Special nerve functions and colour discrimination in workers with long term low level exposure to carbon disulphide

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Abstract

Certain functions of the peripheral and autonomic nervous systems, and colour discrimination were examined in 45 workers (mean age 49; mean exposure to carbon disulphide (CS_2) 20 years) and 37 controls (mean age 48). Conduction velocity and refractory period of the peroneal and sural nerves were determined. The conduction velocity of the slower fibres of the peroneal nerve was measured by means of an improved method that makes use of the refractory period. Function of autonomic nerves was assessed by measuring the variation in heart rate during rest, during deep breathing, and during isometric muscle contraction. Colour discrimination was evaluated by the Lanthony desaturated test. Individual cumulative exposure to CS_2 was calculated on the basis of exposure in the past and individual job history. Mean cumulative exposure was 165 ppm-years. The peroneal nerves of exposed workers showed a decrease (−1·0 m/s) in conduction velocity of the slow fibres and a prolongation (0·1 ms) of the refractory period (mean 1·6 ms) compared with controls. These effects were related to cumulative exposure. No impairment of function of the sural nerve or of colour discrimination was found. The muscle heart reflex was decreased in the exposed group, but this was not related to cumulative exposure. This study has established more firmly that a decrease in conduction velocity of slow motor fibres occurs at low levels of exposure to CS_2. Extrapolation of the results suggests that small effects may occur after 40 years of exposure to concentrations below the present threshold limit value (10 ppm).

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Prolonged occupational exposure to less than 20 ppm carbon disulphide (CS_2) is known to adversely affect peripheral nerves[1–6] and colour discrimination,[7] and a decrease in conduction velocity of slow motor fibres is an early indicator of CS_2 neuropathy.[4 5]

Methods have been described that may be used to improve the early detection of neuropathy induced by CS_2. Studies on diabetic and uraemic patients suggest that prolongation of the refractory period of peripheral nerves may be an earlier symptom of neural impairment than the decrease in conduction velocity.[8 9] Matikainen and Juntunen[10] found that the variation in heart rate in workers suspected of toxic neuropathy was less than in a control group.

The objectives of our investigation were to determine whether a low level exposure to CS_2 affected the peripheral nervous system and colour vision, and to study the feasibility of proposed diagnostic methods.

Subjects

All male workers at a viscose rayon plant who had been exposed to CS_2 for 10 years or more, and a selection of non-exposed workers from the same plant were personally contacted by local occupational health personnel; 87 workers agreed to participate. After exclusion of five workers with other risk factors for neuropathy the analyses concerned 45 exposed workers and 37 controls. The exposed workers (mainly shift workers) had a mean age of 49 (SD 7) and had been exposed to CS_2 for a mean of 20 (SD 9) years. The controls were matched on a group basis with the exposed workers for socioeconomic state, shift work, nationality, and age (48 (SD 7) years); they had all been employed for at least 10 years in the company and had never been exposed to CS_2. Both groups consisted of Dutch, Spanish, and Italian nationals. Ten exposed and eight control workers had worked until 10 years ago in another viscose plant belonging to the same company.

Exposure

All exposed workers were employed in the spinning or bleaching departments. A zoning strategy[11 12] was

used to group the workers with respect to exposure. The exposure concentrations for the zones were determined from personal air samples taken during the past three years (values obtained were 1 ppm for supervisors, 6 ppm for the spinning department, and 12 ppm for the bleaching department). The present situation in the bleaching department has only existed for the past three years; before that two zones were identified with exposure concentrations of 8 and 17 ppm.

For a number of reasons we are confident that the past and present exposures are comparable. The work and the process have hardly changed since 1945. The results from a large number of spot samples taken from 1946 did not indicate a change in exposure level; the effect of the gradual installation of exhausts was counteracted by an increase in production.

Information about exposure in the other plant (where 10 workers had been exposed earlier) was scarce. Workers' exposure was estimated to have been 30 ppm from 1950 to 1960, 25 ppm from 1960 to 1970, and 20 ppm from 1970 to 1976, which was the year the plant closed.

Because present neural impairment is probably due to long term exposure to carbon disulphide, an individual cumulative exposure (E_C) was calculated as the sum of the products of the time spent in different exposure zones and the mean exposure for those zones at those times (ppm-years). In using E_C it was assumed that recent and former exposure contributed equally to present neural impairment. Slight impairment may recur, however, after cessation of exposure,[13-15] and may even recur during low level exposure. For this reason we also allowed for a smaller contribution of past exposure to the actual impairment by weighting the cumulative exposure with half times of 10 and five years (E_{10} and E_5 respectively).

The average cumulative exposure (E_C) of the exposed workers was 165 ppm-years; weighting the exposure with half times of 10 and five years resulted in average weighted exposures of 74 (E_{10}) and 43 (E_5) ppm-years.

Methods

The examinations were performed in 1986 in two warm rooms (25–30°C) in the occupational health centre at the plant. The investigators performing the tests did not know the workers' exposure state. After the examinations the worker, assisted by an investigator, completed a questionnaire on drinking habits, diseases, and exposure to other factors that might cause peripheral neuropathy. All workers were subjected successively to the following tests.

AUTONOMIC NERVE FUNCTION

Before this examination the subjects rested for about 10 minutes in a supine position. Using chest leads a cardiotachogram was recorded on an ink writer. For measurement of forced respiratory sinus arrhythmia (FRSA) the subject was instructed to perform six consecutive maximal inspirations (five seconds) and expirations (five seconds) in one minute; after two minutes rest one repetition followed. We determined the mean of the differences between maximum (inspiratory) and minimum (expiratory) instantaneous (beat to beat) heart rate.[16 17]

Next, for the measurement of muscle heart reflex (MHR) the subject was asked to exert maximum isometric power on a hand dynamometer during five seconds on verbal command with four to five repetitions at intervals of about 90 seconds. Grip force was monitored on an ink writer. We calculated the mean of the differences of the maximum instantaneous heart rate during muscle contraction and the resting heart rate on the basis of three trials in which the exerted power was at least 80% of the subjects' maximum.

The resting arrhythmia (RA) was determined as the standard deviation of the instantaneous heart rate in the 30 second interval immediately preceding the MHR responses. The mean of the standard deviations of three trials was used.

A combined Z score (Z_{aut}) was calculated as being the sum of the individual Z scores of the FRSA and MHR.

ELECTROPHYSIOLOGICAL EXAMINATION

The subject's leg was warmed up to the knee in a barrel of water at 37°C for 10 minutes before electrophysiological testing. All stimuli were 1·2 × voltage that just gave a maximal response. Only surface electrodes were used. The skin temperatures close to the stimulating and recording electrodes were measured.

Motor nerve conduction velocity of the peroneal nerve (MNCV) and amplitude of the compound muscle action potential (CMAP) were determined by electrically stimulating at the head of the fibula and the ankle and recording from the extensor digitorum brevis muscle. All responses were recorded by a Minc-11 laboratory computer and stored on floppy disc.

Motor refractory period (MRP) was determined using a technique adapted from those of Faisst and Meyer[18] and Kimura.[19] Two proximal stimuli with interstimulus intervals (ISI) between 0·5 and 3·0 ms and one distal stimulus were applied (fig 1). The ISI that would result in a test response with an amplitude of 50% was taken as the refractory period (MRP).

Conduction velocity of the slow motor fibres (CVSF) was determined with an antidromic collision technique.[20 21] The ISI that resulted in a response to the proximal stimulus with an amplitude of 80% was taken to calculate the CVSF. To correct for the

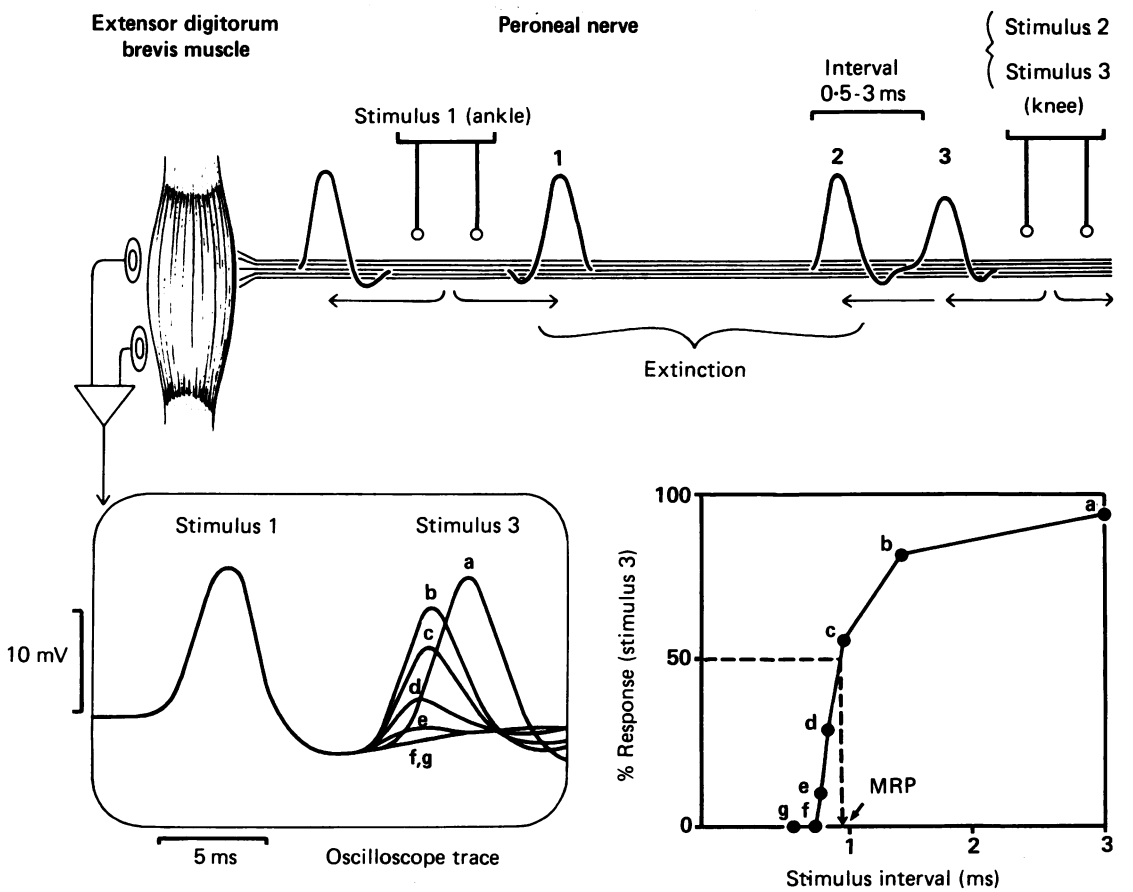

Figure 1 Determination of refractory period of motor nerve fibres. Top picture: extensor digitorum brevis muscle and peroneal nerve with stimulation electrodes. Stimuli are applied in indicated sequence. Response to first proximal stimulus (stimulus 2) collides with antidromic response to distal stimulus, which results in extinction; for this reason only the distal (stimulus 1) and the second proximal (stimulus 3, test) stimuli elicit muscle responses. Bottom pictures; left: oscilloscope trace of seven superimposed combined distal/test muscle responses with different interstimulus intervals (ISI). Right: relative amplitude plotted against ISI. The ISI that would result in test response with amplitude of 50% (MRP) was determined by linear interpolation.

refractory period both 1 ms and the individual MRP were used; the resulting parameters are called $CVSF_1$ and $CVSF_{RP}$.

Sensory nerve conduction velocity (SNCV) and the amplitude of the compound nerve action potential (CNAP) of the sural nerve were determined antidromically by electrically stimulating at the calf and recording at the lateral malleolus. Four to eight responses were filtered and averaged and the results were stored on floppy disc. The SNCV was calculated on the basis of the latency of the first positive peak. The sensory refractory period (SRP) was determined by applying paired stimuli with intervals between 0·5 and 3·0 ms. The ISI that would result in a test response with an amplitude of 50% was taken as the SRP.

COLOUR DISCRIMINATION

Workers were tested with the Lanthony desaturated panel D-15 test, designed to detect mild acquired defects of colour vision.[22 23] In this test subjects have to put 15 faintly coloured caps in order with respect to colour. The caps, if properly ordered, form a series in which colour changes from a faint blue through a faint yellow to a faint lilac. The test was conducted on a black, non-reflecting table illuminated with two fluorescent light sources (Philips type 57) at a distance of 75 cm. The colour confusion index according to Bowman[24] was calculated. As it was expected that loss of colour vision due to exposure would be apparent in cap interchanges due to confounding blue and yellow rather than confounding red and green,[25] separate colour confusion indices were cal-

Table 1 Effects of exposure to CS_2 on nerve function

	Mean value for controls	E–C (90% CI)	Effect of 100 ppm-years cumulative exposure (E_C) (90% CI)
FRSA (b/min)	21·1	−3·37 (−6·33, −0·15)*	−0·75 (−2·06, 0·56)
MHR (b/min)	17·9	−4·09 (−6·91, −0·93)*	−0·92 (−2·16, 0·33)
RA (b/min)	2·4	−0·38 (−0·73, 0·01)	−0·00 (−0·15, 0·14)
Z_{aut}	0·3	−0·51 (−0·86, −0·16)**	−0·12 (−0·25, 0·02)
MNCV (m/s)	47·6	−0·62 (−1·78, 0·55)	−0·39 (−0·83, 0·06)
CVSF (m/s)	42·2	−1·00 (−2·32, 0·31)	−0·73 (−1·23, −0·24)**
$CVSF_{RP}$ (m/s)	45·6	−1·48 (−2·72, −0·24)*	−0·62 (−1·09, −0·15)**
CMAP (mV)	6·8	0·17 (−0·77, 1·11)	−0·05 (−0·41, 0·31)
MRP (ms)	1·6	−0·04 (−0·18, 0·10)	0·05 (0·00, 0·10)*
SNCV (m/s)	52·1	0·03 (−1·56, 1·61)	0·05 (−0·57, 0·67)
CNAP (μV)	9·6	−1·22 (−2·45, 0·24)	−0·03 (−0·66, 0·60)
SRP (ms)	1·3	−0·18 (−0·31, −0·02)	−0·01 (−0·07, 0·06)

*p < 0·05; **p < 0·01.
†E–C = Difference between exposed workers and controls.
Mean E_C = 165 ppm-years.

culated for blue and yellow and for red and green according to a method modified from Bowman.[24]

DATA ANALYSIS

The difference between the exposed workers and the control group was calculated by analysis of covariance. The effect of cumulative exposure was calculated by multiple regression analysis; the control group was included in each analysis. We controlled for age, nationality, and alcohol consumption in all analyses by including them as covariables. For autonomic nerve parameters we also included the Quetelet index; for peripheral nerve parameters we also included body height (except for refractory periods) and skin temperature of the leg. Cumulative consumption of alcohol was calculated in a similar way as cumulative exposure to CS_2. A one sided level of significance of 5%, and a two sided confidence interval of 90% were applied. For non-normally distributed dependent variables, a suitable transformation was made.

Because many confusion indices had a value of zero in the Lanthony test, the data from the exposed workers and the control groups were compared by the Mann-Whitney test. Also, Spearman rank correlation coefficients were computed for confusion indices with age, exposure, consumption of alcohol, and smoking habits. A one sided level of significance of 5% was applied.

Results

NERVE FUNCTION TESTS

In comparing exposed workers with control subjects we excluded three workers who had only been exposed as supervisors and three exposed workers who had not been exposed at all during the past ten years. We found small effects of exposure on the FRSA (−3·4 beats/min), MHR (−4·1 beats/min), Z_{aut} (−0·5), and $CVSF_{RP}$ (−1·5 m/s). The refractory periods were not prolonged (table 1).

The significant differences in FRSA and MHR disappeared if cumulative exposure (weighted) was taken into account (table 1). The effect on Z_{aut} remained for short half times (table 2). For all parameters of autonomic nerve function the absolute t values decreased with longer half times.

For indices of motor nerve function the absolute t values increased with longer half times. The $CVSF_{RF}$ was sensitive to CS_2 exposure irrespective of the half time chosen. Figure 2 shows the relation between $CVSF_{RP}$ and cumulative exposure E_c. Leaving out the subject with the highest E hardly changed the result. With longer half times the $CVSF_1$ and MRP differed significantly between the groups. The MNCV and sensory nerve parameters did not differ between the groups irrespective of the half time chosen.

COLOUR DISCRIMINATION

Three exposed workers did not complete the test for reasons unrelated to exposure. The overall colour confusion index as well as those for red and green and for blue and yellow of the remaining 42 exposed workers did not differ significantly from the corresponding indices of the 37 control workers (p > 0·20). Furthermore, the confusion indices did not

Table 2 t Values for effects of exposure measures on Z_{aut}, $CVSF_{RP}$, and MRP

	E–C†	E_5‡	E_{10}‡	E_C‡
Z_{aut}	−2·43**	−1·72*	−1·69*	−1·43
$CVSF_{RP}$	−1·98*	−1·80*	−2·08*	−2·21**
MRP	−0·50	0·08	0·73	1·67*

*p < 0·05; **p < 0·01.
†E–C = Difference between exposed workers and controls.
‡E_C, E_5, and E_{10} = cumulative exposures with half times of 0, 5, and 10 years.

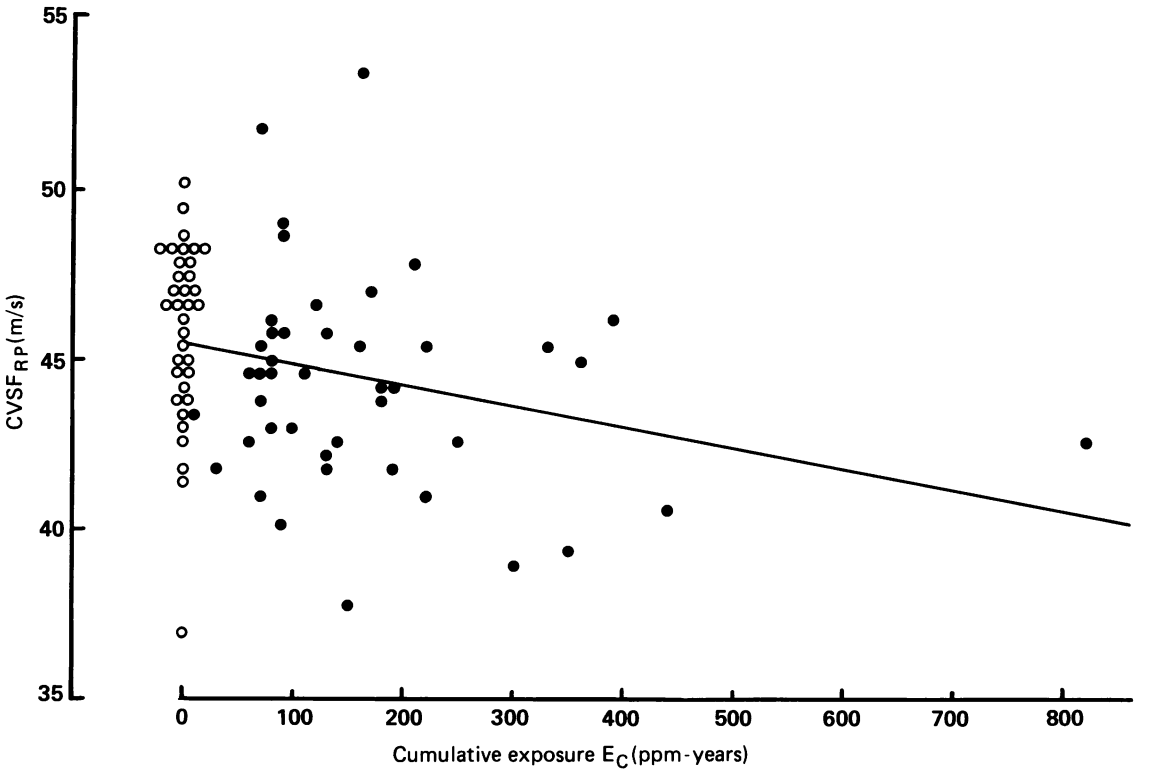

Figure 2 Relation between $CVSF_{RP}$ and cumulative exposure E_C.

correlate with cumulative exposure, or with cumulative exposures with a half life of five or 10 years ($p > 0{\cdot}10$).

With respect to possible confounding factors, no relation was found between exposure and age (owing to the matching procedure), alcohol consumption, or smoking habits. Hence, the small correlations between these factors and the confusion indices had no confounding effect.

Discussion

EXPOSURE

In this investigation we determined cumulative exposure to characterise non-continuous individual exposure in the past. Weighting the cumulative exposure with half times of 10 and five years implies recovery from neural impairment with an exponential time course. No evidence exists to support this but it may be considered as a reasonable assumption to evaluate the relative importance of recent and past exposure. The choice of the half times was based on a report concerned with the recovery of nerve impairment in pathological cases.[13]

A decrease in the statistical significance (t value) of effects when exposure was weighted with longer half times should be interpreted with caution. These differences may be explained by the extent of reversibility of the effects. Another explanation arises from the correlation between age and cumulative exposure (weighted), which increased with longer half times. As a result the statistical significance of an effect due to exposure tends to decrease with increasing half time; hence an increasing significance with longer half times may suggest a non-reversible effect.

Taking these factors into account we believe that with good exposure data the determination of cumulative exposure provides an attractive measure for past non-continuous exposure, because the subject's history of exposure is taken into account. Therefore, we attach more value to our results obtained with cumulative exposure than to the differences between the exposed workers and the control group.

AUTONOMIC NERVE FUNCTION

The autonomic regulation of heart rate is influenced by physical condition, movement, and breathing behaviour during the test. These factors are difficult to control and have contributed to the large scatter in

the individual values; this hampers the detection of an effect.

Contrary to our results, Matikainen and Juntunen[10] found that RA decreased and responses to the provocation tests did not. Their workers had been heavily exposed to many kinds of solvents, however, and had been referred because of suspected poisoning; the MHR was not measured and the FRSA was calculated in a different way.

The significant decrease in MHR and FRSA in exposed workers compared with control subjects disappeared when cumulative exposure was used. The effect may be reversible in a relatively short time or the exposed workers and control subjects differ with respect to a factor not incorporated in the model. We do not know what that factor might be, because all those that seem relevant were taken into account.

For the moment our findings suggest that a slight impairment of the autonomic nerve system as a result of exposure to CS_2 is possible. A well founded interpretation in terms of impairment of health cannot yet be given.

PERIPHERAL NERVOUS SYSTEM

The decrease found in CVSF but not in MNCV or SNCV is in agreement with other studies in which CVSF appeared to be an early indicator of nerve impairment induced by CS_2.[4 5] An important point is that in the measurement of CVSF a correction should be made for the refractory period.[21] Usually a fixed value of 1 ms is substituted for the RP but it is seldom considered that a decrease in the CVSF calculated this way could also be caused by a prolongation of the RP. Using both this method and a correction with the individual MRP, we were able to show that a decrease in the CVSF occurred that cannot be attributed to a prolongation of the RP. The MNCV, SNCV, and response amplitudes showed no decrease. These negative results are not due to large measurement errors because the known influences of age, height, and temperature were all highly significant in our analysis. The exposure to CS_2 was low compared to other studies; moreover we are quite confident that the exposure has been constant over the years, whereas some authors indicate a much higher past exposure.[5]

Measurement of the refractory period concentrated on the nerve fibres with "average" RP. Measuring the fibres with the longest RP may improve sensitivity, but our registrations did not permit this. The MRP appeared to be prolonged with increasing cumulative exposure. Although this may indicate that the increase in the refractory period is at least partially irreversible, the reversibility of this parameter should be studied more extensively. With improvements the measurement of refractory period may be useful in the early detection of impairment of nerves in workers exposed to CS_2 and perhaps in workers exposed to other chemicals..

Impairment of motor nerve fibres was more distinctly related to exposure when increasing half times were used, suggesting that little or no recovery occurs.

COLOUR DISCRIMINATION

Raitta *et al*[7] found an impairment of colour discrimination in workers exposed to CS_2 at a concentration that as a rule remained below the threshold limit value (20 ppm). Such an impairment could not be shown in the present study. One reason could be that the Farnsworth-Munsell test used by Raitta *et al* is more sensitive than the Lanthony desaturated test, although the Lanthony desaturated test is almost as sensitive as the Farnsworth-Munsell test in detecting colour vision loss in workers exposed to solvents.[26] Moreover, the Lanthony desaturated test has been used successfully in epidemiological studies of loss of colour vision resulting from exposure to solvents[26 27] and consumption of alcohol.[28] Thus it seems likely that our negative results are due to the fact that the mean exposure of workers to CS_2 in the study of Raitta *et al* was higher than in our study.

HEALTH

Regarding the significance of the effects on health, the observed changes may reduce reserve capacity to cope with other noxious influences. On this basis, we consider them to be undesirable until it is established that they are not detrimental to health in the long term.

It is possible by extrapolation to calculate roughly the exposure for life time employment (40 years) for which the observed effects would be expected. Mean cumulative exposure of 165 ppm-years gives a concentration of about 4 ppm. It is important to bear in mind, however, as already discussed, that knowledge with respect to the validity of our model is limited. Furthermore, the occurrence of random errors in dependent and independent variables tends to attenuate the magnitude and significance of the regression coefficients resulting in an underestimation of the effect. So, a lifetime exposure below 4 ppm seems required to prevent the small observed effects.

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