233 deaths RR=1.02 for entire cohort Signif. NR	Exposure by years in job. No control for smoking	1981 (205)

Table 10 cont.

Study design	Population studied (follow-up years)	Results	Comments	Year (Reference)
Cohort mortality	43 826 Canadian, retired railroad workers (12 yr)	RR=1.00 (non exposed) RR=1.35 ("probably exposed") ($p<0.013$) RR=1.20 (possibly exp) ($p<0.001$)	Exposure estimated from job at retirement. No control for smoking	1983 (96)
Cohort mortality study	8 684 London bus maintenance workers (8 yr)	701 deaths. RR=1.01 for entire cohort. Signif. N.R.	DE exposure not mentioned. No control for smoking	1983 (180)
Case-control	English-Welsh population. 598 cases, 1 180 controls (5 yr)	RR=1.3 (all DE exposed occup; n=172) S.S. RR= 1.1 ("high" DE exposure; n=32) N.S.	Carc. of bronchus. Occup by death certif. No control for smoking	1984 (45)
Case-control	502 cases & 502 controls (2 yr) 18 US hospitals	RR=1.4 total DE exp. (45 cases) RR=1.9 for heavy equipment repair & operators N.S.	Occup by interview. Control for smoking	1984 (78)
Cohort study Pilot study of mortality	2 519 white males ≥10 yr in rail road work, US	SMR for cohort=87 SMR for DE exposure relative non exposed was 1.42±0.50. N.S.	DE exposure by job classification	1984 (186)
Prospective cancer mortal. study, cohort	34 156 males with potential DE exposure from heavy construction equipment operators union in San Fransisco. National rates	Overall cancer mortality significantly lower than expected. SMR= 93.0 ($p<0.05$) Mortality from lung cancer as expected (309 obs vs 313.44 exp). Increasing trend with duration; SMR=45.3 (<5yr) SMR=107.5 (10-14 yr) Also positive trend with latency; <10 yr; SMR=65.6 >20 yr; SMR=112.2	Lack of adequate work history No adjustments for smoking	1985 (245)
Retrospective cohort mortality and cancer	6 071 Swedish dock workers (20 yr)	1 062 deaths SMR=89 for all causes S.S. SMR=132 for lung cancer mortality (S.S.) SMR=168 for lung cancer morbidity (S.S.)	DE exposure by job record. No control for smoking	1986 (75)

Table 10 cont.

Study design	Population studied (follow-up years)	Results	Comments	Year (Reference)
Case-control	US Teamsters Union, size not reported (2 yr)	7 643 deaths RR=2.26, mechanics RR= 1.54, lorrydrivers RR=1.32, dock workers RR=1.16, others Signif. N.R.	Exposure by job record. No control for smoking	1987 (200)
Case-control	589 cases of lung cancer and 1 035 controls. Northern Sweden population (6 yr)	Professional drivers; OR=1.0 (≥1 yr) (n=72) OR=1.2 (≥20 yr) (n=37) Underground miners; OR=2.7 (≥1 yr)(n=25) OR=9.8 (≥20 yr)(n=16)	Occup by mail questinnaire. Control for smoking	1987 (50)
Cohort	694 Swedish bus company employees (32 yr)	All causes (195 deaths); RR=0.8, Tumours (35 deaths); RR=0.7. No difference between clerks and bus drivers or bus garage workers	Exposure by job record. No control for smoking	1987 (53)
Case-control study of deaths	1 256 lung cancers and matched controls US rail road workers	Workers ≤64 yr at death DE exp. 20 yr: OR=1.41 ($p=0.02$)	DE exposure years by job evaluation (no data). Smoking habits by next-of-kin. DE exposure increases lung cancer risk	1987 (62)
Case-control	506 cases and 721 controls. New Mexico population (excluding bronchio alveolar carcinoma) (3yr)	RR=0.6 for all DE exposure (7 cases) Sign. N.R.	Occup by interview. Control for smoking	1987 (121)
Case-control	1 260 cases, 2 084 controls. French population (4 yr)	RR=1.42, motor vehicle drivers (n=128) RR=1.35, transport equip. operators (n=157) RR=2.14, miners (n=22) RR=1.24, farmers (n=137) S.S.	Occup by quest. Control for smoking	1988 (11)

Table 10 cont.

Study design	Population studied (follow-up years)	Results	Comments	Year (Reference)
Prosepctive mortality study of cancer	461 981 males from 50 states in US (2yr)	DE exposure 1-15 yr: For all causes: RR=0.94. For lung cancer RR=1.05 DE exposure ≥16 yr; all causes RR=1.09, lung cancer RR=1.21. Time trend; 0.05<p<0.1. Lorry drivers; RR=1.03 (no DE) RR=0.87 (DE 1-15yr) RR=1.33 (DE >16yr)	DE exposure by job evaluation (no data).Controlled for smoking, age, other occup. exp.	1988 (16)
Retrospective cohort	55 407 males, whites in work for 10-20 yr rail road workers, US	1 694 lung cancer out of 17 068 deaths; RR=1.45 (p=0.005) for the group with longest duration of DE exposure (30 yr of service)	DE exposure evaluated by industrial hygienists. Mean respirable. part. 71-141 μg/m^3. Adjusted for smoking. DE exposure increases lung cancer risk.	1988 (63)
Population based case-control study	3 726 cancer patients, Montreal, Canada, of whom 651 DE exposure cases	Squamous cell lung cancer OR=1.2 (81 cases) Short-low exp OR=1.5 (n=13) Short-high exp OR=1.6 (n=16) Long-low exp OR=1.0 (n=24) Long-high exp OR=1.1 (n=28)	Higher risk for short exposure than long exp. Control for smoking	1988 (194)
Morbidity cancer	2 465 bus drivers, 87 cancer cases, 3 Danish towns	Morbidity ratio; SMR=85 for lung cancer	Unknown DE-exposure. Cancer Register Control for smoking	1988 (151)
Case-control population	2 291 cases lung cancer, 2 570 controls, hospital studies and general . popul in FL, LA, NJ in US (1-4 yr)	OR=1.5, lorry drivers (>10 yr) OR=2.1, heavy equipm. operators (>10 yr) S.S. OR=2.0 for all motor-exhaust-related occup. (>10 yr).	Occup by interview. Control for smoking. No separation between DE and petrol exposure	1989 (83)
Retrospective cohort	695 bus garage workers Reference general popul. in Stockholm,Sweden	SMR=122 lung cancer SMR=115 general popul.		1990 (76)
Case-control study within the cohort	20 cases, 120 controls	DE (1-10 yr) SMR=97 DE (10-30 yr) SMR=152 DE (>30 yr) SMR=127		1990 (76)

Table 10 cont.

Study design	Population studied (follow-up years)	Results	Comments	Year (Reference)
Case-control	996 cases (dead, lung cancer) and 1 085 contr (dead excl. lung and bladder cancer). Teamster Union, US (2 yr)	All lorry drivers employed after 1959; OR=1.55 Diesel lorry drivers (≥35 yr); OR=1.89 N.S. (56 cases,36 controls)	No DE measurements. Next-of-kin info. Control for smoking	1990 (201)
Case-control	2 584 cases 5 099 controls 18 US hospitals (10 yr)	Cases: males with prim. lung cancer. Self-reported exp: OR=0.90 (1-15 yr) OR=1.04 (16-30 yr) OR=2.39 (>31 yr) Occup with probable exp: OR=0.52 (1-15 yr) OR=0.70 (16-30 yr) OR=1.49 (>31 yr) Lorry drivers: OR=1.83 (1-15 yr) OR=0.94 (16-30 yr) OR=1.17 (>31 yr)	DE exposure by job evaluation (no data). Adjusted for smoking, asbstos, age, education	1990 (15)
	Subanalaysis of 477 cases and 949 controls	DE exposure showed a slight trend of increased risk with increasing duration (p<0.12)due to a high estimate ≥31 yr	Self-reported DE-exposure. Possible recall bias	1990 (15)
Case-control	5 935 lung cancer cases, 3 956 colon & rectum cancer referents	Mining machine operators OR=5.03, Heavy lorry drivers, sales drivers, & farmers OR=2.3, Motor vehicle mechanics OR=1.7	Occupation and tobacco use by tel. interview No DE measurem.	1991 (26)
Case-control	50 lung cancer cases, 154 matched controls (live&dead). Dock workers at Swedish ports	Non-smokers; OR=1.0 (low DE exp) OR=1.6 (medium DE exp) OR=2.9 (high DE exp) Smokers; OR=3.7 (low DE exp) OR=10.7 (medium DE exp) OR=28.9 (high DE exp)	Info by questionaire and next-of-kin	1993 (56)

N.R. = not reported
N.S. = not significant
OR = odds ratio
RR = relative risk
SMR = standardized mortality ratio
S.S. = statistically significant

Burns et al (29) assessed occupational risk factors associated with lung cancer in a case-control study in which colon and rectum cancer was used as referents (controls). Occupation and smoking history was obtained by telephone interview of 5 935 lung cancer cases and 3 956 controls. They observed a significantly increased risk for occupations that are known, from other studies, to present DE exposure. Excavating and mining workers had OR=4.01, farmers OR=2.05, lorry drivers OR=1.88, motor vehicle mechanics OR=1.72, and railroad workers OR=1.27.

Emmelin et al (56) performed a matched case-control study of lung cancer among Swedish dock workers from 15 ports. DE exposure was estimated indirectly from data on annual fuel consumption. Throughout the period studied, there was a system of job rotation, which led to similar exposure levels for all dock workers. There were 53 cases and 176 controls. Smoking history was obtained form mailed questionaires to living controls and next-of-kin of deceased. Using non-smokers with low DE exposure as reference, the odds ratio for lung cancer was 1.6 for medium DE exposure, and OR for high DE exposure was 2.9. For smokers the OR values were 3.7, 10.7, and 28.9, respectively. The results indicate an independent effect of DE exposure and a strong interaction between smoking and DE. See also Table 10.

Silverman et al (196) reported an increased risk for cancer in the bladder among lorry drivers, and also a significant trend with increasing duration of employment as a lorry driver. Furthermore, drivers of diesel lorries experienced a significant elevated risk compared to non-lorry drivers. In another study (195), examining the relationship between employment in occupations with potential exposure to motor exhaust and bladder cancer risk , it was found that lorry drivers and delivery men had a statistically significant, 50 % increase, in risk of bladder cancer. Consideration was taken to occupation, smoking history, alcohol and coffee consumption, and various demographic variables. The trend in risk increased with increasing time of employment as lorry drivers for ≥ 50 years. However, there was no separation between exposure to DE and petrol exhaust.

Wynder et al (249) performed a hospital-based case-control study (194 cases and 582 controls) of men, aged 20 to 80 years, in 18 hospitals in six US cities, from January 1981 to May 1983. The cases were individuals with histologically confirmed primary cancer of the bladder diagnosed within 12 months prior to interview. Controls were individuals hospitalized during the same period with diseases which were not tobacco related. The relationship between exposure to DE and the frequency of bladder cancer was examined. Occupational exposure to DE was estimated by using NIOSH guidelines ("high" exposure ≥20% employees exposed, moderate 10-19% exposed, and "minimal" exposure <10% exposed) with occupational titles defined by industrial hygiene standards as being probable high exposure. Included in this group were bus and lorry drivers, heavy equipment repairmen and operators, railroad workers, and warehouse men. Potential confounders such as age, smoking habit, and socioeconomic staus, were controlled for. There was no association between bladder cancer and employment in occupations with DE exposure, the OR being 0.85 (0.18-4.14, 95% CI) for warehouse men and materials handlers, 0.90 (0.44-1.87, 95%CI) for bus and lorry drivers, 2.0 (0.34-11.61, 95% CI) for railroad workers, and 0.75 (0.16-3.53, 95% CI) for heavy equipment operators and mechanics. Occupations without exposure

Table 11. Epidemiological studies of bladder cancer and DE exposure

Study design	Population studied (follow-up years)	Results	Comments	Year (Ref)
Case-control	356 males and 105 females with newly diagnosed & histol confirmed cancer of lower urinary tract. Local population as controls.	No elevated risk	DE exposure not separated from fumes, dust, dirt and smoke.	1972 (46)
Occupational mortality study	300 000 males ≥ 20 yr in various occupations.	Bus drivers PMR=83 Taxi drivers PMR=117 Lorry drivers PMR=112 Fuel oil lorry PM%=138 all 8 vehicle drivers together PMR=121 (S.S.)	Specific exposure to DE unknown.	1976 (141),
Retrospective hospital-based case-control study	6 434 males,7 515 females with one of 22 types of cancer; matched controls, Buffalo, N.Y.	Elevated but not statistically significant RR (34 cases) of bladder cancer for occupations with probable DE exposure. Miners RR=1.38 Bus, lorry, taxi drivers RR=1.25 Locomotive engineers + fire men RR=1.63	Specific exposure to DE unknown. Adjusted for smoking history.	1977 (51)
Case-control	7 518 cases (males + females), controls not reported (3 yr) US 3rd Natl Cancer Survey	Rel. odds: Transport 1.59 Railway 0.35 Lorry driver 0.91 Miners 1.61	DE exposure not mentioned.	1977 (238)
Population-based case-control	480 males and 152 females with newly diagnosed bladder cancer, matched controls Canada (3 yr).	Elevated risk for those occup. exposed to dust or fumes. Significant increase for those in rail road industry. Not statistically significant risk for DE exposure	Info by interview DE exposure was not separated from exposure to traffic fumes.	1980 (95)
Mortality case-control	347 deaths due to bladder cancer; 347 matched controls (non-cancer deaths).	Oil refining OR=4.46 (n=6) Agriculture OR=1.00(n=38) Mining OR=0.50 (n=7) Transportation OR=1.16 (n=15) Transport equipment, bus and lorry drivers, operators & auto mechanics may have an elevated risk	Specific exposure to DE not known. Not adjusted for smoking.	1981 (71)

Table 11 cont.

Study design	Population studied (follow-up years)	Results	Comments	Year (Ref)
Cohort mortality study	43 826 males pensioners (1965-1977) 17 838 deaths in cohort Canadian railroad workers (12 yr).	No excess risk of death due to bladder cancer after probable exposure to diesel fumes RR=1.03, all deaths (175 cases)	Diesel fume exposure.	1983 (96)
Retrospective cohort	8 684 London bus maintenance workers 701 deaths (8 yr).	RR=1.39 N.S.	No exposure data not controlled for smoking.	1983 (180)
Population-based case-control, morbidity	303 newly diagnosed histologically confirmed cancer of lower urinary tract among male (white) founders. Unknown residents; matched controls (general pop.) Detroit, US (1 yr).	Elevated risk for lorry drivers RR=2.1. Signif. trend with increasing as lorry driver; RR=1.4(<10 yr, n=23) RR=5.5(>10 yr, n=16) (p=0.004) DE-exposed lorry drivers had increased RR compared to non-DE exposed lorrys drivers and non lorry drivers.	Specific exposure to DE not known. Adjusted for con- duration of employment smoking history.	1983 (196)
Case-control death certificate	291 cases, 578 local controls. England and Wales (5 yr).	RR=1.0, all DE exposed occupations (n=68) RR=1.7, "high" DE exposure occup. (n=19) N.S.	DE exposure estimated by job-exposure matrix. No control for smoking.	1984 (45)
Restrospective cohort	501 deaths US railroad workers (15 yr).	RR=0.76 all deaths N.S.	No exposure data Not controlled for smoking.	1984 (186)
Case-control bladder cancer mortality	325 cases, 673 controls (other deaths among residents). White resident of NH&VT, US (5 yr).	OR=1.0 (DE 0 yr; n=197) OR=0.9 (DE1-19 yr; n=5) OR=2.1 (DE 20-29 yr;n=5) OR=3.2 (DE 30-39 yr;n=6) OR=1.7 (DE>40 yr; n=7) Significant trend with increasing year	Self-reported DE-exposure. Next-of-kin information. Controlled for smoking.	1985 (93)

Table 11 cont.

Study design (Reference)	Population studied (follow-up years)	Results	Comments	Year
Case-control hospital	512 cases, 596 hospital controls, Turin, Italy (5 yr)	RR=1.2 lorry drivers (n=16) Signif. N.R.	Info by interview Not adjusted for smoking	1985 (222)
Restrospective cohort	3 243 deaths, US heavy equipment operators (14 yr)	RR=1.18 all deaths regardless of exposure. N.S.	Not controlled for smoking	1985 (245)
Hospital-based fall-kontroll studie	194 cases and 582 controls from 18 hospitals in 6 US cities (2.5 yr)	OR=0.90, bus & lorry drivers (n=10). OR=2.0 railroad workers (n=2). OR=0.75 heavy equip. op. & mechanics (n=2) Jobs meeting NIOSH exposure criteria. OR=0.16 (moderate DE) OR=1.68 (high DE) NS	Info from question-naire. No DE measurements. Adjusted for smoking.	1985 (249)
Case-control Interviews	1 909 white males cases with histol. confirmed carcinoma of urinary bladder. 3 569 white male controls from general popul. US Natl Bladder Cancer Study (1 yr).	RR=2.2, lorry drivers 15-24 yr (n=59) RR=1.7, lorrydrivers 10-14 yr (n=58) RR=1.3, lorry drivers 5-9 yr (n=102) RR=2.0, taxidriver >10 yr (n=16). RR=1.3, bus driver >10 yr (n=16) S.S. for time trend.	Info by inter-view. No DE meaurements. Many confounders for lorry drivers. Controlled for smoking.	1986 (195)
Case-control hospital	117 cases, 117 hospital and 117 neighbourhood controls La Plata, Argentina (2.5 yr)	RR=4.31 lorry & railroad drivers (n=20) S.S. RR=6.22 oil refinery S.S.	Info by interview. When controlled for smoking; RR=4.31 reduced RR=6.22 increased.	1987 (102)
Case-control population	371 cases, 771 local controls, Copenhagen, Denmark (2 yr)	RR=1.55, land transport workers. RR=2.4, lorry, bus & taxi drivers(≥30 yr) (n=9) Signif. time trend; RR=0.7 for 1-9yr (n=11) RR=1.6 for 10-19yr (n=13) RR=3.5 for 20-29yr (n=9)	Info by interview. Controlled for smoking	1987 (106)
Population-based case-control mortality	731 bladder cancer death cases, 1 468 controls (all other deaths), Ohio, US	OR=12.0, lorry drivers ≥20 yr (S.S.) OR=2.2, railroad workers ≥20 yr (S.S.)	DE exp. not mentioned. Not controlled for smoking	1987 (200)

Table 11 cont.

Study design	Population studied (follow-up years)	Results	Comments	Year (Ref)
Population-based case-control	826 cases, 792 controls from the same area in 4 Canadian cities (3 yr)	OR=1.53 (309 men ≥6 mo in DE or traffic fumes; S.S) OR=1.69, DE exposure 8-28 yr (S.S.) Also time trend: OR=0.62 (19 females ever exp.) OR=0.49 (exp. 8-28 yr)	Controlled for smoking	1988 (173)
Long-term prospective mortality (cohort)	62 800 DE exposed from the US Cancer Prevention Study II	RR=1.43 for DE exposure 1-15 yr (n=7) RR=0.94 for DE exposure >15 yr (n=5).	Controlled for smoking	1988 (16)
Morbidity cancer	2 465 bus drivers, 87 cancer cases 3 Danish towns	Morbidity ratio; SMR=204 for bladder cancer	Unknown DE-exposure. Cancer Register Control for smoking	1988 (151)
Register cohort	All men born in Sweden gainfully employed 10 123 cases of bladder cancer, 7 432 among unexposed subjects.	RR=0.98 for DE exp., (n=332)	Swedish Census data, Natl Swedish Cancer Register. Selfreported job. No DE levels or exposure periods	1989 (202)
Mortality and cancer incidence	708 bus-garage workers 1958-1984. Ref. rates, national statist.	SMR=66		1990 (76)
Hospital-based case-control study	136 men with urinary bladder cancer; 272 matched controls with other cancers (except lung cancer) and non-malign diseases	OR=1.1 for possible DE exposure. OR=0.7 probable DE exp. Lorry driving OR=0.5 NS.	No DE measure-ments. Controlled for smoking	1990 (104)
Population-based case-control	256 cases (urothelial cancer), 287 controls All men born 1911-1945 and living in Stockholm 1985-87	RR=1.7 (n=25) for all exposed RR=1.3 (low DE exp) RR=2.2 (moderate DE) RR=2.9 (high DE exp)	Info by questionnaire DE exposure classified by industrial hygienist	1990 (204)

n = number of subjects
NS = not significant
OR = odds ratio
PMR = proportionate mortality ratio
RR = relative ratio
SS = statistically significant

to DE were used as referent group. There was no evidence of a synergistic effect between smoking and employment in an occupation with exposure to DE. The relative odds of bladder cancer, if employed in an occupation with exposure to DE, adjusted for smoking status was 0.87 (95% CI 0.47-1.58). Wynder & Miller (250) conducted an ad hoc study to find out whether lorry driver' s life-style might confound the observed relationship between DE exposure and bladder cancer. The interview with 206 white males, 25 - 60 years old, at a "truck stop" in Bloomsburg, NJ, indicates that long-distance lorry drivers may have rather unusual life-styles with heavy smoking, (76% were smokers and 50 % were smoking >35 cigarettes/day), excessive coffee drinking (70% drank ≥6 cups/day) and eating of fatty food and dairy products, and frequent use of pep pills to keep going. Thus, studies involving lorry drivers must be regarded with caution unless these confounders have been carefully considered.

Iyer et al (104) investigated the effect of DE exposure on bladder cancer risk using data from a large ongoing case-control study of tobacco-related neoplasms at the American Health Foundation. The 136 cases were patients with histologically confirmed primary cancer of the urinary bladder. The 272 controls had a current diagnosis of non-tobacco-related diseases. Exposure to DE was based on job titles and self-reported exposure. Exposure to DE in working and hobby activities was also collected. Occupations were aggregated a priori into three categories of probability of occupational exposure to DE; low probability of exposure (reference exposure), possible exposure, and probable exposure. The self-reported DE exposure was considered separately. However, no actual exposure data were given. There was a slight increase in the crude risk of bladder cancer (OR=1.4; self-reported DE exposure), which after controlling for smoking and educational status was not significant at the 5% CI. Lorry driving, the only specific occupation with enough cases and controls to allow for a separate analysis, did not show an association with bladder cancer.

Steineck et al (203) carried out a population-based case-control study of urothelial cancer in Stockholm, Sweden. The study was based on men born between 1911 and 1945 and living in Stockholm 1985-87. Exposure information was obtained by means of a postal questionnaire. An industrial hygienist classified subjects as being exposed or non-exposed, and he defined the exposure period and rated the annual dose (low, moderate, and high). For 25 DE exposed men (19 controls), the RR of urothelial cancer was 1.7 (0.9-3.3, 95% CI); for low DE exposure RR=1.3 (0.6-3.3, 95% CI), for moderate DE exposure RR=2.2 (0.7-6.6, 95%CI), and for high DE exposure RR=2.9 (0.3-30.0, 95% CI).

See also Table 11.

10.2. Animals

A summary of animal studies is presented in table 12.

The long-term cancer bioassays have been conducted with mice, rats, and hamsters exposed, primarily, to DE by inhalation of diluted DE emission, DEP phase, or filtered DE. The studies in mice gave variable results with the different strains and they do not clearly define a carcinogenic response. Female mice seemed more sensitive than male mice. Female NMRI mice responded to DE for

Table 12. Summary of pulmonary carcinogenicity observed in long-term animal studies after repeated exposures to DE by inhalation

Strain	No	Exposure h/d	Exposure d/w	months	Soot mg/m^3	Lung tumour incidence (%)	Refernce
F344 rats	24	20	7	15(+8)	0 0.25 0.75 1.5	0 1.7 5.0 1.7	110, 236
F344 rats	180	7	5	24	0 2.0	3.3 3.8	123, 124
F344 rats	22 22	8	7	24	0 4.9 (LD)	4.5 42.1[c]	103
F344 rats	288 144	16	5	24(+6)[b]	0 0.7 2.2 6.6	1.4 0.7 9.7[c] 38.5[c]	27
F344 rats	220 220	7	5	30	0 0.35 3.5 7.0	0.9 1.3 0.5 7.5[c]	132, 135
F344 rats	126	16	6	30	0 0.46 0.96 1.84 3.73	0.8 0.8 0.8 3.3 6.5[c]	101
Wistar rats	95	19	5	32	0 4.2	0 15.8[c]	85, 87
F344 rats	123	16	6	30	0 0.1 0.4 1.1 2.3	3.3 2.4 0.8 4.1 2.4	212 (Light-Duty)
F344 rats	123	16	6	30	0 0.5 1.0 1.8 3.7	0.8 0.8 0.8 3.3 6.5[c]	212 (Heavy-Duty)

Table 12 cont.

Strain	No	Exposure h/d	Exposure d/w	months	Soot mg/m^3	Lung tumour incidence (%)	Reference
Strain A mice	-	8	7	~7	6 12	+♀ -♂ -	161
Strain A/J mice	-	20	7	8	0.25 0.75 1.5	- - -	110
Sencar mice	-	8	7	15	6 + 12	+♀ - ♂	110
CD-1 mice♀	-	7	5	24	0.35 3.5 7.1	- - -	123, 124
C 57 BL/6N mice	-	4	4	24	2-4	(+) n.s.	213*
ICR mice	-	4	4	24	2-4	(+) n.s.	213*
NMRI mice♀	96	19	5	28	0 4.2 0[a]	2% 13%[c] 18%[c]	85, 87
Syrian hamster ♂	-	6	5	life-span (~20 mo)	7.3	-	47
Syrian hamster ♂	96	7-8	5	"	3.9	-	85, 87
Syrian hamster (♀ och ♂)	96	19	5	"	4.2	-	85, 87
Syrian hamster (♀&♂, equal no)	312	16	7	24	0.7 2.2 6.6	- - -	27, 28

a) = filtered DE, b) = rats surviving 2 yr DE exposure were maintained for a further 6 mo observation period with no DE exposure, c) = significant differences from control incidence

* F344 rats also negative with similar treatment
n.s. = not significant
CD = coal dust
+ = an increase in lung tumours compared to controls
- = no increase in lung tumours compared to controls

19 hours/day, 5 days/week for 28 months at 4.2 mg soot/m^3 with a signficantly increased lung tumour incidence (32% compared to 13 % in controls), and filtered DE increased the lung tumour frequency by 28% (85,87). Mice given an initial cancer treatment prior to DE exposure showed inconsistent results which appear erratic. Female Strain A mice, exposed for 8 hours/day, 7 days/week, for about 7 months, to DE at 6 mg soot/m^3, showed an increase in the number of lung tumours but not male ones, and at 12 mg soot/m^3 both sexes showed a decrease in the number of lung tumours. Sencar mice exposed for 8 hours/day, 7 days/week for 15 months to DE at 6 mg soot/m^3 followed by 12 mg soot/m^3, responded with an increased lung tumour incidence in females but no effect in males (161). When strain A/J mice were exposed to DE for 20 hours/day, 7 days/week and 8 months at, 0.25, 0.75, and 1.5 mg soot/m^3, the number of tumours tended to decrease with increasing exposure concentrations (111). CD-1 mice were not affected by DE up to 7.1 mg soot/m^3 for 7 hours/day, 5 days/week and 24 months (Mauderlay et al 1987). Another negative study was published by Takemoto et al (213) with C57BL/6N and ICR mice (DE at 2-4 mg soot/m^3 for 4 hours/day, 4 days/week for 24 months). However, 15 F344 rats exposed identically did not develop lung tumours either, which raises some doubts about that particular study. In the study with CD-1 mice, which was negative (135), the weekly exposure dose, i.e. conc x exposure time (CxT), was only 62% of that experienced by the NMRI mice, which was positive (85) (249 vs 399 mg · hr · m^{-3}). The maximum possible weekly soot exposure (CxT) used for C57BL and ICR mice was only 64 mg · hr · m^{-3}, a dose which would not be expected to be carcinogenic in rats. Thus, the question remains whether the trend towards increased lung tumour incidence might become significant with greater exposures or large group sizes.

Rats are more susceptible to DE exposure than mice, with no difference in tumour development between males and females. In the study by Brightwell et al (28), male and female F 344 rats were exposed by inhalation to DE, 2.2 and 6.6 mg soot/m^3 for 24-30 months. Both groups developed a significant increase of lung tumours. However, no increase was observed when rats were exposed to the same DE concentration with the soot fraction removed by filtration. An inhalation study by Mauderly et al (132,135) with different DE exposure concentrations (0.35, 3.5, or 7.0 mg/m^3 for 7 hour/day, 5 day/week for 30 months) resulted in lung tumours in 12.8% of the rats exposed to 7 mg/m^3, in 3.6% of the rats exposed to 3.5 mg/m^3, and did not increase lung tumour incidence in the 0.35 mg/m^3 group (1.3% vs 0.9 % in controls). Heinrich et al (85) exposed female Wistar rats to DE at 4.2 mg soot/m^3, which caused a significant increase in lung tumours, but no increase was observed with filtered DE. The study also included rats injected subcutaneously (sc) with dipentylnitrosoamine (DPN), known to induce exclusively respiratory tract tumours after sc injection. No syncarcinogenic effect with DE exposure was observed. Ishihara (100) exposed male and female F 344 rats to DE from either 1.8 L light-duty (LD) or 11.0 L heavy-duty (HD) engines. A significant increase in lung tumour incidence was only observed in the group exposed to HDE at 3.7 mg/m^3. The differences in response to LDE and HDE are difficult to explain. Most gas concentrations were similar at corresponding soot concentrations. Vapour-phase hydrocarbon concentration was relatively higher for HDE than for LDE, but soot-associated BaP and 1-NP concentration were relatively lower for HDE. In contrast to the tumour incidences,

the extent of epithelial hyperplasia was reported to be greater in lungs of rats exposed to LDE. Takaki et al (212) have done long-term inhalation studies of DE on F344 rats. They only saw carcinogenicity in rats exposed for 16 hour/day, 6 days/week for 30 months to 3.7 mg/m^3 of HDE. They concluded that there was a threshold for the occurence of lung tumours at around 4 mg/m^3 in particle concentration.

The long-term inhalation study by Lewis et al (123,124) with F344 rats, described in Chapter 8.2.2, did not reveal any evidence for increased tumorigenicity in rats.

Four life-span studies with Syrian hamsters exposed by inhalation to DE have all been negative, i.e. no exposure-related increases in lung tumour incidence was observed (see Table 12; 27, 47, 85). In the studies by Brightwell et al (27) and Heinrich et al (86), some hamsters were injected with diethylnitrosoamine (27) or BaP, dibenzanthracene (DBA), or nitrosoamines (86) in order to study a possible cocarcinogenic effect with DE exposure. However, no tumours were observed.

11. Mechanisms of DE-induced Carcinogenesis. Relevance to Risk Assessment

Mauderly et al (134) suggest that DE might exert carcinogenicity via an "initiation - promotion" pathway. The formation of lung DNA adducts indicates the "initial" event, and the progressive accumulation of soot provides "promoting" factors. The chronic DE exposure also causes chronic, active inflammation in the lung, which is followed by cytotoxicity, epithelial proliferation, epithelial metaplasia, and focal fibrosis (85, 87, 89, 133). The increased epithelial cell division increases the probability that a chemical induced DNA damage might be propagated and expressed as transformed preneoplastic or neoplastic cell populations. This reasoning is supported by the fact that the lung tumours are late phenomena. In one study (132,135) more than 80 % of the tumours were observed after 24 months of exposure. In another study (236), the tumour incidence was not increased in rats exposed for only 15 months, although the weekly exposure CxT (concentration x exposure time) exceeded the level that causes cancer in more prolonged exposures. The rats in this study were observed for > 8 months after the end of exposure.

The importance of the inflammatory and proliferative activity is further indicated by the study by Bond et al (17, 18) which shows that DE exposure at 0.35 mg soot/m^3, which did not induce carcinogenicity (132, 135), increased rat lung DNA adducts to levels similar to those which caused an increased tumour incidence (3.5 and 7.1 mg/m^3). The soot level in the former case might be too low to cause the necessary inflammatory and proliferative responses. Furthermore, Bond et al (17, 18) showed that the DE exposure-related lung DNA adduct formation and repair seem to establish a steady-state adduct level during the first few months of chronic exposures.

12. Exposure-Response Relation and Risk Assessment

After the first report on the mutagenic activity of DEP (97), the US EPA was prompted to develop the basis for a new approach to cancer risk assessment for DE emissions (3, 178). However, the limited data available from animal and epidemiologic studies were not sufficient for assessing cancer risks. The estimated human risk was therefore calculated using the comparative potency method, in which the carcinogenic risk of DE emission was estimated by comparison to a known human carcinogen, e.g. coke oven emissions. The following equation was used:

$$EHR_{diesel} = HR_{coke\ oven} \left(\frac{BP_{diesel}}{BP_{coke\ oven}}\right)$$

EHR = estimated human risk
HR = human risk
BP = bioassay potency
RBP = relative bioassay potency $= \left(\frac{BP_{diesel}}{BP_{coke\ oven}}\right)$

RBP was obtained by the ratio of the slopes of the dose responses from the same in vitro or in vivo bioassays.

The estimation of cancer risk of DEP was determined by comparing the carcinogenic and mutagenic potencies of DEP extracts with those of coke oven emission, roofing tar emission, and cigarette smoke tars (all causing lung cancer in humans) in a battery of in vitro mutagenicity and mouse skin tumour initiation and carcinogenicity bioassays.

As a basis for comparing carcinogenic potency, Albert and co-workers at USEPA (3, 178), calculated the "unit risk" for each emission source. The "unit risk" was defined as the lifetime probability of respiratory cancer death due to a constant lifetime exposure of 1 $\mu g/m^3$ benzene-soluble organic equivalent emission in the inhaled air, and it is predicted on the linear non-threshold extrapolation model. Using the comparative potency method, the lifetime risk ranged from 0.20×10^{-4} to 0.35×10^{-4} risk units/μg DEP/m^3 inhaled air for the light-duty diesel automobiles used in the study, and 0.02×10^{-4} risk units/μg DEP/m^3 inhaled air for the heavy-duty diesel engine used.

Recently, Nesnow (148) has followed up this approach by examining the quantitative relationships between the risk of lung cancer in humans, and the formation of mouse skin tumours by four known human complex mixture respiratory tract carcinogens. The aim was to find a method for predicting the human health effects of complex environmental emissions for risk evaluations and risk assessment. Nesnow included DEP fraction as one of the known human carcinogens, the others being coke oven emission, roofing tar emission and cigarette smoke. Male and female SENCAR mice (40 of each sex) were exposed to five doses of each mixture in both the tumour initiation and complete carcinogenesis protocols. No sex difference was observed. Based on the mouse skin papilloma multiplicity data (149), the relative potencies (coke oven = 1.0) were found to be; coke oven: roofing tar: DE: cigarette smoke, 1.0: 0.20: 0.15: 0.0011. When comparing the tumour incidence data (3), the relative values were;

coke oven: roofing tar: DE: cigarette smoke, 1.0: 0.22: 0.16: 0.0027. The human cancer risk was defined as the life time probability of respiratory cancer death due to a constant life time exposure to 1 $\mu g/m^3$ of benzene-soluble organic-equivalent emission of roofing-tar emissions and cigarette smoke. The risk assessment was based on a linear non-threshold extrapolation model reported earlier (3). The unit risk for DE exposure was calculated from rodent inhalation studies with F344 rats by Mauderly et al (132,135), by application of a linearized multistage extrapolation model (2), which gave an estimated risk of 1.2×10^{-5} life time risk/μg particulates/m^3. This was further adjusted for the percentage of organic materials extracted from the particulates (2), using the value from Albert et al (3), giving a human lung cancer unit risk of 0.7×10^{-4} lifetime risk/μg organics/m^3. The induction of papillomas in SENCAR mouse skin by DE was 0.31 papillomas/mouse at 1 mg organics (95% confidence intervals) and TD 25 (dose in mg yielding 25% mice with papillomas was 1.0) (149). The correlation between the mouse skin tumour initiation tumour multiplicity data and the unit risk estimates was quite good, with a correlation constant of 0.95 and a slope of 0.89. The close agreement between the two rankings, which use completely different scientific bases, assumptions, tumour incidences and target tissues, confirms the confidence in the comparative potency assumption and indicates the strengths of using the comparative potency method in risk assessment.

In 1990, an exploratory assessment of the risk of lung cancer associated with occupational exposure to DE among miners was done at NIOSH (254), based on the previously mentioned study with Fischer-344 rats, as reported by Mauderly et al (132,135). The tumour response data were used in the Armitage-Doll multistage model, which allows for an age-dependent exposure effect. The model was fitted to all tumours (benign and malignant), and to malignant neoplasms alone. The potency (unit risk) was estimated from a linear approximation to the results from the multistage model. A biologically equivalent dose was developed to scale the results from airborne exposure in rats to humans, which was adjusted for differences in weight, ventilation rate, deposition fraction, and the percentage of time actually exposed. The authors emphasized, however, that there is a great deal of uncertainty regarding the effects of exposure on lung clearance mechanisms and the deposition rates in humans, and thus, their risk assessment should, at the best, be regarded as an exploratory effort. For miners, the extrapolated life time risk of lung cancer increased from 1 to 2 % per mg/m^3 of particulate phase (DEP). Based on a DEP exposure of 1-5 mg/m^3 the excess risk was estimated to be approximately 1.5-3 in 100, assuming exposure for a working lifetime, i.e. 47 years, and adjusting for body mass (which is proportional to the lung mass /target tissue).

The extrapolation scheme by Oberdörster and Yu (154) from rat to man is based on the lung dosimetry of an inhaled particulate compound, taking into considerations rat specific and human specific deposition and retention characteristics of the particles, to arrive at an equivalent human exposure (EHE). The assumption is made that the same retained (accumulated) dose per g lung (or per unit surface area of the airspaces in the respiratory tract) of man and rat will result in the same effects . This hypothetical model of the pathogenesis of pulmonary effects induced by DE in rats, incorporates both fibrotic and carcinogenic effects, which have been observed in the long-term rat studies

summarized by Ishinishi et al (101). The activation of inflammatory cells is proposed to play a central role, being responsible both for epithelial cell proliferation (important for the carcinogenic response) and for an increased access of particles into the pulmonary interstitium (important for fibrotic response). The particle surface area may be of pivotal importance for activation of inflammatory cells, and a possible role of organic compounds is also indicated in the scheme. The results from these studies indicate that the carcinogenic effect of DE in rats was primarily due to the high concentration of insoluble particles and only to less than 1% due to the carcinogenic effect of the organic compounds associated to the particles. In DE-exposed workers, on the other hand, the lung tumour rate can not be explained by a particle effect alone, as the risk for humans found in the epidemiological study of DE exposed railroad workers was too high (63). If additional epidemiological studies can verify these results, then there must be other mechanisms beside the particle effects, such as effects due to the organic compounds aassociated to the particles or effects due to the gas phase compounds.

Pepelko and Chen (160) have estimated the carcinogenic risk of inhaled DE, based on the hypothesis that the most potent component of DE in inducing tumours is the inorganic carbon core of the DEP.This assumption was based on the fact that carbon black, an agent similar to the DEP core, has been shown to be about as effective as DE in the induction of lung tumours in animals (131). The investigators determined the concentration of inorganic carbon in the target organ by using a comprehensive dosimetry model. The epithelial tissue lining the lung and small airways was assumed to be the most important target site, as tissue damage was most apparent there and tumours appeared to originate there (132, 135). Three chronic animal cancer bioassays, using Fisher 344 rats, were regarded as acceptable (28, 101, 132, 135). When extrapolating from animals to humans, the dose equivalence parameter considered most appropriate was the concentration of particles per unit surface area of the lungs. The dosimetry model developed by Yu and co-workers (251-253), and designed specifically to model DEP retention in rat and human lungs, was used. The model accounts for parameters such as; respiration rates, lung surface areas, particle deposition efficiency, transport of particles to lung-associated lymph nodes, and both normal and decreased clearance rates (due to high exposure concentrations). The results were compared with more conventional methods of estimating human dose. The unit risk estimates, defined as the 95% upper confidence limit of the cancer risk from continuous lifetime exposure to 1µg/ m^3 of DEP, varied between 1.0-$4.6x10^{-5}$ with a geometric mean of $1.7x10^{-5}$.

As mentioned earlier (see Chapter 4) transport by diffusion seems to be an important mechanism in the pulmonary clearance of PAHs. Therefore it seems reasonable to assume that the difference between the distance of diffusion in the respiratory and non respiratory tissues of the lung, might be of importance. In the alveoli, the distance between the air interface and the capillary blood is about 0.5 µm, whereas it probably exceeds 50 µm in the bronchi. Gerde and co-workers (67, 68) have measured the biological half-life for BaP in the alveoli to about 2.3 min, and in the bronchi to about 1.4 hr. A substantial part of the biphasic pattern in the pulmonary clearance of PAHs seems to be due to a rapid penetration into the blood stream of PAHs deposited in the alveolar region, followed by a much slower clearance of PAHs that have escaped mucociliary clearance and have

reached the bronchial epithelium. It has also been shown that the larger the dose, the larger the fraction that will be cleared, unmetabolized, from the lungs (17,18, 64, 65). Thus, when extrapolating from animal studies with very large doses administered, to the human exposure situation with much lower, but more numerous, doses there might be an underestimation of the human risk.

Furthermore, when PAHs are adsorbed or precipitated onto inert dust particles, their retention increases drastically, probably due to formation of large aggregates of dust in the lung (64, 65). Thus, in animal studies, a slow release of the particle-associated PAHs would result in a prolonged exposure to surrounding tissues from a limited number of administrations. However, larger aggregates of inert dust are not likely to form with the much lower doses typical for human exposures. Under such low-dose exposure conditions, particle-associated PAHs will be released rapidly from the particles. Sustained exposure of target tissues to PAHs will result from repeated exposures rather than increased retention. This indicates that the immediate damage done by PAHs as they pass through organs and tissues is more important in determining the outcomes of exposure than the importance of retained particle-associated PAHs on lung cancer. There are some experimental studies supporting these model predictions (90, 209, 211). Furthermore, only low concentrations of PAHs are found in human lungs, even when the lungs are heavily burdened with urban air particles that are expected to carry PAHs at high concentrations (31, 116)

The estimation of human risk using animal data involves a great deal of uncertainty as a number of assumptions are necessary; i.e. regarding the shape of the dose-response curve in the low dose region and the biological differences between animals and humans (2, 3, 48, 49, 81, 139, 160, 165, 198, 253, 254). Furthermore, there are strong indications that high doses of DEP compromise pulmonary clearance mechanisms. The total lung particulate burdens in rats have been reported to be appreciably greater in the high-exposure groups than predicted by extrapolation from the low-dose groups. Long-term exposure to particles have also been shown to decrease the alveolar clearance rate in several experimental studies using radioactive tracers (132, 135). Thus the target tissue dose (biologic dose) will not be directly proportional to air concentration if the carcinogenic potency of deposited particles depends on residence time. Other problems associated with risk assessment for humans, are the fact that workers may be exposed to other sources of particulate matters than DE (in coal mines with total particulate levels ≤ 2 mg/m^3 and in other mines ≤ 10 mg/m^3), and that smoking, chronic obstructive lung disease and emphysema decreases pulmonary clearance of particles. See also Table 13.

Table 13. Summary of unit risk estimates of cancer for working lifetime. Exposures from DE risk assessments

Unit Risk per $\mu g/m^3$	Method	Year (Reference)
1.6×10^{-3}	Comparative potency[a,b] (STT)	1983 (81)
4.7×10^{-5}	Comparative potency[a,b] (STT)	1984 (49)
$2\text{-}3.5 \times 10^{-5}$ light duty 0.2×10^{-5} heavy duty	Comparative potency[a] (STT)	1983 (3)
1.7×10^{-5}	Comparative potency[a,b] (CB)	1986 (2)
0.7×10^{-5}	Multistage model[c] (CB)	1986 (2)
$4\text{-}8 \times 10^{-5}$	Multistage model[b,c] (CB)	1988 (165)
8×10^{-5}	Logistic regression model (CB)	1989 (139)
$1\text{-}2 \times 10^{-5}$ (may underestimate the time risk	Time-to-tumour model[b] (CB)	1990 (198)
$1.0\text{-}4.6 \times 10^{-5}$	Comprehensive dosimetry model[d] (CB)	1993 (160)
5.6×10^{-3}	Epidemiologic analysis[b,e]	1983 (81)
$0.6\text{-}2 \times 10^{-3}$	Epidemiology[f]	1989 (139)

STT = short term tests; CB = cancer bioassay

[a] Based upon comparing the relative potency of extracts of diesel exhaust in bioassays to the potency of other complex mixtures with similar chemical composition (e.g. coke oven emissions) for which epidemiologic information on risk was available.

[b] Reported risk estimates were adjusted to the equivalent for a 47 years of exposure to diesel exhaust (198).

[c] Based upon fit to the quantal form of the multistage model. The paper by Albert et al (2) fits this model to the study by Mauderly et al (135); whereas, the paper by Pott and Heinrich (165) fits this model to the studies by Mauderly et al (135), Brightwell et al (28) and Heinrich et al (85).

[d] Based upon the studies by Brightwell et al (28), Ishinishi et al (101) , and Mauderley et al (135). Extrapolation from animal to human, using, as dose equivalence parameter, the concentration of particles per unit surface area of the lungs. Dosimetry model according to Yu & Yoon (252) and Yu et al (253)

[e] Based upon an upper bound estimate of risk derived from an analysis of the London bus driver study (228).

[f] Based upon the study of rail road workers by Garshick et al (62).

13. Research Needs

- There is a need for specific exposure data related to epidemiological studies in order to make reliable quantitative risk estimates for exposed workers. Furthermore, a biological marker for DE exposure in humans would be valuable.
- The deposition and retention of DEP in the respiratory tract need to be further investigated, as well as the elution of organic compounds from the DEP.
- Some concern should be given to the possible additive or synergistic effect that the gaseous components of DE might have together with the particulate phase.
- The mechanisms causing the discrepancy between the response to DE exposure in different species, including humans, should be elucidated, and a better knowledge about the agents causing the cancer induction, as well as the interaction between the various agents, would be valuable. Such information would be useful also in the future evaluation of chemical hazards due to complex mixtures.
- More studies on the effect of DE exposure on chronic obstructive lung disease are needed, as well as effects on CNS.
- At the present time the best measure of DE exposure is the particulate/soot level. However, a more accurate measure of DE exposure would be valuable.
- Some protective measures have been developed, reducing exposure of workers to DE both inside DE vehicles, and in their surroundings, but greater reductions in exposure should be possible.

14. Discussion and Evaluation

Many of the epidemiological studies are difficult to evaluate as they are lacking exposure data or they have inadequate data, obtained with relatively insensitive and non-specific methods, such as total or respirable dust measured by gravimetry or surrogates such as nitrogen dioxide or carbon monoxide. The occupational groups that are more directly exposed to primarily exhaust, such as car-park attendants and toll-booth workes, are also exposed to petrol exhaust. Also, some of the DE exposed groups (e.g. lorry drivers) have had a special life-style, that is quite different from the general population. Furthermore, occupational cohorts tend to have below-average mortality due to the so called "healthy worker effect", which, however, is of less importance in cancer.

There are a few studies showing some obstructive lung disease, such as emphysema (245), and changes similar to chronic bronchitis (239) or irritation in general. There are also a number of studies in which no such adverse effects were observed. There is one study indicating that long-term exposure of bus-garage workers at "probably" high DE levels might cause organic brain damage (114). However, at the present time there is not enough evidence to suggest that DE exposure may cause serious problems of a more general nature.

The risk for cancer induction, on the other hand, should be regarded as a real risk, even though the opposite has been suggested (115). Cancer induction due to DE-exposure has been extensively studied both epidemiologically and in laboratory studies. Therefore the discussion will focus on this aspect. A large number of epidemiological studies on the association between DE exposure and lung cancer have been published since 1957. The epidemiological studies regarding an increased risk of lung cancer have been performed with several worker populations, covering lorry drivers (15, 16, 29, 50, 51, 83, 140, 181, 201, 238), bus and taxi drivers(7, 50, 51, 140, 151, 168, 228), transport workers (11, 122, 200, 228), railroad workers (62, 63, 96, 110, 184, 186), bus garage workers (50, 76, 168, 180), mechanics and motor vehicle examiners (11, 29, 205, 228), dock workers (56, 75), surface miners and underground miners (11, 29, 50, 231), heavy equipment operators (83, 245), farmers (11, 29) and motorway maintenance workers (10, 128). The results from these studies have varied. Several of the positive studies did not control for important confounders, such as cigarette smoking and exposure to asbestos or other known occupational carcinogens. Non-positive studies, on the other hand, were often lacking statistical power to detect a weak association. Another important factor to bear in mind is the fact that DE measurements are often lacking and, thus, the results are related to more or less accurate DE estimations.

Howe et al (96) found an elevated risk of lung cancer in Canadian railway pensioners, the risk being found in workers exposed to both coal dust and DE. There was no adequate control for smoking habits and asbestos exposure in these studies. Boffetta et al (14-16), on the other hand, found no overall increase in lung cancer risk in any of their studies on subjects employed in occupations likely to have DE exposure relative to subjects never employed in such occupations. However, there was a trend of dose-response effect with increasing duration of self-reported DE exposure, which the authors, however, assumed to be due to reporting bias. There was no significant elevation in risk associated with duration of employment as a lorry driver. In one of the studies (16), the effect of DE exposure on bladder cancer risk was also investigated, with no observed increased risk.

The largest and most thorough epidemiologic studies reported to date are two studies by Garshick and coworkers, a case-control study (62) and a retrospective cohort mortality study (63) of US rail road workers (truck drivers, heavy equipment operators etc). Both studies reported a significant association between exposure to DE and lung cancer among workers who were young when diesel engines were introduced to the railroads and thus had the highest potential for exposure. The association was independent of asbestos exposure in both studies and cigarette smoking in the case-control study.

Two studies, published in 1990 (76, 201), showed an increased lung cancer risk due to increasing accumulative dose of DE. In one study (201) age, smoking habits, and asbestos were taken into account. However, DE exposure was estimated using "next-of-kin" data and the Teamster Union records. In the other study (76), which comprised both a cohort and a case-control study, the DE exposure was assessed by an industrial hygienist. The smoking habits could not be obtained in the cohort study and in the case-control study it was assumed that smoking habits were similar in the two exposure groups. The authors suggested that over 20 years of work in a bus garage, with a DE emisson of 0.9 mg/m^3, might double the lung cancer risk. A study published in 1993 on Swedish dock workers (56) also indicated a dose-response result. However, the DE exposure was only estimated from the fuel consumption. It was, furthermore, shown that smoking might have an additive effect on the lung cancer incidens due to DE exposure.

Due to the fact that a substantial amount of DEP might be ingested, there is also a concern regarding DE induced cancer at other sites than the lung. However, the bladder is the only other organ/tissue that has been shown to be affected in several studies. The worker categories investigated epidemiologically for bladder cancer were buss and taxi drivers (51, 71, 106, 141, 180, 195, 249), long haul drivers and lorry drivers (51, 71, 102, 104, 106, 141, 180, 195, 196, 200, 222, 238, 249), heavy equipment operators (71, 249), bus mechanics (71), locomotive operators (51, 102), railroad workers (95, 96, 186, 200, 238, 249), surface miners and under ground miners (71, 238), and farmesr (71). Silverman and coworkers (195, 196) have, in two separate studies, demonstrated an elevated risk of bladder cancer in DE exposed lorry drivers compared to non-exposed lorry drivers, and the relative risk increased with increasing number of years in the job. However, the large number of confounders associated with the lorry drivers' life style (250) were not controlled for, except smoking. Four more studies (three with control for smoking) have shown a significant increase in bladder cancer, and also a statistically significant time trend. Two of the studies included lorry drivers and rail road workers, one only lorry drivers, and one lorry, bus, and taxi drivers (93, 102, 106, 200). Furthermore, an increase in the relative risk of bladder cancer has been correlated to increasing DE-exposure (204).

When summarizing the positive and non-positive studies, the evidence for lung cancer does support a weak carcinogenic effect, although the evidence is to a large extent circumstantial. Out of the reports shown in Table 10, 21 showed a weak increase in lung cancer risk,. However, twelve of these studies did not report on statistical significans. Three reports showed an increase that was not statistically significant, and seven studies showed no increase in lung cancer risk. Three studies showed a significant trend of increasing cancer risk with increasing duration of DE exposure. In twelve studies, smoking habits were controlled for. The strenght of the evidence for bladder cancer due to DE exposure is similar to the one obtained with lung cancer, i.e. pointig to a weak cancer effect. Out of the reports shown in Table 11, thirteen were positive, i.e. an increased risk for bladder cancer was observed. However, the increase was statistically significant in only five studies. Eight studies showed no increase in bladder cancer risk and two showed a non-significant increase. In four studies, an increasing time trend was demonstrated. Nine of the studies were controlled for smoking. A draw back with

these studies, however, is the low number of cases. Only a few studies had more than 50 cases.

There is little debate on the fact that long-term exposure to high DE concentrations (≥3.5 mg soot/m^3, 7h/day, 5 days/ week, 30 months), increases the frequency of lung tumours in rats (133, 134, 138, 208, 225). However, below this level of exposure there is no clear evidence of an increased tumour incidence. Inhalation studies with mice are inconclusive, as the increase in tumours have been observed in females only, and male mice as well as Syrian hamsters were unaffected by the DE exposures used (161, 207, 213). Mauderly (131) has calculated a DE exposure - lung tumour response relationship from the rat studies. This was possible as all the large positive studies involved similar exposure or exposure - observation periods (30-32 months).The lung tumour incidence (%) in the exposed and control rats were plotted against the weekly soot concentration x exposure time (mg x hr x m^{-3}). The lung tumour incidence of exposed rats diverged from the control incidence at approximately 100 mg x hr x m^{-3}, which led the author to the conclusion that there may be a threshold for response in rats and that the response is not linear.

The rat studies, as well as in vitro genotoxicity studies, indicate that the major carcinogenic potency is associated with the particulate fraction of DE, the filtered gas phase giving no tumours, except in female mice (27, 28, 85, 101, 103). Also the chemical analysis of the particulate fractions demonstrate that a large number of carcinogenic PAHs and nitro-PAHs are associated with the DEP. However, coal dust and carbon black give rise to lung tumours in rats at about the same rate as DE soot (123, 124, 144, 240). Thus the tumour induction in rats may be caused by nonspecific mitogenesis (increased cell proliferation) due to soot-induced inflammation. On the other hand, it has been demonstrated (19, 20, 21, 245) that DNA adduct levels are higher in the lung than in other respiratory tissues in the rat and that these levels are particulary high in the metabolically active type II lung epithelial cells. There is one study, in which female mice developed lung cancer after exposure to filtered diesel exhaust (85). This result might be explained by components of the gas phase of diesel, such as sulfur dioxide and formaldehyde which have cocarcinogenic or carcinogenic activity (84).

Based on NIOSH comprehensive review of the toxicologic and epidemiologic literature regarding carcinogenicity of DE (152), and the OSHA Cancer Policy (158), NIOSH concluded that whole DE should be regarded as "a potential occupational carcinogen". The evaluation done by IARC in 1989 (99) states that there is sufficient evidence for the carcinogenicity in experimental animals of whole DE, and of extracts of DEP, whereas there is insufficient evidene for the carcinogenicity in experimental animals of the gas phase DE. The overall evaluation by IARC states that DE is probably carcinogenic to humans (Group 2A).

The conclusion drawn from the information available to date on DE exposure is that the critical effect for setting occupational exposure limit for DE is cancer.

15. Summary

Beije B. Nordic Expert Group for Documentation of Occupational Exposure Limits.
109. Diesel exhaust. Arbete och Hälsa 1993:35, pp 83-155.

The document presents a literatur survey of the toxic effects of diesel exhaust (DE), and an assessment of the effects to be used as a basis for establishing occupational exposure limits for DE. Studies published between 1988 and 1993 are discribed in more detail, whereas earlier studies are summarized in tables. The emphasis has been put on epidemiological studies when such are available, but also long-term animal studies and short-term tests are presented. The epidemiological studies have revealed a weak increase in both lung and bladder cancer risk. There are some studies indicating obstructive lung disease, but more studies are needed to conclude whether the effects are significant and irreversible. The critical effect for DE is cancer. Further research needs are indicated.

Key words: cancer, diesel exhaust, genotoxicity, lung, lung clearance, obstructive lung disease, occupational exposure limits, reproductive organs, risk assessment, urothelial.

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ETHYLENEBISDITHIOCARBAMATES AND ETHYLENETHIOUREA

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Contents

Abreviations:

Diethylamine = DEA
Electron capture detector = ECD
Ethylenebisdiisocyanato sulfide = EBIS
Ethylenebisdithiocarbamate fungicides = EBDCs
Ethylenethiourea = ETU
Ethyleneurea = EU
Flame photometric detector = FPD
Gas (liquid) chromatography = GLC
High-pressure liquid chromatography = HPLC

Background

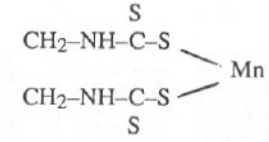
Etylenebisdithiocarbamates (EBDC) belong to a widely used group of organic fungicides. EBDC's constitute five broad spectrum fungicidal compounds, notably mancozeb, maneb, metiram, nabam, and zineb (2). They are used to prevent crop damage, and to protect harvested crops from deterioration, mainly by preventing fungal plant diseases on crucifers, cucurbits, tomatoes, potatoes, and ornamental flowers (130, 155). EBDC's are also widely used in forestry nurseries to protect growing coniferous trees such as pine and spruce against fungal diseases (85). However, nabam and metiram are not used markedly in any of the Nordic countries.

Several reviews have been published on the use, metabolism, fate, and the effects of ethylenethiourea (ETU) in mammals (130, 162). ETU as such is intensively used in the rubber industry where workers may be significantly exposed to the compound (130, 144). ETU is also the major metabolite of all EBDC's and, therefore, all workers exposed to EBDC's are also exposed to ETU due to the biotransformation of EBDC's to ETU (81, 85, 130, 138). Exposure to EBDC's and ETU also takes place via foods which contain EBDC's and ETU as residues. Moreover, EBDC's in foods are partially transformed to ETU during cooking. In Finland, the average exposure to EBDC fungicides via food items was estimated 10 mg annually per capita, i.e. 0.4 µg/kg bw/day in a 70-kg standard man (85). This dose is very low when compared with the recommended allowable daily intake (ADI value) of EBDC's from the foods (81, 85).

EBDC's and ETU are strong goitrogens after single or chronic administration in rodents, and ETU is also a strong rodent thyroid carcinogen after long-term exposure (18, 34, 41, 47, 130). Moreover, recent evidence suggests that mancozeb, one of the EBDC fungicides, may also be a thyroid carcinogen (39). Therefore, exposure to EBDC's, as well as to ETU, has raised much concern and renders the evaluation of the toxicity of both EBDC's and ETU important. In the Nordic countries, the most widely used EBDC fungicides are maneb, mancozeb, and zineb. Toxicity of metiram or nabam are not discussed in this document. However, as workers may be directly exposed to ETU in the rubber industry, or indirectly after exposure to EBDC's in the agriculture and forestry, also a separate assessment of the toxicity and health risks of ETU is included in this evaluation.

1. Physical and Chemical Data

Maneb

Chemical name	1,2-Ethanediylbiscarbamodithioic acid, manganese complex
CAS number	12427-38-2
Synonym	Maneb; 1,2-Ethanediylbis(carbamodithioato)-(2-)-manganese
Formula	C_4-H_6-N_2-S_4.Mn
Structural formula	
Molecular weight	265.3
Melting point	Decomposes before melting
Vapor pressure	$< 10^{-8}$ kPa
Density	1.92
General description.	Yellow or brown crystalline powder or light colored solid crystals; moderately soluble in water, soluble in chloroform and pyridine, insoluble in most organic solvents; stable under ordinary storage conditions but decomposes rather rapidly when exposed to moisture or acids; aqueous suspensions of maneb decompose rapidly; must be stored in sealed original containers, in shaded and well-aerated places; contains small amounts of ethylene-thiourea as impurity

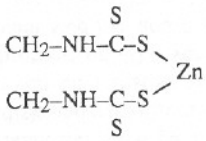
Mancozeb

Chemical name complex)	Carbamic acid, ethylenebis (dithio-, manganese zinc
CAS number	8018-01-7
Synonym	Mancozeb
Formula	C_4-H_6-Mn-N_2-S_4.C_4-H_6-N_2-S_4-Zn
Structural formula	$[S \cdot S \cdot C \cdot NH \cdot CH_2 \cdot CH_2 \cdot NH \cdot C \cdot S \cdot S \cdot Mn]_x$ $[Zn]_y$
Molecular weight	541.03
Melting point	Decomposes before melting
Vapor pressure	Data not available
Density	Varies
General description	Yellow or greenish crystalline powder; stable at room temperature (20 - 25 °C) but decomposes at higher temperatures and when exposed to moisture or acid; very slightly soluble in water, soluble in chloroform and pyridine, insoluble in most organic solvents; contains small amounts of ethylenethiourea as impurity

Zineb

Chemical name	1,2-Ethanediylbis (carbamodithioato) (2-)-zinc
CAS number	12122-67-7
Synonym	Zineb
Formula	C_4-H_6-N_2-S_4-Zn

Structural formula

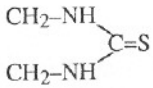


Molecular weight	275.8
Melting point	Decomposes before melting
Vapor pressure	$< 10^{-8}$ kPa at 20 °C
Density	Data not available
General description	White crystalline powder, stable at room temperature but higher temperatures and exposure to moisture or acid causes decomposition; slightly soluble in water (10 mg/l), soluble in carbon disulfide, pyridine and chloroform, not soluble in other organic solvents; contains small amounts of ethylenethiourea as impurity

Ethylenethiourea

Chemical name	N,N'-Ethylenethiourea
CAS number	96-46-8
Synonym	2-Imidazolidinethione
Formula	C_3-H_6-N_2-S

Structural formula



Molecular weight	102.2
Melting point	192-195 °C, decomposes at 200-203 °C
Vapor pressure	Extremely low
Density	Data not available
General Description	White crystalline powder, stable at room temperature at pH 5.0-9.0, decomposes at temperatures above 43 °C and when exposed to moisture or acid; soluble in water (20-30 g/l), slightly soluble in ethyl alcohol, insoluble in acetone, ether, chloroform, and benzene, slightly soluble in acetic acid at room temperature

2 Uses and Occurrence

2.1 Usage

EBDC's maneb, mancozeb and zineb are used in most of the Nordic countries as fungicides in agriculture and forestry (81, 85). They constitute an important group of pesticides used on seeds (130) and crops throughout the growing season. In the Nordic countries, Denmark, Finland, Iceland, Norway, and Sweden, EBDC fungicides are mainly used to protect potato crops against potato late blight, and forestry nurseries against fungal diseases of coniferous trees.

In many countries, major crops sprayed with EBDC fungicides, used alone or in combinations, include broccoli, brussels sprouts, cabbages, cauliflower, eggplants, grapes, lettuce, mushrooms, green onions, pears, peppers, celery, cucumbers, and tomatoes (6, 130, 158). Mancozeb is also used as an insecticide to control psylla, European red mites, and two spotted spider mites affecting pear and apple crops (130). Inspite of the extensive use of EBDC's, no accurate figures exist on their use in most countries. However, in 1976 in Canada the sales of EBDC fungicides were over 300 tons of active ingredients, i.e. more than 35 % of all of the sales of fungicides (130). In Finland, the sales of EBDC formulations containing maneb or mancozeb were 145 tons in 1989. The sales increased by 30 tons, i.e. by 20 %, from 1988 to 1989, when the amount of active ingredients was 105 tons (45). In 1990, the sales of EBDC formulations decreased by 6 tons from 145 to 139 tons with a corresponding 4 % decrease in the sales of the active fungicidal EBDC compounds (46).

Usually the use of fungicides varies from one year to the next according to the weather conditions, e.g. humidity increases the need for fungicides. A good example in this regard is Norway where the annual use of mancozeb between 1967 and 1990 has varied between 20 and 80 metric tons of active ingredient depending on the weather conditions (Kristensen, 1992, personal communication). In Denmark, on the contrary, a slow decrease in the use of EBDC fungicides has taken place over the last few years, i.e. the amounts of EBDC fungicides have decreased from 1460 kg in 1988 to 710 kg in 1992 (Fries, 1992, personal communication). In Iceland, the annual use of EBDC fungicides, mainly mancozeb, has been about 1000 kg (Gudbergsson, 1993, personal communication). In addition to the agricultural uses, maneb and mancozeb are used in the forestry, especially in forestry nurseries to prevent fungal diseases in growing coniferous trees in all of the Nordic countries (see 81, 85, 138).

The most significant exposure to ETU takes place in the rubber industry where it is used as a vulcanizer (130). A source of exposure to ETU is also the use of EBDC's in agriculture and forestry because all EBDC's contain small amounts of ETU as an impurity. EBDC's are also partially biotransformed to ETU in the mammals, the main metabolite of EBDC's, and the main hydrolysis product of the compounds. Moreover, several bacteria and reducing agents, such as glutathione or ascorbic acid, may cause degradation of EBDC's to ETU (161). Cooking of foods, such as spinach, potato and carrot, also causes the transformation of EBDC's partially to ETU. The initial concentration of ETU in foods has varied between 0.07 and 2.7 mg/kg whereas after the cooking the concentrations of ETU had increased about 10-fold (158). Thus, under some special circumstances

exposure via food to both EBDC's and ETU is possible due to the EBDC residues in various food items and drinks. For example, 370 µg of ETU/kg has been found in beer (97).

2.2 Occupational exposure and exposure via foods

Very few data are available on relevant occupational exposure to EBDC fungicides or ETU. In the production areas of a chemical plant, where also bagging and packaging occurred, concentrations between 2.2 and 2.5 mg of mancozeb/m^3 in the ambient air have been found (130). Moreover, Voytenko et al. (164) reported a transient concentration of 2.2-4.5 mg of maneb/m^3 during the preparation of spraying suspensions. However, during spraying of potatoes, an operator of a pneumatic knapsack sprayer was exposed to 0.25-0.55 mg of maneb/m^3 (164). Inhalation rate of 14.4 m^3 of air (30 l/min) by a 70-kg man, during a light 8-hour working day, would result in the production workers in an average respiratory intake up to 0.5 mg of maneb/kg/day provided that EBDC in air consists of inhalable particles and that all inhaled material was effectively transferred to, and absorbed in, the lungs (130). Inhalational uptake is, however, probably less than complete (81, 130), because only particles of the respirable range (0.2-5 µm in diameter) reach the alveolar space, and may ultimately be absorbed. Relatively large particles suspended in the air, which are typical of spray mists, are deposited in the nasopharynx, larynx and airways, cleared to the throat, and mostly swallowed. Therefore, this estimate probably overemphasizes the exposure via the lungs. If the spray operator also weighed 70 kg and inhaled 30 l/min of air during an 8-hour working day, he would inhale 0.05-0.11 mg of maneb/kg/day when exposed to 0.55 mg of maneb/m^3 (164).

Kangas and coworkers (61, 62) reported that the average concentration of maneb in air during an 8-hour working day in a forestry nursery was 0.1-1.3 mg/m^3. The levels of maneb during mixing were 6.7 mg/m^3, and during weighing 12 mg/m^3. Maini and Boni (95) reported, in turn, that the levels in workroom air of mancozeb were 0.04 - 1.8, and those of zineb 0.003 -3.5 mg/m^3. On an average, the levels of mancozeb were about 1 mg/m^3, and those of zineb 1.5 mg/m^3. Nilsson and Nygren (113) reported that the average exposure of potato growers to maneb during preparation and filling of the spraying liquid could be as high as 0.22 mg/m^3. The highest individual exposure was 0.05 mg (50µg)/m^3 for the whole application period. Assuming a 70-kg worker, inhalation rate of 30 l/min, an 8-hour working day, and concentrations of maneb and zineb in air of 1 and 1.5 mg/m^3 respectively, the calculated inhalational exposure to maneb would be 0.21 and for zineb 0.32 mg/kg/day. If one assumes that 10 % of the inhaled dose is absorbed, the biologically significant doses of maneb and zineb were 0.005-0.02 (5-20 µg/kg/day) and 0.03 mg/kg/day (30 µg/kg/day), respectively. Even if only 10 % of the inhaled dose of EBDC's were absorbed in the lungs, a considerable share of the inhaled EBDC's could be transferred through the clearance to the gastrointestinal tract where the absorption could be remarkable. There are no human data on the gastrointestinal absorption of EBDC's or ETU, but animal data suggest that the absorbed amount in the intestine may be considerable (11, 12), and may, therefore, contribute to the uptake of EBDCs and ETU in the occupational environment.

Savolainen et al. (138) found in pine nurseries, with a new mass spectrometric-HPLC method (84, 86), that the levels of maneb were <0.02, 0.03, and 0.77 mg/m^3 in tractor cabin, workers' breathing zone, and during weighing and mixing. The levels of ETU in the respiratory zone were 0.14 and 0.60 µg/m^3, and during weighing 0.87 and 1.81 µg/m^3 for potato field and pine nursery workers, respectively (138). In other studies, the levels of ETU were 0.01 - 0.80 µg/m^3 in the breathing zone of potato field applicators, pine nursery applicators, and nursery field weeders. Ambient air levels of maneb were 21 µg/m^3 after the use of maneb-containing formulations, whereas those of ETU were 843 ng/m^3 (81, 85). This exposure would result, assuming a 70-kg man, ventilation of 30 l/min, and an 8-hour working day, in total inhaled amounts of 302 µg of EBDC and 12 µg of ETU/day, which correspond to doses of 4.3 and 0.17 µg/kg/day of EBDC and ETU, respectively. Acceptable daily intake values for consumers for ETU is 2 µg/kg/day and for EBDC's 500 µg/kg/day. Thus, these estimated occupational exposure values are 8.4 and 0.96 % of the acceptable daily intake of ETU and EBDCs (85). Assuming a 10 % absorption of the inhaled amounts of EBDC's and ETU, the daily doses of EBDC's and ETU were 0.4 and 0.02 µg/kg, respectively. Thus, the range of biologically significant inhalation exposure of EBDC's would range from 0.4 to 30 µg/kg/day. The inhalation exposure of ETU would be 0.002 µg/kg/day.

In the occupational environment, especially dermal exposure to EBDC's, and to ETU, may be significant. A worker spraying EBDC's at an application rate of 2.25 kg/hectare would deposit 22.5 µg of the fungicide/cm^2 exposed skin surface area. Assuming a 70 kg standard man the exposed surface area would be 5040 cm^2, and hence, if uncovered, the dose would be 0.11 g/application. If the fungicide were totally absorbed this would mean a dermal exposure of 1.6 mg/kg bw per exposure. Feldman and Maibach (28, 29) have shown, however, that only 0.88 and 0.3 % of topically administered thiourea or diquat, respectively, penetrate the skin. Dermal absorption of more lipid soluble compounds can be, however, several fold more effective (28, 29, 102). Dermal absorption of EBDC's may be even less than that of ETU because they are poorly water-soluble salts. However, assuming a dermal penetration of 1 % for EBDC's, a calculated dermal dose after the deposition of 0.11 g of EBDC during an 8-hour working day, would result in a systemic dose of 16 µg/kg (28, 29). If 5 - 10 % of the EBDCs are converted to ETU, the dose of ETU would be 0.08 - 0.16 µg/kg bw (130). The estimates of inhalational absorption of EBDC's are 0.25-180 % of the estimates of the dermal absorption of EBDC's, and the corresponding estimate on ETU suggests that inhalational uptake would be about 0.6-1.2 % of the dermal absorption of ETU (81, 85, 138). In spite of the remarkable uncertainty of the significance of the predominating exposure route, recent evidence strongly supports dermal rather than inhalational exposure as the main route of uptake of EBDCs as well as of ETU (81, 85).

EBDC formulations also include 0.02 - 1.3 % of ETU as an impurity. However, after a storage period of 39 days, the amounts of ETU remarkably increase, and the amount of ETU in the formulations increases even 30 fold, up to 14.5 % of the total amount of EBDC in the formulation (8, 9). This is an important consideration because most pesticides are often stored for long time periods.

Dermal penetration of a structural analogue of ETU, notably thiourea, has been shown to be only 0.88 % of the topical dose in humans (28).

In spite of the dermal barrier for absorption, the present data are consistent with the assumption that the major route of exposure of EBDC's in the occupational environment is dermal (28, 29, 81, 85, 130, 138). An important observation is that protective clothing effectively reduces the dermal exposure. Kurttio et al. (85) found that protective clothing reduced the amount of ETU reaching the skin, as compared to the amounts of ETU on the clothes, by 90 - 98%. The situation is likely to be similar when workers are occupationally exposed to EBDC's. These findings emphasize the significance of protective clothing in the reduction of dermal exposure to EBDC fungicides and their impurities.

Exposure to EBDCs via foods

Workers can be exposed to EBDC's in a variety of food in addition to exposure to these compounds in the occupational environment. A number of methods are available to measure these compounds in different food items (112). Maximum residue limits for dithiocarbamates range from 0.1 mg/kg to 10 mg/kg depending on the food item (130). The most widely used procedures for determining EBDC residues in food are those involving spectrophotometric determination of CS_2 liberated following a hot acid digestion of the foodstuff (120, 126, 127, 128, 129, 130, 131, 132, 149). Also, HPLC has been successfully applied for measuring of EBDC residues in food (37, 38, 126). Exposure to EBDCs via food does not usually exceed the allowed residues limits (162) but it adds to the occupational exposure of workers to these compounds.

Exposure to ETU via food

Additional exposure of workers to ETU may take place via food and, therefore, the measurement of ETU in food is important also for assessing the total exposure of workers to EBDCs and ETU. A variety of analytical methods, e.g. paper, thin-layer, gas chromatography, and HPLC are available for this purpose (40, 112, 116, 119, 120). Residues of ETU have been determined extensively in a number of crops and foodstuffs (6, 8, 9, 21, 107, 108, 115). The limit of detection of gas chromatographic methods for ETU has been 0.01 mg/kg (20, 40, 42, 72, 96, 117, 127, 128, 129, 131, 132, 143). HPLC can also be used to detect levels 0.01 mg/kg of ETU in food (27, 76, 91, 97, 111, 114, 146). Even if exposure to ETU via food rarely exceeds the allowed maximum residues limits (162) it adds to the exposure of workers from other sources.

2.3 Determination of ethylenebisdithiocarbamates andethylenethiourea in the occupational environment andbiological samples

Exposure of workers to EBDCs and ETU is possible in the occupational environment through inhalation, and dermally. Therefore, it is important to be able to measure these compounds in occupational air, and in samples reflecting dermal exposure. For biological monitoring, there should also exist the possibility to measure important metabolites of EBDCs in human fluids, mainly in the urine (85).

Analysis of ethylenebisdithiocarbamates in air

Levels of EBDCs have been measured in the air of occupational environments. Samples of maneb have been collected from the respiratory zone of workers who are spraying maneb in pine nurseries. For the sampling, a personal pump equipped with a membrane filter has been used. Maneb in the samples is processed by boiling it in diluted hydrochloric acid and CS_2, liberated during the processing, is analyzed with a gas chromatograph equipped with a flame photometric detector (FPD) (61, 62). Maini and Boni (95) collected maneb and mancozeb in workroom air by using a portable pump and membrane filters. Maneb was processed by heating it in 37 % HCl and by measuring the release of CS_2 by using a gas chromatographic method applying ECD (95).

Nilsson and Nygren (113) collected air samples from the respiratory zone of potato growers exposed to maneb. They used membrane filters and portable air pump. The filters were wet-ashed by heating in 2 ml of nitric acid:hydrochloric acid:water; 2:1:1. The samples were evaporated to dryness and dissolved in water. The manganese in the samples was analyzed with an atomic absorption spectrophotometer the absorbance being measured at 279.5 nm. Savolainen et al. (138) also collected maneb samples from the breathing zone of pine nursery workers with a portable pump on membrane filters. Maneb on clothes and skin was collected using patch samples attached to the skin or clothes of the workers, and the samples were then dissolved in strong nitric acid and analyzed for manganese with an atomic absorption spectrophotometer (113, 138). Concentrations of 0.03 mg/m^3 of maneb in air could be analyzed (138). A provisional occupational exposure limit value for zineb has been 5 mg/m^3, and this value has been only rarely exceeded (62, 95, 138).

Analysis of ethylenethiourea in air, on the skin, and in urine

Because EBDCs are salts insoluble in water and most organic solvents and lipids, they are not likely to be found in biological fluids. This renders biological monitoring of exposure to EBDCs practically impossible by measuring the EBDCs themselves. Instead, metabolites of EBDC's in urine or blood are the best candidates for biological monitoring of EBDC exposure. However, biological monitoring of exposure to EBDCs or directly to ETU has not been possible until recently because suitable and sensitive enough methods to measure metabolites of EBDCs have not been available. The use of diethylamine (DEA) in the urine as an indicator of exposure of workers to EBDCs was not possible because the limit of detection for DEA was too high (62). Kurttio et al. (84) have applied a new HPLC method, with a limit of detection of 0.1 ng/l, for the determination of ETU in biological samples. The identity of the compounds is ultimately identified by applying thermospray liquid chromatography-mass spectrometry (86). This method has been successfully used for biological monitoring of exposure to EBDCs by measuring ETU in air in the breathing zone, on the skin, and in urine (81, 85, 138).

3 Toxicokinetics

3.1 Uptake

3.1.1 Uptake by inhalation

The solubility of EBDCs both in water and lipids is low, and this probably decreases the absorption of the inhaled amounts of EBDCs which have reached the alveolar regions of the lung. In the occupational environment, all EBDC fungicides occur as solvent-particle-air suspension, or as a suspension in which also water is present. There are no data on the particle size distribution of these suspensions, and, therefore, the assessment of the proportion of inhalable particles is not possible. For the sake of estimation, inhalational uptake can be assumed to exceed dermal uptake 10-fold, taking into consideration the low solubility of ETU and especially of EBDCs. Feldman and Maibach (28, 29) have provided evidence that the dermal penetration of thiourea is about 1 %, and probably the same figure can be used for ETU as well, and with certain reservations for EBDCs as well. This would mean that about 10 % of the inhaled amounts of these compounds would be absorbed from the lungs. This estimation does not, however, take into account the amount of EBDCs and ETU which are transferred from the lungs through the clearance into the gastrointestinal tract, and which also has an impact on the absorption of EBDCs and ETU. These figures for dermal and inhalational uptake have, however, been used in this document when assessing the inhalational and the dermal uptake of EBDCs and ETU.

There are no experimental animal data on the uptake by inhalation of mancozeb, maneb, zineb, or ethylenethiourea.

Maneb. Maini and Boni (95) have reported that levels of dithiocarbamates, notably thiram, in factory work room are below the provisional occupational threshold limits (ACGIH, 1984-1985) of 5 mg/m^3. The recovery of their method for maneb-containing formulations was 86.5 $\pm$ 21.6%. In other studies, the average exposure to maneb during a working day has been from 0.03 mg/m^3 (30 µg/m^3) (138) to 1.3 mg/m^3 (62). Assuming an 8-hour working day, a 70-kg man, and minute ventilation of 30 l/min this would result in an inhaled dose which would vary between 8 and 270 µg/kg/day. With an assumption of 10 % inhalational uptake this would result in a biologically significant dose range of 0.8-27 µg/kg/day. Nilsson and Nygren (113) found that time-weighed average exposure to maneb during an 8-h working day was 0.05 mg/m^3. Assuming a 70-kg man, and 30 l/min minute ventilation this exposure would result in a daily dose of 13.2 µg of maneb/kg, and the size of the biologically significant dose would be 1.3 µg/kg/day. Exposure to 0.03 mg of maneb/m^3 of air would result, again assuming a 70-kg man, minute ventilation of 30 l, and an 8-hour working day, in an inhalational dose of 7.8 µg/kg, and a biologically significant dose of 0.8 µg/kg with the 10 % absorption of the compounds in the lungs. At the same time, the concentrations of ETU in the urine were 0.9-1.5 µg/l, indicating that only a small share of ETU in the urine can be explained by inhalational uptake of maneb, and by its biotransformation to ETU (138). Kurttio et al. (85) have calculated that the concentration of maneb in air was 21 µg/m^3. Based on an average ventilation of

30 l/min during an 8-hour working day the inhaled dose of maneb would be 302 µg/day, i.e. 4.2 µg/kg bw the biologically effective dose being 0.4 µg/kg. These figures may explain only a small part of the concentrations of ETU in the urine (85). The available data on exposure to maneb suggest, however, that inhalational uptake of the compound plays a minor role in the exposure as indicated by the time of the onset (3 h) of the urinary excretion of ETU after the cessation of exposure (81, 85). Another explanation for the delayed absorption of maneb, and its slow elimination as ETU in urine, may also be the slow transfer of the inhaled maneb through clearance to the instestine.

Mancozeb. Maini and Boni (95) reported concentrations of mancozeb between 0.042 and 1.78 mg/m^3. This would result, assuming a 70-kg man, and 30 l/min ventilation during an 8-hour working day, in an inhalational exposure which would vary between 8 and 372 µg/kg, and a biologically active dose between 0.8 and 37 µg/kg of mancozeb. Kurttio and Savolainen (81) and Kurttio et al. (85) measured excretion of ETU in urine in exposed potato field workers exposed to 56 % mancozeb formulation together with a 80 % maneb formulation. Even though it is not possible to exactly assess the proportion of inhaled maneb and mancozeb in the formation and excretion of ETU, they may have had a small impact on the excretion of ETU in the urine. However, dermal exposure probably plays a major role during the exposure to both maneb and mancozeb because the time of the onset of the urinary excretion of ETU after the cessation of the exposure was 3 h, or more, in these workers. Nilsson and Nygren (113) measured inhalational exposure to mancozeb, and found that the time-weighted average concentration in the breathing zone was 0.05 mg/m^3, and that the highest concentrations was 0.22 mg/m^3. These results are in agreement with earlier studies on the exposure to mancozeb (81, 85, 95).

Zineb. Maini and Boni (95) reported concentrations of zineb between 0.042 and 3.48 mg/m^3. Assuming a 70-kg man, 8-hour working day, and 30 l minute ventilation this exposure would result in a dose between 8 and 720 µg/kg/day, and in a biologically significant dose of 0.8 and 72 µg/kg/day. The high concentration of zineb probably overemphasizes the amounts zineb in air because the peaks are usually transient.

Ethylenethiourea. Inhalational exposures to ETU in pine nursery and potato field workers were 0.14 and 0.6 µg/m^3 in the respiratory zone during the working day, and 0.87 and 1.81 µg/m^3 during weighing and mixing of maneb formulations (138). Because the urinary elimination of ETU was 1523 ng for potato field workers within 3 h after the cessation of the exposure, and 866 ng for pine nursery workers, inhalational exposure to ETU may explain only a small share of the amount of ETU eliminated in the urine. In another study, where exposure of potato farmers to maneb and its ETU impurity was studied, exposure to ETU during the 4 h exposure period was 0.07 µg/kg, with a calculated biologically active dose of 0.007 µg/kg. Elimination of ETU after the exposure of potato farmers began 3 h after the cessation of the exposure. The small inhaled amounts of ETU may slightly contribute to the amounts of ETU excreted in the urine (85). Kurttio and Savolainen (81) found that exposure of potato field applicators, pine nursery applicators, and pine nursery field weeders to ETU was 0.14 - 0.8, 0.6, and 0.01 µg/m^3, respectively. Levels of ETU varied between 0.9 and 1.8 µg/m^3 during the weighing of EBDC formulations. The resulting biologically significant

inhaled dose of ETU probably contributed very little on the amounts of ETU in the urine which varied between 498 and 3746 ng/24 h.

3.1.2 Uptake through the skin

Very little is known of the dermal absorption of EBDCs or ETU. Feldman and Maibach (28, 29) have shown, however, that 0.88 % of a topical dose of thiourea is absorbed through the skin. It is probably safe to use the figure for assessing of the dermal penetration of ETU, and with a remarkable uncertainty for EBDCs as well.

Maneb. There are no direct measurements of the uptake of maneb through the skin in humans. However, Savolainen et al (138) measured ambient exposure to maneb and ETU, and the excretion of ETU, in pine nursery workers. The results suggest that inhalational exposure neither to maneb nor to ETU explains the amounts of ETU excreted in the urine, i.e. the amount of ETU in urine exceeded the amounts which could have derived even from a complete inhalational absorption of maneb or ETU. Therefore, there are circumstantial data to indicate that dermal exposure may significantly account for the amounts of maneb entering into the body. These data are supported by later studies in which the exposure of potato farmers and pine nursery workers to maneb and ETU were explored (81, 85).

Mancozeb. No direct evidence on uptake of mancozeb through the skin is available in humans or experimental animals. However, when workers in potato farms or pine nurseries were exposed to mancozeb, the estimated concentrations of mancozeb or maneb were 21 $\mu g/m^3$. Because the length of the working day was 4 h the average inhalational dose could have been 151 µg/day, i.e. 2.2 µg/kg assuming a mean ventilation of 30 l/min and 100 % absorption (85). During a 22 day period the daily urinary excretion of ETU in these workers was between 0.05 and 0.5 µg of ETU/mmol creatinine. This is in agreement with the skin as the main route of entry of mancozeb. Similar results were also obtained in another study with pine nursery and potato farm workers (81).

Zineb. There are no data available on the uptake of zineb through the skin in humans or experimental animals.

Ethylenethiourea. There are no experimental animal data on the uptake of ETU through the skin. Feldman and Maibach (28, 29) have shown that 0.88 % of topical dose of thiourea penetrates human skin. It is probably safe to use the same figure for the assessment of dermal penetration of ETU in workers. Kurttio et al. (85) analyzed in detail dermal exposure to ETU in 29 potato farmers. In this study exposure of the skin was evaluated by using patch samples on the clothing and on the corresponding places on the skin. The concentrations of ETU on the clothes were between 5 and 45 ng/cm^2 on the arms, thigh, chest and back. The concentrations of ETU on the corresponding places on the skin were between 0.05 and 5 ng/cm^2. The contamination rates for ETU on the clothing on the back, chest, shoulders, and forearms were 5, 2, 9, and 14 ng/cm^2 an hour. The contamination rates in the corresponding areas of the skin were 0.07, 0.19, 0.39, and 0.17 ng/cm^2 an hour. Therefore, only 1.2 - 10 % of the ETU that contaminated the clothes was able to reach the skin under these occupational circumstances.

3.1.3 Uptake from the gastrointestinal tract

Urinary elimination of ETU subsequent to exposure to EBDC fungicides and ETU provide evidence that ETU in the ambient air may enter the body also via the gastrointestinal tract even if direct data on gastrointestinal absorption of EBDCs or ETU are lacking (81, 85, 138). There are, however, no direct evidence on the absorption of EBDCs or ETU through the intestine. Nevertheless, EBDCs and ETU may reach the intestine from the lungs through the clearance, first by entering the mouth and the nasopharynx, and then by being swallowed. Alternatively, these compounds may contaminate the hands of the workers, be transferred to the mouth, and also thereby contribute to the absorption from the gastrointestinal tract of EBDCs and ETU in the occupational environment.

Maneb. There are no data on the uptake of maneb from the gastrointestinal tract in humans. Brocker and Schlatter (11) have shown, however, that orally administered [^{14}C]-maneb is rapidly degraded in the intestine of rats, and that the excretion of the radioactivity is almost complete within 3 days. More specifically, the amount of [^{14}C]-maneb excreted in urine varied between 33 and 57 %, whereas the amount of [^{14}C]-maneb excreted in the feces was 40 - 63 %. In the exhaled air, 0.24 - 0.60 % of the dose of ^{14}C radioactivity was excreted. These results provide evidence that a remarkable share of maneb entering into gastrointestinal tract is absorbed, and subsequently excreted in urine. Also, studies by Brocker and Schlatter (11) with [^{54}Mn]-maneb showed that manganese forms complexes with intestinal cations. This complex formation which occurs, for instance, during simultaneous administration of Fe(III), Zn(II), Hg(II), or Cu(II) salts, significantly reduces the excreted ^{14}C activity in urine and exhaled air. Thus, cations in the food may also reduce gastrointestinal absorption of maneb. Kurttio et al. (82) have also shown that nabam, a close structural water-soluble analogue of maneb, was rapidly and effectively absorbed from the gastrointestinal tract, and a 14 - 24 % of the dose of nabam, given in drinking water, was excreted in the urine as ETU during a 28-day administration period. The water-solubility of nabam is likely to increase its absorption from the gastrointestinal tract.

Mancozeb and Zineb. There are no data on the uptake of mancozeb or zineb through the gastrointestinal tract in humans or experimental animals.

Ethylenethiourea. There are no human data on the gastroinstestinal absorption of ETU. There are, however, data on the gastrointestinal absorption of ETU in experimental animals. Two female rhesus monkeys and four Sprague-Dawley (SpD) rats were given [^{14}C]-ETU by gastric intubation, and the rate of excretion was evaluated for 48 h. Of all of the radioactivity in the rhesus monkeys 47 - 64 % was excreted in the urine, and only 1.5 % in the feces during the 48 h observation period. During the same period, 82 % of the ^{14}C radioactivity was excreted in the urine of rats, and only 1.3 % in the feces (1). These data indicate that ETU, when given by intragastric gavage, is rapidly absorbed from the gastrointestinal tract in rhesus monkeys and SpD rats. The absorption was almost complete in the rats, and about half of the dose was absorbed in the monkeys. Ruddick et al. (133) showed that when [^{14}C]-ETU was given as a single oral dose to pregnant rats peak radioactivity was reached within 2 h, and within 24 h 72.8 % of the radioactivity had been excreted in the urine, indicating again that gastrointestinal absorption of ETU from the intestine in rats is rapid and almost complete. The study also provided evidence that only a very small amount of the

administered ETU had been biotransformed. Kurttio et al. (82) gave ETU to Han:Wistar rats in drinking water for 28 days at dose levels of 100, 200, or 300 mg/l (10.6, 17.6, or 23.4 mg/kg/day), respectively. In these rats, the proportion of the dose of ETU excreted as ETU increased as a function of daily ETU dose. The mean percentages of the doses of ETU excreted as ETU during the exposure were 25 %, 36 %, and 49 % at the mean daily doses of 10.6, 17.6, and 23.4 mg/kg, respectively.

3.2 Distribution

Maneb. There are no data on the distribution of maneb in humans. The main metabolites of maneb in the urine of rats were ETU, DEA, and ethylenebisdiisocyanato sulfide (EBIS) (11, 12). Once formed, the water-soluble metabolites of maneb are probably evenly distributed into the water-phase of the body, and excreted into the urine.

Mancozeb. There are no data available on the distribution of mancozeb in humans or experimental animals.

Zineb. There are no data on the distribution of zineb in humans. In experimental animals, zineb is rapidly biotransformed to water-soluble metabolites (10, 59, 94, 103, 159). The formation of water-soluble metabolites suggests that a share of zineb is evenly distributed in the body after biotransformation. However, a remarkable portion of orally administered zineb is excreted as such in feces (11, 59).

Ethylenethiourea. There are no data on the distribution of ETU in humans. Six hours after an oral administration of [^{14}C]-ETU to pregnant rats, the radioactivity was mainly distributed in the kidney (25 %), blood (25 %), liver (23 %), and the fetus (12 %) (133). Forty-eight hour after oral administration of [^{14}C]-ETU to rhesus monkeys, 47-64 % of ^{14}C radioactivity was found in the urine, 0.4-0.6 % in the feces, 8-15 % in the muscles, 2-4 % in the blood, 1.5-3 % in the skin, and 0.9-1.2 % in the liver. In rats, the radioactivity was evenly distributed in the water phase, and rapidly eliminated in the urine after oral administration (1). Kato et al. (63) studied in detail the distribution of ETU in pregnant rats. They found that ETU was strongly accumulated as a function of time in the thyroid of the pregnant rats whereas the distribution of ETU in all the other tissues was rather even.

3.3 Biotransformation

Maneb. Human data on the metabolism of maneb are scanty. However, studies with workers provide evidence that much of the absorbed maneb is biotransformed to ETU, and excreted as ETU in the urine (81, 85, 86, 138). Autio and Pyysalo (4) found that 10 % of the radioactivity of [^{14}C] maneb orally administered to male mice was present in urine after a 22 h collection time. Jordan and Neal (59) administered [^{14}C]-maneb, dissolved in olive oil, orally to male ND/4(S)BR mice. About 16 % of the radioactivity in the urine was present as [^{14}C]-ETU. The radioactivity in the urine after administration of maneb was mainly present as unknown polar products. Maneb was metabolized in smaller amounts also to EBIS and DEA. However, 91 % of the dose of maneb was found unchanged in the feces. Thus, the amount of ETU in the urine was about 1.3 % of

the orally administered dose of [^{14}C]-maneb (59). Engst and Schnaak (23) reported the formation of ethylenethiuram-monosulfide from maneb in rats. They (24, 25) also demonstrated in vitro the formation of ETU, EBIS, ethylenethiuram-monosulfide, and DEA from maneb. Engst et al. (26) showed also that many of the metabolites of maneb, e.g. ethylenebisthiuram-monosulfide, ethylenebisthiuram-disulfide, and ETU inhibited peroxydases and ureases in rats and mice in a dose-dependent fashion.

Mancozeb. There are data on exposed workers to indicate that absorbed mancozeb is at least partially metabolized to ETU and excreted in the urine in man (81, 85). There are no experimental animal data on the metabolism of mancozeb. By analogy to maneb it is likely, however, that mancozeb is also metabolized to various water-soluble metabolites found in the urine of maneb-exposed rodents.

Zineb. There are no human data on the metabolism of zineb. Jordan and Neal (59) showed that 90.4 % of the radioactivity in [^{14}C]-zineb orally administered to male mice was found unchanged in the feces, and 9.6 % as water-soluble metabolites in the urine. Of the water-soluble compounds found in the urine, ETU constituted 15.2 %, ethyleneurea (EU) 1 %, unidentified polar products 81 %, and other unidentified products 2.7 %. Engst and Schnaak (23) found in rat urine ETU, EBIS, and ethylenebisthiuram-monosulfide, and they confirmed these results also by applying an in vitro approach (24, 25). In agreement with the findings of Engst et al., (26) Lowy et al. (94) proposed that zineb is an effective inhibitor of liver mixed function oxidase in rats. Moreover, zineb and its close structural analogue nabam, at a dose of 1 mmol/kg body weight, caused a reduction in the biotransformation of aminopyrine and aniline in the microsomes of rats. Both fungicides reduced the levels of P-450, and induced the formation of inactive P-420 (104). Villa et al. (159) demonstrated in rabbits that zineb inhibited both liver gammaglutamyltransferase and alkaline phosphatase activities. Borin et al. (10) showed, in agreement with Miladi et al. (104), that zineb inhibited the metabolism of aminopyrine and aniline and caused the denaturation of P-450 to P-420. EBIS also caused a similar inhibition of enzyme activities whereas ETU was without an effect. Also Meneguz and Michalek (103) have shown that zineb inhibits the activity of microsomal mixed function oxidase system both in rat and mouse liver. In agreement with the findings of Borin et al. (10), ETU did not demonstrate this effect.

Maneb, mancozeb, and zineb seem to inhibit their own metabolism. Even if the mechanism of this inhibition is not known, there are data to indicate that these compounds may inhibit the function of the mixed function oxidase system, the main route of their biotransformation, by denaturating the active P-450 to inactive P-420 (10, 103, 104).

Ethylenethiourea. There are no human data on the metabolism of ETU in humans. Savolainen and Pyysalo (139) showed that the degradation of ETU in mice involves oxidation at the sulfur atom, giving 2-imidazolin-2-yl sulfate as the major product. Iverson et al. (56) found in cat and rat that after a 24 h collection period the main metabolites after [^{14}C]-ETU exposure both in the cat and rat were imidazoline, ethyleneurea (EU), 4-imidazolin-2-one, and unchanged ETU. Also, S-methyl-ETU was found in the urine of both species. The metabolism of ETU was, however, much more extensive in the cat than in the rat thereby

possibly explaining the lack of teratogenic effects in the cat (56). Kobayashi et al. (74) have later identified also 1-methylthiourea in the urine of rats exposed to 200 mg/kg of ETU orally. In pregnant rats, [^{14}C]-ETU was very rapidly absorbed, and the radioactivity was excreted in the urine. Metabolites of ETU were not, however, identified in this study (63). ETU has not been shown to affect the activity of the enzymes of the liver mixed function oxidase system in rats, mice, or rabbits (103, 159).

3.4 Elimination

3.4.1 Elimination by kidneys

Maneb. Urinary excretion of ETU has been reported in workers inhalationally and dermally exposed to maneb-containing EBDC formulations in potato farms or in pine nurseries (138). Urinary elimination of ETU after exposure to ambient air levels of maneb ranging from 0.03 to 0.77 mg/m^3 was 866 ng/l/h at 3 h, and 395 ng/l/h at 40 h after the cessation of exposure. Because the levels of ETU in the air were 0.6-1.8 µg/m^3, excretion of ETU was probably not because of inhalational exposure to ETU, but rather due to dermal or inhalational exposure to maneb (138). Kurttio et al. (85) found that the most probable route of absorption of maneb in occupationally exposed workers is the skin. In this study, the urinary elimination half-life was about 100 h, but this value was obtained with a misleading urinary sample collection strategy which did not allow the estimation of the early phases of urinary elimination of ETU (85). A detailed analysis of urinary elimination of ETU after exposure to maneb or mancozeb revealed a urinary half-life of 32-37 h for ETU. The results were also in agreement with the assumption that skin was the most important route of exposure (81).

Brocker and Schlatter (12) found that the urinary excretion of ^{14}C radioactivity in rats followed first-order kinetics, i.e. was not modified by the size of a single oral dose of [^{14}C]-maneb between 23 µg and 1.4 mg/kg, i.e. the share of the radioactivity excreted in the urine being stable, 48.8 ± 12.6 %. They (11) also proposed that the gastrointestinal absorption of maneb may be smaller at small rather than at high doses because intestinal cations may inhibit the absorption of maneb. In another study, more that 90 % of the ^{54}Mn radioactivity of orally administered [^{54}Mn]-maneb was excreted within 35 h in the feces of rats. However, 33 - 57 % of ^{14}C radioactivity given as [^{14}C]-maneb orally to rats was excreted in the urine within 72 h. Fecal excretion of [^{14}C]-maneb was between 66 and 43 %, and exhalation of the radioactivity was between 0.24 and 0.6 % of the amount of the radioactivity (11). These data provide evidence that maneb is degraded in the intestine and, partially, rapidly absorbed and excreted in the urine. However, the Mn-containing moiety of the molecule is not readily absorbed from the intestine. When [^{14}C]-maneb was orally administered to mice, the majority (91 %) of the radioactivity was excreted in the feces, and only 9 % in the urine within 48 h (59). About 15 % of the radioactivity excreted in the urine was ETU, and the rest unidentified polar compounds. Also Autio and Pyysalo (4) have shown that the main urinary metabolite of orally administered [^{14}C]-maneb in mice is ETU. Thus, the absorption and elimination of maneb seem to be affected by decomposition of the compound in the intestine, and to depend on the dose,

and animal species. Available data indicates, however, that the main metabolite of absorbed maneb is ETU.

Mancozeb. Kurttio and Savolainen (81) and Kurttio et al. (85) have found that workers that are exposed to mancozeb-containing formulations excrete ETU in urine with a half-life of 32 - 37 h after the cessation of a 4 h exposure period (see also above for maneb). The data indicated that the most important route of exposure is likely to be dermal because of the delayed onset of the urinary excretion of ETU, and because small inhalational exposure to any of the EBDC fungicides applied could not explain the amount of urinary ETU excretion. There are no data on the urinary elimination of mancozeb in experimental animals.

Zineb. There are no data on the urinary excretion of zineb in humans. Jordan and Neal (59) have shown, however, in rats that 90.4 % of orally administered [^{14}C]-zineb is excreted in the feces and 9.6 in the urine within 48 h after a single dose of zineb. Of the urinary metabolites, about 15 % consisted of ETU, 1 % of EU, and the rest of polar and other unidentified products. Camoni et al. (16) measured ETU in the urine of rats that had received 50 mg of zineb/kg orally. Urinary excretion of ETU peaked at 24 h following the administration; at that time 52 % of the total urinary ETU had been excreted. Of the 50 mg/kg dose of zineb, 5.2 % was excreted as ETU, 86 % in the urine, and 14 % in the feces of rats. Kurttio et al. (82) have shown that during a 28 d administration of nabam, a water-soluble analogue of zineb, 14 - 21 % of the orally administered nabam was excreted as ETU. Moreover, the share of urinary ETU of the dose of nabam decreased as the dose of nabam increased, perhaps because nabam inhibits its own metabolism. This finding tallies with other results which have shown that EBDCs inhibit their own hepatic metabolism (10).

Ethylenethiourea. In workers, a 4-hour exposure to maneb and its ETU impurity has been estimated to produce an inhalational dose of 100 µg of maneb, and 2 µg of ETU in pine nurseries and potato farms (138). Dermal exposure to maneb and mancozeb, as well as ETU was, however, much more pronounced under these occupational circumstances, and resulted in a dermal exposure of several hundred micrograms of maneb, and several micrograms of ETU. Urinary elimination of EBDCs and ETU could be followed as urinary ETU which displayed a delayed urinary elimination with a half-life of 32-37 h (81). For the details of the elimination kinetics of ETU, see the chapter on the elimination of maneb and mancozeb by kidneys (above).

Newsome (110) showed that 65 % of orally administered [^{14}C]-ETU was excreted in the urine in rats within 24 h after a single oral dose, whereas the share excreted in urine in guinea pigs was 45 %. When [^{14}C]-ETU was administered orally to mice, 53 % of the radioactivity was found in the feces, and 47 % in the urine, after 48 h. Of the radioactivity in the urine, 52.3 % was as ETU, 12.1 % as EU, and 37.2 % other unidentified polar products (59). Brocker and Schlatter (11) showed that when [^{14}C]-ETU was given orally to rats, the radioactivity excreted in the feces was complete within 10 - 15 h, and of the excretion in the urine within 15 - 20 h. This indicates a good gastrointestinal absorption of ETU, and an elimination half-life of 4-7 h both in the feces and urine, respectively. When ETU was given to rats in drinking water for 28 d, the proportional urinary excretion of ETU increased from 25 to 49 % of the administered dose as the daily dose increased from 10.6 to 23.4 mg/kg, thus contradicting the findings that ETU does

not affect its hepatic biotransformation (103, 159). When [^{14}C]-ETU was given to pregnant rats, its elimination from the blood of the dams was practically complete within 48 h; 85 % of the dose had been excreted in the urine indicating an elimination half-life from the blood of 8 h, and that of urinary elimination of about 8 -9 h (63, 133). A similar result was obtained by Brocker and Schlatter (11), also in rats. In rhesus monkeys the primary way of orally administered [^{14}C]-ETU was elimination via urine because 47 - 64 % of the dose was excreted via the kidneys. In rats, on an average 82 % of the ETU dose was excreted in the urine within 48 h. The excretion rate of ETU in the monkey was 0.24 - 0.41 % of the dose per ml. No excretion rate of ETU had been calculated for the rats (1). Kobayashi et al. (74) have also identified 1-methylthiourea among the urinary metabolites of ETU, and Savolainen and Pyysalo (139) have proposed that 2-imidazolin-2-yl sulfate may be the main urinary metabolite of ETU in mice. This finding has not been confirmed by other investigators, the results of whom provide evidence that ETU is excreted mainly in the urine as such (1, 59, 63, 82). Iverson et al. (56) have identified imidazoline, EU, as well as unchanged ETU among the urinary metabolites of ETU.

3.4.2 Elimination through the gastrointestinal tract

Maneb. There are no data on the elimination of maneb through the gastrointestinal tract in humans. Brocker and Schlatter (11) have shown that orally administered maneb is rapidly degraded in the intestine of rats, the excretion being almost complete within 3 d. After the administration of 4 - 10 mg of [^{54}M]-maneb to female rats, about 40 % of the radioactivity was excreted in the feces. Gastrointestinal absorption was almost complete (> 95 %) within 20 h after the administration. They (11) also concluded that gastrointestinal absorption of maneb does not correlate with the size of the administered dose because cations naturally occurring in the food may decrease the intestinal absorption of oral doses of maneb. Jordan and Neal (59) found in mice that, after an oral dose of 0.05-0.25 mmol/kg of [^{54}Mn]-maneb, 91 - 93 % of the dose was excreted in the feces within 48 h after administration. Autio and Pyysalo (4) reported that 50 % of [^{54}Mn]-maneb orally given to mice was excreted within 22 h, and 18 % of the radioactivity was found in the feces.

Mancozeb. There are no data on the excretion of mancozeb through gastrointestinal tract in humans or experimental animals.

Zineb. There are no human data on the elimination of zineb through the gastrointestinal tract. Jordan and Neal (59) have shown that about 90 % of a dose of 0.25 mmol/kg of orally given zineb is excreted in the feces within 48 h after the administration to mice.

Ethylenethiourea. There are no human data on the elimination of ETU through gastrointestinal tract. When [^{14}C]-ETU was given by gastric intubation to Sprague Dawley rats or rhesus monkeys, only 1.5 % of the radioactivity was found in the feces within 48 h after the administration of 40 mg/kg of [^{14}C]-ETU (1). In pregnant Wistar rats, the elimination of orally administered [^{14}C]-ETU through the gastrointestinal tract was 0.5 % of an oral dose of 100 mg/kg (63). Ruddick et al. (133) found in pregnant rats after a single oral dose of 240 mg/kg of [^{14}C]-ETU, that less than 10 % of the radioactivity was excreted in the feces. Iverson et al. (56) provided evidence that after a dose of [^{14}C]-ETU less than 20

% was excreted in the feces in rats and cats. Newsome (110) found in the rat and guinea pig that within 48 h after an oral administration of 20 mg/kg of ETU, only 1.06 % was excreted in the feces of the rat, and 0.78 % in the feces of the guinea pig. On the contrary, Jordan and Neal (59) found that 51.8-53.3 % of ETU was excreted in the feces of mice after an oral dose of 0.05-0.25 mmol/kg within 48 h.

Exact assessment of the mechanisms of the gastrointestinal excretion of EBDCs and ETU is not possible to carry out because studies on the biliary excretion, an important route of excretion for many compounds, are completely lacking. This issue could be explored by studying gastrointestinal excretion of these compounds e.g. both after oral and intravenous administration of the compounds to be studied.

3.5 Factors affecting the metabolic model

Brocker and Schlatter (11) have shown that cations e.g. in the food may markedly inhibit the absorption of maneb. By analogy, the same applies to mancozeb and zineb because both can chelate cations. They (12) found that urinary elimination of maneb follows first-order kinetics after a single dose, i.e. the elimination is not dependent on the dose at a dose range between 23 µg and 1.4 g/kg. Kurttio et al. (86) found, however, that prolonged peroral administration nabam, as well as that of other EBDC fungicides (10, 26, 104) and thiurams (35, 36, 135), decreases its own elimination as ETU at high doses, possibly due to inhibition of its own hepatic biotransformation. In fact, nabam and zineb inhibit several hepatic enzymes as well as the biotransformation of aniline and aminopyrine (10, 104). Moreover, Meneguz and Michalek (103) found that zineb inhibits the hepatic mixed function oxidase system in rats and mice. Kurttio et al. (86) did not find, however, effects of ETU on its own urinary excretion. This is in agreement with the findings of Meneguz and Michalek (103) that ETU, at a single dose between 50 and 600 mg/kg, causes a dose-dependent increase in hepatic microsomal aniline hydroxylase activity without affecting aminopyrine-N-demethylase activity or the total content of microsomal P-450 enzyme. Moreover, all of the identified metabolites of zineb, notably ETU, EBIS, ethylenebisthiuram disulfide, and ethylenebisthiuram-monosulfide dose-dependently inhibited peroxydases and ureases in vitro (26). What the significance of these findings is for the biotransformation of EBDCs and ETU, remains to be elucidated.

3.6 Biological monitoring

In this paper, biological monitoring is the measurement of a pesticide or its metabolites in the body fluids of exposed workers, and the assessment of the absorbed dose, by using information on the pharmacokinetics of the pesticide both in humans and experimental animals. Compared to occupational hygienic monitoring of the concentration of the pesticide in the ambient air (58, 81, 85, 138) biological monitoring has the advantage of providing an integrated estimation of the absorbed dose through all exposure routes, ie. the skin, gastrointestinal tract, and the lungs (137). Because factors such as fluctuations in the air concentrations of a chemical, physical exercise, and pulmonary ventilation all have an impact on the absorption of a pesticide in the occupational environment (136), biological monitoring is, in most cases, superior to hygienic

monitoring of occupationally exposed workers (137). In the case of EBDC fungicides, biological monitoring of exposure to these compounds must take place by measuring metabolites of EBDC fungicides, preferentially in the urine, because the parent compounds themselves are insoluble in physiological fluids and most solvents (Chapter 1; 137).

The setting of occupational exposure limits for EBDC fungicides, and other similar compounds, may even be irrelevant to their exposure assessment. The reason for the probable uselessness of occupational exposure limits of EBDCs and ETU is that dermal rather than inhalational exposure to their airborne aerosols predominates. Because of the dominance of the dermal exposure route, the minor significance of inhalational exposure, and the unknown relationships between dermal exposure, absorption, and resulting biological dose, occupational exposure limits are of limited value. The assessment of exposure to these compounds should be based on their biological monitoring, preferentially as ETU in the urine. The focus should be on the timing of the sample collection after the cessation of exposure, and on the accepted levels of ETU in urine at the accepted time point (see 137).

Maneb. Measurement of maneb in workroom air has been dealt with in Chapter 2. Maneb, as such, cannot be measured in biological fluids because of the insolubility of the compound, and because of its rapid degradation e.g. in the gastrointestinal tract (11). Attempts have been made to measure DEA in human urine, but the levels have been under the limit of detection (62). Kurttio et al. (84, 86) used a sensitive GC and GC-MS method for the determination of ETU in biological fluids such as urine. This method (sensitivity of 0.1 µg/l) has been successfully applied in the measurement of ETU in urine of workers exposed to maneb (81, 85, 138).

Mancozeb. Available evidence indicates that the main metabolite of mancozeb is ETU (81, 85), and, therefore, measurement of ETU in the urine can be applied for biological monitoring of workers exposed to mancozeb (81, 85).

Zineb. The main metabolite of zineb in rodents is ETU (16, 23, 24, 25, 59). Even if the metabolism of zineb has not been studied in man, ETU is probably the most suitable metabolite of zineb to be used for the biological monitoring of exposed workers (84, 86).

Ethylenethiourea. The main compound found in the urine after an exposure to ETU is ETU both in experimental animals and man (1, 56, 63, 81, 85, 110, 133, 139). Even if ETU is mainly excreted as such in the urine, and even if ETU is the main metabolite of the parent compounds in the EBDC group (88, 97, 98, 125), also other metabolites of both ETU and EBDC fungicides, such as thiourea, methylthiourea, and EU, have been found after exposure to ETU or EBDC fungicides (23, 24, 25, 74, 75). Because ETU is eliminated as such in the urine, measurement of ETU in urine is the most suitable means for biological monitoring of workers exposed to ETU (81, 84, 85, 86, 137).

4 General toxicology

4.1 Toxicological mechanisms

Toxicological mechanisms of the EBDC fungicides maneb, mancozeb, and zineb, and of their metabolite, ETU are not well known. The same statement applies also to the structural analogues of the EBDCs, notable thiurams (135). Common to EBDC's, ETU, and thiurams is the great number of target organs of their toxicity with different mechanisms of toxicity. Main targets of the toxic actions of EBDC fungicides and ETU are the function of hypophyseal-thyroid axis, thyroid itself, developmental toxicity, mainly teratogenicity, and sensitization of the skin.

Laisi et al. (87) showed in rats that a single dose of EBDCs maneb and zineb, and ETU distort the humoral activity of the thyroid gland. They concluded that maneb (20 - 200 mg/kg i.p.) and zineb (70 - 500 mg/kg i.p.) inhibit rat TSH secretion through an action on the endogenous TRH at the hypothalamic or pituitary level. In this study, ETU (100 - 500 mg/kg i.p.) did not have an effect on serum TSH levels. None of the compounds had an effect on serum T_3 or T_4 levels. The proposed mechanism of action was the inhibition of dopamine-beta-hydroxylase which leads to the accumulation of dopamine (DA) and depletion of adrenaline in the brain. Increased brain DA, as well as inhibitors of dopamine-beta-hydroxylase such as disulfiram and diethyldithiocarbamate decrease TSH secretion in the pituitary after a single high dose (154). On the other hand, Kurttio et al. (83) found that nabam and ETU decreased serum T_4 levels, and correspondingly increased serum TSH levels, after a prolonged administration of the compounds for 28 d in drinking water. Both induced ultrastructural alterations in thyroid morphology. The authors concluded that the ultrastructural alterations may be connected with, and proceed through morphological changes, in the thyroid after long-term administration of EBDC fungicides or ETU. The target of these effects is the thyroid in which the inhibition of accumulation of iodine causes excessive stimulation of TSH from the pituitary. These observations are in agreement with findings which suggest that continuous administration of maneb or zineb cause the stimulation of thyroid by TSH, combined with a decreased ability of thyroid to concentrate iodine lead to thyroid hyperplasia (53) and cancer (18, 34, 39).

ETU is well known for its teratogenic actions in rodents (71) but the mechanisms of this action are not known. However, alterations in thyroid function as the primary target of the teratogenic actions of ETU, and of EBDC fungicides through ETU, cannot be excluded. Some authors have also found that zinc-containing EBDCs are less likely to induce teratogenicity in rodents than those not containing zinc moiety in the molecule (122) and others (89) have suggested that the chelation of zinc, an important cofactor of many enzymes, by EBDCs not containing zinc moiety in the molecule may be behind the teratogenic effects of EBDCs.

4.2 Acute toxicity

The acute toxicity of EBDC fungicides and ETU has been studied systematically. In most cases the data are, however, old, and e.g. the purity of the compounds

may have had affected the resulting toxicity of the compounds. In Table I are shown the LD_{50} values for the EBDC fungicides maneb, mancozeb, zineb, and ETU in rodents. The available data indicate that the acute oral toxicity of all EBDCs and ETU is low (86).

Compound	Animals species	LD_{50} (mg/kg)	References
Maneb	Rat	4500 - 4800	26, 90
	Mouse	>8000	90
Mancozeb	Rat	12500 - 14000	52
Zineb	Rat	5200 - 9400	52
	Mouse	6500 - 9200	52
Ethylenethiourea	Rat	1830	33

5 Organ effects

5.1 Effects on skin, mucous membranes and eyes

There is one case report on maneb in which maneb-induced pellegra-like allergic contact dermatitis was associated with extensive vitiligo patches present in the face, sides of neck, trunk, forearms, and genitalia (93). In addition, only sensitizing effects of EBDC fungicides and ETU have been described (13, 109).

5.2 Effects on lungs

There are no human or experimental animal data on the effects of maneb, mancozeb, zineb, or ETU on lungs. However, a metabolite of ETU, notably thiourea (TU), significantly increased the permeability of lung vasculature to Evans Blue dye when given intraperitoneally. Moreover, the increase of vascular permeability in response to TU in mature rats was associated with corresponding increases in lung and plasma histamine levels (32).

5.3 Effects on gastrointestinal tract

There are no human data of maneb, mancozeb, zineb or ETU on gastrointestinal tract. For exploring effects of EBDC fungicides and ETU on the autonomic nervous system control of the gastrointestinal tract, 4 daily intraperitoneal doses of 200 mg/kg of maneb, zineb, or ETU were given to male Wistar rats. Maneb, zineb, and ETU all decreased acetylcholinesterase (AChE) and non-specific cholinesterase (nsChE) activity in the wall of gastrointestinal tract of exposed rats (136). These alterations indicate an effect of EBDCs on the autonomic innervation of the intestine, and they may influence the motility of the intestine in exposed rats. Periquet and Derache (121) found that intragastric dosing of nabam caused a severe inflammation of the intestine in rats after 28 d.

5.4 Effects on liver

There are no human data on the effects of EBDCs or ETU on liver functions or morphology. Borin et al. (10) have shown, in agreement with Miladi et al. (104), that zineb inhibits the metabolism of aminopyrine and aniline, and denaturates P-450 to P-420. EBDCs or ETU have not caused morphological alterations in the liver of experimental animals.

5.5 Effects on kidneys

There is one case report on maneb-induced renal failure after occupational exposure. A 62-year old man had spread maneb on about 200 m^2 garden, and was subsequently taken to the emergency clinic with complaints of oliguria. diarrhea, and hoarseness. The clinical chemical data suggested acute renal failure; the serum levels of BUN, creatinine, and potassium were 1.4 g/l, 0.14 g/l, and 5.8 mEq/l, respectively. The ST segment depression in V_{4-6}, reciprocal ST segment elevation in V_{1-3}, and inverted T waves in V_5 and V_6 were recorded on ECGs. Both the renal failure and the ECG abnormalities disappeared after hemodialysis. The authors concluded that a possibility exists that maneb caused the acute renal failure (77). Kurttio et al. (86) did not find alterations in renal functions or morphology when rats were exposed to 50, 100 or 200 mg of nabam/l drinking water for 28 d. On the contrary, when rats were exposed for the same time to 100, 200 or 300 mg of ETU/l drinking water, an increased number of lysosomes and myelin figures as well as vacuolization and edema were found in the cytoplasm of the epithelial cells of proximal tubules (86). Moreover, Periquet and Derache (121) found that high doses of nabam (210 - 490 mg/kg/ day for 28 days, in the diet) cause renal necroses and severely impair renal function in rats. Together these results suggest that kidneys are not likely to be sensitive target organs of EBDC or ETU toxicity, and the case report on maneb-induced acute renal failure in man may be a coincident as well.

5.6 Effects on blood and blood forming organs

There is one case report on a farmer who had been exposed to zineb for 4 h during a morning (123). Physical examination revealed a severe condition with a yellowish and slate-blue color of the skin and sclerae, and dark brown urine. There was an increased number of Heinz bodies in erythrocytes, and normoblastic hyperplasia in the bone marrow. The following day the patient became semicomatose, and cyanosis increased. On 4th day his condition became critical, and the haemoglobin dropped to 4.7 g/l, but the condition was stabilized with blood transfusion and cortisone. The patient was discharged two weeks after the hospital admission. During a control visit 4 months later he was in a good condition. Later studies revealed that when whole blood was incubated with 5 mg/ml of zineb it increased the formation of Heinz bodies. The authors concluded that exposure to zineb may be associated increased formation of Heinz bodies and acute hemolytic anaemia. There are no other human or experimental animal data on the effects of EBDC fungicides or ETU on blood or blood forming organs. The

significance of this case report remains to be verified. There are no data on individual susceptibility to hematopoietic effects of EBDCs or ETU in man.

5.7 Effects on central nervous system

Acute intoxication of a worker due to exposure to maneb and zineb has been described in a case report (49, 50). A healthy 42-year old male sprayed an EBDC formulation containing both maneb and zineb on a cucumber plantation twice during a week. Behavioral changes appeared after he walked through the plantation after the first application, and they consisted of loss of consciousness, convulsions. Right hemiparesis with diffuse slow rhythm in the electroencephalogram (EEG) occurred after the second exposure. Both the behavioral and central nervous system symptomatology disappeared within a few days. An EEG was normal two weeks later. Because reliable exposure information is not available the significance of this occurrence remains to be elucidated, and cannot be evaluated at this stage. There are no other data on neurotoxic effects in humans of EBDC fungicides or ETU.

Thuránszky et al. (153) found that disulfiram and thiurams such as tetramethylthiuram disulfide caused a significant reduction of the orientation hypermotility and at the same time a depression of the subcortical EEG activity. The authors concluded that inhibition of dopamine-beta-hydroxylase was involved in these effects of thiurams not found in rats exposed to EBDC fungicides maneb or zineb. Komulainen and Savolainen (80) studied whether cerebral dopaminergic D_2-receptor binding is affected by exposure of rats to maneb, nabam, zineb, or ETU. It appeared that none of these compounds caused alterations in cerebral D_2-receptor binding at doses which were already overtly toxic.

Khera and Tryphonas (70) found, in turn, in the rat that a single dose of 15 or 30 mg/kg of ETU induced early histologic changes in the fetal CNS when ETU was given on the 13th day of the pregnancy. Histologic study revealed the presence of karyorexis in the germinal layer of basal lamina of CNS extending from the thoracic spinal cord to the telencephalon 12 h after treatment with 30 mg/kg of ETU. In the brain, the ventricular lining was focally denuded, neuroepithelial cells were arranged in the form of rosettes, and the nerve cell proliferation was disorganized. Khera (68) used monocell layers, containing a mixture of neuronal and non-neuronal (primary glial) cells, obtained from growing cells dissociated from trypsinized fetal brains of 19 day pregnant rats, to explore the neurotoxicity of ETU. ETU caused necrosis in the monocell layers in vitro, and a marked depression in the formation of neurites and fascicles without any noticeable change in the non-neuronal cells. In an in vivo study an orally administered single dose of 30 or 45 mg/kg of ETU induced necrosis of neuroblasts in fetal CNS after 18 or 24 h after dosing (68).

5.8 Effects on peripheral nervous system

There are no human or experimental animal data on the neurotoxic effects of maneb, mancozeb, zineb, or ETU.

5.9 Effects on thyroid gland and hypophyseal functions

Maneb, mancozeb and zineb. There are no data on the effects of maneb, mancozeb or zineb on thyroid gland or hypophyseal functions in man. However, Laisi et al. (87) found in rats that maneb (20 - 200 mg/kg i.p.) significantly decreased the cold stimulated TSH response while it had no effect on the TRH-stimulated TSH secretion. Zineb (70 - 500 mg/kg i.p.), in turn, significantly decreased the TSH burst induced by cold-stimulation. Maneb or zineb did not affect serum T_3 or T_4 levels. It seemed that maneb and zineb inhibit rat TSH secretion after a high single dose through an action on the endogenous TRH at the hypothalamic or pituitary level. The authors suggested that the mechanism could be the inhibition of dopamine-beta-hydroxylase in the brain (see 106). Kurttio et al. (83) found that nabam, a structural analogue of maneb and zineb, decreased serum T_4 levels, and increased serum TSH levels when given in drinking water to rats for 28 days at doses between 8.4-30.5 mg/day. Short-term exposure to nabam did not, however, cause morphological alterations in the thyroid gland.

Ivanova-Chemishanska et al. (53) gave to rats for 3 d a 0.1 LD_{50} dose of either maneb, mancozeb or zineb. The LD_{50} of these compounds were not given in the paper. EBDCs caused a diminution of the follicular diameter from about 50 µm in the control rats to 40 µm in the exposed rats. The histological picture was similar for all chemicals studied, but it was most conspicuous in maneb-treated animals. The most notable finding in electron microscopy was the dilatation of the granular endoplasmic reticulum in the follicular cells in the rats treated with maneb, mancozeb, or zineb. The main conclusion of the study by Ivanova-Chemishanska et al. (53) was that subacute treatment of rats with maneb, mancozeb, or zineb led to an increased functional activity of the thyroid gland. The intrafollicular hyperplasia and the formation of microfollicles led to markedly increased thyroid weight in maneb- or mancozeb-treated rats. These are likely to be processes which occur later, after the functional limits of the gland have been reached.

Zineb-treated rats did not show thyroid hyperplasia, perhaps due a protective action of zinc e.g. on enzymatic oxidation of iodine. Ivanova et al. (51) gave rats a single peroral dose 3.5 g/kg of maneb or 2.4 g/kg of zineb. Maneb caused a 80 %, and zineb a 90 % reduction in the accumulation of I^{131} in the thyroid. This antithyroid effect of maneb and zineb can well explain their effects on the development of follicular hyperplasia after a prolonged exposure. In another study Ivanova-Chemishanska et al. (54) gave zineb peroral doses, equivivalent to 1 or 10 % of the LD_{50} dose of zineb, to rats for 4.5 months. The higher dose of zineb caused thyroid hyperplasia in the exposed rats. The changes were consistent with a long-term stimulation of the thyroid by TRH (see also 83, 87). The findings with a single high dose and long-term administration of maneb or zineb are consistent with the assumption that the EBDC fungicides maneb, mancozeb and zineb have a strong antithyroid action, perhaps both at the thyroid and hypothalamic-pituitary level. However, in the same group, increases up to 80 % were found in the uptake of I^{131} by the thyroid 5 d after the dosing of zineb to the rats, suggesting that the effect of EBDC's on the thyroid activity is transient. Szépvölgyi et al. (150) gave male Wistar rats 0, 10, 50, 75, 113, 169, 253, or 359 mg/kg of mancozeb in the diet for 12 weeks. Mancozeb induced dose-dependently thyroid hyperplasia in the exposed rats which was associated with a decreased content of iodine relative to

thyroid weight, and protein-bound iodine. The study clearly showed that thyroid is the target organ of mancozeb.

Ivanova-Chemishanska et al. (55) have also studied the effects of 4.5 month administration of 1 % and 10 % of the LD_{50} dose of zineb on the blood levels of gonadotrophic and thyrotropic (TSH) hormone. Zineb dose-dependently increased the blood levels of both hormones. These increases were associated with morphological changes in the pituitary, e.g. increased number of basophilic cell in the anterior pituitary as an indication of increased pituitary activity. The changes in the morphology in the pituitary and increased levels of gonadotrophic hormone were associated with a complete or nearly complete loss of Leydig cells in male gonads, and replacement of the theca cells by fibrocytes in female gonads. In females, the interstitial cells were most sensitive to the effects of zineb, and were affected already by a 0.002 LD_{50} dose of zineb. The authors suggest that the pituitary changes may be secondary to the peripheral effects of zineb. In the light of more recent observations (83, 87) this conclusions may not be entirely correct. Therefore, also the effects of zineb on the gonads of both male and female rats have been reported in this chapter.

Ethylenethiourea. Smith (145) studied the effects of ETU on the thyroid functions of workers during manufacturing of the compound. Clinical examinations and thyroid function tests were carried out over a period of three years on eight process workers and five mixers and on matched controls. The results show that the exposed mixers, but not the exposed process workers, had significantly lower levels of total thyroxine (T_4) than the controls. One mixer had an appreciably raised level of serum TSH. This is the only report of the effects of ETU on thyroid functions in humans.

Laisi et al. (87) could not demonstrate effects of ETU on the humoral activity of the pituitary-thyroid axis after a single dose in the rats, whereas Kurttio et al. (83) found that peroral administration of ETU for 28 d in drinking water remarkably decreased serum T_4, and increased serum TSH levels. Similarly, O'Neil and Marshall (118) found, in a 90-d feeding study in Sprague Dawley rats, that ETU elevated serum TSH levels, and the serum T_3/T_4 ratio due to a partial inhibition of thyroid hormone synthesis. Moreover, ETU also significantly decreased the serum T_4 levels. The results showed a decreased uptake of [I^{131}] by the thyroid both in the male and female rats. ETU, in fact, inhibited the utilization of monoiodinated tyrosyl residues, and significantly reduced the active synthesis of T_3 and T_4 prohormones. No evidence was found in this subchronic rat study for the inhibition by ETU of T_4 to T_3 monodeiodination, or an interference with the normal feedback mechanisms of thyroid hormones on TSH secretion. ETU was given to Sprague Dawley rats for 90 d at 1 ppm (0.05 mg/kg/d), 5 ppm (0.25 mg/kg/d), 25 ppm (1.2 mg/kg/d), 125 ppm (6.1 mg/kg/d) or 625 ppm (31.2 mg/kg/d) in the diet. The two highest dose levels of ETU caused a very marked decrease in serum T_3 and T_4 levels, and in the same groups of rats, the serum TSH levels were dose-dependently increased. Rats which ingested 625 ppm (31.2 mg/kg/d) of ETU in the diet also exhibited a decrease in the iodine uptake by the thyroid. Moreover, thyroid hyperplasia was found in rats which ingested 125 (6.2 mg/kg/d) or 625 (31.2 mg/kg/d) ppm of ETU in the diet. Thus, the no-effect level of ETU towards functional or morphological changes in the thyroid was 25 ppm (1.2 mg/kg/d) (30).

Graham and Hansen (33) gave ETU to male Osborne-Mendel rats in the diet at 50 ppm (2.5 mg/kg/d), 100 ppm (5 mg/kg/d), 500 ppm (25 mg/kg/d), or 750 ppm (37.5 mg/kg/d) for 30, 60, 90 and 120 d. The acute oral LD_{50} for ETU was 1380 - 2560 mg/kg (mean 1830 mg/kg). ETU dose-dependently decreased animal weight gain, increased the thyroid to body weight ratio, as well as the weights of the thyroids in the exposed rats. The three highest dose levels of ETU decreased [I^{131}] iodine uptake by the thyroid, and the two highest dose levels caused thyroid hyperplasia. The no-effect levels of ETU towards functional or morphological changes in the thyroid was 50 ppm (2.5 mg/kg/d). Results from another study (34) with male and female Charles river rats, with exposure time of 12 months and doses of ETU between 5 (0.25 mg/kg/d) and 500 ppm (25 mg/kg/d) essentially confirmed the results of the earlier study by Graham and Hansen (33) with Osborne-Mendel rats. Kameda (60) reported goitrogenic effects in dogs after 6 months' of administration of ETU in drinking water at 1 g/l. During the exposure, the weight of the thyroids of the dogs increased about 30-fold, and the follicles became lined by stratified epithelium. The follicles were also essentially devoid of colloid already 1 month after the beginning of the administration of ETU. In another study (3), groups of male and female Sprague-Dawley rats were fed diets containing 75, 100 or 150 ppm (3.7, 5, or 7.5 mg/kg/d) of ETU for 7 weeks. Some of the rats were killed 2, 3, or 4 weeks after the end of the administration of ETU. During ETU exposure, there was a dose-dependent decrease in body weight gain, increase in the thyroid weight, and decrease of serum T_4 levels. These changes partially disappeared after ETU was removed from the diet indicating a reversibility of ETU-induced thyroid effects, at least after a short-term administration.

Newsome et al (112) gave 25, 50 or 100 ppm (1.2, 2.5, or 5 mg/kg/d) of ETU to male Sprague-Dawley rats for 28 d. In this study ETU had only a slight effect on thyroid weight, but it increased the thyroid/body weight ratio. However, in this study ETU was given together with NaBr (0 -2000 ppm; 0 - 100 mg/kg), and the joint toxicity of the compounds may have modified the effects of ETU on the thyroid. There were no signs of augmentation of ETU-induced thyroid effects by NaBr.

Existing evidence indicates that thyroid is the principal target organ of EBDC fungicides and ETU. The ETU-induced functional and morphological changes in the thyroid seem to be, at least partially, transient. The no-effect level for the antithyroid effects of ETU was 1.2 mg/kg/day in rat.

6 Immunotoxicology and Allergy

There are no data on the immunotoxic effects of maneb, mancozeb, zineb or ETU in humans besides skin sensitization. In one 3-week study with male Wistar rats, zineb at a dose level of 2500 ppm (125 mg/kg/d) decreased the relative thymus weight (163). There are no other data on the immunotoxicity of any of the EBDC fungicides or ETU.

Nater et al. (109) reported several cases of allergic sensitization due to exposure to maneb in the occupational environment. Patch testing with maneb-related compounds did not reveal a regular pattern of cross sensitization. Matsushita et al.

(100) found an association with exposure to EBDC fungicides and allergic contact dermatitis in an epidemiological study carried out in rural areas of Japan from 1968 to 1970 among 216 patients with contact dermatitis. Maneb, mancozeb and zineb induced sensitization with a high incidence both among female (17 %) and male (23 %) agricultural workers exposed to these EBDC fungicides. Lisi and Caraffini (93) reported a 68-year old agricultural worker with a very severe pellagroid dermatitis after an exposure to maneb-containing fungicides. Burry (14) reported several cases of contact dermatitis among agricultural workers exposed to mancozeb-containing formulations in Australia. The findings were verified by using patch testing. Kleibl and Rácková (73) also reported several severe cases of cutaneous allergic reactions towards mancozeb and zineb among agricultural workers in Czechoslovakia. The patients were patch-tested with 0.002 % solution of mancozeb and zineb, and the reactions among the exposed individuals were all positive whereas no positive reactions were found among the controls. Matsushita et al. (99) have shown, by using guinea pig maximization test, that maneb, mancozeb, and zineb had a strong potency for allergenicity. The cross sensitization among maneb, mancozeb and zineb was also strong. Sensitizing properties of maneb, mancozeb and zineb were considered much greater than those of thiurams. e.g. disulfiram, thiram, ferbam or ziram in guinea pig maximization test (101).

Matsushita et al. (99) found that ETU also has sensitizing properties in guinea pig maximization test, but the sensitizing properties of ETU were much weaker than those of maneb, mancozeb, or zineb. Moreover, there is no strong cross sensitization between ETU and maneb, mancozeb, or zineb. There are also human data on the sensitizing properties of ETU. Rudzki et al. (134) showed that among 200 individuals patch tested, ETU showed only very weak or not at all sensitizing properties. There is, however, one case report by Bruze and Fregert, (13) on ETU-induced contact dermatitis which showed also cross sensitization with maneb.

Based on experimental animal and human data, maneb, mancozeb, and zineb are potent sensitizers whereas ETU is a weak sensitizer. In the occupational environment, sensitizing properties of maneb, mancozeb, and zineb deserve attention.

7 Genotoxic effects

Maneb. Moriya et al. (105) studied mutagenicity of pesticides in bacterial reversion assay system using 5 Salmonella typhimurium tester strains (TA 100, TA 98, TA 1535, TA 1537 and TA 1538) and Escherichia coli strain WP2 hcr. The studies were carried out with and without metabolic activation. Maneb proved negative in all these studies. Likewise, maneb proved negative in a series of identical studies carried out by Choi et al. (19). Maneb did not have either the ability to induce complete or partial chromosome losses in Drosophila melanogaster males (160). Kawachi et al. (64) found that maneb did not induce mutations when tested with Salmonella typhimurium TA 100 and TA 98 strains. However, there was an indication of increased mutagenicity with metabolic activation in rec assay applying Bacillus subtilis. Results of maneb mutagenicity in all the other applied test systems, i.e. the chromosome aberration and sister

chromatid exchange (SCE) test of hamster lung fibroblast cells in vitro, human embryo fibroblast cell tests (chromosome aberrations, SCEs), rat bone marrow cell test for chromosome aberrations in vivo, and silk worm test were negative. Klopman et al. (79) showed that maneb did not have an effect in Salmonella typhimurium histidine reversion assay, Escherichia coli, or Bacillus subtilis assays. However, maneb showed some activity in Saccharomyces cerevisiae assay. Moreover, studies by Hodgson and Lee (43) suggested that ferbam, thiram, maneb and zineb may have the same, or similar, subcellular target, and presumably a common mechanism of toxicity in Chinese hamster ovary cells.

Mancozeb. Georgian et al. (31) determined cytogenetic effects of mancozeb in human lymphocytes in vitro and in rat bone marrow cells in vivo. A dose-dependent increase of chromosomal aberration frequency was found at concentrations of 1, 2, 4, 10, 20 and 40 µg/ml of mancozeb when administered during the last 24 h in 72-h human blood cultures. The two lowest concentrations of mancozeb did not induce chromosomal aberrations whereas the highest concentration was associated with a high level of heavily damaged cells, with despiralated, shattered and concomitantly separated chromatids. Mancozeb was given to Wistar rats as a single intraperitoneal dose of 2.5, 5 or 10 mg/kg, and the bone marrow cells were collected 24 h afterwards. Almost all of the rats died after the highest dose, but the two lower doses induced dose-related clastogenic effects, mainly of chromatid type. When the rats were given mancozeb for 280 d at a dose of 200 ppm in the diet with an average daily dose of 1.7 mg/kg, a significantly increased number of bone marrow cells with chromosomal damages were found.

In a well designed study, Jablonická et al. (57) analyzed chromosome aberrations and SCEs in short-term cultures of peripheral lymphocytes of 44 workers occupationally exposed to mancozeb during the production of the pesticide. The results suggested that exposure to mancozeb was associated with a slight increase, from 1.1 % in the controls to 2.07 % in the exposed workers, in the frequencies of cells with structural chromosome aberrations. However, Moriya et al. (105), by applying bacterial reversion assay systems, including Salmonella typhimurium tester strains TA 100, TA 98, TA 1535, TA 1537, and TA 1538, could not find signs of mutagenicity of mancozeb in these test systems. The results obtained by Choi et al. (19) were identical to those obtained by Moriya et al. (105). There are, however, data to suggest that mancozeb may have some genotoxic potential, not only in experimental animals but in humans as well.

Zineb. Moriya et al. (105) could not find mutagenic activity when zineb was studied in a bacterial reversion assay system applying Salmonella typhimurium tester strains TA 100, TA 98, TA 1535, TA 1537, and TA 1538. Identical results with zineb were obtained by Choi et al. (19) in similar test systems. Klopman et al. (79) analyzed the mutagenicity of zineb, and observed positive results when applying Saccharomyces cerevisiae tester strain, whereas no mutagenicity was found when Salmonella typhimurium histidine reversion assay, Bacillus subtilis assay, or Escherichia coli assays were applied. Shiau et al. (141) found, in turn, that zineb was strongly mutagenic in Bacillus subtilis strain TKJ6321, but not in Salmonella typhimurium strains, with or without metabolic activation. These results provide evidence that zineb may have slight mutagenic potential. Hodgson and Lee (43) found, by using Chinese hamster CHO-K1 cells, that zineb displays cytotoxic potential in this test system.

Ethylenethiourea. Seiler (140) found, by applying Salmonella typhimurium strain his G-46, that ETU caused a slight dose-dependent increase in the mutation frequency in this tester strain. Teramoto et al. (152) investigated the mutagenic potential of ETU in the mouse dominant lethal test. No increase in post-implantation losses (dominant lethal mutations) were found. When ETU was given together with $NaNO_2$, a significant increase was seen in the pre-implantation losses. These findings suggested that mutagenic alterations may also be induced in the mouse germ cells by an interaction of $NaNO_2$ with ETU. Autio et al. (5) found that ETU induced mutagenicity in vitro when Salmonella typhimurium tester strain TA 1950 was used. When the same Salmonella typhimurium tester strain was used in a host-mediated assay, an increase of mutagenicity was also found. The results suggested that the oxidation of the sulfur atom in ETU decreased the mutagenic potential of ETU - the mutagenicity of 2-imidazolin-2-yl was less than that of ETU. ETU exhibits mutagenic potential in some test systems, but is not a clear-cut mutagen.

8 Carcinogenicity

Maneb, mancozeb, zineb. There are no adequate data on the carcinogenic effects of maneb, mancozeb, or zineb in humans or in experimental animals (47, 65, 157). Börzsönyi et al. (15) has summarized short-term genotoxic effects of maneb and zineb; there are data to suggest that both compounds display a weak mutagenic potential in different test systems. Animal carcinogenicity data on both compounds are inadequate, according to the evaluation of the International Agency for Research on Cancer (48). There are , however, recent data which show that mancozeb dose-dependently increases the incidence of thyroid follicular carcinomas in a 24-month study with Cr1:CD BR rats at a dose level of 750 ppm in the diet (37 mg/kg/d). An increase of thyroid carcinomas was not found in Cr1:CD-1(1CR)BR VAF mice in a 18 month carcinogenicity study in which the high dose was 1000 ppm (100 mg/kg/d) (39). This is an important finding; by analogy to the other EBDC compounds, well designed studies on the carcinogenicity of maneb and zineb with rats and mice are required.

Ethylenethiourea. There are no data on carcinogenic effects of ETU in humans (144) but strong evidence of the carcinogenicity of ETU in experimental animals exists (148). IARC (48) has classified ETU as a probable carcinogen to humans (Group 2B) (see 15, 157). To date, there are data to indicate that workers can be exposed to ETU in work places, mainly in the rubber industry (44, 147) and in the agriculture and forestry (81, 85, 138).

Innes et al. (47) found an increased incidence of hepatomas in mice when a peroral dose of 215 mg/kg/d of ETU was administered for 18 months. Ulland et al. (156) administered 175 or 350 ppm (8.7 mg/kg/d or 17.5 mg/kg/d) of ETU in the diet to Charles River CD female rats for 18 months. Thereafter the rats were followed for six additional months. There was a dose-dependent increase of both follicular and papillary thyroid cancers, with pulmonary metastases, and of related lesions such as thyroid solid-cell adenomas and hyperplastic and simple goiter. The first tumor was found after 68 weeks; most cancers occurred after 18 - 24 months when the study was terminated.

Chhabra et al. (18) conducted a chronic toxicity and carcinogenicity study with ETU using F344/N rats and B6C3F1 mice of both sexes. The authors were interested whether incorporation of perinatal exposure, in addition to the conventional exposure of young adult animals for 2 years, enhances the sensitivity of the bioassay in the identification of the carcinogenic potential of chemicals when compared to the conventional exposure of animals for 2 years. The studies were designed to determine 1) the toxic and carcinogenic effects of dietary ETU in rats and mice receiving perinatal exposure up to 8 weeks of age followed by control diet for 2 years; 2) the effects of ETU in rats and mice receiving exposure for 2 years beginning at the age of 8 weeks, and 3) the effects of combined perinatal/adult exposure. During the perinatal period, rats were exposed to dietary ETU concentrations from 9 to 90 ppm (0.45-4.5 mg/kg/d) and adult exposure concentrations of ETU in the diet ranged from 33 to 330 ppm (1.65 to 16.5 mg/kg/d). In the mice, the perinatal exposure concentrations in the diet ranged from 33 to 330 ppm (3.3 to 33 mg/kg/d), and in the adults the concentrations were 100 to 1000 ppm (10 to 100 mg/kg/d). The perinatal only exposure was not carcinogenic to rats or mice while perinatal/adult combination exposures to ETU were carcinogenic both in rats and in mice. The carcinogenic effects of ETU were generally similar by adult and perinatal/adult combination protocols except that incidences of the thyroid tumours were slightly higher in the rats receiving the perinatal/adult combination of ETU exposure in the diet. The thyroid gland was the major site of ETU carcinogenicity both in rats and mice. The liver and pituitary glands were other major sites of ETU carcinogenicity in mice. In this study, the exposure to ETU for 9 months and for 2 years generally decreased serum T_4 levels and increased serum TSH levels both in rats and mice. These hormonal changes correlated with morphological changes in the thyroid gland after 9 months or 2 years of ETU exposure, suggesting that ETU carcinogenicity may be due to an imbalance of thyroid-pituitary homeostasis (41).

Based on the available evidence on the thyroid carcinogenesis, United States Environmental Protection Agency's Science Advisory Panel has concluded that 1) thyroid follicular cell tumours may arise from long-term disturbances in thyroid-pituitary hormonal feedback under conditions of reduced circulating thyroid hormone and elevated thyroid stimulating hormone (TSH); 2) the steps leading to these tumours are expected to show threshold, such that the risks of tumour development are minimal when thyroid pituitary homeostasis exists; and 3) models that assume thresholds may be used to assess the risks of certain thyroid follicular cell tumors where there is evidence of thyroid-pituitary imbalance (2).

9 Reproduction and Teratogenicity

Maneb, mancozeb, zineb. There are no data on the reproductive or teratogenic effects of maneb, mancozeb or zineb in humans. Chernoff et al. (17) found that a dose of 384 mg/kg/d of maneb, given during the period of organogenesis in the rat, causes several malformations, such as hydrocephalus and cleft palate. A single oral dose of maneb was given to pregnant rats on day 11 or 13 of organogenesis. Maneb induced congenital anomalies in 12-100 % of the fetuses at a dose range of 1-4 g/kg. The maximum dose of maneb at which no anomalies were found was

500 mg/kg in rats (122). Larsson et al. (89) found similar teratogenic effects, also in rats, with maneb and mancozeb at a dose range of 400-1320 mg/kg on the day 11 of the pregnancy. The teratogenicity of maneb was more pronounced than that of mancozeb. Administration of zinc displayed a clear-cut antiteratogenic effect. Bleyl (7) reported that when maneb was given to pregnant rats in combination with ethanol, an increased embryotoxicity as well as retardation of the skeletons and postnatal growth in F_1 generation was observed. A single oral dose of zineb to pregnant rats on the day 11 or 13 of pregnancy caused severe malformations in 12-100 % of the fetuses at doses of 2-4 g/kg. The maximum dose at which zineb was without a teratogenic effect was 1 mg/kg (122). Sing and Spencer (142) fed zineb to pregnant Sprague-Dawley rats on days 6 - 15 during pregnancy at doses of 500, 1000, 1500, 2000 or 2500 ppm in the diet (daily doses 12, 21, 30, 38, and 55 mg/kg). Zineb did not have an effect on ovarian weight of pregnant rats, but it decreased ovarian protein, and increased glycogen content in a dose-dependent fashion. Zineb also decreased fetal weight and maternal weight gain in a dose-dependent fashion.

Ethylenethiourea. Smith (144) did not find an increased incidence of congenital abnormalities among children of 699 female workers occupationally exposed to ETU in rubber industry.

There are, however, data on the teratogenicity of ETU in animals. When ETU was given to pregnant mice from the 7th to 14th d of pregnancy at a dose of 600 mg/kg/d it decreased the total number of pregnant mice, and the number of mice which survived throughout the pregnancy. This dose of ETU also decreased the number of pregnant mice which delivered pups (124). Teramato et al. (151) gave a single oral dose of 250 mg/kg and 2000 mg/kg of ETU to rats and mice, respectively. ETU, given on the 14th d of pregnancy, was teratogenic in rats. It reduced the fetal weight severely without increasing mortality. All the fetuses had externally visible malformations such as cranial meningocele, mandibular micrognathia, cleft palate, omphalocele, anal atresia, and oligodactyly. Skeletal malformations were detected in the lumbar vertebrae, sternebrae, ribs, clavicles, and long bones of extremities. Surprisingly, ETU was not teratogenic in mice even at a high dose. Chernoff et al. (17) studied ETU-induced malformations in rats, mice, guinea pigs, and hamsters. ETU, at a dose of 40 mg/kg, induced hydrocephalus and encephalocele in rats. ETU did not, however, induce malformations in hamsters at a dose of 100 mg/kg/d. In the mouse, slight skeletal malformations were observed at a dose of 200 mg/kg/d. ETU-induced malformations were not observed in guinea pigs at a dose of 100 mg/kg/d, either. Khera (67) found, however, that high doses of ETU given to Swiss-Webster mice induced hindpaw deformities in mice. Single oral doses of ETU given to the dams were 1600, 2000, or 2400 mg/kg, and it was given on d 12 of the pregnancy. Most significant defects were hindpaw ectrodactyly, syndactyly, and polydactyly, and cleft palate. Khera and Shah (69) reported that zinc acetate did not reduce ETU-induced anomalies in rats. The doses given to dams in this study were 80, 120, or 160 mg/kg. There are also data on the developmental toxicity of ETU in the CNS in rats (see Chapter 5.7; 70).

Khera (66) observed reduction of ETU-induced teratogenicity in rats after co-administration of sodium nitrite. Khera et al. (71) found increased number of malformations in pups of hamsters which were given a single intragastric dose of

600, 1200, 1800, or 2400 mg/kg of ETU on the 11th d of pregnancy. None of the doses induced maternal toxicity but the highest dose exhibited marked fetal toxicity. The ventricular system of the brain and the cerebellum were among the most sensitive sites for malformations. Daston et al. (22) have shown that ETU is teratogenic to the rat embryo in vitro at concentrations of 40 - 200 µg/ml. Korhonen et al. (78) have shown that ETU is teratogenic in the chicken embryo. Lewerenz and Bleyl (92) have shown that ETU, given orally to pregnant rats as a single oral dose of 1 - 50 mg/kg on day 17, 18, 19, or 20 of pregnancy, impair the progeny survival after maternal treatment with doses of 10 mg/kg of ETU or more. At 6 months of age, 16 and 26 % of the surviving pups from the dams treated with 10 or 20 mg/kg of ETU had hydrocephalus.

These data indicate that EBDCs have a clear-cut teratogenic potential in experimental animals at high doses. However, ETU is a more potent teratogen than the EBDCs, and rat is especially sensitive to the teratogenic effects of ETU.

10 Relation between Exposure, Effect and Response

It is difficult to obtain an exact conception of the dose-effect and dose-response relationships in humans for maneb, mancozeb, zineb, and ETU because the human data of most of the compounds are so scanty or completely lacking. Adequate human data are available, in fact, only on the sensitizing effects of these compounds, but in this context dose-effect and dose-response evaluation is problematic. Also, in the occupational environment, the exposure to maneb, mancozeb, zineb or ETU takes place mainly through the skin, but there is almost a complete absence of studies in humans or experimental animals applying a detailed analysis of the characteristics of this exposure route, cutaneous hypersensitivity studies excluded.

Mainly effects on the thyroid, carcinogenicity, and skin sensitization of maneb, mancozeb, zineb and ETU have been studied. In the following, dose-effects and dose-responses for each compound separately have been presented. Critical effects are presented in Tables 2. - 5.

Most important effects of maneb were directed towards thyroid gland, functional activity of the thyroid, and thyroid morphology. Maneb was also an extremely strong skin sensitizer, but dose-effect or dose-response relationship is difficult to establish for sensitizing effects.

Most important effects of mancozeb are directed towards thyroid functions and morphology.

Like maneb, mancozeb is also an extremely strong skin sensitizer. Moreover, mancozeb has also slight mutagenic potential, similar to its structural analogue maneb. Also the most significant effects of zineb are directed towards thyroid function and morphology, and serum T_4 levels decrease, and TSH levels increase. Recent evidence also suggests that mancozeb is a thyroid carcinogen in rat. Zineb is also a strong skin sensitizer, and it has shown some mutagenic activity in the yeast.

The most marked effects of ETU are directed towards thyroid gland; thyroid hyperplasia, decreased serum thyroid hormone levels, increased serum TSH, and after long-term exposure, thyroid follicular carcinoma are the key-effects of ETU.

Moreover, ETU is a strong teratogenic compound which causes central nervous system malformations at low doses.

Table 2. Summary of effects of maneb in experimental animals.

Daily exposure	Species	Route	Effect	Reference
20 mg/kg (single dose)	Rat	i.p.	decrease of cold-induced TSH secretion	87
384 mg/kg (organogenesis during pregnancy)	Rat	oral	malformations	17
2400 mg/kg (single dose)	Rat	oral	inhibition of [I^{131}]-uptake by the thyroid	51
1200 mg/kg twice weekly for 4.5 months	Rat	oral	inhibition of [I^{131}]-uptake, thyroid hyperplasia, increased thyroidweight	54

Table 3. Summary of effects of mancozeb in experimental animals.

Daily exposure	Species	Route	Effect	Reference
50 mg/kg 12 weeks	Rat	oral	Decreased thyroid [I^{131}], thyroid hyperplasia	150
75 mg/kg 12 weeks	Rat	oral	Increased thyroid weight	150
37.5 mg/kg/d 24 months	Rat	oral	Increased incidence of thyroid adenomas and carcinomas	45

Table 4. Summary of effects of zineb in experimental animals.

Daily exposure	Species	Route	Effect	Reference
70 mg/kg (single dose)	Rat	i.p.	Inhibition of cold-stimulated TSH secretion	87
900 mg/kg 3 daily doses	Rat	oral	Follicular hyperplasia secretion	53
90-900 mg/kg Twice a week for 4.5 months	Rat	oral	Thyroid hyperplasia, increased serum TSH, basophilia of adenohypophysis	55
2000 mg/kg on day 11 or 13 of pregnancy	Rat	oral	Malformations	119

11 Needs for Further Research

There is an urgent need to develop reliable, sensitive, and practical methods for biological monitoring of exposure to maneb, mancozeb, zineb and other EBDC fungicides as well as ETU. To date, there are methods for biological monitoring of ETU in urine and other biological fluids (81, 84, 85, 86, 138), but they require further development to be suitable for routine use in biological monitoring of exposed workers.

Understanding the mechanisms of absorption is vital for reliable biological monitoring. Dermal penetration shall be studied in detail to find out the relationship between the amounts of EBDCs and ETU deposited on the skin, and ultimately absorbed into the blood stream. Data on the particle size of EBDC aerosols in essential for the estimation of the exact role of inhalation in the assessing of exposure to EBDCs and ETU. Details of metabolism of EBDC fungicides and ETU require further clarification. This is important because information on toxicokinetics of these compounds is the only firm basis for their biological monitoring.

Mechanisms of thyroid effects of maneb, mancozeb, zineb and ETU should be worked out. All of these compounds are known strong goitrogens which decrease serum levels of thyroid hormones, elevate serum levels of TSH, and cause thyroid hyperplasia. Moreover, ETU at very low doses also causes follicular carcinomas of the thyroid. Understanding of the effects of these compounds on the thyroid and pituitary-thyroid will possibly allow us to understand the associations between the goitrogenic and carcinogenic effect. Also, association between goitrogenic effects and effects secondary to this effect may be delineated with this

Table 5. Summary of effects of ethylenethiourea (ETU) in experimental animals.

Daily exposure	Species	Route	Effect	Reference
1000 mg/kg (single dose)	Rat	i.p.	Inhibition of cold-stimulated TSH secretion	87
10.6 mg/kg for 28 d	Rat	oral	Decreased serum T_4 and increased TSH levels	83
5 mg/kg for 90 days	Rat	oral	Decreased serum T_4, increased TSH, and thyroid hyperplasia	118
40 mg/kg during pregnancy	Rat	oral	Hydrocephalus and encephalocele and other cerebral malformations	17
12.5 mg/kg for two years	Rat	oral	Thyroid follicular carcinoma, thyroid hyperplasia, decrease in T_4 and increase in TSH	18

important piece of information. Moreover, especially ETU is a strong teratogen, possibly due to its effects on the thyroid. This association should also be clarified. There are convincing data on the thyroid teratogenicity of ETU. Moreover, recent data suggest that also mancozeb may display carcinogenic potential in rats. Considering that all EBDCs are partially biotransformed to ETU, and the structural and metabolic analogy between all of the EBDC fungicides, well-designed carcinogenicity studies on maneb, mancozeb, and zineb are required.

Epidemiological human studies should be carried out to clarify the associations between exposure to EBDCs and ETU and their effects on the thyroid and reproduction, as well as their teratogenicity and carcinogenicity in workers.

12 Discussion and Evaluation

EBDCs are an important group of fungicidal pesticides. They are also a good example of compound in exposure to which dermal absorption predominates, even though the relative significance of dermal and inhalational exposure is not known. Because the dermal exposure route predominates, occupational threshold limit values are probable of limited value in the assessment of exposure, and risks derived therefrom. Because the main urinary metabolite of these compounds, ETU, is known, and can be measured, it can be utilized for the protection of workers exposed to EBDCs or ETU. ETU in the urine should be measured and

compared to a reference value at given time points after the cessation of exposure. The value of this approach is that it gives an integrated picture of biologically relevant exposure through all exposure routes. Other means by which workers can be protected from becoming exposed, is to give clear-cut guidelines on spraying equipment, and instructions for proper protective clothing.

Ethylenebisdithiocarbamates maneb, mancozeb, and zineb, and their degradation product and main metabolite ETU are used as fungicides in agriculture and forestry nurseries. Moreover, they are used as accelerators in the rubber industry. In most cases there are only few reports of their toxicity in humans. Most information is derived from animal and in vitro experiments. Even though different EBDCs exhibit somewhat different toxic effects, by and large, the common features of EBDCs, rather than differences in toxicity, clearly dominate. Common to all EBDCs is their metabolism, ETU as the main metabolite. Due to this analogy, a finding on one of the compounds can, and should, applied to some degree to the other compounds in the group. With this background, the finding that mancozeb increases the incidence of thyroid carcinomas in rat is important, and shall be clarified in detail.

Maneb. The acute toxicity of maneb is low, and mainly directed towards the thyroid gland. After single and repeated doses maneb decreases serum T_4, increases serum TSH levels, and causes thyroid hyperplasia. Maneb has the potential to induce mutations in bacterial test systems, but data on the carcinogenicity of maneb are inadequate. It induces malformations in rats at high doses, and is a very strong skin sensitizer which cross reacts with mancozeb and zineb. Goitrogenicity and skin sensitizing properties are the critical effects of maneb.

Mancozeb. The acute toxicity of mancozeb is low as that of maneb. The most important target organ of mancozeb is the thyroid where mancozeb causes hyperplasia, and distorts thyroid functions, e.g. decreases serum T_4 levels and increases serum TSH levels. Mancozeb has a low potential to induce point mutations in bacterial test systems. There are also data to suggest that mancozeb is carcinogenic in experimental animals. Mancozeb is a strong skin sensitizer which cross reacts with other EBDC fungicides. Antithyroid, and possible carcinogenic effect, and skin sensitizing properties are the critical effects of mancozeb.

Zineb. The acute toxicity of zineb is low and mainly directed towards the thyroid gland. It causes thyroid hyperplasia after long-term administration, but already a single dose of zineb decreases serum T_4 and increases serum TSH. Zineb has been mutagenic in the yeast. Data on the carcinogenicity of zineb are scanty. Zineb is a strong skin sensitizer, and cross reacts with other EBDC fungicides. Antithyroid and skin sensitizing properties are the critical effects of zineb.

Ethylenethiourea. ETU also has thyroid as its major target organ. Low doses of ETU decrease serum thyroid hormone levels and increase serum TSH. These functional changes are associated with marked thyroid hyperplasia. The most important effect of ETU is its thyroid carcinogenicity. ETU does not have, however, any marked potential to produce mutations in in vitro bacterial test systems. ETU has also a clear-cut potential to cause teratogenic effects in rodents.

Among rodents, rat is a sensitive strain, whereas mice, guinea pigs, and hamsters are more resistant. Thyroid carcinogenicity is the critical effect of ETU.

The critical toxic effects of EBDCs and ETU, notably the thyroid toxicity and carcinogenicity, the potential to cause severe malformations, and the strong sensitizing potency, deserve serious consideration and cautiousness for the protection of exposed workers. However, the exposure to these compounds in the occupational environment is usually negligible. In most cases, one can find a several hundred to several thousand fold margin of safety between the doses to which the workers can be exposed in the work places or via food, and the doses at which toxic effects have been found in experimental animals. Sensitizing property of these compounds is, in fact, the only exception to this rule. Therefore, in practice, the exposure to EBDCs or ETU is likely to be of minor toxicological significance in most cases in the work situation, and the same applies also to the exposure to these compounds via food. The toxic hazards of EBDCs and ETU can most likely be prevented, or minimized, in most occasions if proper protection is being applied.

13 Summary

K. Savolainen. 108. Ethylenebisdithiocarbamates and ethylenethiourea. Nordic expert group for documentation of occupational exposure limits. Arbete och hälsa 1993: 35, pp 157-206.

A critical survey of the literature relevant for the discussion of an occupational exposure limit for ethylenebisdithiocarbamate fungicides and ethylenethiourea is given.

Maneb. The critical targets of exposure to maneb are toxic effects of the compound on the thyroid gland, and its sensitizing and cross sensitizing properties.

Mancozeb. The critical effects of mancozeb are directed towards the thyroid gland. Critical effects are also possible carcinogenicity, and skin sensitizing properties of mancozeb and its cross reactivity with other compounds of the fungicides of the same group.

Zineb. The critical target organ of zineb is the thyroid gland. Critical effects of zineb are also its sensitizing properties, and cross reactivity with maneb and mancozeb.

Ethylenethiourea. The critical effects of ETU are directed towards the thyroid gland where ETU induces thyroid hyperplasia and follicular carcinomas at low doses.

The present occupational exposure limit values for ethylenebisdithiocarbamates are based on their sensitizing properties, on their effects on the thyroid gland, and possible carcinogenic effects in rodents. The main emphasis in the occupational exposure limit value for ETU is in its goitrogenic and thyroid carcinogenic properties. There is a need to emphasize the significance of biological monitoring, and to reduce the exposure to EBDCs and ETU rather than develop more detailed occupational exposure limits. It is important, therefore, to delineate dermal absorption, and the metabolic pathways of EBDC fungicides and ETU for the development of biological monitoring methods. Moreover, understanding of the association between the effects of EBDC fungicides and ETU on thyroid, and ETU-induced thyroid follicular carcinomas is crucial for adequate risk assessment.

Key words: Maneb, mancozeb, zineb, ethylenethiourea, occupational exposure limit value, organ effects, effects on thyroid gland, thyroid carcinoma, malformations, skin sensitization, cross sensitization.

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LIMONENE

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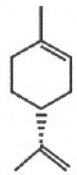
Contents

1. Chemical and Physical Data

d-Limonene

CAS No.:	5989-27-5
Systematic name:	(R)-1-methyl-4-(1-methylethenyl) cyclohexene
Synonyms:	(R)-(+)-limonene
	(+)-limonene
	(R)-limonene
	(R)-(+)-p-mentha-1,8-diene
Formula:	$C_{10}H_{16}$
Structure:	
Molecular weight:	136.23
Vapour pressure (20°C):	0.19 kPa*
Density (20°C):	0.8411
Melting point:	-74.35°C
Flash point:	48°C
Boiling point:	175.5-176.0°C
Optical rotation (α) (20°C):	+125.6°

1ppm = 5.56 mg/m^3 1mg/m^3 = 0.177ppm

* Calculated from data in literature.

d-Limonene is a colourless liquid with an odour of citrus fruits. It is soluble in organic solvents but practically insoluble in water. It is inflammable (9, 17, 45, 70, 86, 99, 131).

d-Limonene is the main constituent in oil of citrus fruits, but is also present in many other essential oils such as oils of caraway, dill, fennel and celery and in turpentine. It is obtained as a by-product from the citrus juice industry. After

mechanical extraction of the juice, the orange (or other citrus fruits) skins are treated with alkali (0.15 - 0.30% calcium hydroxide) and pressed. The press liquor is evaporated by steam distillation and the d-limonene is recovered from the condensate. The purity of the distillate is 90 - 98%. The main inpurities in d-limonene are other monoterpenes such as myrcene, α- and β- pinene, sabinene, Δ3-carene. Autooxidation of limonene readily occurs which gives a variety of oxygenated monocyclic terpenes (carvone, limoneneoxide, carveol and hydroperoxides of limonene). This gives the liquid a yellow colour. If the oxidation process continues polymers are created and the liquid will become viscous (68, 69, 110, 119).

l-Limonene

CAS No.:	5989-54-8
Systematic name:	(S)-1-methyl-4-(1-methylethenyl)cyclohexene
Synonyms:	(S)-(-)-limonene
	(-)-limonene
	(S)-limonene
	(S)-(-)-p-mentha-1,8-diene
Formula:	$C_{10}H_{16}$
Structure:	
Molecular weight:	136.23
Vapour pressure (20°C):	0.19 kPa*
Density (20°C):	0.8422
Melting point:	-74.35°C
Flash point:	48°C
Boiling point:	175.5-176.0°C
Optical rotation (α) (20°C):	-122.1°
1ppm = 5.56 mg/m^3	1mg/m^3 = 0.177 ppm

* Calculated from data in literature.

Description see above for d-limonene.

l-Limonene is found mainly in pine-needle oils and turpentine but also in, for example, spearmint and peppermint. It is obtained by purification of isolated monoterpenes from certain pine needle oils, or synthetically from pinene by acid catalysis (9, 17, 45, 70, 86, 100, 131).

Dipentene

CAS No.:	138-86-3
Systematic name:	1-methyl-4-(1-methylethenyl)cyclohexene
Synonyms:	dl-limonene
	p-mentha-1,8-diene
	4-isopropenyl-1-methyl-1-cyclohexene
	cinene
	cajeputene
Formula:	$C_{10}H_{16}$
Molecular weight:	136.23
Vapour pressure (20°C):	0.19 kPa*
Density (21°C):	0.8402
Melting point:	-95.9°C
Flash point:	43°C
Boiling point:	175.5-176.0°C
1ppm = 5.56 mg/m^3	1 mg/m^3 = 0.177ppm

* Calculated from data in literature.

Description see above for d-limonene.

Dipentene is the racemic mixture of d- and l-limonene and can be obtained by mixing equal parts of these. Dipentene of high purity (95%) can be manufactured by thermal isomerization of α-pinene (9, 17, 45, 70, 86, 131).

2. Occurrence and Use

2.1. Use

d-Limonene

Because of the importance of replacing chlorinated hydrocarbons, chlorofluorocarbons (CFC) and other organic solvents with substances thought to be less toxic, industry has shown great interest in d-limonene-containing products. d-Limonene is used in products for degreasing metals (content of limonene 2 - 30%) before industrial painting and for cleaning assemblies (content of limonene 50 - 100%). It is also used for cleaning in the printing industry (content of limonene 30% - 100%) (15) and in paints as a solvent. d-Limonene has replaced xylene in many histological laboratories for preparation of histological and cytological specimens.

d-Limonene is used for flavoring in provisions and as perfume. The concentration of d-limonene ranges between 0.005% to 1% (99). It is often added to perfumes due to a declared effect of quenching the allergenicity of other related compounds (99).

d-Limonene might be added to terapeutic transdermal delivery systems to enhance the penetration of the active substance (96, 97, 103, 147).

d-Limonene can be used as an antifungal agent (93).

l-Limonene

l-Limonene is used as a fragrance material. The concentration of l-limonene ranges between 0.005% to 1%. However, d-limonene is the main product used.

Dipentene

Dipentene is used in many products for the same reason given above for d-limonene.

Limonene (no enantiomeric form given) has antimicrobial, antiviral, antifungal, antilarval, and insect attractant or repellent properties (115).

According to the product register from the Swedish National Chemical Inspectorate from August 1992 a total amount of 11-120 metric tons of d-limonene is used in 24 different products in Sweden. The corresponding figures for dipentene are 110-1700 metric tons and 49 different products. No use of l-limonene was reported.

d-Limonene

Function	Number	Conc.(%)	Total amount (metric tons)
Paint and laquer	4	17-40	0.84-8.4
Cleaning material, incl. car cleaner	7	0.1-14.3	1.9-17
Degreasing material	9	5-99	6.8-83
Washing liquid & solvents	2	99	0.99-9.9
Tensides	2	10-80	0.18-1.8
Total	24		11-120

Dipentene

Function	Number	Conc.(%)	Total amount (metric tons)
Paint and laquer	5	1-10	0.14-5.7
Cleaning material	6	1.5-100	0.2-5.0
Degreasing,washing liquid	6	2-100	3.5-32
Solvents	3	1-100	1.1-11
Flotation material and friction material	2	5-25	0.60-10
Paint, laquer, paint remover, and glue	15	1-30	100-1500
Polishing material	4	1-10	0.016-0.22
Paint thinner	6	1-100	0.26-11
Other	2	4-6	4-50
Total	49		110-1700

2.2. Air concentrations in work environment

d-Limonene. Air concentrations were measured when degreasing metals before industrial painting in two factories. The products used for degreasing usually consist of a solution of water, limonene and tensides in various concentrations. In the first factory the details were dipped into a built-in bath containing 6% d-limonene. Air concentrations measured around the bath averaged 0.9 - 6 mg/m^3. In the second factory the degreasing was performed manually in an open bath containing 2-3% d-limonene, but also by high-pressure washing, using a product containing 7% d-limonene. Air concentrations measured during the whole day averaged 50 mg/m^3, while the concentrations raised to 80-100 mg/m^3 when the manual handling was carried out. Air concentrations measured during the high-pressure washing averaged 400 mg/m^3.

Air concentrations were also measured when cleaning electronic assemblies in a small built-in bath containing 46% d-limonene. The average concentration around the bath was 10 mg/m^3, but concentrations of 200 mg/m^3 were measured during manual handling (13).

Air concentration measurements in printing work averaged 69.7 mg/m^3 (stationary samples) and 22.7 mg/m^3 (personal samples) in one study (16), while in the second study only concentrations about 2 mg/m^3 were found. (13). The products in the first study consisted of about 50% d-limonene, 15% glycol ethers, 15% natural oils and 5% tensides.

l-Limonene. No data found.

Dipentene. The total air concentrations in four joineries of the terpenes α- and β-pinene, Δ^3-carene and limonene varied between 19-123 mg/m^3. The ratio between the different terpenes was 10:0.8:4:0.2. Thus the content of limonene was very low (31).

Air concentration of limonene was measured at 16 different work places in Norway during 1985 - 1992 according to data from the National Exposure Database at the National Institute of Occupational Health, Oslo, Norway. Most of the measurments were performed during printing work. The arithmetic mean for all measurements was 28 mg/m^3 (range 0-886 mg/m^3) (41).

2.3. Air concentrations in housholds

Personal exposure and indoor-outdoor air concentrations of limonene (the enantiomeric form not given) were determined in households in Los Angeles. Mainly two consecutive 12 h samples were collected (6 AM-6 PM, 6 PM-6 AM). Daytime arithmetic mean personal exposure and indoor concentration were both determined to 4x10^{-2} mg/m^3, while the corresponding value for outdoor concentration was found to be 0.2x10^2 mg/m^3. Breath samples taken in the evening, and the following morning and evening, showed considerable stability even though personal air exposures were more variable. The median concentrations in breath were 1.2-2.4x10^{-2} mg/m^3 (139).

2.4. Methods for analysis of air concentrations

Diffusive samplers with charcoal are recommended for analysis of air concentrations of terpenes. The terpenes are desorbed with carbondisulfid and analysed by a gas chromatographic method (85). This method has been used with sampling rates from 7.2 ml/min (32), 30 ml/min (122) and 50 ml/min for long-time sampling, and also 250 ml /min for short-time sampling (13).

3. Kinetics

3.1. Uptake

3.1.1 Uptake by inhalation

d-Limonene is taken up easily from the alveoli, as suggested by a relatively high solubility in human blood. The partition coefficient blood/air in vitro is 42 (37). The relative uptake of d-limonene in human volunteers, exposed for 2 hours during light physical exercise (50 W) to 10, 225 or 450 mg/m^3 d-limonene, has been determined to, in average, 68% for the two higher concentrations and 63% at the lowest exposure level (38) .The concentration of d-limonene in arterial blood was found to increase rapidly at the beginning and to level off towards the end of the exposure (38).

3.1.2. Uptake through the skin

d-Limonene. The data on uptake by skin absorption are very sparse. In an experimental study on dermal uptake of d-limonene the subject was immersing one hand in a glass jar with d-limonene (98%) during 2h. The concentration of d-limonene in blood was found to be low compared to inhalation exposure (10 mg/m^3, 50 w). 140 min after start of exposure the concentration of d-limonene in

the arterial capillary blood of the non-exposed hand was approx. 1.4 x 10^{-6} mol/l (36).

The effects of a single dermal application of a commercial insecticidal dip containing d-limonene (78.2%) and tenside polysorbate 80 - a polyoxyethylene derivate - were studied in cats. Different groups of cats were thoroughly wetted with the dip in different concentrations of limonene in water (6.5 x 10^{-2} mol/l-1 mol/l). No measurments of the uptake were performed. A systemic toxicological effect was seen at higher concentrations. However, the effect of uptake by licking and inhalation must be taken into consideration as well as an eventual effect of other components in the product (58).

d-Limonene is used as a penetration enhancer of drugs in transdermal delivery systems (103, 130). The effect of which is considered due to lipid disruption in stratum corneum (147).

l-Limonene. The percutaneous uptake of limonene from a bath with different concentrations of pine-needle oil was studied in man by estimation of the concentration in the expired air. The enantiomeric form is not given, but according to literature, mainly l-limonene is present in pure needle oil. By injecting known amounts of terpenes intravenously, the proportion eliminated unchanged in expired air was determined. The authors calculated that the absorption rate of limonene (from a 0.01% water solution) was about 0.02 µl/cm^2 x h (2 x 10^{-4} l/m^2 x h), which is of the same order of magnitude as for oxygen, radon and carbon dioxide and 100 times larger than for water (113).

Dipentene. The percutaneous absorption of dipentene (together with menthol, camphene, isoborneolacetate and α-pinene) as a constituent of a foam bath was measured in mice using radioactivly labeled ingredients. The concentration of dipentene in the bath was 1.9x10^{-5} mol/l (2.62 µg/ml). The exposure time (5 - 40 min) was varied as well as the area of the shaved, depilated skin (1.5 - 6 cm^2). Blood levels were a direct function of the size of the skin area involved. A maximum blood level was obtained after 10 minutes exposure giving a value of about 11 x 10-2 mg dipentene/l blood when an area of 6 cm^2 was exposed (121).

3.1.3.Uptake from the gastrointestinal tract

The absorption of d-limonene from the gastro-intestinal tract is rapid. Following oral administration of 800 mg 14C-labelled d-limonene /kg bw to rats, the blood level of radioactivity reached a maximum 2 hours after administration and declined after maintaining high levels for 10 hours. 24 hours after the administration the level of radioactivity in the blood was negligible (60).

3.2. Distribution

After absorption d-limonene disappears rapidly from blood. This is mainly due to the distribution to different tissues and the biotransformation. The blood clearance in human after exposure to 450 mg/m^3, measured up to 21 hours after termination of exposure, has been determined to 1.1 l/kg x hour (38). A high solubility of d-limonene in olive oil (calculated partition coefficient oil/blood=140) as well as a

long half-life in blood, in the slow elimination phase, indicates affinity to adipose tissues (37, 38).

In a study in rat, maximal radioactivity was measured within two hours after oral administration of 800 mg 14C-labeled d-limonene/kg bw (60). The concentrations in the liver, kidney and adrenal were higher than concentrations in other tissues. The radioactivity in the liver was highest one hour after administration and equivalent to 2.5% of the administered radioactivity (60). Tissues other than the liver, kidney and blood contained less than 0.2% at any time examined (60). Negligible amounts in all tissues were found 48 hours after administration (60).

Species differences in renal disposition and protein binding of d-limonene has been observed. In rats, d-limonene exhibits sex-dependent renal disposition (82, 146). 24 hours after administration of d-limonene by gavage, the renal concentration of d-limonene equivalents has been reported to be about 3 times higher in male rats than in female rats (80, 82). The total amount of d-limonene equivalents in male rat kidney represented about 0.15 % of the administered dose, and of this approximately 40% was bound reversibly to the male-rat specific protein α2u-globulin (80, 82). No significant interaction between d-limonene equivalents and female rat kidney proteins was reported (80,82).

In mice, no sex difference in the renal disposition of d-limonene has been observed. The amount of d-limonene equivalents in the kidney 24 hours after an oral dose accounted for about 0.02% of the administered dose and there was essentially no binding to kidney proteins or any interaction between d-limonene equivalents and the major urinary protein in male mouse (MUP) (79,80). Binding studies in vitro has confirmed that MUP does not bind d-limonene-1,2-oxide (80).

3.3. Biotransformation

The biotransformation of d-limonene has been investigated in several species. There are some different pathways in the metabolism, including oxidation at the 8,9-double bond or the 1,2-double bond, oxidation of methyl groups to hydroxyl and further to carboxylic acid derivatives, ring hydroxylation at the C-1 and C-6 position, and glycine or glucuronide conjugation. Species differences in plasma and urinary metabolites have been observed.

In human, d-limonene is converted, to a large extent, to p-menth-1-ene-8,9-diol (uroterpenol; see fig 1: M-II) and excreted as a glucuronide (see fig 1; M-VI) in the urine (76, 125). The 8,9-diol is believed to arise from the corresponding d-limonene-8,9-epoxide (76,141). The percentage of the amount excreted as d-limonene-8,9-diol and its glucuronide in man, was in one study (76) reported to be about 25-30% of an orally administered dose. In other species (see fig 1), that pathway accounted for about equal or a smaller part of the amount excreted in the urine (76).

Oxidation at the 1,2-double bond also has been described. In an abstract (20) limonene-1,2-diol was reported to be present as a human circulating metabolite. In studies in vitro the formation of the 1,2-epoxide was determined in rats and mice, when liver microsomes were incubated with d-limonene (79, 141). The results indicated that cis-d-limonene-1,2-oxide was a minor metabolite both in rats and

mice, representing less than 1% of the total d-limonene metabolized (79). In mice small amounts of the trans-isomer of the epoxide also was found (79, 80).

An alternative pathway of biotransformation, include perillic acid (see fig 1; M-III) and metabolites derived through perillyl alcohol and perillic acid (see fig 1; M-IV, M-VII, M-VIII, M-IX) (76, 105). About 30-50% of an orally administered dose, was in one study (76), excreted in the urine as perillic acid and metabolites derived through this pathway in rodents, while only 7-11% of the dose in man could be accounted for in this way. Perillic acid, dihydroperillic acid and their methyl esters are among the compounds reported to be present in plasma in rat and human after administration of d-limonene or orange oil, containing 95 % d-limonene (19, 20). The former two were reported as major circulating metabolites in both species (20).

Other metabolic pathways have also been reported to occur . Ring hydroxylation of d-limonene at the C-6 position (see fig 1; M-X, M-XI) and to some extent at the C-1 position (rat) has been observed (76, 105). In humans, less than 7% of the dose in one study (76) was identified as a triol, probably p-menth-1-ene-6,8,9-triol (see fig1; M-XI), in the urine after peroral administration of d-limonene (76). Limonene-6-yl acetate has been reported to occur in human urine, after ingestion of pine oil containing 6% limonene (unspecified) and is also most likely a metabolite of limonene (77). Further, metabolites obtained after oxidation of the C-10-methyl group (see fig 1; M-I, M-V) has been reported to be present in the urine after administration of d-limonene, but only in small amounts (~1% in man) (76).

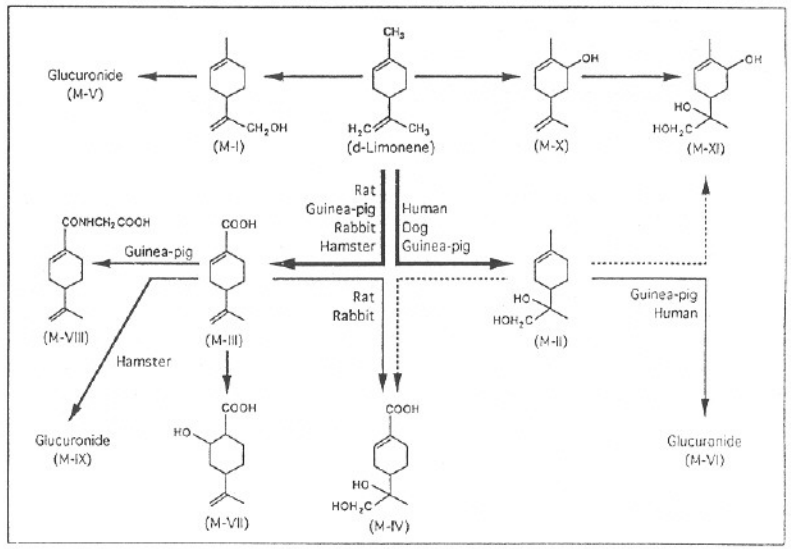


Fig 1. Some metabolic pathways of d-limonene (76).

3.4. Elimination

Three different phases of elimination of d-limonene have been observed in human blood (38). The half-times in blood reported after exposure by inhalation for 2 hours to 450 mg d-limonene/m^3 are approximately 3 minutes, 33 minutes and 750 minutes (38) .

d-Limonene seems to be metabolized to a very large extent. The metabolites have been found to be excreted mainly in the urine. Male human subjects, given 1.6 g d-limonene (^{14}C) per os, were found to excrete 55 -83% of the radioactivity in the urine in 2 days (76). When the elimination of d-limonene was investigated in a study (38) on human volunteers exposed by inhalation (450 mg/m^3 for 2 hours), about 1% of the total uptake was found to be eliminated unchanged in the expired air after end of exposure, while approximately 0.003% was eliminated in the urine. The excretion of metabolites was not investigated.

After oral administration of 14C-labelled d-limonene to animals 60-95% of the radioactivity has been reported to be excreted in the urine during 2-3 days, mostly during the initial 24 hours (60, 73, 76). Faecal excretion accounted for less than 10% of the dose during 2-3 days and only 2% of the administered radioactivity was found, within 48 hours, in expired CO2 (60, 73, 76). In bile duct cannulated rats about 25% of an administered dose (14-C) was excreted in bile within 24 hours (60).

4. General Toxicology

Limonene is easily oxidized by the athmospheric oxygene to a variety of products. However, there are few studies concerning the effects of d-limonene (limonene) on the organism, where this fact has been taken into consideration. The effects studied might therefore in part be due to other compounds than limonene itself and/or its metabolites.

4.1. Toxicologic mechanisms

An effect of the level of cholesterol in serum and liver has been observed at peroral administration of d-limonene to animals (4, 104, 145). Explanations for the decreased levels of cholesterol observed in rats, might be an increased catabolism or a decrease of gastro-intestinal absorption of cholesterol (4). However, a decrease in hepatic 3-hydroxy-3-methylglutaryl-coenzym A (HMG-CoA) reductase activity has also been reported (14, 104). It is possible that the cholesterol-suppressive action, as well as the anticarcinogenic action of d-limonene (or metabolites) found in animal studies, at least to some extent are expressed at the level of HMG-CoA reductase (104). HMG-CoA reductase is an enzyme active in the biosynthesis of mevalonate, which is a precursor of isoprenoid molecules for example cholesterol. Tumor cells as well as normal cells need mevalonate products to grow (19, 35, 117).

Isoprenes, derived through mevalonate, have been demonstrated to be required for modification of proteins mediating cell growth and division, such as ras proteins, to achieve activation (19, 55, 83, 117). Activated ras proteins are capable

of stimulating cell growth (19). Ten to 20 percent of all human tumours as well as a large percentage of carcinogen- induced mammary tumours have been shown to have activated ras genes (117, 149). d-Limonene, and even more metabolites such as perillic acid, dihydroperillic acid and perillyl alcohol have been shown in cells in vitro to selectively inhibit isoprenylation of ras-like small proteins (19, 48, 51). This observation provides one explanation for the mechanism of action against chemically induced tumours in vivo, such as mammary tumours (19, 48). The fact that d-limonene inhibits protein isoprenylation in more than one cell type may explain its efficacy against other tumour types as well (19). The effective chemopreventive and chemotherapeutic activity of d-limonene at subtoxic doses in vivo could be caused by the greater dependence of malignant cells, compared to normal cells, on one or more isoprenylated growth control associated proteins (19).

Other mechanisms believed to be important for the anticarcinogenic effect of d-limonene are effects on enzymes involved in the metabolic activation or detoxification of carcinogens or in the repair of DNA adducts (90, 91, 126, 143, 144). These mechanisms could be of importance especially when d-limonene is administered during the initiation stage (47, 90). An increase in the content and activity of phase I hepatic detoxification enzymes, such as for example cytochrome p-450, has been reported in some studies with limonene (4, 5, 90). Recently, an induction of phase II conjugation enzymes, such as glutathione transferase and UDP-glucuronyl transferase also was described (48, 92, 150). These increased activities of phase II enzymes has been shown in studies with the carcinogen 7, 12-dimethyl-benz(a)anthracene (DMBA) to correlate well with an increase in urinary excretion of DMBA and DMBA-derived metabolites and with a significant decrease in DMBA-DNA adduct level in liver, lung, spleen and kidney (21, 48, 90, 92). When 5% d-limonene was given in the diet for 2 weeks prior to administration of DMBA, the d-limonene-fed rats excreted twice the amount of radiolabeled DMBA and/or DMBA-derived metabolites in the urine compared to control rats. There was approximately a 50% decrease in DMBA-DNA adduct level in the tissues measured 24 h after carcinogen exposure (21, 90).

d-Limonene has been shown to cause a renal disease, known as α2u-globulin nephropathy. This nephropathy, which occurs only in adult male rats, has also been observed following exposure to a number of other industrial and environmental chemicals (1, 2, 53, 87, 101, 146). The disease is characterized by a sequence of renal changes, observed using light microscopy. First, an extreme hyaline droplet (α2u-globulin) accumulation within the cytoplasm of proximal convoluted tubule (PCT) epithelial cells, primarily in the P2 segment, is noticed. This is caused already by acute dosing, but is reversible if exposure ceases after a short time period (1). With continued exposure cytotoxicity, necrosis and exfoliation of individual epithelial cells, as well as, a compensatory cellular proliferation is caused. At subacute administration, a granular cast formation (necrotic cell remains) in the outer zone of the renal medulla and dilation of the affected tubule segment is observed. As a late effect, linear mineralization of tubules within the renal papilla and an increased severity and earlier onset of the multiple cortical alterations normally present in ageing rats (especially males), collectively classified as chronic progressive nephrosis, can be seen (1, 30, 65, 66, 106, 123, 129, 146). In addition to these successive pathological changes,

characterizing the α2u-globulin nephropathy, renal tubule hyperplasia and neoplasia may develop.

The first step in this chain of events seems to be the accumulation of α2u-globulin. α2u-Globulin is the major urinary protein in male rats of all strains tested, except one, the NBR strain, but not in female rats (23, 30, 80, 87, 106, 108, 109). In other species, including man, this protein has not been reported to occur (2, 23, 87, 98, 129). The observed increased accumulation of α2u-globulin in renal tubular cells in male rats, after exposure to d-limonene, is thought to depend on the formation of a reversible complex between α2u-globulin and a metabolite of d-limonene, cis-d-limonene-1,2-oxide (11, 81, 82, 87). The formation of this complex is accompanied by a change in the renal metabolism of α2u-globulin (probably an induced defect in the renal lysosomal degradation of α2u-globulin), leading to the accumulation of large lysosomes (hyaline droplets) in the epithelial cells of the proximal convoluted tubules (23, 67, 81, 87, 98). This excessive protein accumulation within the epithelial cells is considered to lead to lysosomal disruption and cell injury (65, 88, 129).

The increased renal tumour formation observed in male rats after chronic administration of d-limonene, is thought to have its origin in the elevated rates of cellproliferation in tubule epithelial cells, caused by the recurrent cytotoxicity. Proximal tubule cell proliferation has been shown to be a persistent phenomenon in long-time exposure to compounds causing α2u-globulin accumulation, while neither hyaline droplet accumulation nor cell proliferation has been observed some days after end of exposure, at short-time administration (24, 123, 124). This mechanism, which assumes a threshold, could involve an increased rate of spontaneous mutational events and/or a promotion /progression of initiated cells (24, 30, 46, 87, 95, 123, 128, 129).The assessment of the genotoxic properties of d-limonene by a battery of tests, that has shown that d-limonene is essentially non-genotoxic (3, 15, 34, 43, 56, 94, 95, 102, 126, 132, 140, 141), support the idea of an epigenetic mechanism. The absolute sex-specificity, the involvement of hyaline droplet accumulation in the early nephrotoxicity, as well as the low incidence of renal tumours recorded for d-limonene are other facts that suggest that d-limonene act via another mechanism than the "classical" renal carcinogens such as the nitroso compounds (30, 42). Furthermore, d-limonene has been reported in promotion studies to cause cell proliferation and to promote tumours in the male rat kidney in a conventional strain, the F344 rats, but not in the α2u-globulin deficient NBR strain (24). This study thereby forms a basis for the hypothesis that α2u-globulin have a crucial role in the development of neoplastic (and nonneoplastic) kidney lesions.

4.2. Acute toxicity

Acute toxicity for d-limonene, expressed as LD_{50}-values, is indicated in Table 1. The acute oral LD_{50} for l-limonene in rats has been reported to be about the same as for d-limonene, that is >5 g/kg (Moreno, 1975, cited in 100). The LD_{50}-values (d-limonene and l-limonene) at dermal application on rabbits has also been reported to exceed 5g/kg (Moreno 1972, cited in 99; Moreno,1975, cited in 100).

Table 1. Median lethal dose (LD_{50}) values for d-limonene in animals (134).

Species	Sex	Route	LD 50 (g/kg bw)
mice	male	perorally	5.6*
mice	female	perorally	6.6*
rats	male	perorally	4.4
rats	female	perorally	5.2
mice	male	intraperitoneally	1.3*
mice	female	intraperitoneally	1.3*
rats	male	intraperitoneally	3.6
rats	female	intraperitoneally	4.5
rats	male	intravenously	0.125*
rats	female	intravenously	0.110*

*The value is probably given as ml/kg bw and has not been recalculated to g/kg bw.

4.3. Chronic toxicity

Slightly lower mean body weights, but no compound-related clinical signs, were observed during a 2-year NTP study (see Chapter 8), in the high-dose groups of male and female rats, dosed with 150 and 600 mg d-limonene/kg bw/day respectively and in female mice dosed with 1000 mg d-limonene/kg bw/day (high-dose group)(95). In female rats (high-dose group) the survival after week 39 was also significantly reduced. Mean body weights in the low-dose groups and in the male mice high-dose group (500 mg/kg bw/day) were similar to those of the controls (95).

5. Organ Effects

5.1. Effects on skin and mucous membranes

5.1.1. Irritancy

d-Limonene is considered a skin irritant (18, 40). Older reports ascribe occupational cutaneous diseases to citrus fruits, but they are not very conclusive. The exposure was complex with many factors contributing to the dermatitis. The cutaneous hazards also included extraneous allergens, mechanical trauma, irritation and secondary infections from wet work. Most cases reported were irritant contact dermatitis (8, 10, 120). Skin reactions among consumers of citrus fruits are seldom seen by dermatologists since the problems disappear when the fruits are avoided (62).

In an experimental study on dermal uptake of d-limonene the subject was immersing one hand in a glass jar of d-limonene (98%) during 2h. Painful itching and burning occured within a few minutes after start of exposure, and increased throughout. Itching decreased at the end of the exposure while burning continued to increase for 10 more minutes. The dorsal skin was moderately erythematous and swollen. The swelling was gone at 1.5 h post-exposure, but 6 h after end of

exposure a severe purpuric eruption appeared, which was maximal after 1-2 days and remained for several weeks (36).

Skin irritancy of d-limonene was demonstrated in a dose-response study in rabbits (97). Irritancy was also studied in cats dipped in various concentrations in water of an insecticidal dip consisting of d-limonene 78.2% and a tenside (polysorbate 80 - a polyoxyethylene derivate). Normal usage concentration 6.5 x 10^{-2} mol/l of limonene in water and a control dip containing only polysorbate 80 in water gave no abnormal signs. When raising the concentration of the dip skin symptoms appeared. Erythematous and excoriated skin was seen in the peritonal region and scrotum of male cats that were dipped in the highest concentration of the dip corresponding to a concentration of 1 mol/l of d-limonene. Histopathology showed a mild to moderate, acute epidermitis, with multifocal erosions. Several of the more severe lesions obtained from the most concentrated solution comprised multifocal ulcerations of the overlaying epidermis with expensive infiltration of neutrophils and mononuclear inflammatory cells, and with necrotic debris in the subjacent dermis. The authors presume that the superficial acute necrotizing epidermal component of the dermatological condition was secondary to dermal infiltration and self-trauma (58).

Eight healthy subjects were exposed to d-limonene (10, 225 and 450 mg/m^3) in an exposure chamber. The subjects experienced no irritation in the eyes and nose. (38).

5.1.2. Allergy

Reports on allergic contact dermatitis where d-limonene is the only causative agent are not found. d-Limonene is considered the principal sensitizer in citrus species, but the incidence of contact allergy from these fruits is unknown (62). d-Limonene is included among the fragrance allergens (22, 138). Of 179 patients with contact allergy to fragrances two reacted to d-limonene (116). Contact allergy to both d- and l-limonene is described in a patient originally sensitized to turpentine (25). Allergic contact dermatitis from dipentene in paint thinner and honing oil has been described (12, 112).

A human maximization test with d-limonene was carried out in 25 volunteers, but no contact sensitization was provoked (49).

In a comprehensive study using guinea pig methods, contact sensitization to limonene (the enantiomeric form not given) was obtained in three out of four tested methods (71). No significant sensitization was obtained when d-limonene was tested in mice (89). Experimental studies in guinea pigs with d-limonene of high purity (98%) gave no significant sensitization. However, after prolonged air exposure (2 months) to the solvent, new experiments gave a clear sensitization. Chemical analysis of the sensitizing d-limonene identified the oxidation products: limonene oxide, carvone, and carveol. All but carveol were found to be potent sensitizers in animal experiments (68, 69).

A case of immune-mediated dermatopathy in a dog due to d-limonene is reported (44). The dog was dipped with an insecticidal dip containing 78.2% d-limonene. The dog subsequently developed a bullous skin disorder which rapidly progressed to severe coalescing necrotizing dermatitis with large areas of skin sloughing, which was diagnosed as toxic epidermal necrolysis. However, no histopathology was performed.

d-Limonene used as a quenching agent is discussed in Chapter 6.

5.2. Effects on the respiratory tract

Eight healthy subjects were exposed to d-limonene (10, 225 and 450 mg/m^3) in an exposure chamber; 10 mg/m^3 served as control level. The work load, 50 W, was chosen to imitate the physical activity generally found in physically light industrial work. The subjects experienced no irritation in the eyes and nose nor in the larynx and lower respiratory areas. A statistically significant decrease in vital capacity was observed after exposure to d-limonene at 450 mg/m^3 compared to 10 mg/m^3. The change in vital capacity was of low magnitude (-2%) and probably of no functional significance. No change was seen in other lung function varaibles: forced expiratory volume after one second, peak expiratory flow, residual volume, total lung capacity, mean expiratory flow at 50% vital capacity and airway resistance (38). In a study of the air exposure and symptoms of workers in four joineries the prevalence of symptoms from eyes and nose was more pronounced in the workers than in a reference group. The prevalence of symptoms from larynx and lower respiratory areas was in accordance with that of the reference group. However, the symptoms were mainly considered to be caused by exposure to wood-dust. No reduction of the lung function was seen in the workers in the studied joineries (31).

Occupational asthma was found in a worker exposed to concentrated perfume consisting mainly of isoamylacetate, limonene, cinnamaldehyde and benzaldehyde. No specific measurements of the components in air were performed (63).

The effects of d-limonene, squalene and phytol on the lungs of hamsters and rats have been studied (84). Groups of rats and hamsters received weekly intratracheal instillations of 0.2 ml of either of the substances. Autopsy was performed on animals found dead after a few instillations. After the sixth instillation, the animals were sacrified at intervals. Twenty-four hours after the third instillation there was destruction of bronchial and bronchiolar epithelium with alveolar hemorrhage. Six weeks after the last instillation the lung of the hamster was enlarged, pale and vesiculated; some showed subpleural blebs. The alveolar spaces were distended and the septum atrophic in areas. Scanning electronmicroscopy revealed fenestrated alveolar walls. The lesions were more acute in the rats and most of them died within a few hours after instillation of the substances. The reference cited is just a congress abstract and no full report is found. The author does not discuss the effects of each different substance in the abstract.

5.3. Hepatic effects

Data on humans are scarce. In animals d-limonene has been shown to affect the content and activity of liver enzymes, liver weight, the level of cholesterol and bile flow.

Slightly prolonged hexobarbital induced sleeping time has been observed in mice at single oral administration of 3 ml d-limonene/kg bw (133). A significant

increase in cytochrome P-450 and δ-aminolevulinic acid (ALA) synthetase activity has been reported in rat, 24 hours after a single oral dose of 1200 mg d-limonene/kg (4). At the dose 600 mg/kg, only a transient increase (less than 24 hours) in δ-ALA-synthetase activity was noted (4). In another study (5) a small but significant ($p<0.05$) increase in the content of cytochrome P-450 was observed in female rats at the dose level 40 mg/kg/day, following administration of three daily intraperitoneal injections of unspecified limonene.

A significant increase in the content of total cytochrome P-450 and in activity of epoxide hydratase (EH) has recently been reported in rats after administration of 5% d-limonene in the diet for 2 weeks (90). Increased EH activity was seen as well, when 1% d-limonene was administered (90). An increase in glutathione transferase and UDP glucuronyl transferase, i.e. phase II detoxification enzymes, has also been described in an abstract after administration of 5% limonene in the diet (92). In mice, induction of glutathione-S-transferase was observed when 20 mg limonene was given by gavage once every two days for three days (150).

After repeated peroral treatment of rats with 400 mg d-limonene/kg for 30 days, an 20-30% increase in the content and activity of liver enzymes (cytochrome P-450, cytochrome b5, aminopyrine demethylase, aniline hydroxylase) as well as a slight increase ($p<0.05$) in relative liver weight and hepatic phospholipid content has been observed (4). An increase in relative liver weight (significant at 300 mg/kg bw) was also reported in rats after peroral administration of 75-300 mg d-limonene/kg bw, 5 or 20 times (66). In another study in rats (146), with administration of 2-75 mg d-limonene/kg bw/day by oral gavage, 5 days/week for 91 days, a significant increase in relative liver weight was observed at 75 mg/kg. No light-microscopicy histopathological changes in the liver were reported in the studies (66, 146) and the increased relative liver weights might reflect a microsomal induction (66, 146). Activity and content of liver enzymes was not investigated in the two latter studies.

In dogs, an increase in serum alkaline phosphatase levels and an increase in serum cholesterol (35%) after administration of 1.2 ml d-limonene/kg bw/ day by gavage (~1000 mg/kg bw/day) for up to 6 months has been reported (145). At a lower dose (~100 mg/kg bw/day) no treatment-related differences were noticed. In a 28-day pilot test slightly increased absolute and relative liver weights but no evidence of treatment-related injury at histopathological examination was observed after administration of 1.2 ml/kg bw/day (145).

A 49% decrease in liver cholesterol and an 8% decrease in serum cholesterol has been reported in rats after daily oral doses of 400 mg d-limonene/kg for 30 days (4). A decrease in the LDL cholesterol level in serum as well as a 45% decrease in hepatic 3-hydroxy-3-methylglutaryl-coenzym A (HMG-CoA) reductase activity was reported in another study in rats (104), following administration of 1% d-limonene in the diet, which provided the rats with a daily intake of approximately 150 mg d-limonene for three months. Significant inhibition of hepatic HMG-CoA reductase activity has also been observed in rats after single intubation with 409 mg limonene (unspecified) /kg bw (14).

Oral single administration of 8.5-850 mg d-limonene/kg to rats resulted in a marked increase in bile flow, significant from the dose level 85 mg/kg and with a dose response correlation (72).The hydroxyl metabolites, p-mentha-1,8-dien-10-

ol, p-menth-1-ene-8,9-diol and p-mentha-1,8-dien-6-ol induced much higher increases in bile flow than the parent compound (72).

In human, d-limonene as a 97% preparation, infused directly into the biliary system (5-29 ml d-limonene/infusion), has been shown to dissolve or disintegrate retained gallstones (cholesterol stones) (59, 61). However, during the infusion, transient elevations of serum aminotransferases (ASAT,ALAT) and alkaline phosphatase were noted (61). When d-limonene was given perorally at a dose of 20 g to five adult male volunteers, no biochemical changes in the liver (for example total protein, total bilirubin, total cholesterol, ASAT, ALAT, alkaline phosphatase) were observed (59).

When d-limonene was injected as a 97% preparation into the biliary tract of cats, acute or chronic inflammatory damages of various degrees in the hepatobiliary tissues were observed (118).

A different toxic response in the liver has been observed at long-term administration of d-limonene to mice (95). Hepatocytes containing three or more nuclei and cytomegaly occurred at increased incidences in male mice, but not in female mice, at peroral administration of 500 mg d-limonene /kg bw 5 days/week for 2 years (95).

5.4. Renal effects

Evidence regarding renal effects in humans from exposure of d-limonene is almost lacking. However, in one study (59) minor amounts of proteinuria was reported to occur transiently when five adult male volunteers received 20 g d-limonene orally as a single dose. The significance of this finding is unknown.

Histopathological evidence of renal toxicity of d-limonene in male rats have been reported in several studies, while female rats, male rats of a strain that do not synthetize α2u-globulin, and other mammals such as mice, hamsters, guinea pigs, dogs and monkeys seem to be refractory to a condition like the α2u-globulin nephropathy (2, 23, 64, 95, 106, 129, 145, 146). Indeed, minor changes consisting of protein casts in the renal tubule of male and female dogs were reported in a Japanese study, when 1.2 or 3.6 ml d-limonene/kg bw/ day was administered orally for 6 months (135), but the significance of those findings remains unclear. Moreover, in a recent study with dogs (145), administered d-limonene by gavage twice daily for 6 months (100 or 1000 mg/kg bw/ day), no treatment-related histopathological changes in the kidneys were observed, although increased kidney weights (females only) and increased relative kidney weights (females, males) were reported at the highest dose level.

In the male rat, signs of the nephrotoxic syndrome have been reported already 24 h after an oral dose of d-limonene (82, 146). Exacerbation of hyaline droplet formation was observed in Sprague-Dawley and Fischer 344 rats after a single oral dosage of ≥41 mg d-limonene/kg bw (82, 146), while a dose of 14 mg/kg bw did not significantly increase the hyaline droplet content (82). At the dose 200 mg/kg, an increase in the content of α2u-globulin was also determined (146).

Dose-related increase in hyaline droplet formation in the PCT epithelial cells associated with renal accumulation of α2u-globulin was also present in a study (95) on male F344/N rats dosed with 75 - 1200 mg d-limonene/kg by gavage once per day for 14 days over a 21-day period. Similar changes were noted in another

study (66) on male Fischer 344 rats after administration of 75, 150 or 300 mg d-limonene/kg bw by gavage for 1 or 4 weeks (5 days/week). Increased severity of chronic nephrosis were present, at all dose levels, in the kidneys of the treated Fischer 344 rats, killed after 4 weeks, while granular casts were observed especially at the highest dose level (after 4 weeks) (66). Significantly increased relative kidney weights were also observed after administration of 5 or 20 doses of 300 mg d-limonene/kg bw (66).

In a study in male Sprague-Dawley rats, given d-limonene by gavage for 14 days at a dose level of 300 mg/kg/day, accumulation of hyaline droplets in PCT epithelial cells and increased levels of α2u-globulin in the kidney were observed. A minimal grade of tubular cell degeneration/necrosis was noted at histopathological examination. In the urine from all treated rats the kidney-type-α2u-globulin occurred, indicating loss of kidney-processed α2u-globulin as a result from exfoliation of proximal tubule cells after injury (114).

In a subchronic study on Fischer 344 male rats, an increase in both size and number of hyaline droplets was observed after 8 days of treatment by oral gavage at a dose level of 10 mg d-limonene /kg bw (146). An increased severity of chronic nephrosis appeared after 91 days at dose levels ≥30 mg/kg bw, while granular casts in the outer zone of the medulla, and significantly increased relative kidney weights were present at the highest dose level, i.e. 75 mg/kg bw at the end of the study (91 days) (146). The content of α2u-globulin was not investigated at any dose level. A no-observed-effect level (NOEL) for nephrotoxicity in this 91 - day study was determined to be 5 mg/kg bw (146).

In studies (64, 95) where 150-2400 mg d-limonene/kg bw/day was administered per os for 3 months, renal alterations consisting of chronic nephrosis and granular casts in the outer stripe of the medulla were formed in the male rats in a dose-related manner, except at the highest dose-level, where the alterations were somewhat similar to those in the low-dose-group and the vehicle control. At the highest dose level (2400 mg/kg bw/day) hyaline casts and dilation were noticed within the tubules/ducts of the cortex/medulla and hyaline material also occasionally within the glomerular space. No differences between controls and d-limonene-treated animals regarding hyaline droplet accumulation within the cytoplasm of the PCT epithelial cells were observed at any dose level. The absence of an increase in hyaline droplets is peculiar but might be explained by the fact that 3 days elapsed between the time of the last dose and when the animals were killed. Studies with decalin (another chemical that causes the male-rat-specific nephropathy) have shown that cytoplasmic protein reabsorption droplets resolve rapidly after cessation of exposure (1).

d-Limonene has also been tested in a long-term study (95). Consistent with the results of short-term studies, the kidney was shown to be the target organ in male F344/N rats. Male rats dosed with 75 or 150 mg d-limonene/kg bw /day 5 days/week by gavage for 2 years showed a couple of dose- and compound-related kidney lesions, including increased severity of spontaneous age-related nephropathy, increased incidences of conditions including mineralization of the renal medulla and papilla, focal hyperplasia of the transitional epithelium overlying the renal papilla, and proliferative lesions of the renal tubular cell epithelium (hyperplasia and neoplastic changes). No compound-related effects in the kidneys were observed in mice or female rats.

5.5. Gastrointestinal effects

Vomiting and nausea were reported in a Japanese study in dogs, orally administered 0.4, 1.2 or 3.6 ml d-limonene /kg/day for six months (135). Nausea, vomiting, diarrhea, pain in the upper abdomen and transient elevations of serum amylase have also been reported in humans, when d-limonene was infused as a 97% preparation (5 - 29 ml d-limonene/infusion) into the biliary system (59, 61). When five adult volunteers received d-limonene orally, as a single dose of 20 g, diarrhea and tenesmus was experienced (59).

5.6. Effects on heart and blood vessels

d-Limonene, when applied topically to rabbit ears, is an effective local vasodilating agent (78).

5.7. Haematologic effects

No data were found.

5.8. Effects on the nervous system

In a recent study (38) where 8 human volunteers were exposed by inhalation for 2 hours to 10, 225 or 450 mg/m^3 of d-limonene, no subjective CNS-related effects attributed to the exposure were experienced.

However, in studies on animals some effects resembling CNS-effects have been reported to occur. If these effects reflect a general toxic response or depend on a real involvement of the CNS can not be judged. An oral dose of 3 ml d-limonene/kg to mice and rats was reported to depress the CNS and cause decreased spontaneous motor activities (133). Hypothermia was also reported to occur in mice (133). Lethargy and excessive lacrimation was reported in a 13-week gavage study in male and female rats at the dose levels 1200 mg/kg/day and 2400 mg/kg/day. The highest dose level was also lethal to many of the animals. In mice, decreased activity was observed at the doses 1000 and 2000 mg/kg/day (95).

In cats an increased salivation of short duration and very mild muscle tremors were observed after soaking the animals with a water solution of an insecticidal dip containing 78.2% d-limonene (58). The concentration of d-limonene was about 6.5×10^{-2} mol/l water. At a concentration around 3×10^{-1} mol/l d-limonene in water the cats also suffered from mild ataxia and hypothermia. All the clinical signs of intoxication deteriorated even more at higher concentrations (~1 mol/l), but were always resolved within 7 hours (except the skin lesions) (58). In these cases the possible neurotoxicity can not be clearly associated with d-limonene because many insecticides contain small amounts of cholinesterase inhibitors.

6. Immunotoxicity and Allergy

6.1. Immunotoxicity

Forced intragastric feeding of BALB/c mice with d-limonene was associated with significant immunosuppression. Decreased responses occurred in primary and secondary antibody responses and in T- and B-cell mitogen-induced proliferation responses. Evidence was presented that d-limonene had polyclonal activator action (33). Competitive inhibition at the receptor level was suggested as a mechanism of suppression in a study where d-limonene treatment (in vivo) reduced non-immune immidate contact reactions to cinnamaldehyde in humans (50).

d-Limonene is used in a process named quenching in the perfume industry to suppress delayed contact sensitivity caused by other terpenes especially citral and cinnamaldehyde (99). Histological studies and radiolabel experiments demonstrated a quenching effect of d-limonene to some extent in animals sensitized to citral, although no difference in the reactivity was seen at challenge testing (6, 54).

The results in a recent animal study (7) lend little credibility to earlier reports of quenching phenomena in delayed contact hypersensitivity respones. d-Limonene gave no quenching of the response to cinnamaldehyde in animal studies. No evidence was found for the quenching effect of d-limonene on the elicitaion phase of carvone and limonen oxide in animal studies (69).

6.2. Allergy

No reports on type I allergy to limonene are found.

Regarding type IV allergy (contact allergy) see 5.1.2

7. Mutagenicity and Genotoxicity

d-Limonene and limonene unspecified has been tested in vitro in bacterial tests with strains TA1535, TA1537, TA 1538, TA 98, TA 100, UTH 8414 and UTH 8413 of Salmonella typhimurium, with or without the addition of a mammalian metabolic system. The mutation frequency was not found to be increased in the dosing intervals used (0.3-3333 µg/plate) (15, 43, 56, 141), but limonene was reported to be toxic to the bacteria in doses ≥400 µg/plate (43). Neither the 1,2-epoxide nor the 8,9-epoxide of d-limonene showed mutagenic activity to any of Salmonella typhimurium TA98, TA 100, TA 1535, TA 1537, and TA 1538 strains in the presence or absence of S9 mix (140, 141). However, they showed considerable cytotoxicity. Lethality to Salmonella typhimurium TA 100 was reported at the dose 1.0 µmol/plate (140, 141).

Negative, inconclusive or questionable responses (the overall evaluation was negative) were also reported with d-limonene in the dosing interval 5-100 nl/ml, in the L5178Y mouse lymphoma cell mutation assay, performed with or without S9 (94). In some of the experiments lethality was noticed at concentrations ≥50 nl/ml.

When tested in vivo in the mouse spot test, with injection into the peritoneal cavity of 215 mg/kg/day days 9-11 during gestation, d-limonene was not reported to be mutagenic (34).

With the Chinese hamster ovary cell assays for chromosome aberrations (ABS) and sister chromatid exchanges (SCE), d-limonene was not judged to induce cytogenetic damage at the doses tested, i.e. up to 100 µg/ml for ABS without S9, up to 500 µg/ml for ABS with S9 and up to 162 µg/ml for SCE (+S9,-S9). Doses above listed concentrations were toxic (3, 95).

Limonene (unspecified) did not induce malignant morphologic celltransformation in the dose range tested (0.1-100 µg/ml) in the Syrian hamster embryo cell system (102). When Rauscher murine leukemia virus-infected F344 rat embryo cells were treated with subeffective doses of 3-methylcholanthrene (MCA) and subsequently with unspecified limonene, inhibition of cell transformation was observed at the dose 15 µg/ml, but not at a lower concentration of limonene (1.5 µg/ml) (132). When limonene (15 µl/ml) was used without initiation with MCA, no cell transformation was noticed (132). In a recent study in vitro (126) d-limonene (21.9 µM) was shown to inhibit the formation of transformed rat tracheal epithelial cell colonies, following exposure to the carcinogen benzo(a)pyrene.

8. Carcinogenicity

In a cancer bioassay within the National Toxicology Program (95), male rats were dosed orally with 75 or 150 mg d-limonene/kg bw/day 5 days/week for 2 years. The results of these studies showed a dose-related increase in renal tubular cell hyperplasia and renal tubular cell adenomas /adenocarcinomas combined. Eight out of 50 rats in the low-dose group and 11 out of 50 rats in the high-dose group were found with renal tubular cell tumours. No renal lesions or renal tumours were observed in d-limonene-treated female rats (300 and 600 mg/kg/day), male mice (250 and 500 mg/kg/day) or female mice (500 and 1000 mg/kg/day) (95). Thus, under the conditions of these 2-year gavage studies, there was "clear evidence" of carcinogenic activity of d-limonene in the kidney for male rats. There was "no evidence" of carcinogenic activity of d-limonene for female rats or male or female mice (95). In an older study (127), where the occurrence of pulmonary tumours in mice was studied, no increase in the incidence of lung tumours was found after intraperitoneal injections of 200 mg or 1000 mg d-limonene/kg bw 3 times a week for 8 weeks.

In an initiation-promotion assay (24), using 500 ppm EHEN (N-ethyl-N-hydroxyethylnitrosamine) administered in the drinking water for two weeks as the initiator and d-limonene administered by gavage for 30 weeks (150 mg/kg bw/day, 5 days/week) as the promotor, an increase in cell proliferation of P2 cells and increased incidence and mean number/rat of atypical tubules and atypical tubule cell hyperplasia was observed in F344 rats and in the F344 control group treated only with d-limonene. A 10-fold increase in the incidence of renal adenomas in the F344 group (in comparison with the initiation control group) was also noted after d-limonene promotion, while the reported incidences and mean numbers/rat of observed kidney lesions in the α2u-globulin deficient male NBR

rat, demonstrated that d-limonene had no effect on renal neoplasia in that strain (24). In the same study (24) a significantly lower incidence of liver tumours was found in the F344 rats treated with EHEN and d-limonene, when compared to treatment with EHEN alone.

In a study in mice, where 0.05 ml d-limonene was fed by stomach tube once weekly for one year, after the administration of safrole, tannic acid or methylcholanthrene (injected subcutaneously on days 1, 7, 14 and 21 after birth), a significant reduction of the total number of mice bearing tumours compared to the groups not treated with d-limonene was reported (148).

In another study, where 0.2 mmol (27 mg) d-limonene was given to mice by gastric intubation once a week for a total of eight times, one hour before they were given 20 mg/kg bw of the carcinogen N-nitrosodiethylamine (NDEA) perorally, d-limonene was found to effectively decrease the numbers of forestomach tumours and to a lesser extent pulmonary adenomas (143). Markedly reduced pulmonary adenoma formation and completely inhibited occurrence of forestomach tumours was also exhibited when 25 mg d-limonene was administered to mice perorally as an occasional dose or twice a week for 8 weeks, one hour prior to a single or repeated administration of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (142). In contrast with these findings are the results of an older experimental study in mice (39) where d-limonene, administered perorally as 40 weekly doses of 0.05 ml after pretreatment with polyethylene glycol 400 or 50 µg benzo(a)pyrene (BP) by stomach tube, was found to be highly irritating and possibly weakly carcinogenic for the forestomach epithelium. However, orange oil administered in the same way was shown to be more effective than d-limonene in causing tumours of the forestomach (39).

A significant decrease in lung tumour incidence has been observed in mice given weekly intravenous injections of approximately 1 mg d-limonene for 16 weeks after a single subcutaneous injection of 0.5 mg dibenzpyrene (DBP) (57). The formation of fibrosarcomas in mice after a single subcutaneous injection of 25 µg DBP was also found to be significantly slower by treatment with d-limonene (totally 0.2ml) subcutaneously (57).

When limonene (unspecified) was topically (10 mg/application) coapplicated with benzo(a)pyrene (5µg/application) three times weekly for 440 days on mouse skin, a weak inhibitory activity on skin tumours was reported to occur (137). In some other experiments (107) orange oil, containing more than 90% d-limonene, have been reported to act as tumour promoter in the two-stage mouse skin tumour assay (but not to be carcinogenic when applied alone). However, when purified d-limonene (99.7%) was tested more recently on mouse skin (0.2 ml twice a week for about 40 weeks), after initiation with 51 µg DMBA, only a minor promotional activity ($p<0.05$) for skin tumours was noticed at week 34 post initiation (28). Orange oil was considered to be a stronger (but still weak) promoter (28). It was concluded, that the promotional activity of orange oil could not be accounted for by d-limonene (28). When 1% d-limonene or 1% orange oil was added in the diet, no promotional activity for skin tumours was noted (28).

Many studies in rats elucidating the effects of d-limonene on chemically induced mammary tumours have been published. The incidence of mammary tumours and the average number of mammary tumours/animal was found to be

effectively reduced, when a diet containing 5% d-limonene was fed 2 weeks before the initiation with a single intravenous dose of nitrosomethylurea (30-50 mg/kg) and to the end of the experiment at 23 weeks. The same result was obtained when the d-limonene containing diet was given only during the promotion/progression stage. There was no effect on tumour incidence or tumour number when d-limonene was administered only at the initiation stage, that is 2 weeks prior to and one week after carcinogen administration (47, 91).

In studies with the indirect -acting carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) as the initiator (50-65 mg/kg as a single oral dose), d-limonene, given in a quantity of 5% in the diet during the initiation stage or 1% or 0.1% throughout the experiment (from 1-2 weeks before the DMBA-administration), has been shown to significantly increase the latency for mammary tumours and at the higher dose levels to reduce the number of mammary tumours (21, 26, 29, 91, 111). 0.5% -1% d-limonene in the diet only during the initiation period was not found to significantly affect the mammary tumour latency or multiplicity (21, 111). On the contrary two metabolites of d-limonene (p-menth-1-ene-8,9-diol and p-menth-1,8-diene-6-ol) were reported to be effective in preventing DMBA-induced mammary cancer, when given at the 1%-level in the diet during the initiation stage (21). Administration of 5% d-limonene during the promotion/progression stage only (65 mg DMBA/kg as initiator), has not been found to be as effective as administration during initiation, but has been reported to cause a significant reduction in the average number of mammary tumours/animal (29).

After comparison with the results of the initiation-promotion studies with DMBA or NMU and d-limonene also the results in the 2-year NTP study (see above), showing a dose-related decrease in the occurrence of mammary gland fibroadenomas, adenomas, cystadenomas or adenocarcinomas (combined) in female rats, seem to be of some importance despite the reduced survival observed in the high-dose group (95).

In addition to the chemopreventive effects of d-limonene, a chemotherapeutic effect has been noticed. Regression of mammary tumours was reported in a study in rat with administration of 0.1% or 1% d-limonene in the diet before the administration of DMBA and for 27 weeks afterwards (26). In two later studies (27, 52) rat primary mammary carcinomas induced with DMBA (50-130 mg/kg perorally) or NMU (50 mg/kg iv) was found to regress and fewer subsequent tumours to occur, when animals bearing tumours were treated with 10% d-limonene in the diet. When 2.5%, 5%, 7.5% or 10% d-limonene was added to the diet of DMBA-treated rats for a minimum of 15 weeks, significant regressions (complete) of primary mammary tumours was observed at the two highest dose levels, while a complete regression of secondary tumours was noted at dose levels ≥5% (52). A metabolite of d-limonene, perillyl alcohol, has been reported to be even more effective in causing regression of mammary tumours (51).

9. Reproduction Toxicology

The effects of d-limonene on the development of rat fetuses was investigated in a study with oral administration of 591 mg or 2869 mg d-limonene/kg from day 9 to 15 of gestation. At the higher dose, maternal toxicity (decreased body weights,

several deaths) was observed. In the offspring, delayed ossification and decreased weights of thymus, spleen and ovaries was reported in the high-dose group (136). In a study in mice (74) d-limonene was administered orally at the dose levels 591 mg/kg or 2363 mg/kg for 6 days from day 7 to 12 of gestation. Maternal toxicity demonstrated by a significant reduction in body weight gain was observed in the high-dose group. At the same dose level, a significantly increased incidence of skeletal abnormalities and delayed ossification in the offspring was noted. When pregnant rabbits were given doses of 250, 500 or 1000 mg d-limonene /kg orally from day 6 to day 18 of gestation, maternal toxicity was evident in the mid-dose group (temporary decrease in body weight gain) and in the high-dose group (death, decrease in body weight gain). No dose-related anomalies in the fetuses were observed (75).

No biologically significant differences in serum estradiol and prolactin levels or duration of estrus cycle were found in rats, intubated with a single dose of the carcinogen DMBA (65 mg/kg) and then put on a diet containing 1% or 5% d-limonene (29).

10. Correlation between Exposure, Effect and Response

There are few reports concerning effects of limonene in human.

The only published study with exposure by inhalation is a study , in which 8 human volunteers were exposed to 10, 225 or 450 mg d-limonene/m^3 for 2 h, during light physical exercise (50W). This study showed a small but statistically significant decrease in vital capacity after exposure to d-limonene at the highest exposure level, but no significant changes in the other lung function variables measured. The change in vital capacity was probably of no functional significance. The subjects did not experience any irritative symptoms or symptoms related to the central nervous system, but these conclusions were based only on subjective ratings.

d-Limonene is considered a skin irritant. Immersing one hand in d-limonene (98%) for 2 h gave painful itching and burning which occurred within a few minutes and increased throughout. The dorsal skin was moderately erythematous and swollen. The swelling was gone at 1.5 h post-exposure, but 6 h after stop of exposure a severe purpuric eruption appeared, which was maximal after 1-2 days and remained for several weeks. The blood concentration of d-limonene measured was low compared to that achieved by inhalation (10 mg/m^3, 50W).

Reports on allergic contact dermatitis in human where d-limonene is the only offending agent are not found. Experimental studies in guinea pigs with d-limonene of high purity (98%) gave no significant contact sensitization, but after prolonged air exposure of the solvent new experiments gave a clear sensitization. Chemical analysis of the air-exposed d-limonene identified different oxidation products of which limonene-1.2-oxide and carvone were found to be potent sensitizers in guinea-pigs.

Inhalation studies of limonene in animals are missing. However, there are many studies in which limonene (mostly d-limonene) has been administered in other ways, usually per os. These results are summarized in Table 2.

Table 2. Some dose-effect data* for animals exposed to d-limonene.

Exposure	Species	Effect	Reference
5.2 g/kg p.o.	rat (female)	LD_{50}	134
3 ml/kg p.o. once	mouse rat	decreased motor activity, hypothermia, potentiation of hexobarbitalinduced sleeping	133
1200 mg/kg/ day p.o. 13 weeks	rat	lethargy, lacrimation	95
1200 mg/kg p.o. once	rat	induction of enzymes	4
~1000 mg/kg/ day p.o. up to 6 months	dog	increase in serum alkaline phosphatase and cholesterol, increased kidney weight	145
1000 mg/kg/ day p.o. 13 weeks	mouse	decreased motor activity	95
~1000 mg/kg/ day p.o. 28 days	dog	increased liver weight	145
600 mg/kg p.o. once	rat	increase in δ ALA-synthetase activity	4
500 mg/kg/ day p.o. 2 years	mouse	hepatocytes containing three or more nuclei, cytomegaly	95
1 % in the diet (~ 500 mg/kg/day) 3 months	rat	decrease in HMG-CoA reductase activity and serum LDL cholesterol	104
1 % in the diet (~ 500 mg/kg/day) 2 weeks	rat	induction of enzymes	90
**409 mg/kg p.o. once	rat	inhibition of HMG-CoA reductase activity	14

Table 2 cont.

Exposure	Species	Effect	Reference
400 mg/kg/ day p.o. 30 days	rat	induction of enzymes, increase in relative liver weight, decrease in cholesterol	4
0.4 ml/kg/ day p.o. 6 months	dog	vomiting, nausea	135
300 mg/kg p.o. 5 or 20 times	rat	increase in relative liver weight	66
85 mg/kg p.o. once	rat	increase in bile flow	72
75 mg/kg/ day p.o. 91 days	rat	increase in relative liver weight	146
**40 mg/kg ip 3 times	rat	induction of enzymes	5

*Data concerning the male-rat specific nonneoplastic and neoplastic kidney lesions are not included.
**Optical isomer of limonene is not specified.

11. Research Needs

d-Limonene is easily oxidized by athmospheric oxygene. Experimental studies concerning its sensitizing effect in skin have shown that d-limonene itself is not allergenic but that allergenic compounds are formed during autooxidation. An eventual effect of different oxidation products has not been taken into consideration when investigating other health aspects of d-limonene (limonene). The effects studied might therefore in part be due to other compounds than limonene itself and its metabolites and further studies where this is taken into account are needed.

Studies on the work exposure of limonene are few and no measurements of its oxidation products formed by air exposure have been performed. New methods to detect and determine the concentrations of these substances in air must be developed.

There are few data regarding the effects on the eyes and the respiratory organs. More information regarding lung function and irritative effects at exposure by inhalation of limonene and compounds formed by air oxidation is important. Furthermore, there is a research need for eventual CNS effects.

It has been shown that d-limonene can affect the liver in animals. However, there are no studies using exposure by inhalation, where the effects on the liver

have been investigated and thus such studies should be performed. Experimental studies to determine eventual effects of limonene on the reproduction at exposure by inhalation are also needed.

12. Discussion and Evaluation

Limonene is considered a skin irritant. Older reports ascribe occupational skin diseases, mainly irritant contact dermatitis to citrus fruits in the citrus fruit canning industry. However, the exposure was complex with many factors contributing to the dermatitis. d-Limonene in high concentrations gives marked irritancy as shown in experimental studies on human and animals.

d-Limonene is considered the principal sensitizer in citrus species, but the incidence of contact allergy from these fruits is unknown. It is also included among the fragrance allergens. A few case reports on contact allergy to d- and l-limonene as well as to dipentene are found, but the purity of the compounds are not investigated. Reports on allergic contact dermatitis where d-limonene is the only offending agent are not found. Experimental studies have shown that d-limonene itself gives no significant contact sensitization, while air oxidized d-limonene gives contact allergy. These studies should also be valid for l-limonene and dipentene, since d- and l-limonene are the two enantiomeric forms (mirror images) of the same molecule. Dipentene is a mixture of d- and l-limonene. Therefore, oxidation products in d-limonene and not limonene itself might be the offending agents in the clinical studies. The handling and purity of d-limonene is thus critical for its allergenicity.

Data on uptake of d-limonene through the skin are very sparse. The blood concentration obtained after dermal exposure of one hand of one subject to d-limonene at a high concentration was low compared to that achieved by inhalation (10 mg/m^3, 50W).

The information regarding human at exposure to d-limonene by inhalation is based on one study. A slight decrease in vital capacity, probably of no functional significance, was noted in a recent study. Based on this information the critical effect in human could possibly be an effect on the respiratory system, but more information is needed before this can be stated.

Limonene has been shown to affect the liver in animals at peroral and intraperitoneal administration. An increase in the content and activity of different liver enzymes, increased liver weights as well as a decrease in the hepatic HMG CoA reductase activity was among the effects recorded. A change of the metabolism of well-known carcinogens has been determined experimentally. This change in the metabolic pattern was concluded to be one of the reasons for the observed anticarcinogenic effect of d-limonene. Based on available information concerning the effects on the liver, the critical effect of d-limonene in animals, except in the male rat, is considered to be the liver effects. Induction of liver enzymes has been reported to occur at a dose level of 40 mg/kg/day at intraperitoneal administration. At peroral administration of somewhat higher doses an increase in the relative liver weight has also been observed. However, no toxic effects on the liver have been recorded at these dose levels or at dose levels more than ten times higher. Thus, the observed effects in the liver are probably

signs of a physiological adaptation. A recalculation to the human situation indicates that this physiological response also might be present in humans after inhalation of limonene at exposure levels about 350 mg/m^3 or above.However, there are no studies involving inhalation exposure in which effects on the liver have been investigated. Therefore, the liver effects cannot with certainty be stated as the critical effect in human.

It is well documented that d-limonene can affect the kidney in male rats. However, for human these effects on the kidney are not relevant. The reason for this is, that the protein α2u-globulin, which is considered to be the origin of the observed kidney lesions not has been reported to occur in any other species than the rat (males). Furthermore, the special kind of nephrotoxicity (including increased incidence of renal tumours) shown to be present in the kidneys of the male rat at exposure to d-limonene has not been observed in experiments with any other animals.

13. Summary

Karlberg A-T, Lindell B. Nordic Expert Group for Documentation of Occupational Exposure Limits. 107 Limonene. Arbete och Hälsa 1993:35, pp 207-246.

There are few data regarding toxicity of limonene in human. A slight decrease in vital capacity at a concentration of 450 mg d-limonene/m^3 (2 h) was observed in one study. Skin exposure to d-limonene in high concentrations gives a marked irritancy of the skin. Contact allergy has been shown in animal studies with air oxidized d-limonene. d-Limonene itself gave no significant sensitization in these studies. Liver effects are the critical effects in animals, except in male rats. In the male rat, d-limonene causes damage to the kidney and renal tumours. The male-rat specific protein α2u-globulin is considered to have a crucial role in the development of the neoplastic as well as the nonneoplastic kidney lesions. d-Limonene has been studied in a battery of short term tests in vitro and found to be nongenotoxic.

Key words: allergy, cancer, dipentene, α2u-globulin, kidney, limonene, liver, lung function, occupational exposure limits, skin irritancy.

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Appendix I. List of permitted or recommended maximum levels of limonene in air

Country	Mg/m3	Ppm	Comments	Year	Ref
Denmark	-	-		1988	1
Finland	-	-		1987	2
Iceland	-	-		1989	3
Netherlands	-	-		1989	4
Norway	-	-		1989	5
Sweden	150	25	NGV HS	1990	6
	300	50	KTV		
USA (ACGIH)	-	-		1991-92	7
(NIOSH)	-	-			8

HS = skin sensibilisation
NGV= nivågränsvärde
STV = short time value

References to Appendix 1

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Summary

Beije B, Lundberg P (eds). Criteria documents from the Nordic Expert Group 1993. Arbete och Hälsa 1993:35, pp 1-254

The Nordic Expert Group is a standing committee with the task of producing criteria documents on health effects of occupationally used chemicals. The documents are meant to be used by the regulatory authorities in the five Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The volume consists of English translations of the criteria documents which have been published in a Scandinavian language during 1993.

Key words: Criteria document, Crystalline silica, Diesel exhaust, Ethylenebisdithiocarbamates, Ethylene thiourea, Limonene, Nordic Expert Group, Occupational Exposure Limit.

Sammanfattning

Beije B, Lundberg P (eds). Kriteriedokument från Nordiska Expertgruppen 1993. Arbete och Hälsa 1993:35, pp 1-254.

Den Nordiska Expertgruppen är en arbetsgrupp med uppgift att producera kriteriedokument om hälsoeffekter av kemiska ämnen i arbetsmiljön. Dokumenten skall användas av tillsynsmyndigheterna i de fem nordiska länderna som ett vetenskapligt underlag vid fastställande av hygieniska gränsvärden.

Volymen omfattar en engelsk översättning av de kriteriedokument som har publicerats på ett skandinaviskt språk under 1993.

På engelska.

Nyckelord: Dieselavgaser, etylenbisditiokarbamater, etylentiourinämne, hygieniska gränsvärden, kristallint kisel, kriteriedokument, nordiska expert-gruppen.