Methods for Assessing the Effects of Mixtures of Chemicals
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Mixed Exposures: Assessment and Evaluation of Some Carcinogenic and Non-carcinogenic Effects

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ABSTRACT

Several examples of mixed human exposures in the occupational and general environment are given. While in all instances an adverse influence on human health can be assumed, the possibilities of providing dose–response relationships vary among the examples. A dose–response curve concerning exposure to methyl mercury and the risk for development of neurological signs of poisoning in humans seem to be best established; however, the influence of combined intake of selenium is uncertain. Concerning a relationship between general air pollution in urban areas and respiratory disease, dose–response relationships for humans exist but are very uncertain. Evidence presented during recent years demonstrates effects of low concentrations of air pollutants on asthmatics in laboratory experiments and supports the notion that sensitive subgroups deserve additional attention in epidemiological studies. Combined effects of sulphur dioxide with cigarette smoke and with specific sulphates indicate the need for refined dose measurements in epidemiological studies including detailed data on active and passive smoking. Occupational arsenic exposure has been related to incidence of lung cancer; however, the important combined effect of cigarette smoking has been revealed recently and a different dose–response relationship would therefore be expected if arsenic exposure alone were to be considered. Concerning general air pollution and respiratory cancer, epidemiological evidence is very uncertain and experimental techniques have been designed for estimating cancer risk from samples of general air pollution or sources of such pollution. The Ames *Salmonella* assay has been established as a useful tool but which can, however, only give a qualitative indication of hazard. Combinations of mutagenicity and other short-term bioassays and long-term carcinogenicity studies in animals may provide a means of making some kind of quantitative predictions about human risk from exposure to air pollution mixtures.

1 INTRODUCTION

There are several methodological difficulties related to the assessment of complex exposure situations which occur frequently in the general environment. An important example of a complex mixture of chemical agents is the exposure to air pollution in the general and working environment. This paper will discuss a few specific examples which may illustrate the usefulness of a combination of research methods.

2 NON-CARCINOGENIC EFFECTS

2.1 General Air Pollution and Respiratory Illness

2.1.1 General Aspects

The general air pollution situation as it occurs in urban areas in relation to rural ones is a good example of a complex exposure situation. Because of the general concern for effects related to air pollution, considerable research effort has been devoted to sorting out possible exposures and associated risks. An increased mortality on a day-to-day basis was recorded in episodes of high pollution (WHO, 1979) in the Mease Valley in 1930, in Donora in 1948, and in London in 1952. During the four days of the episode in London in 1952 approximately 4000 more deaths were recorded (among the 8 million inhabitants) than would have been expected. In several subsequent studies an effect on acute mortality and morbidity has been recorded and related to air pollution concentrations. However, there are several difficulties in the epidemiological assessment of effects of air pollution on respiratory disease. It has also been difficult in studies in experimental animals to reproduce the effects observed in human beings. The effects studied by epidemiological methods include acute respiratory symptoms such as cough with or without phlegm, irritation of the throat or nose, and more severe effects such as obstructive lung disease including chronic bronchitis. Epidemiological evidence has been related to chemical analyses including determination of sulphur oxides and smoke (measuring the blackening of a filter paper). Such air pollution analyses were performed in London during the 1950s and reflected the predominant use of coal under primitive combustion conditions such as those prevailing with domestic use of coal in individual stoves. It is evident that a number of other compounds were present in the air pollution at that time and that the measurement of sulphur dioxide and smoke levels was only an index of the air pollution situation. The index substances were related to the epidemiological registration of disease occurrence and certain dose–response relationships were established. However, it was already evident at that time that interactions with other agents, such as air humidity and temperature, infectious agents (viruses and bacterial agents), cigarette smoking (active and passive

cigarette smoking), as well as occupational exposures, may have influenced the relationship (WHO, 1979). It has also been recognized that other pollutants, e.g. nitrogen oxides (Lindvall, 1984; WHO, 1977), can occur in cities in concentrations that cause adverse effects in humans.

2.1.2 Relationship between Exposure to Sulphur Dioxide, Particles, and Short-term Effects on Mortality or Morbidity

Available evidence concerning the correlation between air pollution concentrations and mortality or morbidity in respiratory illness has been compiled by WHO (1979) and by Ericsson and Camner (1983), and the reader is referred to these documents for a more detailed description and evaluation of this subject. A dramatic example of increased mortality was recorded in London in 1952 when air pollution levels were very high (maximum value for central area: sulphur dioxide 3700 $\mu g/m^3$, smoke 4500 $\mu g/m^3$). Persons who died during these episodes were predominantly those with pre-existing cardiovascular or respiratory disease, which to a large extent had been induced by factors other than the air pollution. It is obvious that the short episodes could not have caused the chronic illnesses, but rather acted as the agent that caused an extra stress to kill the individual. The persons who died in these cases were thus those belonging to an extremely sensitive group of the population.

According to WHO (1979), an increase in mortality among the elderly and the chronically sick is to be expected at exposure levels of 500 $\mu g/m^3$ of sulphur dioxide and smoke at the same time (24-hour average). Worsening of the condition of patients with existing respiratory disease is expected at still lower levels (about 250 $\mu g/m^3$) of sulphur dioxide and smoke or total suspended particulates (WHO, 1979).

2.1.3 Sulphur Oxides and Particulates: Long-term Exposure in Humans

The effects of long-term exposure to air pollutants are more complicated to assess than acute effects, especially since the mechanism of the development of chronic respiratory disease and its relation to duration of exposure is not clarified. It has been demonstrated that smoking is more strongly related to symptom rates than air pollutants. Social and economic factors may also be of importance. Detailed comments on the various studies have been given by WHO (1979) and by Ericsson and Camner (1983).

Occupational exposures to sulphur dioxide have previously been correlated to adverse effects on the respiratory system only to a limited extent (WHO, 1979). Recent studies (Stjernberg *et al.*, 1985) demonstrate a higher incidence of chronic obstructive lung disease in workers exposed to sulphur dioxide than in controls. The effect was particularly prominent among smokers. A multiplicative interactive effect was indicated between smoking and exposure to sulphur dioxide.

Children are more sensitive to acute respiratory infection, more easily develop symptoms in relation to bronchoconstriction, and are usually not influenced by the direct effects of smoking. Studies in children may therefore provide a particularly useful data base for the evaluation of the influence of air pollution on the respiratory tract. A detailed discussion of exposure–effect relationships for children is given by Ericsson and Camner (1983). One study by Holma *et al.* (1979) emphasizes that factors such as parental smoking habits and socio-economic status must be taken into account when evaluating effects of air pollution in children.

According to WHO (1979), long-term exposure to sulphur dioxide and smoke at 100 $\mu g/m^3$ each is expected to lead to increased respiratory symptoms among samples of the general population (adults and children) and increased frequencies of respiratory illnesses among children.

2.1.4 Experimental Studies in Humans Involving Sulphur Oxides Alone or in Combination with Other Substances

A number of studies have been performed both in asthmatics and in healthy persons where respiratory effects after exposure to sulphur dioxide have been measured (see Ericsson and Camner, 1983). In healthy persons no effects have been reported at concentrations of sulphur dioxide lower than 2–3 mg/m^3. However, when asthmatics were exposed during exercise to 700 $\mu g/m^3$ or more, airflow resistance was increased (Sheppard *et al.*, 1981). Without exercise, these sulphur dioxide levels did not influence airway resistance.

Experiments have also been performed with combined exposures to sulphur dioxide and other substances that occur as air pollutants, i.e. particulate matter, ozone and nitrogen dioxide (Table 1). There is limited evidence concerning interactions between these pollutants. Water aerosol increased the effect of sulphur dioxide. Inert dust was reported to influence, but did not potentiate, the effects of sulphur dioxide. A combined exposure to sulphur dioxide and ozone was shown to have a greater effect on respiratory physiology than ozone alone.

2.1.5 Animal Studies

A number of animal experiments have also been made with the aim of investigating whether similar effects to those observed in humans could be induced by sulphur dioxide alone, by particulate materials alone, or by combinations of sulphur dioxide and particulates. Amdur (in Casarett and Doull, 1975) summarized the effects of sulphur dioxide as a 'bronchoconstriction reflected by increased airflow resistance which is accompanied by a decreased compliance and increase in respiratory rate.' Effects of exposure to low levels (3 $\mu g/m^3$) are variable (see review by Ericsson and Camner, 1983). Amdur (1974) reported that about 10% of normal experimental animals appear to be

Table 1 Experimental studies in humans: effects of combined exposure to sulphur dioxide and particles or gases

Combination	Duration	Subjects	Results
1.4 mg/m^3 SO_2 + saline or water aerosol	15 min	9 normals	Water aerosol but not saline increased influence on $MEFR_{50}$ (Snell and Luchsinger, 1969)
1.1 mg/m^3 SO_2 + 0.79 mg/m^3 O_3	2 hours	8 normals (exercise)	Combined exposure caused more pronounced effects on MMFR and lung volumes (Hazucha and Bates, 1975)
2.6 or 13 mg/m^3 SO_2 + 2 or 10 mg/m^3 'inert dust'	5 hours	16 normals	Combined exposure influenced the effect of SO_2 (decreased nasal mucous flow rate, increased nasal airflow resistance and $FEV_{1.0}$) (Andersen *et al.*, 1974, 1981)
1.0 mg/m^3 SO_2 + 0.77 mg/m^3 O_3 + 0.1 mg/m^3 H_2SO_4	2 hours	19 normals	Small reduction in FVC, $FEV_{1.0}$ and MMFR which might be slightly greater than O_3 alone (Kleinmann *et al.*, 1981)
1.1–49 mg/m^3 SO_2 + NaCl, O_3	30 min to 2 hours	asthmatics and normals	Five studies showing no clear interaction (evaluation by Ericsson and Camner, 1983)

Data taken from detailed compilation by Ericsson and Camner (1983).

'sensitive'. Higher levels of sulphur dioxide (28 mg/m^3) give rise to morphological changes in nasal ciliated mucosa in mice (Giddens and Fairchild, 1972).

Effects on defence mechanisms, such as mucociliary clearance, are noted after long-term exposure to low levels of sulphur dioxide (3 mg/m^3) in rats and dogs (Ericsson and Camner, 1983).

2.1.5.1 Particulate Matter Studies on particulate matter in animals have focused on effects of sulphates. The relative irritant potency of various sulphates as observed in such experiments varies considerably, sulphuric acid being the most irritating (Amdur *et al.*, 1978a,b). These observations thus give support to the assumption that sulphuric acid and some sulphates are more potent irritants than sulphur dioxide (review by Ericsson and Camner, 1983). The measurement of 'suspended sulphates' as done in some epidemiological studies appears to have little toxicological meaning in the absence of a specification of the chemical composition and size distribution of the sulphates.

2.1.5.2 Combined Effects of Sulphur Dioxide with Particles or Other Gases Evidence from animal experiments using combined exposures has been

summarized in Table 2. A high relative humidity sodium chloride aerosol potentiated the response to sulphur dioxide. The enhancement is probably related to the dissolution of the sulphur dioxide in the aqueous sodium chloride droplets and the transfer to lower airways. Soluble salts of some metals potentiated the response in flow resistance even at low levels of sulphur dioxide (0.6 mg/m^3). Sulphur dioxide, together with sulphuric acid or some sulphates (e.g. copper sulphate), has affected pulmonary function and morphology.

The relative contribution of sulphur dioxide and sulphuric acid to the effects is not clear but sulphuric acid probably plays a greater role. Interaction effects on the morphology of trachea in hamsters have also been noted after exposure to sulphuric acid in combination with coal particles. No interaction effects have been noted after exposure to different combinations of sulphur oxides, nitrogen oxides and raw or irradiated automobile exhaust, or after sequential exposure to sulphur dioxide or sulphuric acid in combination with ozone.

In summary, although there are great differences in findings in various animal experiments, it may be justified to conclude that differences in the potency of various sulphates seem to have been demonstrated as well as the possibility of a combined effect (whether additive or potentiating is difficult to say at this point) of sulphur dioxide with sulphuric acid and possibly certain other sulphates.

2.1.6 Summary of Studies Related to Respiratory Illness and General Air Pollution

In previous evaluations, for example the one published by WHO (1979), it was considered that neither the epidemiological evidence from occupational exposure situations nor animal data were very helpful in providing support for the epidemiological observations of an increased mortality and morbidity in respiratory illness related to high air pollution exposure, i.e. a combination of sulphur oxides and particulate matter. Evidence collected during recent years has changed the situation to a certain extent. In the occupational setting it has been demonstrated that exposure to sulphur dioxide together with cigarette smoking has given rise to an elevated incidence of chronic bronchitis. Smoking is an important factor also in the general population, since cigarette smoking is known to be strongly correlated to the incidence of chronic bronchitis. Air pollution in the general environment thus can be regarded as acting upon a population already suffering the influence of pollution from cigarette smoke. The experimental studies with low concentrations of sulphur dioxide in humans demonstrate effects only in asthmatics and give further support to the assumption that in epidemiological studies the increased mortality and morbidity seen at exposure levels around 200–1000 $\mu g/m^3$ of sulphur dioxide and particulate matter are related to an effect on persons already suffering some illness of the respiratory tract. The epidemiological recordings of increased mortality in London in 1952 correspond to $LD_{0.005}$ (5 persons in 100 000) when expressed in toxicological

Table 2 Effects of combined exposure to sulphur dioxide with sulphuric acid or other particles or gases

Concentration	Duration	Species	Results
2.9 mg/m^3 SO_2 + 1 mg/m^3 NaCl	1 hour	Guinea-pig	Potentiation of pulmonary flow resistance at high RH > 80% (McJilton *et al.*, 1973, 1976)
0.6–285 mg/m^3 SO_2 + 1 mg/m^3 of soluble metal salts (Mn(+II), Fe(+II) and V(+V), CMD < 0.1 μm) or +8–10 mg/m^3 of insoluble aerosols (e.g. MnO_2, Fe_2O_3 and carbon, $\leq$ 5 μm)	1 hour	Guinea-pig	Soluble metal salts, but no insoluble aerosols, potentiated the response in pulmonary flow resistance at 0.6–57 mg/m^3 SO_2 (Amdur and Underhill, 1968)
13.4–14.1 mg/m^3 SO_2 + 0.8–0.9 mg/m^3 H_2SO_4 (90% < 0.5 μm)	21 hours/day 225 or 620 days	Dog	After 225 days, residual volume was more reduced and after 620 days, several functional parameters (e.g. increased airway resistance) tended to be more affected than after exposure to SO_2 or H_2SO_4 alone. Dogs previously exposed to NO_x were less affected (Lewis *et al.*, 1969, 1973)
0.3–14 mg/m^3 SO_2 + 0.1–1.0 mg/m^3 H_2SO_4 (MMD: 0.5–3 μm) + 0.5 mg/m^3 fly ash (MMD: 4–6 μm)	78 weeks	Monkey	Bronchial mucosa was affected after exposure to mixtures containing 2.9 mg/m^3 SO_2 and 1 mg/m^3 H_2SO_4. The effects were probably due to H_2SO_4. No effects on pulmonary function (Alarie *et al.*, 1975)
1.1 mg/m^3 H_2SO_4 + 1.5 mg/m^3 carbon (→ acid-coated carbon aerosol (mean diameter: 0.4 μm))	3 hours	Hamster	A potential effect on the tracheal epithelium at 24 and 48 hours after combined exposure (Schiff *et al.*, 1979)
1.4 mg/m^3 H_2SO_4 + 1.5 mg/m^3 carbon (→ acid-coated carbon aerosol (mean diameter: 0.4 μm))	3 hours/day 5 days/week up to 20 weeks	Mouse	The mixture caused greater changes on the immune system (e.g. reduced resistance to respiratory viral infection than carbon alone (Fenters *et al.*, 1979))
0.9 mg/m^3 H_2SO_4 (VMD: 0.23 μm) + 0.2 mg/m^3 O_3	3 hours	Mouse	Exposure to O_3 before H_2SO_4 caused an additive response in susceptibility to respiratory infections (Gardner *et al.*, 1977)
Nine additional studies compiled by Ericsson and Camner (1983) showing no clear interactions			

Data from review by Ericsson and Camner (1983).

terminology. This means that those who died belonged to an extremely sensitive group in the general population, probably those suffering from various diseases of the respiratory and cardiovascular system.

In animal experiments, combined effects of sulphur dioxide and sulphuric acid mist have been demonstrated. For certain other sulphates an effect can also be assumed, but for sulphates with low irritability and for other 'inert' particles, available evidence does not demonstrate any clear influence on the effect of sulphur oxides.

In summary, both data from experiments in animals and humans and epidemiological data from occupational exposures to sulphur dioxide have provided information of importance for the interpretation of observed increases in mortality and morbidity among the general population when air pollution levels (including sulphur dioxide) were high. Experiments in humans have demonstrated the greater sensitivity of asthmatics to sulphur dioxide compared with healthy individuals. Epidemiological studies have indicated a combined effect of cigarette smoking and air pollution. In addition to sulphur dioxide, certain sulphates, particularly sulphuric acid, increase the irritating effect on the respiratory tract.

Although there are still considerable uncertainties in interpreting the epidemiological evidence of increased mortality and morbidity in the general population in response to an increased level of sulphur dioxide and particulates, recent evidence has indicated some factors that may be of importance:

(1) Epidemiological evidence relates to effects on an extremely sensitive subgroup of the population ($LD_{0\cdot05}$).
(2) Experimental studies in humans have not been performed on sick people until recently.
(3) Animal experiments have mainly been performed on small groups of healthy animals.
(4) Measurements of 'particulate matter' and 'sulphates' include a mixture of active and inactive components.
(5) Other air pollutants (e.g. nitrogen dioxide) have not been considered.

2.2 Exposure to Metallic Compounds with Special Reference to Methyl Mercury–Selenium Interactions

2.2.1 Methyl Mercury–Selenium Interactions

Based on experience from cases of methyl mercury poisoning in Japan and in an Indian population in Canada, and particularly from the evidence from the extensive epidemic in Iraq in 1972, the dose–response relationships for effects of

methyl mercury on the central nervous system of adult humans is relatively well known (Bakir *et al.*, 1973). By use of a metabolic model and taking into consideration interindividual differences in susceptibility, dose–response relationships for long-term exposure have been extrapolated (Nordberg and Strangert, 1976, 1978, 1985). Data on hair concentration of mercury in pregnant women have been related to observations of neurological deficits in the children born to these women. Such sets of observations have allowed preliminary dose–response relationships for effects on the foetus to be proposed (Berlin, 1983; Marsh *et al.*, 1980).

Data from animal experiments may suggest that the dose–response relationships may be changed by simultaneous exposure to selenium. Since marine fish contains considerable concentrations of selenium and in some instances (tuna, swordfish) also methyl mercury, it has been suggested that methyl mercury consumed by this route might represent less of a risk than that calculated from the poisoning epidemic in Iraq where selenium intakes were low (Ganther and Sunde, 1974; Ganther *et al.*, 1972, Ohi *et al.*, 1976).

Dietary selenite and vitamin E diminished the mortality in quail exposed to methyl mercury (Welsh and Soares, 1976). In rats, Welsh (1976) showed that vitamin E and other antioxidants had a protective effect.

It was considered by the Task Group on Metal Interactions (see Nordberg *et al.*, 1978) as well as in a more recent review (Berlin, 1983) that the evidence for this protective effect is not yet sufficient to modify the dose–response relationships for humans. However, such an effect should by no means be excluded.

2.2.2 Factors Influencing the Toxicity of Other Metals

The dose–response relationships of other metals may be modified by dietary factors such as the calcium and protein content. A low calcium content may increase the absorption of both lead and cadmium. A similar relationship is valid in relation to the iron status of the individual. For example, it has been demonstrated that the gastrointestinal absorption of cadmium can be substantially elevated in individuals with iron deficiency (Flanagan *et al.*, 1978). In addition to the interaction between selenium and methyl mercury mentioned above there are several other metallic compounds whose toxicity and/or metabolism seems to be influenced by selenium. This is valid for inorganic mercury, cadmium, and arsenic (Nordberg *et al.*, 1978, 1986).

The general comment can be made that experience from experimental animals seems to agree fairly well with the human evidence, at least for some animal species. Further comments relating the experience from exposure to metallic compounds to other exposure situations will be given in the general summary.

3 SOME CARCINOGENIC EFFECTS

3.1 Respiratory Cancer in Workers at a Swedish Non-ferrous Smelter

3.1.1 The Role of Arsenic and Cigarette Smoking

A high incidence of respiratory cancer has been documented in workers who are exposed to arsenic at a non-ferrous smelter in Sweden (Wall, 1980). This high incidence has been demonstrated in a case–control study (Pershagen *et al.*, 1981) to be related to the combined effect of cigarette smoking and exposure to arsenic at certain sites in the smelter. In Table 3 it is demonstrated that the effects of exposure to arsenic in the occupational environment act as a potentiating agent on the carcinogenic effect of cigarette smoking. In the group of non-smokers there is also an increased risk of respiratory cancer.

Although it is considered (IARC, 1980) partly on evidence from the above-mentioned type of smelter that inorganic arsenic compounds are respiratory carcinogens in humans, there has been no animal evidence to support this epidemiological observation until recently. A long-term carcinogenicity assay in animals was therefore set up which employed the intratracheal route of administration. Since it had been suggested (Rossman, 1981; Rossman *et al.*, 1977) that the carcinogenicity of arsenic might be due to an influence on certain DNA repair mechanisms, combined exposure with benzo[*a*]pyrene, a known respiratory carcinogen (Saffiotti *et al.*, 1972a), was included for comparison. According to the method developed by Saffiotti *et al.*, (1968), Syrian golden hamsters were given 15 weekly intratracheal instillations of arsenic trioxide (0.25 mg arsenic) alone or in combination with benzo[*a*]pyrene. All animals in this study were given carrier dust (charcoal carbon) and a small amount of sulphuric acid (Pershagen *et al.*, 1984). The results of the study are shown in Figure 1, where the calculated probabilities for the observation of cancer in the respiratory tract of hamsters are displayed. It can be seen from the figure that there is no clear

Table 3 Standardized rate ratios for death from lung cancer among non-smoking and smoking smelter workers in different arsenic exposure categories

Exposure	Non-smoker	Smoker
No arsenic	1.0	4.9
Arsenic		
High SO_2	1.8	6.5
High arsenic	1.2	14.7
Roaster worker	4.4	22.0
Total	3.0	14.6

Modified from Pershagen *et al.* (1981).

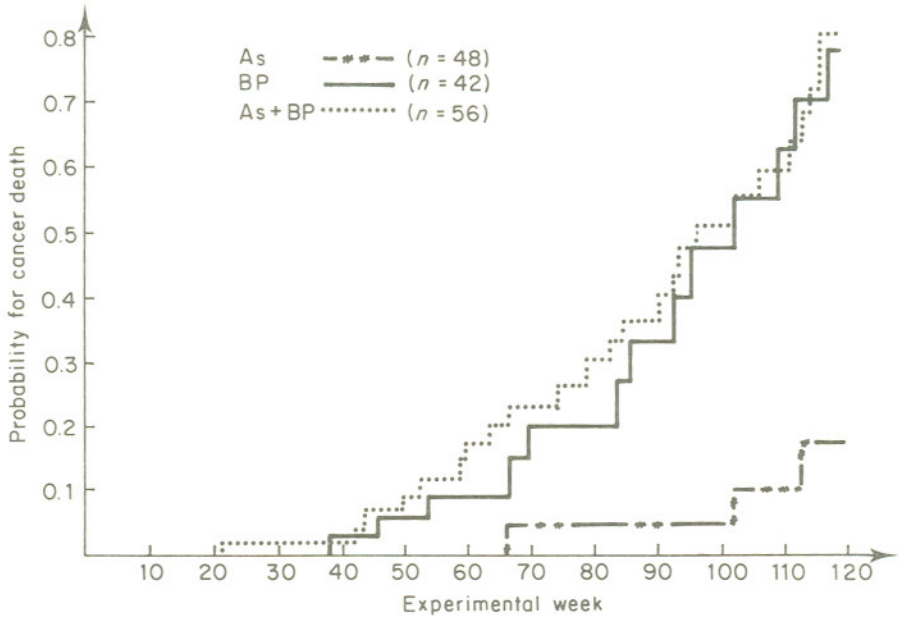

Figure 1 Probabilities for observation of cancer in the respiratory tract at death of hamsters given intratracheal instillations of arsenic trioxide (As) and/or BP (benzo[*a*]pyrene). $n =$ Number of animals in the respective groups after 15 weekly instillations. In the control group (where no cancers were found) $n = 3$. From Pershagen *et al.* (1984). *Reproduced by permission of Academic Press*

difference between the benzo[*a*]pyrene and the arsenic plus benzo[*a*]pyrene groups. In the arsenic group there were three animals with respiratory cancer but no such cancers occurred in the control group. There were also 19 benign tumours (papillomas, adenomas, and adenomatoid lesions) in the arsenic-treated animals while only four such tumours were observed in controls ($P < 0.01$). The observation of the three cancers, in combination with the excess occurrence of benign tumours of the respiratory tract, provides strong evidence that arsenic trioxide can cause lung cancer.

The use of a carrier dust was shown to enhance the retention of arsenic in the lung which may have increased the tumour response. Sulphuric acid, which was also given to all groups (including controls), may also have played a role. Although there was no evident interaction between benzo[*a*]pyrene and arsenic according to the overall calculated probability of cancer occurrence, there was a tendency to an interaction with regard to induction of adenomatous tumours.

3.1.2 Possible Role of Other Metallic Compounds for Respiratory Cancer in Smelter Workers

Autopsy samples from lung, liver, and kidney were obtained (Brune *et al.*, 1980) from dead persons who had worked at the same smelter where the epidemiological studies (Pershagen *et al.*, 1981; Wall, 1980) were carried out. By means of multielement analysis (neutron activation and atomic absorption spectrometry) it was demonstrated (Brune *et al.*, 1980) that an elevated concentration of a number of elements was present in the lungs of smelter workers compared with controls. These elements were antimony, arsenic, cadmium, chromium, lead, lanthanum and selenium. The largest difference in relation to the reference group was noted for antimony and arsenic. Concentrations of arsenic and antimony were approximately the same for persons who died during exposure compared with persons who died several years after retirement (Gerhardsson *et al.*, 1982; Wester *et al.*, 1981). The data thus indicate that the biological half-time of arsenic and antimony in the human lung is long.

For several metallic compounds there seems to be a positive relationship between the biological half-time in pulmonary tissues and carcinogenicity to the respiratory tract (e.g. chromates, nickel compounds). Airborne dust was collected from a work area at the smelter that was believed to cause exposure to arsenic. The elemental composition of this dust was analysed and found to contain approximately 20% arsenic and 2% antimony. The dust was neutron irradiated and shipped to our laboratory where it was instilled intratracheally in hamsters and the retention in the lung was studied by external measurements (Leffler *et al.*, 1984). A longer biological half-time of antimony was observed both in the form of antimony trioxide and in the form of actual industrial dust compared with arsenic trioxide and arsenic in the actual dust. Although more data are needed to draw any valid conclusions, these observations taken together suggest that the relatively high antimony values in workers' lungs may be explained by the considerably longer retention of antimony in pulmonary tissues compared with arsenic.

In accordance with the hypothesis mentioned, that metallic compounds with long pulmonary retention times tend to be carcinogenic, one might speculate that antimony may play a role as an additional risk factor besides arsenic, cigarette smoking, and possibly sulphur oxide for the excess of respiratory cancer observed in non-ferrous smelter workers.

3.2 Respiratory Carcinogenicity of Particulates from Oil- and Coal-fired Power Plants

Concern has been expressed over the possible carcinogenicity and mutagenicity of particulates emitted from coal- and oil-fired power plants and boilers

(Cederlöf *et al.*, 1978; Chrisp *et al.*, 1978; Holmberg and Ahlborg, 1983; Rall, 1980). In connection with an expected increase in coal usage in Sweden, it was considered of value to evaluate and compare the possible mutagenicity and carcinogenicity of emissions from coal- and oil-fired power plants and boilers as a part of the Swedish Coal Health Environment Project (1983). This programme has now been completed and a brief summary of the results will be given here.

3.2.1 Sampling from Coal- and Oil-fired Plants

Tests were carried out on samples from 17 different plants representing the two different fuels and various combustion and flue gas cleaning technologies. Samples of the flue gases which included both the particle and the gaseous phase (Alfheim *et al.*, 1983) were taken in a consistent way from all the plants. The extracts were tested for mutagenicity in the Ames *Salmonella* bioassay and extracts from eight plants were also tested on V79 hamster cells. Other tests, including sister chromatid exchange (SCE) in a macrophage-like cell line P388D1, influence on chromosome length in human lymphocytes, *in vivo* test on *Drosophila melanogaster*, and long-term carcinogenicity test by intratracheal instillation in Syrian golden hamsters, were performed only on selected samples of particulate material. Such particles were sampled from the stack with a dilution tunnel probe which simulated the cooling and dilution process that takes place in the flue gas plume (Ahlberg *et al.*, 1983).

3.2.2 Results of Mutagenicity Testing of Solvent-extracted Samples

In Figure 2 the results from the Ames tests (Alfheim and Møller, 1983) in these studies are displayed together with results from studies performed on wood and peat burning (compiled by Swedish Coal Health Environment Project, 1983). Although there is a large variation among stationary combustion devices of the same size using the same fuel, there seems to be a tendency that larger plants release less mutagenicity per MJ than smaller plants. However, what this probably means is that larger plants in general have better supervision of combustion efficiency, which is the important factor for reduction of mutagenicity. No mutagenicity was detected with the Ames test from nine out of the 17 samples from oil- and coal-fired boilers and power plants.

Of the samples tested for mutagenicity in V79 cells, four were negative and four were positive (Jenssen and Magnusson, 1983). The V79 mutagenicity assays were not performed on the sample from the plant that was most mutagenic in the Ames *Salmonella* assay. It is difficult to evaluate whether there is a correlation between the V79 and the *Salmonella* mutagenicity assay.

Comutagenic effects of the extracts from several of the flue gas samples together with benzo[*a*]pyrene were tested in the Ames *Salmonella* assay. For most samples a diminished mutagenicity of benzo[*a*]pyrene was noted, probably because of bacteriotoxic compounds in the extract (Alfheim and Møller, 1983).

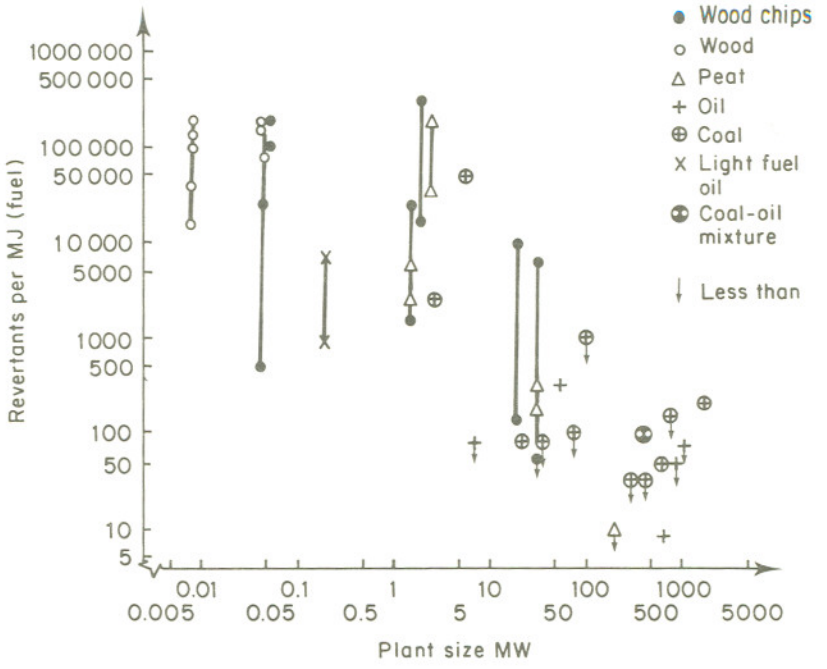

Figure 2 Mutagenicity of solvent extracts from the flue gases measured by the *Salmonella* assay. Relationship to plant size. From Swedish Coal Health Environment Project (1983). *Reproduced by permission of Swedish State Power Board.* MW = megawatt fuel power

Also, the extracted samples tested in the V79 hamster cell system were tested after pretreatment of the cells with a direct mutagen, methylnitrosourea. Comutagenic effects were noted for samples primarily from the oil combustion plants (Jenssen and Magnusson, 1983).

3.2.3 Tests Performed on Intact Particulate Material

To mimic the conditions in the flue gas plume, particulate samples from some of the plants were collected after cooling and dilution of the flue gases with air.

3.2.3.1 Tests on Drosophila and on a Macrophage-like Cell Line Two samples from plants showing low mutagenicity of the flue gas extracts were given to *Drosophila* in their feed. There were no mutagenic effects recorded (Jenssen and Magnusson, 1983).

Five samples from plants showing low or no mutagenicity of the flue gas extracts were used in a test where a macrophage-like cell line (P388D1) was exposed to the particles in culture. The particles are engulfed by the cells, and the effect assessed was the number of SCEs. There was a slight effect in two samples

from coal-fired plants. One of these samples also displayed a certain activity when tested for influence on chromosome length in human lymphocytes (Andersen, 1983a, b).

3.2.3.2 Long-term Carcinogenicity Assay On two samples, one from an oil-fired power plant and the other from a coal-fired power plant, long-term carcinogenicity assays were performed by intratracheal instillation of the suspended particulate materials in Syrian golden hamsters. In some groups the exposure was combined with benzo[*a*]pyrene and control groups were included. Fifteen weekly instillations were given and the animals were exposed and autopsied according to an established protocol (Saffiotti *et al.*, 1968). Results of an evaluation after 75 weeks are seen in Figure 3, where the probabilities for animals to die with malignant tumours are plotted at various times of observation (Persson *et al.*, 1983).

The particles as such were not carcinogenic during this observation time. This is in accord with their lack of mutagenic properties in the short-term tests. (The solvent extracts of the flue gas samples from both plants were negative in the *Salmonella* assay and the particles were negative in the SCE test on P388D1 cells.) When there was combined exposure with benzo[*a*]pyrene, clear differences among the different types of particles were detected (Figure 3). The particles from oil combustion, when combined with benzo[*a*]pyrene, displayed a marked carcinogenicity which was evident 20 weeks after the first instillation. Other types of particles did not differ from benzo[*a*]pyrene alone (at the 75-week observation period).

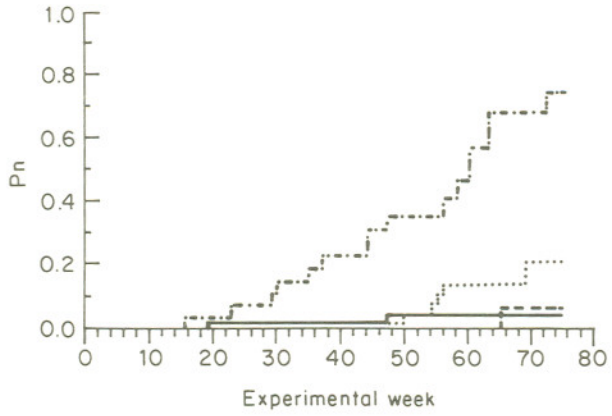

Figure 3 Probability (Pn) of observation of cancer at death in various groups after 75 weeks of observation; the study is still on-going. (. . .), E (benzo[*a*]pyrene 4.5 mg); (---), F (coal fly ash 4.5 mg + benzo[*a*]pyrene 4.5 mg); (- · -), G (oil soot 4.5 mg + benzo[*a*]pyrene 4.5 mg); (——), H (ferric oxide 4.5 mg + benzo[*a*]pyrene 4.5 mg). From Persson *et al.* (1983).

3.2.4 Aspects of the Usefulness of Methods for Assessment of Mutagenicity and Carcinogenicity of Flue Gas Samples

The classical approach when trying to assess the carcinogenicity of air pollution is epidemiology and long-term carcinogenicity assays in animals. Although there is a great deal of uncertainty in the epidemiological evidence, it is generally considered that combustion products of fossil fuels in ambient air (in combination with cigarette smoking) have been responsible for cases of lung cancer in humans (Cederlöf *et al.*, 1978; Holmberg and Ahlborg, 1983). It is not possible to assess exactly which chemical substances are most important. However, products of incomplete combustion (e.g. benzo[*a*]pyrene, a compound that has been established as an animal and human carcinogen) are considered to be of importance. Other compounds known to be animal or human carcinogens have also been identified in combustion effluents and the cancer risk associated with these compounds for populations living in the vicinity of the plants may be assessed by extrapolation from experimental or epidemiological data. However, only a small fraction of the compounds emitted to the air can be evaluated in this way.

All carcinogens in combustion effluents are by no means known. This makes it attractive to use biological tests of the complex mixture for the evaluation of mutagenicity and carcinogenicity. Short-term mutagenicity tests can be used for the screening of mutagens in different fractions of the complex mixture. In fact, this approach has shown that the majority of mutagens in combustion effluents are chemically unidentified.

Evidence from testing pure chemicals indicates a good qualitative correlation between carcinogenicity in animals and mutagenicity in the Ames *Salmonella* assay (McCann *et al.*, 1975; Purchase *et al.*, 1976; Rinkus and Legator, 1979) although this may not be valid for certain classes of chemicals. On the assumption that such a correlation would also exist for complex mixtures like combustion effluents, the Ames *Salmonella* assay has become an established tool for screening mutagenic chemicals in effluents (Ramel, 1983).

Only in a few cases have complex mixtures been assessed both in short-term bioassays and in long-term whole animal studies. The evidence collected in the Swedish Coal Health Environment Project (1983) provides some evidence in relation to this problem; no mutagenicity or carcinogenicity could be detected in the two samples collected from coal- and oil-fired plants.

One drawback with mutagenicity assays performed on solvent extracts is that primarily organic compounds are evaluated. The possibility of using other test systems with whole particles has been demonstrated in the above project; however, these programmes have not been sufficiently extensive to allow conclusions concerning the usefulness of these short-term methods.

The importance of particles as a cofactor for respiratory carcinogenicity has been documented for various metallic oxide particles (review by Nordberg and

Andersen, 1981; Saffiotti *et al.*, 1972a,b). Interactions of this kind were demonstrated also in the long-term carcinogenicity study in hamsters performed in the Swedish Coal Health Environment Project (Figure 3). Particles from oil combustion (large hollow carbonaceous structures) were very carcinogenic when coated with benzo[*a*]pyrene. Such was not the case for the small, round silicate particles from coal combustion that were coated with benzo[*a*]pyrene.

It is generally recognized that short-term tests (e.g. Ames *Salmonella* assay) are useful for screening combustion effluents for potential carcinogenicity. However, a quantitative correlation to carcinogenicity in animals and humans cannot be expected (Holmberg and Ahlborg, 1983; Ramel, 1983). A more generally valid system for estimating human risk from bioassay data would also have to include bioassays that allow whole particles to be tested such as long-term inhalation or instillation in animals and possibly tissue culture systems, in addition to assays based on extraction in organic solvents. This consideration is in accordance with the conclusions of a symposium concerning bioassays and air pollutants (Holmberg and Ahlborg, 1983).

4 GENERAL SUMMARY AND CONCLUSIONS

Examples have been described in the present paper of a few specific situations both in the general environment and in the occupational setting which involve exposure of humans to a number of chemical substances simultaneously or in sequence. Epidemiological studies are available in all instances to support the notion that the various exposure situations may influence human health. The studies vary in their ability to provide dose–response relationships for humans.

Among the examples described in this paper the dose–response curve concerning exposure to *methyl mercury from food and the risk of neurological signs* in human populations seems to be the best established. Animal experiments demonstrate similar effects to those found in humans, at similar tissue levels. The toxicological mechanism of action has been partly revealed in such experiments. When it comes to combined exposure, the influence of selenium has been demonstrated in animal experiments but available evidence is at present inadequate for extrapolation to humans. When experiments have been performed on animal species that are similar to humans with regard to methyl mercury metabolism and toxicity, an extrapolation of selenium–methyl mercury interaction to dose–response relationships for humans should be possible.

A large number of epidemiological studies have been published concerning *general air pollution in urban areas and respiratory disease* (chronic bronchitis) or mortality. Evaluations concerning the concentrations of sulphur dioxide and particles expected to give rise to an increased risk of adverse health effects have been presented. However, dose–response curves calculated from available data for the mixed exposure and its components are not well established scientifically. Evidence from experiments on asthmatics has been presented during recent years

and indicates that respiratory effects can be induced at relatively low exposure levels of sulphur dioxide. This is in accordance with the fact that mortality increases during episodes with high air pollution levels in persons with pre-existing respiratory disease. Evidence mainly from animal experiments has indicated that combined effects with certain types of sulphate particles (but not with other sulphates) may be of importance.

For scientifically valid dose–response curves to be established for sulphur dioxide and particulate air pollutants, epidemiological evidence is needed that includes adequate dose descriptions for sulphur dioxide and specific irritating particles and detailed information concerning smoking habits in the groups particularly sensitive to air pollutants. Information concerning other air pollutants that may have similar effects should also be included. In view of the long time it takes to develop chronic bronchitis or other respiratory diseases, long follow-up times are necessary for studies relating air pollution levels to incidence of such diseases. Although some of the reported epidemiological studies included evidence of the kind specified, most of them were inadequate in one or several aspects.

A quantitative dose–response relationship relating *arsenic exposure* to *risk of respiratory cancer* has been presented. However, this linear relationship was calculated before the interaction between arsenic exposure in non-ferrous smelters and cigarette smoking had been detected. With the knowledge available at present it should be possible to recalculate the relationship and break it up into the smoking and arsenic components. The 'arsenic' component might need to be further broken down in the future into true arsenic and other chemical substances (e.g. antimony).

Predictions about quantitative relationships between *air pollution and respiratory cancer* are very uncertain. Epidemiological evidence is difficult to use because of several deficiencies in dose descriptions and the influence of a number of confounding factors.

Because of the difficulties with the epidemiological evidence, a considerable effort has been devoted to the construction of a system of experimental techniques that would allow qualitative and quantitative predictions about human cancer risk from samples of general air pollution, as well as from the sources of this pollution (e.g. various combustion effluents). The Ames *Salmonella* assay has been established as a useful tool for this purpose but it can only give a qualitative indication of hazard. Combinations of mutagenicity, other short-term bioassays, and long-term carcinogenicity assays in animals may provide a means of making some kind of quantitative predictions about human risk.

5 REFERENCES

Ahlberg, M., Berghem, L., Nordberg, G., Persson, S.-Å., Rudling, L., and Steen, B. (1983). Chemical and biological characterization of emissions from coal- and oil-fired plants. *Environ. Health Perspect.*, **47**, 85–102.

Alarie, Y., Krumm, A. A., Busey, W. M., Ulrich, C. E., and Kantz, R. J. (1975). Long-term exposure to sulfur dioxide, sulfuric acid mist, fly ash, and their mixtures. Results of studies in monkeys and guinea pigs. *Arch. Environ. Health*, **30,** 254–262.

Alfheim, I., and Møller, M. (1983). *Mutagenicity in Emissions from Coal- and Oil-fired Boilers as Detected by the Salmonella/Microsome Assay*. Technical Report 60. Coal Health Environment Project, Swedish State Power Board, Stockholm.

Alfheim, I., Bergström, I. G. T., Jenssen, D., and Møller, M. (1983). Mutagenicity in emissions from coal- and oil-fired boilers. *Environ. Health Perspect.*, **47,** 177–187.

Amdur, M. O. (1974). The long road from Donora. Cummings Memorial Lecture. *Ind. Hyg. Assoc. J.*, **34,** 589–597.

Amdur, M. O., and Underhill, D. (1968). The effect of various aerosols on the response of guinea pigs to sulfur dioxide. *Arch. Environ. Health*, **16,** 460–468.

Amdur, M. O., Dubriel, M., and Creasia, D. A. (1978a). Respiratory response of guinea pigs to low levels of sulfuric acid. *Environ. Res.*, **15,** 418–423.

Amdur, M. O., Bayles, J., Ugro, V., and Underhill, D. M. (1978b). Comparative irritant potency of sulfate salts. *Environ. Res.*, **16,** 1–8.

Andersen, O. (1983a). Effects of coal combustion products and metal compounds on sister chromatid exchange (SCE) in a macrophage like cell line. *Environ. Health Perspect.*, **47,** 239–254.

Andersen, O. (1983b). *Effects of Metal Compounds on Chromosome Length*. Technical Report 64. Coal Health Environment Project, Swedish State Power Board, Stockholm.

Andersen, I., Lundqvist, G. R., Jensen, P. L., and Proctor, D. F. (1974). Human response to controlled levels of sulfur dioxide. *Arch. Environ. Health*, **28,** 31–39.

Andersen, I., Mølhave, L., and Proctor, D. F. (1981). Human response to controlled levels of combinations of sulfur dioxide and inert dust. *Scand. J. Work Environ. Health*, **7,** 1–7.

Bakir, F., Damluji, S. F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N. Y., Tikriti, S., Dhahir, H. I., Clarkson, T. W., Smith, J. C., and Doherty, R. A. (1973). Methylmercury poisoning in Iraq. *Science*, **181,** 230–241.

Berlin, M. (1983). *Health Effects in Connection with Mercury Emission from Coal Burning*. Technical Report 79. Coal Health Environment Project, Swedish State Power Board, Stockholm (in Swedish with English summary).

Brune, D., Nordberg, G., and Wester, P. O. (1980). Distribution of 23 elements in the kidney, liver and lungs of workers from a smeltery and refinery in north Sweden exposed to a number of elements and of a control group. *Sci. Total Environ.*, **16,** 13–35.

Casarett, L. J., and Doull, J. (Eds.) (1975). *Toxicology. The Basic Science of Poisons*. Macmillan, New York, London: 768 pages.

Cederlöf, R., Doll, R., Fowler, B., Friberg, L., Nelson, N., and Vouk, V. (Eds.) (1978). Air pollution and cancer: risk assessment, methodology and epidemiological evidence. Karolinska Institute, Symposium. *Environ. Health Perspect.*, **22,** 1–12.

Chrisp, C. E., Fisher, G. L., and Lammert, J. E. (1978). Mutagenicity of filtrates from respirable coal fly ash. *Science*, **199,** 73–75.

Ericsson, G., and Camner, P. (1983). Health effects of sulfur oxides and particulate matter in ambient air. *Scand. J. Work Environ. Health*, **9,** Suppl. 3, 1–52.

Fenters, J. D., Bradof, J. N., Aranyi, C., Ketels, K., Ehrlich, R., and Gardner, D. E. (1979). Health effects of long-term inhalation of sulfuric acid mist–carbon particle mixtures. *Environ. Res.*, **19,** 244–257.

Flanagan, P. R., McLellan, J. S., Haist, J., Cherian, G., Chamberlain, M. J., and Valberg, L. S. (1978). Increased dietary cadmium absorption in mice and human subjects with iron deficiency. *Gastroenterology*, **74,** 841–846.

Ganther, H. E., and Sunde, M. L. (1974). Effect of tuna fish and selenium on the toxicity of methylmercury. A progress report. *J. Food Sci.*, **39,** 1.

Ganther, H. E., Goudie, C., Sunde, M. L., Kopecky, M. J., Wagner, P., Oh, S. H., and Hoekstra, W. G. (1972). Selenium: relation to decreased toxicity of methylmercury in diets containing tuna. *Science*, **175,** 1122.

Gardner, D. E., Miller, F. J., Illing, J. W., and Kirtz, J. M. (1977). Increased infectivity with exposure to ozone and sulfuric acid. *Toxicol. Lett.*, **1,** 59–64.

Gerhardsson, L., Brune, D., Nordberg, G. F., and Wester, P. O. (1982). Antimony in lung, liver and kidney tissue from deceased smelter workers. *Scand. J. Work Environ. Health*, **8,** 201–208.

Giddens, W. E., and Fairchild, G. A. (1972). Effects of sulfur dioxide on the nasal mucosa of mice. *Arch. Environ. Health*, **25,** 166–173.

Hazucha, M., and Bates, D. V. (1975). Combined effect of ozone and sulphur dioxide on human pulmonary function. *Nature*, **257,** 50–51.

Holma, B., Kjaer, G., and Stokholm, H. (1979). Air pollution, hygiene and health of Danish schoolchildren. *Sci. Total Environ.*, **12,** 251–286.

Holmberg, B., and Ahlborg, U. (Eds.) (1983). Consensus report. Mutagenicity and carcinogenicity of car exhausts and coal combustion emissions. *Environ. Health Perspect.*, **47,** 1–30.

IARC (1980). Some metals and metallic compounds. In *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 23. International Agency for Research on Cancer, Lyon.

Jenssen, D., and Magnusson, I. (1983). *Mutagenicity in Emissions from Coal- and Oil-fired Boilers. Investigations in Drosophila and Mammalian Cells.* Technical Report 61. Coal Health Environment Project, Swedish State Power Board, Stockholm (in Swedish with English summary).

Leffler, P., Gerhardsson, L., Brune, D., and Nordberg, G. F. (1984). Lung retention of antimony and arsenic in hamsters after intratracheal instillations of industrial dust. *Scand. J. Work Environ. Health*, **10,** 245–251.

Lewis, T. R., Campbell, K. I., and Vaughan, T. R. (1969). Effects on canine pulmonary function. *Arch. Environ. Health*, **18,** 596–601.

Lewis, T. R., Moorman, W. J., Ludmann, W. F., and Campbell, K. I. (1973). Toxicity of long-term exposure to oxides of sulfur. *Arch. Environ. Health*, **26,** 16–21.

Lindvall, T. (1984). *Health Effects of Nitrogen Dioxide and Oxidants.* Final Report 1984-12-20. National Institute of Environmental Medicine, Stockholm, Sweden.

Marsh, D. O., Myers, G. J., Clarkson, T. W., Amin-Zaki, L., and Tikriti, S. (1980). Fetal methylmercury poisoning: clinical and toxicological data on 29 cases. *Ann. Neurol.*, **7,** 348–353.

McCann, J., Choi, E., Yamasaki, E., and Ames, B. N. (1975). Detection of carcinogens and mutagens in the *Salmonella* microsome test: assay of 300 chemicals. *Proc. Natl. Acad. Sci. (USA)*, **72,** 5135–5139.

McJilton, C. E., Frank, R., and Charlson, R. (1973). Role of relative humidity in the synergistic effect of a sulfur dioxide–aerosol mixture on the lung. *Science*, **182,** 503–504.

McJilton, C. E., Frank, R., and Charlson, R. (1976). Influence of relative humidity on functional effects of an inhaled SO_2–aerosol mixture. *Am. Rev. Respir. Dis.*, **113,** 163–169.

Nordberg, G. F., and Andersen, O. (1981). Metal interactions in carcinogenesis: enhancement, inhibition. *Environ. Health Perspect.*, **40,** 65–81.

Nordberg, G. F., and Strangert, P. (1976). Estimation of a dose–response curve for long-term exposure to methylmercuric compounds in human beings taking into account variability of critical organ concentration and biological halftime. In Nordberg, G. (Ed.) *Effects and Dose–Response Relationships of Toxic Metals.* Elsevier, Amsterdam.

Nordberg, G. F., and Strangert, P. (1978). Fundamental aspects of dose–response relationships and their extrapolation for non-carcinogenic effects of metals. *Environ. Health Perspect.*, **22,** 97–102.

Nordberg, G. F. and Strangert, P. (1985). Risk estimation models derived from metabolic and damage parameter variation in a population. In Vouk, V. B., Butler, G. C., Hoel, D. G., and Peakall, D. B. (Eds.) *Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems*. SCOPE 26/SGOMSEC 2, pp. 477–491. John Wiley & Sons, Chichester, New York.

Nordberg, G. F., *et al.* (1978). Factors influencing metabolism and toxicity of metals: a consensus report by the Task Group on Metal Interactions. *Environ. Health Perspect.*, **25,** 31–41.

Nordberg, G. F., *et al.* (1986). Factors influencing effects and dose–response relationships of metals. In Friberg, L., Nordberg, G. F., and Vouk, V. B. (Eds.) *Handbook on the Toxicology of Metals*, pp. 175–205. Elsevier, Amsterdam.

Ohi, G., Nishigaki, S., Seki, H., Tamura, Y., Maki, T., Konno, H., Ochial, S., Yamada, H., Shihamura, Y., Mizoguchi, I., and Yagyu, H. (1976). Efficacy of selenium in tuna and selenite in modifying methylmercury intoxication. *Environ. Res.*, **12,** 49–58.

Pershagen, G., Wall, S., Taube, A., and Linnman, L. (1981). On the interaction between occupational arsenic exposure and its relationship to lung cancer. *Scand. J. Work Environ. Health*, **7,** 302–309.

Pershagen, G., Björklund, N., and Nordberg, G. F. (1984). Carcinomas of the respiratory tract in hamsters given arsenic trioxide and/or benzo[*a*]pyrene by the pulmonary route. *Environ. Res.*, **34,** 227–241.

Persson, S.-Å., Ahlborg, M., Berghem, L., Bergman, F., Könberg, E., and Nordberg, G. (1983). *Long-term Carcinogenicity Test on Syrian Golden Hamsters—Comparison between Particulate Emissions from One Coal- and One Oil-fired Power Plant*. Technical Report 65. Coal Health Environment Project, Swedish State Power Board, Stockholm (in Swedish with English summary).

Purchase, I. F. H., Longstaff, E., Ashby, J., Styles, J. A., Anderson, D., Lefevre, P. A., and Westwood, F. (1976). Evaluation of six short-term tests for detecting organic chemical carcinogens and recommendation for their use. *Nature*, **264,** 624–627.

Rall, D. P. (Chairman) (1980). Report of health and environmental effects of increased coal utilization. *Environ. Health Perspect.*, **36,** 135–154.

Ramel, C. (1983). Advantages of and problems with short-term mutagenicity tests for the assessment of mutagenic and carcinogenic risk. *Environ. Health Perspect.*, **47,** 153–159.

Rinkus, S. J., and Legator, M. S. (1979). Chemical characterization of 465 known or suspected carcinogens and their correlation with mutagenic activity in the *Salmonella typhimurium* system. *Cancer Res.*, **39,** 3282–3318.

Rossman, T. G. (1981). Effect of metals on mutagenesis and DNA repair. *Environ. Health Perspect.*, **40,** 189–195.

Rossman, T. G., Meyn, M. S., and Troll, W. (1977). Effects of arsenite on DNA-repair in *Escherichia coli*. *Environ. Health Perspect.*, **19,** 229–233.

Saffiotti, U., Cefis, F., and Kolb, L. H. (1968). A method for the experimental induction of bronchogenic carcinoma. *Cancer Res.*, **28,** 104–124.

Saffiotti, I., Montesano, R., Sellakumar, A. R., and Kaufman, D. G. (1972a). Respiratory tract carcinogenesis induced in hamsters by different dose levels of benzo[*a*]pyrene and ferric oxide. *J. Natl. Cancer Inst.*, **49,** 1199–1204.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G. (1972b). Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo[*a*]pyrene and ferric oxide. *Cancer Res.*, **32,** 1073–1081.

Schiff, L. J., Bryne, M. M., Fenters, J. D., Graham, J. A., and Gardner, D. E. (1979).

Cytotoxic effects of sulfuric acid mist, carbon particulates, and their mixtures on hamster tracheal epithelium. *Environ. Res.*, **19,** 339–354.

Sheppard, D., Saisho, A., Nadel, J. A., and Boushey, H. A. (1981). Exercise increases sulfur dioxide-induced bronchoconstriction in asthmatic subjects. *Am. Rev. Respir. Dis.*, **123,** 486–491.

Snell, R. E., and Luchsinger, P. C. (1969). Effects of sulfur dioxide on respiratory flow rates and total respiratory resistance in normal human subjects. *Arch. Environ. Health*, **18,** 693–698.

Stjernberg, N., Eklund, A., Nyström, L., Rosenhall, L., Emmelin, A., and Strömqvist, L.-H. (1985). Prevalence of bronchial asthma and chronic bronchitis in a community in northern Sweden; relation to environmental and occupational exposure to sulphur dioxide. *Eur. J. Respir. Dis.*, **67,** 41–49.

Swedish Coal Health Environment Project, Final Report, Main Part (1983). *The Health and Environmental Effects of Coal.* Swedish State Power Board, Stockholm.

Wall, S. (1980). Survival and mortality pattern among Swedish smelter workers. *Int. J. Epidemiol.*, **9,** 73–87.

Welsh, S. O. (1976). Influence of vitamin E on mercury poisoning in rats. *Fed. Proc.*, **35,** 761–766.

Welsh, S. O., and Soares, J. H. (1976). The protective effect of vitamin E and selenium against methyl-mercury toxicity in the Japanese quail. *Nutr. Rep. Int.*, **13,** 43–48.

Wester, P. O., Brune, D., and Nordberg, G. F. (1981). Arsenic and selenium in lung, liver and kidney tissue from dead smelter workers. *Br. J. Ind. Med.*, **38,** 179–184.

WHO (1977). *Oxides of Nitrogen.* Environmental Health Criteria 4. World Health Organization, Geneva.

WHO (1979). *Sulfur Oxides and Suspended Particulate Matter.* Environmental Health Criteria 8. World Health Organization, Geneva.