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The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and The Dutch Expert Committee on Occupational Standards

# 133. Tetrachloroethylene (PER)

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Nordic Council of Ministers

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# Preface

An agreement has been signed by the Dutch Expert Committee on Occupational Standards (DECOS) of the Health Council of the Netherlands and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). The purpose of the agreement is to write joint scientific criteria documents, which could be used by the national regulatory authorities in both the Netherlands and in the Nordic countries.

The document on health effects of tetrachloroethylene was written by Karel de Raat, TNO Food and Nutrition Research, the Netherlands, and has been reviewed by DECOS as well as by NEG.

The joint document is published separately by DECOS and NEG. The NEG version presented herein has been adapted to the requirements of NEG and the format of Arbete och Hälsa. The editorial work and technical editing has been carried out by Jill Järnberg, scientific secretary of NEG, at the National Institute for Working Life in Sweden.

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G.J. Mulder
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DECOS

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Chairman
NEG

# Abbreviations

| | |
|---|---|
| ATP | adenosine triphosphate |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferase |
| ASP | aspartate aminotransferase |
| BAEP | brain stem auditory evoked potentials |
| CNS | central nervous system |
| ECD | electron capture detection |
| EEG | electroencephalogram |
| EPA | US Environmental Protection Agency |
| FID | flame ionisation detection |
| GC | gas chromatography |
| HSE | UK Health and Safety Executive |
| γ-GT | γ-glutamyl transferase |
| $LD_{50}$ | lethal dose for 50% of the exposed animals at single administration |
| LDH | lactic acid dehydrogenase |
| MS | mass spectrometry |
| NIOSH | US National Institute for Occupational Safety and Health |
| NOAEL | no observed adverse effect level |
| NTP | National Toxicology Program |
| 5'-NU | 5'-nucleotidase |
| OR | odds ratio |
| PDA | protein-droplet accumulation |
| PER | perchloroethylene, tetrachloroethene |
| PMR | proportional mortality ratio |
| SG-6-P | serum glucose-6-phosphatase |
| SGOT | serum glutamic oxaloacetic transaminase |
| SGPT | serum glutamic pyruvic transaminase |
| SIR | standardised incidence ratio |
| SLDH | serum lactic acid dehydrogenase |
| SMOR | standardised mortality odds ratio |
| SMR | standardised mortality ratio |
| SOCT | serum ornithine carbamyl transferase |
| SPMR | standardised proportional mortality ratio |
| STEL | short-term exposure limit |
| TBAR | thiobarbituric acid-reactive substance |
| TCA | trichloroacetic acid |
| TCE | trichloroethanol |
| TLV | threshold limit value |
| TWA | time-weighted average |

# Contents

# 1. Introduction

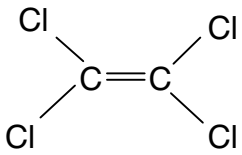
At ambient temperatures, tetrachloroethylene (perchloroethylene, PER) is a colourless liquid with an ethereal odour. PER is a commercially important chlorinated hydrocarbon solvent and chemical intermediate. The compound causes a reversible depression of the central nervous system, which has lead in the past to its use as a human anaesthetic.

A criteria document on tetrachloroethylene was written for the Nordic Expert Group for Criteria Documentation for Health Risks from Chemicals (NEG) in 1979 (203). The present document is a co-production between NEG and the Dutch Expert Committee on Occupational Standards (DECOS) hereafter called the committees.

# 2. Identity, properties and monitoring

## 2.1 Identity

*2.1.1 Structure*




*2.1.2 Chemical names and synonyms/registry numbers*

| | |
|---|---|
| IUPAC name: | tetrachloroethene |
| Common name: | tetrachloroethylene |
| CAS registry number: | 127-18-4 |
| RTECS: | KX3850000 |
| UN: | 1897 |
| EEC: | 602-028-00-4 |
| EINECS: | 204-825-9 |
| Synonyms: | carbon dichloride, ethylene tetrachloride, per, perc, perchloroethylene, 1,1,2,2-tetrachloroethylene, PCE |

## 2.2 Physical and chemical properties (1, 124, 132)

| | |
|---|---|
| Molecular formula: | $C_2Cl_4$ |
| Molecular weight: | 165.83 |
| Boiling point (100 kPa): | 121°C |
| Freezing point (100 kPa): | -22.4°C |
| Density (20°C): | 1.62 g/ml |
| Vapour pressure (20°C/100 kPa): | 1.9 kPa |
| Percentage of vapour in saturated air (20°C/1 bar): | 1.8 |
| Vapour density (air=1; 100 kPa): | 5.8 |
| Solubility in water: | 150 mg/l |
| Solubility in organic solvents: | completely soluble in ethanol and diethylether |
| Physical form: | liquid |
| Odour: | ethereal |
| Odour threshold | 5 ppm (34.5 mg/m$^3$) |
| Conversion factors 25°C, 1 atm: | 1 ppm = 6.89 mg/m$^3$<br>1 mg/m$^3$ = 0.145 ppm |

## 2.3 Analytical methods

*2.3.1 Environmental monitoring*

Several methods are proposed for the determination of the concentrations of gaseous PER in air. The compound is always collected by adsorption. Sorbents used are: activated charcoal or Tenax[1], the former being desorbed by elution with organic solvents (e.g. carbon disulfide), the latter by elution of the heated sorbent with an inert gas, followed by condensation. The desorbed material is fractionated by gas chromatography (GC). Detection and quantification are based on flame-ionisation detection (FID) or mass spectrometry (MS), while the identification of the compound is based on retention time and mass spectra.

*ISO method 9486: (E)*

A known volume of air is passed through a glass or metal tube packed with activated charcoal. The organic vapours are adsorbed onto the charcoal. The collected vapours are desorbed by using a suitable solvent and analysed with a GC equipped with a FID or another suitable detector. This method can be used for the measurement of concentrations of airborne vapours of PER between approximately 1 mg/m$^3$ and 1 000 mg/m$^3$ (about 0.2 ml/m$^3$ to 200 ml/m$^3$) when 10 litres of air are sampled. Organic components, which have the same or nearly the same retention time as PER in the GC analysis will interfere. Proper selection of GC columns and program conditions will minimise interference (131).

[1] A polymeric material used for the sorption of gaseous organic compounds from air. Desorption can be achieved by heating; i.e., without the need of dissolving the sorbed compounds in a solvent.

*NEN method 2947/2964*
Air is drawn through a tube with two sections, both containing activated coconut charcoal to adsorb gaseous PER. The compound is subsequently desorbed with carbon disulfide (containing an internal standard) and is determined by GC, using FID. The method was validated over a range of 2.5-1 600 mg/m$^3$ and has a detection limit of 238 $\mu$g/m$^3$ (61, 64).

*NEN method 2948/2965*
The sample is collected by adsorption on Tenax (200 mg) and analysed by thermal desorption of volatile components into a GC, using FID. The method is validated over the range of 0.02-400 mg/m$^3$ and has a detection limit of 0.1 $\mu$g/m$^3$ (62, 65).

*NEN method 2950*
The sample is collected on an indicator tube and analysed by reading the colour change. The method has been validated over a range of 140-1 150 mg/m$^3$. The coefficient of variation was 25% (63).

*NIOSH method S335 and S336*
Air is drawn through a tube with two sections, both containing activated coconut charcoal to adsorb gaseous PER. The compound is subsequently desorbed with carbon disulfide (containing an internal standard), followed by GC with FID. A calibration curve is employed and a correction is applied for desorption efficiency. This method is validated over the range 655-2 750 mg/m$^3$, using a 3 litres sample (24.5 °C, 101 kPa). The coefficient of variation for the total method over the above range was 5.2%. The limit of detection depends on the analyte (206).

*IARC method 5*
Air is drawn through a tube with two sections, both containing activated coconut charcoal to adsorb the gaseous compound. The compound is subsequently desorbed with carbon disulfide (containing an internal standard), followed by GC, using FID. A calibration curve is employed, and a correction curve is applied for desorption efficiency. This method is validated over a range of 136-4 060 mg/m$^3$ using a 3 litres sample. The breakthrough volume is 21 litres at 2 750 mg/m$^3$. The detection limit depends on the analyte and lies normally in the useful range (171).

*IARC method 12*
Air is drawn through a cartridge containing 1-2 grams of Tenax. The cartridge is placed in a heated chamber and purged with an inert gas, which transfers the volatile compound from the cartridge onto a cold trap and subsequently onto a high-resolution (capillary) GC column, which is held at low temperature (e.g. –70°C). The column temperature is then increased and the component eluting from the column is identified and quantified by MS. Component identification is normally accomplished by a library search routine, using GC retention times and mass-spectral characteristics. The limit of detection is generally in the order of 0.1-1.0 $\mu$g/m$^3$ (233).

*BIA method 8690*
The "Berufsgenossenschaftliches Institut für Arbeitssicherheit" has published a method using Dräger active coal tubes, type B and GC using FID. The limit of detection is 1.2 mg/m$^3$ for an air volume of 40 litres (246).

*2.3.2 Biological monitoring*
For biological-monitoring purposes, the concentrations of PER are determined in expired air or blood. Concentrations in expired air can be determined in the same manner as those in ambient air. PER is removed from blood or tissues by evaporation or by extraction with organic solvents. Evaporated PER can be concentrated with Tenax before analysis with GC/MS or GC with electron-capture detection (ECD); analysis can also be performed without prior concentration (head-space analysis). The solvent extracts are also analysed by GC/MS or GC with ECD.

*IARC method 24*
This method can be used for the determination of PER in expired air. The breath sample is dried over calcium sulphate and led through a Tenax GC cartridge. The adsorbed PER is subsequently thermally desorbed and led into a GC/MS. The detection limit of the method is 0.33 $\mu$g/m$^3$, and the linear range for the analysis depends mainly on the adsorption breakthrough-volume and on the sensitivity of the MS (221).

*IARC method 25*
This method is suitable for the determination of PER in blood and tissues. The volatile PER is recovered from a blood sample by warming the sample and passing an inert gas over it. Tissues are first macerated in water, then treated in the same manner as blood. PER is trapped on a Tenax GC cartridge, then recovered by thermal desorption and analysed by GC/MS. For a 10 ml blood sample, the limit of detection is about 3 ng/ml. Detection limits of about 6 ng/g are typical for 5 g tissue samples. Upper limits for these samples equal approximately 104 times lower limits (220).

*IARC method 27*
PER concentrations in blood can be determined with this method. The specimen is extracted with n-hexane and the concentration of PER in the organic phase is determined by GC, using ECD. The limit of detection is 5 $\mu$g/l (219).

*DFG method 1*
Method for the determination of PER in blood. An organic matrix is prepared from the sample. The volatile compound is removed from the matrix by increasing the temperature. The headspace of the matrix is then analysed with GC (ECD). The detection limit is 1.2 $\mu$g/l (6).

# 3. Sources

## 3.1 Natural occurrence

PER is reported to be produced by algae and one micro-algae (124).

## 3.2 Man-made sources

*3.2.1 Production (3, 123, 124, 132)*

World production of PER amounted to 680 kilotonnes in 1972, and to 1 000 kt in 1974. For 1979 the annual production is estimated to be 50-100 kt in Eastern Europe, about 55 kt in Japan, and 250 kt in Western Europe. Germany, France, Italy, and the United Kingdom are major European producing countries; Austria, Scandinavia, Spain, Switzerland, and Benelux being minor ones. In 1981, annual production in the USA was estimated to be about 350 kt. The most recent production estimates are presented by IARC (124). They amount to 280, 83 and 169 kt for Western Europe, Japan and the United States, respectively. The data in this publication show a decreasing trend for PER production over the last 5-10 years.

PER was first prepared in 1821 by Faraday by thermal decomposition of hexachloroethane. The original commercial production method involved a four-step process starting from acetylene and chlorine. Nowadays, PER is produced mainly by oxyhydrochlorination, perchlorination, and/or dehydrochlorination of other hydrocarbons or chlorinated hydrocarbons. Raw materials include 1,2-dichloroethane, methane, ethane, propane, propylene, propylenedichloride, and various other chlorinated materials such as 1,1,2-trichloroethane.

PER is produced in the following grades: purified, technical, US Pharmacopoeial, spectrophotometric, and dry cleaning. The dry-cleaning grade meets the specifications for technical grade, differing only in the amount of stabiliser added to prevent decomposition. Stabilisers, which include amines or mixtures of epoxides and esters, are added to prevent decomposition by hydrolysation. Thus stabilised, PER is transported in tanks and drums.

*3.2.2 Use (3, 132)*

PER is a commercially important chlorinated-hydrocarbon solvent and chemical intermediate. It is mainly used as a solvent for cleaning and for vapour degreasing in metal-cleaning. It is also used for processing and finishing in the textile industry, as an extraction solvent, as an anthelminthic, as a heat-exchange fluid, in grain fumigation, and in the manufacture of fluorocarbons.

PER has been found in air, soil, surface water, seawater, sediments, drinking water, aquatic organisms, and terrestrial organisms. Industrial spillage is the main source of environmental pollution with PER, while distribution is to a large extent determined by evaporation from surface water.

**Table 1**. Use of PER in kilotonnes; reproduced from (124).

| Year | Metal cleaning (vapour degreasing) | Metal cleaning (cold cleaning) | Dry cleaning | Precursor of chemical synthesis | Other |
|---|---|---|---|---|---|
| *Western Europe* | | | | | |
| 1980 | 71 | 10 | 150 | 34 | 20 |
| 1984 | 61 | 5 | 133 | 36 | 15 |
| 1987 | 50 | 5 | 122 | 65 | 15 |
| 1990 | 45 | 5 | 115 | 60 | 10 |
| *Japan* | | | | | |
| 1980 | | 10 | 26 | | 20 |
| 1983 | | 12 | 23 | | 29 |
| 1987 | | 11 | 25 | | 63 |
| 1990 | | 13 | 20 | | 69 |
| *United States* | | | | | |
| 1971 | | 50 | 163 | 32 | 38 |
| 1974 | | 54 | 193 | 39 | 45 |
| 1977 | | 59 | 181 | 39 | 28 |
| 1980 | | 45 | 172 | 50 | 59 |
| 1984 | | 34 | 136 | 70 | 39 |
| 1987 | | 27 | 127 | 84 | 14 |
| 1990 | | 16 | 111 | 45 | 6 |

An overview of use-patterns, presented originally in (124), is reproduced here as Table 1.

# 4. Exposure

## 4.1 Environmental levels

*4.1.1 Water (3, 123, 124, 132)*

Rainwater has been found to contain up to 150 $\mu$g PER/m$^3$. Average and maximum concentrations in seawater samples were 12 $\mu$g/m$^3$ and 2 600 $\mu$g/m$^3$ respectively, while the maximum concentration in sediments was 4 800 $\mu$g/m$^3$. Surface water from the Atlantic Ocean contained 0.2-0.8 $\mu$g PER/m$^3$. In Western Europe, levels of 10-46 000 $\mu$g/m$^3$ were found in ground water. In the Netherlands, maximum levels of 22 000 $\mu$g/m$^3$ were measured. Concentrations up to 473 $\mu$g/m$^3$ were found in surface water samples taken from Lake St. Clair (Canada/Michigan).

PER was detected in the influent of a sewage-treatment plant at a level of 6 200 $\mu$g/m$^3$; concentrations in the effluent of the plant before and after chlorination amounted to 3 900 $\mu$g/m$^3$ and 4 200 $\mu$g/m$^3$ respectively. The compound has also been detected in the effluents of chemical production plants, an oil refinery, and textile plants.

In Germany, the United Kingdom, and the USA, municipal drinking water contained an average of 1 300 $\mu$g PER/m$^3$, or less. The maximum concentration found in a drinking-water survey in 100 cities in Germany was 35 300 $\mu$g/m$^3$ in 1977, the average being 600 $\mu$g/m$^3$.

*4.1.2 Food*

An overview of PER concentrations in food is presented in Table 2.

*4.1.3 Air*

*Indoor and ambient air*

Median level of PER in about 400 Dutch homes was 4 $\mu$g/m$^3$, while maximum levels varied between 49 and 205 $\mu$g/m$^3$. A median outdoor level of 2 $\mu$g/m$^3$ was measured in this study (163).

In a study performed in Turin, Italy (120 samples taken during 10 consecutive days, 24 hours each, during approximately one year; 31 measurements during winter and 28 during summer), it was found that contamination of air was higher in winter than in summer, the mean atmospheric concentrations being 8.70 $\mu$g/m$^3$ and 4.75 $\mu$g/m$^3$, respectively. It was found that the indoor/outdoor concentration ratio was higher in winter that in summer, median concentration ratios being 2.15 and 1.38 respectively (93).

It has been estimated that 80-85% of the PER used annually in the United States is released into the atmosphere. A major portion of the atmospheric releases is attributed to evaporative losses during dry cleaning. Other atmospheric emissions result from metal-degreasing, production of fluorocarbons, and other chemicals, use in textile industry, and miscellaneous solvent-associated applications (3).

In Germany, annual mean levels of 6 ppb (41 $\mu$g/m$^3$) and 10 ppb (69 $\mu$g/m$^3$) were detected downwind of a chemical laundry and a rubber factory, respectively (132).

According to the Toxics Release Inventory 1988 (an annual compilation of information on the release of toxic chemicals by manufacturing facilities in the United States (207)), an estimated total of at least 32.3 million pounds of PER was released into the air from manufacturing and processing facilities in the United States.

General-population exposure from inhalation of ambient air varies widely with location. While background levels lie generally in the lower ppt range (1 ppt = 6.9 ng/m$^3$) in rural and remote areas, values in the higher-ppt and lower-ppb range (1 ppb = 6.9 $\mu$g/m$^3$) are found in urban and industrial areas and areas near point sources of pollution (3).

Surveys of the air in 9 cities in the USA showed concentrations between 0.2 and 52 $\mu$g/m$^3$, with averages between 2 and 4 $\mu$g/m$^3$. In 14 cities in Germany average concentrations were between 1.7 and 6.1 $\mu$g/m$^3$ (132).

**Table 2.** Concentrations of PER in food products, adapted from (3, 124, 132).

| Country | Food samples | Concentration ($\mu$g/kg) |
|---|---|---|
| Switzerland | Milk and meat products | 3-3 490 |
| United Kingdom | Dairy products | 0.3-13 |
| | Meat | 0.9-5 |
| | Margarine | 7 |
| | Oils | 0.01-7 |
| | Instant coffee | 3 |
| | Tea | 3 |
| | Fruit and vegetables | 0.7-2 |
| United Kingdom | Olive oil (81 out of 98 samples) | <10 |
| | Olive oil (17 samples) | 1-17 |
| United States/Pennsylvania, | Tap water | 0.0004 |
| samples from a food-processing plant | Chinese style sauce | 0.002 |
| | Quince jelly | 0.0022 |
| | Crab apple jelly | 0.0025 |
| | Grape jelly | 0.0016 |
| | Chocolate sauce | 0.0036 |
| United States | 93 out of 231 samples | 13 (1-124) |
| | Cereals | 22 (1-108) |
| | Corn oil | 21 |
| | Pork and beans | 2 |
| | Peas | 2 |
| | Onion rings | 5 |
| | Fried potatoes | 9 |
| | Baked goods | 12 (3-48) |
| | Peanut butter | 3 |
| | Pecan nuts | 120 |
| | Dairy products | 9 (2-30) |
| | Milk chocolate | 20 |
| | Meat products | 13 (1-124) |
| | Baby foods | 2.5 (1-5) |
| | Bananas | 2 |
| | Grapes | 1 |
| | Avocados | 14 |
| United Kingdom | Fish | 0.3-11 |
| | Fish liver | 1-41 |
| | Molluscs (dry weight) | 4 (1-15) |
| United States | Clams | 3 |
| | Oysters | 10 |
| Germany, supermarket near dry-cleaning shop | Margarine | 110 |
| | Herb butter | 7 |
| | Butter | 21 |
| | Flour | 25 |
| | Corn starch | 36 |
| | Cheese spread | 36 |
| Germany, in dry-cleaning shop | Fruit sherbet | 2 |
| | Chocolate-coated ice cream | 1 330 |
| | Chocolate- and nut-coated ice-cream | 4 450 |
| | Ice-cream confection | 18 750 |
| Germany, in apartment above dry-cleaning shop | Butter | 58 000 |

*Workplace air*

Exposure levels for organic solvents at Dutch workplaces were measured by the Dutch Ministry of Social Affairs and Employment (55). During cleaning activities in dry-cleaning establishments, metal industries (cleaning machinery parts and degreasing activities), and offset-printing offices, breathing zone air levels of up to 51 ppm (352 mg/m$^3$), 39 ppm (269 mg/m$^3$), and 16 ppm (110 mg/m$^3$) were observed, respectively.

A US National Institute for Occupational Safety and Health (NIOSH) survey of 44 dry cleaning facilities showed exposures for machine operators to range from 4.0 ppm (28 mg/m$^3$) to 149 ppm (1 027 mg/m$^3$). Geometric mean exposures for machine operators, pressers, seamstresses, and in front counter-areas were 22, 3.3, 3.0, and 3.1 ppm (152, 23, 21 and 21 mg/m$^3$), respectively. A study of the dry cleaning industry in the United Kingdom indicated exposure levels similar to those observed in American studies (3).

An 8-hour time-weighted average (TWA) of up to 4 000 mg/m$^3$ can occur in dry-cleaning establishments. In the United Kingdom, over 90% of 493 8-hour measurements in 131 dry-cleaning establishments revealed concentrations below 680 mg/m$^3$, and over 50% of these samples revealed concentrations below 200 mg/m$^3$. Similar results were obtained in a survey of 46 dry-cleaning establishments in Germany (132).

## 4.2 Human exposure

*4.2.1 General population*

The most important routes of exposure to PER for members of the general population appear to be inhalation of the compound in ambient air and ingestion via drinking water. Available data indicate that dermal exposure is not important for most people.

The breath of residents, living above 12 dry-cleaning shops in the Netherlands, was found to contain a mean concentration of 5 mg/m$^3$, while the breath of residents, living adjacent to the shops, contained 1 mg/m$^3$ (132).

In Turin, Italy, blood samples of 30 volunteers (15 females, 15 males) contained a mean concentration of 1.33 $\mu$g/l (1 330 $\mu$g/m$^3$) and 0.46 $\mu$g/l (460 $\mu$g/m$^3$) during winter and summer, respectively (93).

In the USA the average daily intake by the inhalation route, assuming ambient concentrations of 0.3-2.5 ppb (2.1-17.3 $\mu$g/m$^3$) and inhalation of 20 m$^3$/day, is estimated to be 41-204 $\mu$g/day. The average daily intake from water, assuming concentrations of 0.3-3 $\mu$g/l and ingestion of 2 litres water/day, is estimated to be 0.6-6 $\mu$g/day (3).

In Switzerland and Germany total daily intakes via food were calculated to be 160 $\mu$g/day and 87 $\mu$g/day, respectively (132).

*4.2.2 Occupational population*

NIOSH estimated that nearly 500 000 United States' workers are at risk of exposure to PER in over 20 000 dry-cleaning establishments and in a large

number of other industries producing or using the chemical. The National Occupational Exposure Survey (NOES), conducted by NIOSH from 1981 to 1983, came to an estimation of 688 110 workers employed at 49 025 plant sites, which were potentially exposed to PER in the United Sates during this period.

Between 1977 and 1979, breathing-zone air samples were collected from 144 workers at 44 out of an estimated 25 000 dry-cleaning establishments in the USA. Machine-operators experienced the highest exposures, with 8-hour TWAs between 27 and 1 010 mg/m$^3$. Machine-operators in 9 plants had 8-hour TWA exposures exceeding 340 mg/m$^3$, while in 7 plants 15-minute peak exposures exceeded 680 mg/m$^3$. Other workers were exposed to a maximum 8-hour TWA of 251 mg/m$^3$.

At a railway works, where PER was used as a cleaning agent, 94% of 104 8-hour measurements exceeded 680 mg/m$^3$ with peaks up to 1 290 mg/m$^3$ (132).

### 4.3 Summary

The environmental levels of PER vary strongly, depending on matrix and location. Surface water from the Atlantic showed relatively low values of 0.2-0.8 $\mu$g/m$^3$, while other seawater concentrations may reach levels as high as several mg/m$^3$. Similar concentrations have been found for marine sediments. Ground water may reach even higher levels; in this matrix concentrations between 10 and 49 000 $\mu$g/m$^3$ have been observed. Average drinking-water concentrations lie around 1 000 $\mu$g/m$^3$. However, much higher values (35 500 $\mu$g/m$^3$) have been measured.

Background ambient-air concentrations amount to a few ng/m$^3$. In urban areas, 1 000 times higher levels may be encountered, in particular downwind of certain industries.

The general population is exposed by inhalation of ambient and indoor air and via the food. For inhalation a range of 41-204 $\mu$g/day is reported. The intake via the food shows similar values. Much higher levels are observed for people living near dry-cleaning shops.

When PER is used in occupational settings, concentrations in air vary strongly, depending on process, amount used, and hygienic measures. Most observations for dry cleaning and metal degreasing lie within a range from several tens to several hundreds of mg/m$^3$.

## 5. Kinetics

### 5.1 Absorption

*5.1.1 Respiratory*

*Humans*

Respiratory absorption of PER has been investigated in a number of human-volunteer studies (16, 81, 108, 192, 194, 214, 215, 222, 257, 260). Alveolar retention was determined by Monster *et al.* (192) and Fernandez *et al.* (81) by

**Table 3.** Blood/air, tissue/air and tissue/blood human partition coefficients for PER (89).

| Blood and tissue/air partition coefficient | | Tissue/blood partition coefficient | |
|---|---|---|---|
| Blood/air | 12 | | |
| Fat/air | 1 450 | Fat/blood | 125 |
| Kidney/air | 59 | Kidney/blood | 5 |
| Muscle/air | 70 | Muscle/blood | 6 |
| Liver/air | 61 | Liver/blood | 5 |

comparing concentrations in inhaled air and exhaled air. Results of these studies show the alveolar retention to decrease from about 90% immediately upon the start of exposure, to about 50% after an exposure period of 8 hours. Over a period of 4 hours, lung clearance decreased from 6.7 to 3.8 l/minutes (192). These rather high absorption values are to be expected, in view of the high solubility of PER in blood and adipose tissues (194) and the partition coefficients of the compound (89), which are listed in Table 3. The decrease of retention with duration of exposure is caused by the concomitant increase of the PER blood concentrations.

Benoit *et al*. (16) investigated retention during steady-state absorption (defined as the absorption when the concentration of PER in exhaled air in unit time is constant) in 7 human volunteers. They found retention coefficients ranging from 0.78 to 0.93 when the subjects were exposed to concentrations of 50-92 ppm (345-634 mg/m$^3$).

The actual absorption of PER by the respiratory tract equals the product of retention and ventilation volume (volume of breathed air). Monster *et al*. (192) calculated the human absorbed amounts, which are listed in Table 4 based on their experimental observations.

The strong stimulating effect of exercise on absorption shown in this table was also found by Hake and Stewart (108). These authors reported a fourfold increase in PER blood concentrations upon an exercise of 30 minutes.

The influence of the fat content of the body on absorption has been investigated by Monster *et al*. (192). Their results show that the absorption depends stronger on lean body mass than on the amount of adipose tissue.

**Table 4**. Estimated absorption of PER by 6 human volunteers exposed for 4 hours to 72 or 144 ppm (469 or 992 mg/m$^3$) PER at rest, or for 4 hours to 142 ppm (978 mg/m$^3$) PER combined with 2 half-hour periods of exercise equal to 100 W (192).

| Subject | Absorption in mg | | |
|---|---|---|---|
| | 72 ppm (469 mg/m$^3$) rest | 144 ppm (992 mg/m$^3$) rest | 142 ppm (978 mg/m$^3$) rest + exercise |
| A | 370 | 670 | 1 060 |
| B | 490 | 940 | 1 500 |
| C | 530 | 1 000 | 1 400 |
| D | 500 | 1 210 | 1 510 |
| E | 390 | 880 | 1 320 |
| F | 450 | 970 | 1 120 |

*Experimental animals*

Pegg *et al*. (218) exposed rats for 6 hours to 10 ppm (69 mg/m$^3$) or 600 ppm (4 134 mg/m$^3$) of $^{14}$C-labeled PER and determined the radioactivity in exhaled air, urine, and carcass. As in humans, excretion occurred largely via exhalation (Section 5.4). As the exhaled air could not be analysed during exposure, the study does not allow a quantitative estimation of the respiratory absorption. However, it did show that considerable absorption occurs, as 8.9 and 467.1 $\mu$mol PER equivalents of radioactivity was recovered for the 10 ppm and 600 ppm exposure group respectively, from exhaled air, faeces, urine and carcass together, during a period of 72 hours after termination of exposure. Schumann *et al*. (244) reported, that under comparable conditions, 2.44 $\mu$mol could be recovered over a period of 72 hours after 6 hours of respiratory exposure of mice to 10 ppm (69 mg/m$^3$) of PER. Again, this value only shows that considerable respiratory absorption does indeed occur. It cannot be used to derive a reliable quantitative estimate for absorption.

*5.1.2 Dermal*

*Humans*

Stewart and Dodd (258) investigated the dermal absorption of PER in 5 human volunteers. One of the thumbs of the subjects was kept immersed in the solvent for 40 minutes, during which inhalation of the solvent was excluded. The mean peak concentration in alveolar air amounted to 0.31 ppm (2.14 mg/m$^3$) and was reached less than 10 minutes after termination of exposure. The mean concentration decreased to 0.20 ppm (1.38 mg/m$^3$) over a period of 5 hours; the overall mean was 0.23 ppm (1.59 mg/m$^3$). The absence of data on the concentration of PER and its metabolites in urine and blood prevents the dermal absorption to be estimated on the basis of these results. However, comparison with the results of human respiratory absorption studies (Section 5.1.1) makes clear that the alveolar concentrations observed in this study, would have been reached by respiratory exposure to about 0.2-0.6 ppm (1.4-4.1 mg/m$^3$). It may, therefore, be concluded that a much longer dermal exposure to fluid PER or a much larger exposed skin surface would be necessary to achieve a body burden that equals the body burden observed in human respiratory absorption studies (Section 5.1.1). However, assuming that the skin area of the thumb is 15 cm$^2$, the maximum alveolar air concentration of 0.31 ppm from the 40-minute exposure can be used to calculate the alveolar air concentrations occurring when 2 000 cm$^2$ (hands and fore-arms) of skin is exposed for 1 hour. The calculation would be as follows: $(2\,000/15)\cdot(60/40)\cdot 0.31 = 62$ ppm. Based on a respiratory absorption under steady-state conditions of 50% this would correspond to a respiratory exposure of 124 ppm. It is then assumed that the relative respiratory absorption is not concentration dependent, that the toxicokinetics is not exposure-route dependent and that dermal absorption is linearly related to surface and exposure time.

Aitio *et al*. (2) investigated the blood-concentration of PER after dermal exposure in 2 human volunteers. A hand was immersed in the solvent to the wrist for 5 minutes. Respiratory exposure was prevented. The blood concentration appeared to depend strongly on the arm used for blood sampling. Concentrations were

much lower in the contralateral arm (the hand of which was not immersed) than in the ipsilateral arm (the arm with the immersed hand). It took more than 2 hours for the concentrations in both hands to become equal. This difference suggests that the low concentrations of PER observed in the exhaled air by Stewart and Dodd (258) are not necessarily solely determined by poor dermal absorption, but also by tissue absorption after PER has penetrated through the skin.

The very slow decrease of the alveolar-air concentration in Stewart and Dodd's study is important in this context. Comparison with the respiration studies (Section 5.1.1) suggests higher alveolar elimination rate after exposure via inhalation. The comparison of dermal and respiratory absorption can, therefore, not only be based on mean and maximum concentrations in the alveolar air; the overall area under the elimination curve should be taken into account as well. The available data do not allow for such a comparison. Nevertheless, the committees regard it as probable, that the influence of differences in elimination rate will not be so strong as to refute the conclusion above, i.e. respiratory exposure will much sooner lead to high body burdens than dermal exposure.

The dermal absorption of PER vapour was investigated by Riihimäki and Pfäffli (234). The amount of PER exhaled by 3 volunteers after exposure to 600 ppm (4 134 mg/m$^3$) of the compound via inhalation and via the skin together and via the skin alone was compared. Exposure lasted 3.5 hours and concentrations in exhaled air were followed up to 50 hours after termination of exposure. It appeared that the amount of PER vapour, which entered the body via the skin was only 1.1% of the amount absorbed via inhalation. A dermal (with respirator) absorption of 48 mg was found, compared to an estimated respiratory absorption of 4.22 g. The authors concluded that the contribution of dermal absorption to the body burden is strongly exceeded by respiratory absorption.

*Experimental animals*

Tsuruta (272) has investigated the dermal absorption of PER in mice. An amount of 0.5 ml of PER was applied to 2.9 cm$^2$ of clipped skin of the abdominal region for a period of 5-15 minutes. The compound was determined by GC in the expired air and an extract of the blended whole body. The sum of PER in expired air and whole body was regarded to be equal to the absorbed amount of PER. They found a total absorption of 177 $\mu$g over a period of 15 minutes, 173 $\mu$g of which was eliminated via the exhaled air. This amount is equivalent to 24.4 nmoles/min/cm$^2$.

The same author (273) investigated the dermal absorption in an *in vitro* system with rat skin, and compared the results with those of his *in vivo* study with mice. A clear linear correlation was found between the results obtained with the two systems for a series of halogenated compounds. However, *in vitro* absorption rates were markedly lower. In particular this holds for PER, which was absorbed at a 44-fold lower rate (0.067 nmoles/min/cm$^2$) in the *in vitro* system than in the *in vivo* system.

Jakobson *et al*. (134) investigated the dermal absorption of PER in guinea pigs. These authors followed the concentration in the blood (carotid artery) of the anaesthetised animals during exposure. One, two or four 3.1 cm$^2$ areas of clipped

skin were exposed to 1 ml of solvent for 6-12 hours. A maximum blood concentration of 1.1 $\mu$g/ml was found after 0.5 hour, followed by a slow decrease to 0.63 $\mu$g/ml after 6 hours of exposure. Elimination from the blood after termination of exposure was not investigated. This prevents quantitative conclusions to be drawn on the dermal absorption of PER, because the blood concentrations are the resultant of dermal absorption and elimination through excretion and biotransformation, excretion via the exhaled air being by far the most important process which contributes to the elimination of PER.

*5.1.3 Oral*

*Humans*

A number of human oral PER poisonings have been described (3, 148), most of them being the result of the use of the compound as an anthelminthic drug. The systemic nature of the effects observed (Section 6.1.2) and the presence in blood and urine of PER or its metabolites trichloroacetic acid (TCA) and trichloroethanol (TCE), show that PER is readily absorbed by the human gastrointestinal tract.

However, these studies do not allow for more quantitative conclusions, because data on exposure or concentrations in biological samples were too incomplete and simultaneous respiratory and dermal exposure and absorption may have contributed to effects or concentrations in biological samples.

*Experimental animals*

Studies with experimental animals indicate a rapid and virtually complete absorption of PER following oral administration (3, 38, 45, 85, 189, 218).

## 5.2 Distribution

*5.2.1 Respiratory*

*Humans*

Inhalation experiments with human volunteers by Stewart *et al*. (257) show that upon repeated exposure (101 ppm (696 mg/m$^3$); periods of 7 hours per day for 5 consecutive days), the PER concentration in the expired air increases compared to one exposure of 7 hours. The explanation for this phenomenon is accumulation of the compound in the fat tissues of the body. This accumulation will give rise to an increase of blood concentrations, which in their turn will lead to an increase of the PER concentrations in the expired air.

Accumulation was also indicated by the long decay period of the concentrations in expired air after termination of exposure (257). After 10 days, the expired air still contained more than 1 ppm (6.89 mg/m$^3$). Prolonged decay is also revealed by other human volunteer studies; although in most of them shorter exposure periods were applied, while exposure was not repeated (81, 106, 192).

The accumulation is predicted by kinetic modelling (106). It is largely the result of the very high fat/blood partition coefficient, the much lower air/blood partition coefficient (89; Table 3) and the relatively poor perfusion of the fat tissues, which together result in a relatively slow absorption by, and removal from the fat tissues,

compared to the exchange of PER between blood and air in the lungs and between blood and non-fat tissues and well perfused tissues. The model of Guberan and Fernandez (106) predicts that half of the body burden of a 70-kg person after 8 hours of respiratory exposure to 100 ppm (690 mg/m$^3$) of PER, will be present in the fat tissues.

No tissue analyses have been performed in human volunteer studies. However, two case studies of fatal respiratory PER poisonings give an impression of the amounts of PER that can occur in tissues after short-term respiratory exposure to high concentrations. Brain and lung tissue, blood, stomach content, and urine of a deceased dry cleaner were analysed by Lukaszewski (169) with GC/MS. The following concentrations were found: brain, 36 mg/100 g; lungs, 0.3 mg/100 g; blood, 4.4 mg/100 ml; urine, and stomach content, below detection limit. Levine *et al*. (165) found 240 mg/kg in the liver, 71 mg/kg in the kidneys, 69 mg/kg in the brain and 30 mg/kg in the lungs of a deceased dry cleaner.

*Experimental animals*

Savolainen *et al*. (240) studied the distribution of radioactivity in rats following respiratory exposure to 200 ppm (1378 mg/m$^3$) of radiolabeled PER for 4 days, 6 hours per day. PER appeared to be primarily distributed in the fat tissues, in particular the perirenal fat. A 145 times higher concentration were found in the perirenal fat (4495 nmol/g after 6 hours at day 4) than in the blood (31 nmol/ml after 6 hours at day 4; corresponding concentrations for cerebrum, cerebellum, lungs and liver were 143, 92, 74 and 161 nmol/g respectively).

Pegg *et al*. (218; Section 5.1.1) found that respiratory exposure of rats to 10 or 600 ppm (69 or 4134 mg/m$^3$) of $^{14}$C-labeled PER for 6 hours resulted in 4.3 and 2.2 percent of the radioactivity being present in the carcass respectively, the distribution being investigated 72 hours following termination of exposure. The distribution over various tissues is presented in Table 5.

The fact that these authors did not find a predominant distribution towards the fat tissue, can most probably be attributed to the 72 hours between termination of exposure and determination of distribution. During this period a large part of the PER accumulated in the fat will most probably be eliminated by expiration. The radioactivity in the other tissues may be due to bound metabolites (Section 5.3).

**Table 5.** Distribution of radioactivity ($\mu$mol-eq/g) over various rat tissues 72 hours after respiratory exposure to $^{14}$C-labeled PER (218).

| Tissue | 10 ppm (68.9 mg/m$^3$) | 600 ppm (4134 mg/m$^3$) |
|---|---|---|
| Liver | 0.0047 | 0.096 |
| Kidney | 0.0018 | 0.167 |
| Fat | 0.0018 | 0.082 |
| Brain | ND[a] | ND |
| Lung | 0.0012 | 0.066 |
| Heart | 0.0009 | 0.045 |
| Adrenal | ND | ND |

[a] ND - not determined.

Schumann *et al*. (244) compared the distribution upon respiratory exposure in rats and mice (same exposure conditions as in (218)): 10 ppm (68.9 mg/m$^3$) resulted in 3% of the recovered radioactivity in the carcass 72 hours after termination of exposure. Much higher binding to the liver tissue was observed for the mouse than for the rat (maximum difference: 9.2 times per gram hepatic protein). In the mouse, excretion in urine was predominant over excretion in the expired air. In the rat, the reverse was found. This difference is caused by a much faster oxidative metabolism (Section 5.3) in the mouse. Consequently, less unmetabolised PER will be accumulated in the fat tissues and subsequently eliminated by expiration in this species.

Ghantous *et al*. (92) studied the distribution of PER and trichloroethylene and metabolites in pregnant mice by means of whole-body autoradiography and GC, with special emphasis on the foeto-placental unit. A strong accumulation and retention of radioactivity in the amniotic fluid was observed, which could be identified as TCA. The results suggest that this compound is partly formed from the parent compound after the latter has been transported to the foeto-placental unit. Furthermore, absorption of radioactivity was observed in brains, fat tissues, nasal mucosa, blood, liver, kidneys and lung. Volatile radioactivity (assumed to be the parent compound) was distinguished from non-volatile radioactivity (assumed to represent polar metabolites). The former accumulated in particular in fat tissues and the brains. Immediately after exposure (which lasted 10 minutes) non-volatile compounds were found in liver, kidneys, lungs, nasal mucosa and blood. A strong increase was observed within a period of 4 hours, during which non-volatile radioactivity did also appear in the eyes and the intestinal tract.

### *5.2.2 Dermal*

*Humans*

Accumulation of PER in fat tissues upon dermal absorption (to fluid PER) is suggested by the low decay rate of the concentration in the expired air which was observed by Stewart and Dodd (258) in human volunteers. As in the case of respiratory exposure, the low decay rate can be explained by the low perfusion of the fat tissues combined with the high fat/blood partition coefficient and the much lower air/blood partition coefficient.

Slow decay was also observed by Riihimäki *et al*. (234) after dermal exposure to a PER vapour concentration of 600 ppm (6 134 mg/m$^3$) during 3.5 hours.

*Experimental animals*

Tsuruta (272) determined the presence of PER in the whole body and in expired air of mice which were dermally exposed to the compound for 15 minutes. They found 173 $\mu$g to be retained by the body; 3.95 $\mu$g was expired during the exposure period of 15 minutes.

Further pertinent experimental data on the distribution of PER after dermal exposure were not found in the literature.

*5.2.3 Oral*

*Humans*

No studies were found, which provide information on the distribution in humans after oral exposure.

*Experimental animals*

Pegg *et al*. (218) found retentions of 3.3 and 1.2% of recovered radioactivity in the carcasses of rats 72 hours after oral administration (by gavage) to 1 and 500 mg PER/kg body weight, respectively. The distribution obtained, demonstrated the preference of PER for fat tissues for the 500 mg/kg body weight group. Even 72 hours after termination of exposure, the fat tissues showed the strongest radioactivity. In case of the 1 mg/kg body weight group, no clear differences were found between liver, kidneys and fat, probably, because the fat contains unmodified PER, which is eliminated by expiration, while the other tissues may also contain bound metabolites (Sections 5.2.1 and 5.3).

In the study of Schumann *et al*. (244) 0.5% of the recovered radioactivity was found to be retained in the carcasses of mice, 72 hours after a single gavage exposure to 500 mg/kg body weight. Again a much stronger hepatic-protein binding is observed for the mouse, which is most probably the result of the higher metabolism rate in this species.

In their drinking-water study, Frantz and Watanabe (85) found a retention value for the carcass of 0.9% 72 hours after a 12-hour-long period in which rats received a saturated solution of PER as drinking water.

Vemmer *et al*. (281) investigated the distribution of PER in pigs which received PER in their food. Their results confirm the high affinity of PER for fat tissues, compared to well perfused tissues as liver, kidney and muscles.

Marth (175) investigated the transport of PER through the body with mice after oral exposure. Two separate mechanisms were identified. PER absorbed to the phospholipid cell membrane of the red blood cells, which leads to an increased fragility of these cells and premature destruction. The fragments of these cells, loaded with PER, are phagocytised in the spleen, resulting in the accumulation of PER in this organ. Besides the red blood cells, PER is transported by the chylomicrons in the blood to the adipose tissue. Thereby, the compound inhibits lipoprotein lipase, and thus the breakdown of the chylomicrons.

Dallas *et al*. (44) investigated the time dependence of the PER concentrations in various organs in rats and dogs after a single bolus of 10 mg/kg body weight, which was administered by gavage. The results of this study are summarised in Table 6. The species differences, which are revealed by Table 6 are attributed by the authors to a markedly higher rate and magnitude of exhalation and metabolism in the rat.

**Table 6.** Toxicokinetic parameters in rat and dog after a single oral gavage treatment with 10 mg PER/kg body weight (44).

*Dog*

| Tissue | Area under the curve ($\mu$g·min/ml) | Half-time (min) | $C_{max}$ ($\mu$g/g) | $T_{max}$ (min) |
|---|---|---|---|---|
| Liver | 1 851 | 2 448 | 6 | 60 |
| Kidney | 1 606 | 1 572 | 5 | 60 |
| Fat | 55 838 | 494 | 43 | 720 |
| Heart | 1 849 | 1 775 | 6 | 60 |
| Lung | 1 001 | 2 289 | 2 | 60 |
| Muscle | 1 907 | 1 625 | 3 | 60 |
| Brain | 3 238 | 4 641 | 11 | 60 |
| Blood | 782 | 865 | 2 | 90 |

*Rat*

| Tissue | Area under the curve ($\mu$g·min/ml) | Half-time (min) | $C_{max}$ ($\mu$g/g) | $T_{max}$ (min) |
|---|---|---|---|---|
| Liver | 1 673 | 331 | 12 | 10 |
| Kidney | 1 057 | 395 | 6 | 10 |
| Fat | 49 964 | 695 | 36 | 360 |
| Heart | 806 | 396 | 3 | 15 |
| Lung | 627 | 342 | 2 | 60 |
| Muscle | 798 | 310 | 2 | 60 |
| Brain | 1 377 | 327 | 5 | 15 |
| Blood | 332 | 384 | 1 | 15 |

$C_{max}$: maximum concentration
$T_{max}$: time at which the maximum concentration is reached

## 5.3 Biotransformation

The biotransformation of PER has been reviewed by Dekant *et al*. (50), Clement International Corporation (38), European Centre for Ecotoxicology and Toxicology of Chemicals (69), and Agency for Toxic Substances and Disease Registry (ATSDR) (3).

### *5.3.1 Metabolites detected in humans*

The toxicokinetic studies with human volunteers, which are described in the foregoing sections of this chapter, unambiguously show that the major part of absorbed PER is not transformed by metabolism, but excreted in unchanged form via exhalation, regardless of exposure route and exposure conditions (16, 81, 106, 108, 190, 192, 222, 234, 257, 258, 260). Compared to the amounts of PER absorbed, the metabolised amounts can justly be called small (195).

The major metabolite detected in human blood and human urine is TCA (81, 125, 129, 191, 192, 194, 225, 252, 296). The study of Ohtsuki *et al*. (212) with dry cleaners and workers in textile processing plants (removal of glue from silk cloth) indicated that saturation of biotransformation occurred upon respiratory exposure to 100 ppm (690 mg/m$^3$). Exposure to 50 ppm (345 mg/m$^3$) for 8 hours resulted in

the excretion of less than 2% of the compound via urine in metabolised form. Lauwerys *et al*. (162) did not find TCA in urine of laundry workers exposed to 8.9-37.5 ppm (61.3-258.3 mg/m$^3$), while PER could be detected in expired air (0.2-10 ppm or 1.4-68.9 mg/m$^3$, 30 minutes after work) and blood (0.4-3.1 mg/l, 30 minutes after work).

Some authors reported the occurrence of other metabolites in human urine instead of, or next to TCA. Ikeda *et al*. (129) found TCE and TCA in urine of volunteers exposed to PER: equal amounts of both metabolites in workers exposed to 20-70 ppm (138-482 mg/m$^3$) of PER, while an increase of exposure to 200-400 ppm (1 378-2 756 mg/m$^3$) resulted in a TCE/TCA ratio of 2/3.

Monster (191, 194) demonstrated TCA in blood and urine of dry cleaners and metal cleaners; in the latter biological fluid he found TCE as well. TCE in urine of PER-exposed humans is not necessarily a metabolite of PER. Often humans are exposed to a combination of chemicals, among them trichloroethylene (191). The latter compound is present as impurity in most commercial PER batches used for cleaning purposes. Even rather low concentrations of this compound in PER might lead to detectable TCE concentrations in urine, as trichloroethylene is metabolised to a much greater extent than PER (75% versus 2%; 190). Also the PER batches used in volunteer studies might be contaminated to a sufficiently high level with trichloroethylene, to give rise to TCE in urine.

The study of Meuling and Ebens (186) can be referred to as an example in this context. These authors could fully explain the presence of TCE in urine of laundry workers from the presence of trichloroethylene as an impurity in PER, the estimated exposure to this impurity and literature data on the formation rate of TCE from trichloroethylene. Moreover, it is doubted whether biotransformation of PER does indeed yield TCE as metabolite (296). Finally, it is doubted whether the presence of TCE can be deduced from results based on the Fujiwara reaction (231), as is done in some studies.

LaFuente and Mallol (160) found an enhanced excretion of thioethers in occupationally exposed women. They speculate this to be the result of the conjugation with glutathione of an epoxide formed from PER. Exposure to other compounds, which gave rise to an increase of thioethers could not be excluded.

Birner *et al*. (20) investigated the biotransformation of PER in 4 humans occupationally exposed during dry cleaning to 50 ppm (345 mg/m$^3$). GC/MS analysis of urine samples at the start and the end of the workweek revealed the presence of TCE, TCA and *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-*L*-cysteine. The concentrations of the latter compound were found to be more than 1 000 times lower than those of the first two (2.2-14.6 pmol/mg creatinine versus 13.5-65 nmol/mg creatinine). Whereas the concentrations of TCA/TCE increased during the workweek, those of *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-*L*-cysteine did not change. However, a clear increase was found for the concentrations of the latter compound when the daily working period increased from 4 to 8 hours.

*5.3.2 Metabolites detected in experimental animals*

In most studies with experimental animals, the majority of the PER absorbed after oral ingestion, inhalation or dermal exposure was found to be eliminated unchanged via the exhaled air (Section 5.1 and Section 5.4). However, the ratio between the amount of exhaled unchanged PER and the amount of metabolised PER appeared to be species dependent. In particular, mice metabolise PER to a much greater extent than other investigated species. This is clearly revealed by the studies in which binding of radioactivity to liver proteins is investigated (244). According to Stott and Watanabe a 6 hours respiratory exposure to 10 ppm (68.9 $mg/m^3$) leads in the rat to a total metabolised amount of 10.5 $\mu$mol/kg liver protein, whereas in the mouse this amount is 89.5 $\mu$mol/kg liver protein (263).

The major metabolite identified in blood and urine of experimental animals is TCA. Besides, several other metabolites have been found. An overview of the results of studies with experimental animals, which indicate the identity of PER metabolites, is presented in Table 7.

Very pure PER was used in the studies of Dekant *et al*. (48), which would refute the hypothesis that the presence of TCE is only the result of trichloroethylene being an impurity of PER.

The absence of TCA in the urine samples of the study of Pegg *et al*. (218) is striking. While exposure of rats in other studies gives clearly rise to TCA, only oxalic acid was found in the study of Pegg *et al*. (218).

*5.3.3 Metabolites detected in vitro*

The results of studies in which the biotransformation of PER has been investigated *in vitro* (i.e., in isolated cells, tissues or organs) are summarised in Table 8.

*5.3.4 Biotransformation pathways*

The presence of TCA and other metabolites in body fluids, blood and tissues of man and other animals can be explained by assuming the biotransformation of PER to occur via two different and unlinked metabolic pathways.

*Epoxidation*

The most important pathway (in terms of amount of PER metabolised) starts with the epoxidation of PER by cytochrome P450 (i.e. mixed function oxidases) to tetrachloro-oxirane (first postulated by Yllner (295)). The presence of oxalic acid in urine is consistent with the formation of the oxirane. The oxirane has, however, never been detected in metabolism studies. It is probably rapidly converted to other, more stable compounds. Apparently, its half-time is too short to allow its detection in the experimental systems used so far.

Involvement of cytochrome P450 is indicated by the work of Costa and Ivanetovich (42, 43), who demonstrated a clear-cut stimulation of biotransformation by pretreatment of animals with inducers of this enzyme.

**Table 7.** Metabolites of PER detected in body fluids and tissues of experimental animals upon exposure to PER.

| Species | Exposure | Blood | Urine[a] | Details | Reference |
|---|---|---|---|---|---|
| Mouse | Inhalation, 2.5 h<br>0.5-1 g/kg | TCA | TCA (52%)<br>oxalic acid (11%)<br>dichloroacetic acid (traces) | 20% excretion via urine, 70% excretion via exhaled air<br>Postulates the formation of an epoxide, which is rearranged to trichloroacetyl chloride. No monochloroacid, formic acid or TCE were found. | (295) |
| Rat | Oral gavage<br>$^{36}$Cl-labelled PER | | TCA, chloride | | (45) |
| | Inhalation, 5 h/day<br>for 3 days, 740 ppm<br>(5 099 mg/m$^3$) | | TCA (0.08-0.11 mg/ml)<br>oxalic acid (1.08-1.62 mg/ml)<br>ethylene glycol (0.52-0.83 mg/ml) | | |
| Rat | Oral,<br>7.5 mmol/kg bw | | TCA (2.8, 15.8 and 24.8 mmol/24 h/ animal) | Animals were pretreated with inducers of cytochrome P450. The three amounts excreted in urine come from vehicle treated, phenobarbitone-treated and Aroclor-1254-treated rats. | (198) |
| Rat | Oral,<br>1 or 500 mg/kg bw | | Oxalic acid (0.13 and 2.7 mmol) | Excretion followed for 72 h. A minor HPLC peak was not identified. | (218) |
| | Inhalation, 6 h<br>10 and 600 ppm<br>(69 and 4 134 mg/m$^3$) | | Oxalic acid (0.27 and 2.3 mmol) | No TCA found. | |
| Rat | Oral, 6.0 mmol/kg bw<br>chronic exposure | | | Rats and mice showed comparable HPLC chromatogram, indicating the absence of qualitative differences in metabolism. HPLC peaks were not identified. Part of the radioactivity was exhaled as $CO_2$, 2.08% and 1.45% of the dose for mice and rats, respectively. | (189) |
| Mouse | Oral, 5.4 mmol/kg bw<br>chronic exposure | | | | (189) |

**Table 7.** Cont.

| Species | Exposure | Blood | Urine[a] | Details | Reference |
|---|---|---|---|---|---|
| Mouse | Oral, up to 2 g/kg bw for 6 weeks | | TCA (up to 100 mg/kg/day) | Oxalic acid, ethylene glycol and TCE could be excluded. Saturation of metabolism was observed. | (31) |
| Rat | Oral, 800 mg/kg bw, [14C]-PER | | Percentage of urinary radioactivity Oxalic acid (8%), Dichloroacetic acid (5.1%), TCA (54%), Trichloroacetylaminoethanol (5.4%), TCE (8.7%), Trichlorovinylacetyl-cysteine (1.6%), Conjugate of TCA (1.8%) | | (48) |
| Mouse | Oral, 800 mg/kg bw, [14C]-PER | | Percentage of urinary radioactivity Oxalic acid (2.9%), Dichloroacetic acid (4.4%), TCA (57.8%), Trichloroacetyl-aminoethanol (5.4%), TCE (5.7%), Trichlorovinylacetylcysteine (0.5%), Conjugate of TCA (1.3%) | | (48) |
| Rat | Inhalation, 6 h 400 ppm (2 756 mg/m$^3$) | TCA (max of ≈7 mg/ml after 3 h) | | | (210) |
| Mouse | Inhalation, 6 h 400 ppm (2 756 mg/m$^3$) | TCA (max of ≈130 mg/ml after 4 h) | | | (210) |
| Mouse | Inhalation, 10 min 5 mmol/4 mice | | TCA, traces of TCE, | TCA was also found in the amniotic fluid, maternal plasma and foetuses. | (92) |
| | Intraperitoneal, 0.6 mmol/mouse | | TCA, traces of TCE | | |

[a] Does not exclude the presence of other metabolites, unless indicated otherwise in the column Details.

**Table 8.** Metabolites of PER detected *in vitro*.

| Species/organ/ tissue/cell | Experimental set up | Metabolites detected | Details | Reference |
|---|---|---|---|---|
| Rat/liver/ homogenate | PER was added as vapour to the carbogen (to the saturation level) used for oxygenation of the blood by which the liver was perfused; perfusate and homogenate were analysed for metabolites. | TCA, 10-15% (of absorbed PER) in perfusate and 3-5% bound to liver. | Not detected were dichloroacetic acid, chloral, glucuronides and TCE (GC). | (26) |
| Rat/liver/ microsomes | Microsomal fraction of rat liver was incubated with PER (3.3 mM); the incubation mixture was analysed for metabolites. | TCA, up to 2.5 mmol/mg protein. Trichloroacetyl moiety covalently bound to protein. | Marked stimulation of biotransformation upon the use of microsomes of rats treated with inducers of cytochrome P450. No other metabolites detected (Fujiwara reaction and GC) | (42) |
| Rat/liver/ hepatocytes | Rat hepatocytes were incubated with PER; the incubation mixture was analysed for metabolites. | TCA, 0.08 nmol/106 cells/min | Rats were pretreated with phenobarbital to induce cytochrome P450. No other metabolites detected (Fujiwara reaction and GC). | (43) |

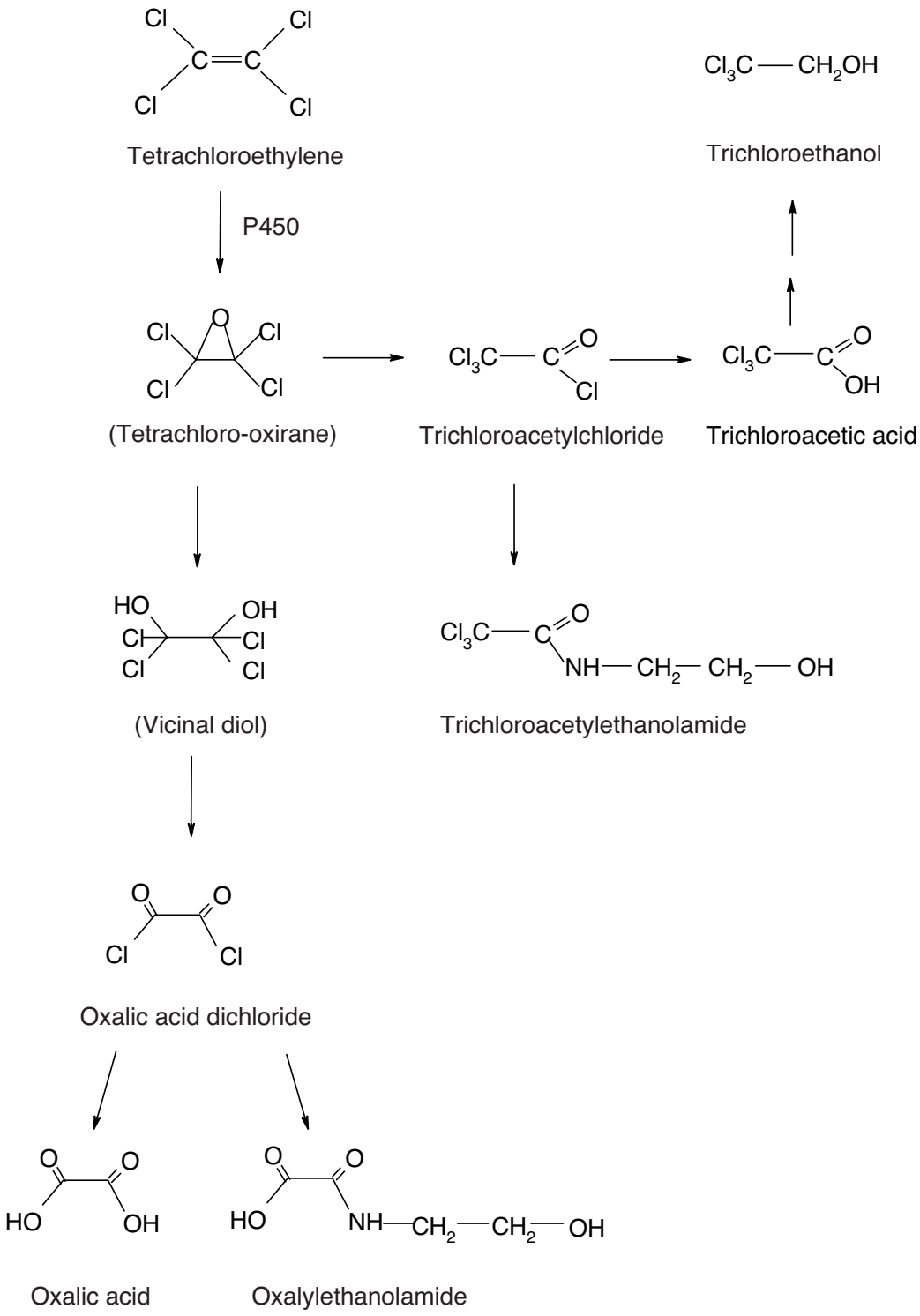



**Figure 1.** Oxidative biotransformation pathway of PER, adapted after (3).

The oxirane undergoes rearrangement to trichloroacetyl chloride, which compound is subsequently oxidised to TCA. TCE could be formed through the reduction of TCA.

Dekant *et al*. (50) identified trichloroacetylethanolamide, as another, minor metabolite, which is most probably formed via trichloroacetyl chloride by the reaction of the latter compound with phosphatidylethanolamine.

The presence of oxalic acid can be explained by the hydration of the oxirane by epoxide hydratase, leading to the vicinal diol, which is subsequently converted via oxalic acid dichloride to oxalic acid. A side product of this pathway is oxalyl-ethanolamide, which is formed through the reaction of oxalic acid dichloride with phosphatidylethanolamine.

The biotransformation of PER via the oxirane is largely located in the liver, which is expressed in the binding of metabolites to macromolecules and the role of this organ as target for PER toxicity and carcinogenicity.

The epoxidation pathway is depicted in Figure 1.

*Conjugation*

Originally it was believed that PER is only metabolised in mammals via epoxidation (126).

More recently, however, it became clear that direct conjugation of PER with glutathione plays a role as well (48, 49, 50). This is not to say that this pathway is important in quantitative terms. The amount conjugated is rather small compared to the amount epoxidised (102, 104). The importance of this pathway lies in the fact that it offers one of the two explanations for the occurrence of kidney tumours in male rats, the other being protein-droplet formation due to binding of PER to $\alpha_{2u}$-globulin.

Conjugation of PER takes place in the liver (50, 102, 104, 209). The conjugate is converted to 1,1,2-trichlorovinylcysteine by glutamylpeptidase and cysteine-glycinase, followed by transformation to the mercapturic acid 1,1,2-trichlorovinyl-N-acetylcysteine by the enzyme N-acetyltransferase (48). 1,1,2-trichlorovinyl-cysteine may however also be converted by β-lyase in the kidneys (47, 49, 103) to dichlorothioketene and dichlorothioacetyl chloride (47) and dichloroacetic acid (69, 104). It is assumed that the first two of these products are cytotoxic and bind to the DNA (5, 49, 104).

So far, conjugation of PER could not be detected in human liver. The β-lyase catalysed cleavage of trichlorovinylcysteine is more important in rats, in particular males, than in mice and humans (69, 102, 104).

The conjugation pathway will lead to an increase of the thioether concentrations in urine, thereby providing an explanation for the results of LaFuente and Mallol (160). The study by Birner *et al*. is the only one providing direct evidence for glutathione conjugation products in human urine (20).

The conjugation pathway is depicted in Figure 2.

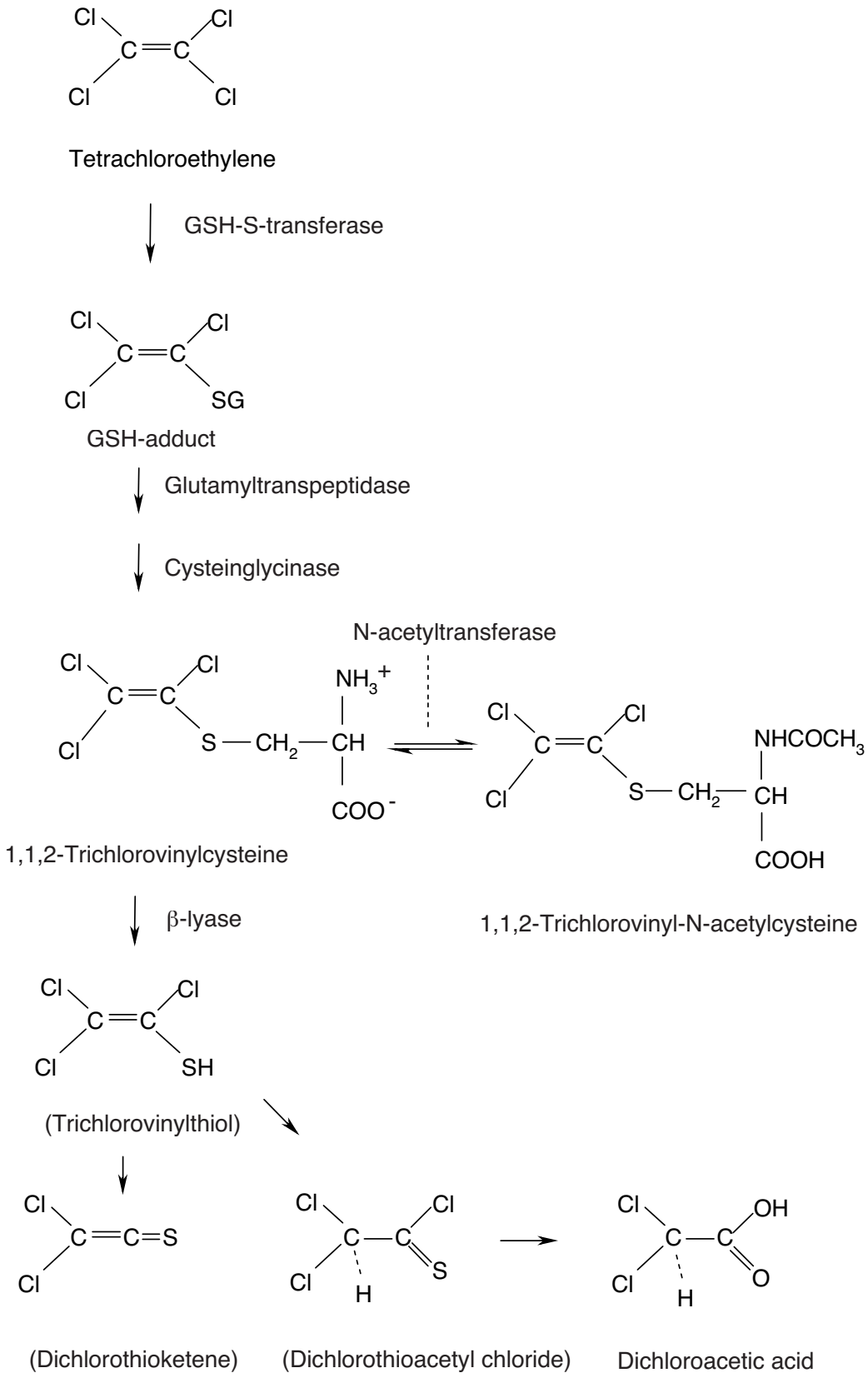



**Figure 2.** Conjugative biotransformation pathway of PER, adapted after (3).

## 5.4 Elimination

*5.4.1 Humans*

The foregoing sections of this chapter reveal that in humans, PER is primarily and slowly eliminated as such via exhaled air. On the basis of animal experiments it may be assumed that a fraction will be exhaled as $CO_2$. Minor fractions will also be excreted in the urine as TCA, possibly TCE, oxalic acid and mercapturic acid. A number of studies which provide quantitative information about the elimination of PER in humans, are summarised in Table 9. It should be emphasised, that in most experiments listed in Table 9, the elimination was measured by determining concentrations of PER in exhaled air or blood. The presence of exhaled metabolites was not taken into account. As is demonstrated with experimental animals (189, 218), $CO_2$ may be one of the exhaled metabolites. The study of Morgan *et al*. (197) is the only one which provides a complete balance, as $^{38}Cl$-labelled PER was used. Nevertheless, also in this study, part of the inhaled "material" may be exhaled undetected in the form of a chlorine-free compound. This notwithstanding, the studies based on the analysis of PER alone (e.g. 81, 192, 193, 284, 286) clearly show that the contribution of metabolites to the respiratory exhalation can only be small to insignificant.

The human elimination pattern found for PER can be explained from the slow metabolism of the compound (Section 5.3), together with its air/blood and blood/fat partition coefficients (89, 190) and the poor perfusion of the adipose tissue (191). Generally, two elimination phases are discerned for solvents like PER (18). A relatively rapid phase, which represents the clearance of the PER already present in the blood at the moment the exposure terminates. The rate-limiting factor of this phase is the air/blood partition coefficient. When the PER already present in the blood is depleted, the blood/fat partition coefficient becomes the rate-limiting factor, because it is much lower than the air/blood partition coefficient.

The slow metabolism of PER allows this compound to be absorbed by the fat tissues before significant biotransformation to more polar components can occur. Furthermore, being much slower than the elimination of PER in the blood via the lungs, biotransformation does not contribute to any great extent to the elimination of the compound in the blood. Finally, the poor perfusion of the adipose tissue is assumed to be important in this context, because it adds to the slow elimination of PER from this tissue, and thus to the slow elimination in general (191).

Monster *et al*. (192) discern a phase between the two mentioned here above. Analysis of their results lead them to estimate a separate half-time for the muscles, between the relatively short one for the vessel-rich compartment of the body and the long one for the poorly perfused adipose tissue.

**Table 9.** Elimination of PER and its metabolites by humans.

| Exposure route | Elimination route | Eliminated compounds | Experimental conditions | Half-time (h)[a] | Details | Reference |
|---|---|---|---|---|---|---|
| Inhalation | Exhalation<br>Blood<br>Urine | PER | Volunteers exposed to 194 ppm (1 337 mg/m$^3$) for 83 or 187 min, to 101 ppm (696 mg/m$^3$) for 183 minutes or to 393 ppm (2 708 mg/m$^3$) for 210 min. | | No half-time estimated; within 30 min no detectable levels in blood or urine; total clearance from exhaled air took up to 400 h. | (260) |
| Dermal | Exhalation | PER | 4 volunteers kept their thumbs immersed in PER for 40 min. | 10 | Estimated by comparison of peak concentration (0.31 ppm (2.4 mg/m$^3$)) reached after termination of exposure with subsequent concentrations, see (74). | (258) |
| Inhalation | Exhalation | $^{38}$Cl-compound(s) | Volunteers inhaled approximately 5 mg in one breath. | 1.2 and 8.4 (see next column; estimation by author) | No half-time was estimated; however, the following elimination rates were observed: 0.6%/min and 0.1%/min after 5 min and 60 min respectively; after 60 min 15% of the dose had been eliminated. | (197) |
| | Urine | $^{38}$Cl-compound(s) | | | Excretion rate was less than 0.01%/min | |
| Inhalation | Exhalation | PER | 7 h experimental exposure to 100 ppm (690 mg/m$^3$) per day for 5 days; 17 volunteers. | 65 | Estimated half-time (126) from elimination curves obtained by (257). | (126, 257) |
| Inhalation | Urine | Trichlorocompounds determined with Fujiwara reaction | Occupational exposure; 26 workers. | 144 | | (126) |

**Table 9.** Cont.

| Exposure route | Elimination route | Eliminated compounds | Experimental conditions | Half-time (h)[a] | Details | Reference |
|---|---|---|---|---|---|---|
| Dermal | Exhalation | PER | Dermal exposure of volunteers to 600 ppm (4 134 mg/m$^3$) vapour of PER for 3.5 h; respiratory exposure precluded | 1, 6 and 72 | Three half-times were determined; 2 h post exposure, 3-8 h post exposure and 72 h post exposure respectively. | (234) |
| Inhalation | Exhalation | PER | Exposure of 6 volunteers for 4 h to 72-144 ppm (496-992 mg/m$^3$) at rest and for 4 h with two periods of 30 min exercise to 142 pm (978 mg/m$^3$) | 12-16, 30-40 and 55 | The half-times were observed 20, 50 and 100 h after termination of exposure respectively; the 3 half-times were assumed to represent the vessel-rich compartment of the body (blood), the muscles and the adipose tissue. | (192) |
| | Blood | TCA | | 75-80 | | |
| Inhalation | Exhalation | PER | Occupational exposure; 32 workers 340 mg/m$^3$ (50 ppm) | | No half-time could be estimated for respiratory elimination; however, during the weekend the concentration in exhaled air decreased to about 15% of the mean inhaled concentration in the previous workweek. | (194) |
| | Blood | TCA | | 90 | Estimated from comparison of 2 concentrations, one at the end of the previous and one at the beginning of the next workweek. | |
| | Urine | TCA | | 65 | | |

**Table 9.** Cont.

| Exposure route | Elimination route | Eliminated compounds | Experimental conditions | Half-time (h)[a] | Details | Reference |
|---|---|---|---|---|---|---|
| Inhalation | Exhalation | PER | 5 volunteers exposed to 55 ppm (379 mg/m$^3$) for 90 min. | 1 | The authors explain the short half-time found by assuming that they only observed the clearance of the compound from the blood and not from body tissues. | (16) |
| Inhalation | Exhalation | PER | 1 volunteer exposed to air in a dry-cleaning shop; subsequent determination of concentrations in exhaled air in a clean-airchamber for 10 h. | 21 | | (99) |
| Inhalation | Exhalation | PER | 340 mg/m$^3$ (50 ppm). | 21 | | (284, 285) |

[a] The long half-time of TCA may be related to the strong protein binding of this compound.

*5.4.2 Experimental animals*

Studies with experimental animals in general confirm the results of human studies in a qualitative sense, i.e. most studies point to exhalation of unchanged PER as the most important elimination pathway (Section 5.4.1). Species differences can be attributed to biotransformation, the most notable example being the mouse (244). This species has a much more rapid oxidative biotransformation than other species investigated, including man (Section 5.3), leading to a large part of the absorbed PER being eliminated as TCA.

## 5.5 Possibilities for biological monitoring

The feasibility of biological monitoring of human exposure to PER has been investigated by several authors (106, 130, 135, 162, 191, 193, 194). Four biological exposure parameters may in principle be used: the concentrations of PER in the exhaled air and in blood, and the concentrations of TCA in blood and urine.

In his review paper Monster (191) mentions two extra parameters: the concentrations of TCE in urine and blood. However, as has been put forward before, it is doubtful whether this parameter really reflects exposure to PER. TCE might be a metabolite of trichloroethylene, which occurs as impurity of PER or is used in the same room as PER (252, Kezic S. Personal communication).

The PER concentrations in exhaled air show a strong linear correlation with the concentrations in blood (192)[2] and can thus be used as a reliable, non-invasive parameter for the concentrations in blood.

The latter concentrations are directly determined by the amount of PER absorbed in the various tissues. Analysis of PER decay rates for exhaled air and blood, shows that, besides the blood itself (or the vessel-rich compartment), two other compartments have to be discerned: muscle tissue and fat tissue (192). The contributions of these compartments to the concentrations in exhaled air will depend on the exposure history of the subject in question and on the time elapsed since termination of exposure.

As has been put forward in the previous section, the PER originally present in the blood is eliminated rather rapidly. If the period between monitoring and termination of exposure becomes relatively long, only the adipose tissue will contribute. In between, the muscle compartment contributes to the elimination.

The slow elimination rate of the PER which is present in the adipose tissue, combined with the long time necessary for the concentrations in this tissue to reach equilibrium (81, 241, 257), makes it possible to use the PER concentrations in exhaled air and blood as parameters for a TWA of exposure over several days, in particular when the first (rapid) elimination phase has completed before taking the samples.

It may be expected that biotransformation will not affect the reliability of biological monitoring studies, which are only based on PER concentrations in

[2] These authors found the blood-exhaled-air ratio to be 23 in a study with volunteers.

blood and exhaled air, as the amount metabolised is only marginal (192, 193). Furthermore, it has unambiguously been shown that exhalation of the unaltered compound is by far the most important elimination route; most percentages mentioned in the literature approach 100% (81, 108, 192).

However, the slow biotransformation of PER does not imply that the concentrations of metabolites cannot be used for biological monitoring purposes. PER exposure gives rise to clearly detectable TCA in blood and urine. Part of the PER, which is already present in the blood, which is eliminated from the muscle compartment, or which is eliminated from adipose tissues will be metabolised. The concentrations of the metabolites in blood and urine will, therefore, reflect the concentrations in the various compartments, and, thereby, the history of exposure.

Nevertheless, there are four reasons to use PER concentrations for biomonitoring instead of metabolite concentrations: 1) The part of PER metabolised does not correlate linearly with PER concentrations in blood. At certain PER concentrations in the air (about 100 ppm (690 $mg/m^3$)) the metabolic capacity seems to reach saturation (212). This means that TCA concentrations are not a reliable measure for exposure to higher concentrations; 2) TCA itself does also accumulate upon repeated exposures to PER, which complicates the relation between solvent and metabolite (192); 3) The use of TCA concentrations will lead to an extra source of noise, due to the interindividual variation of metabolic capacity; 4) PER will in many cases be contaminated with trichloroethylene, while the latter compound or trichloroethane will in some cases be used concurrently with PER in the same room. Being the major metabolite of trichloroethylene or trichloroethane as well, TCA simultaneously indicates exposures to all three compounds (252, Kezic S. Personal communication). Moreover, while biotransformation of PER is only marginal compared with exhalation of the unchanged compound, it is a much more important elimination pathway for trichloroethylene (190). It is impossible to discriminate exposures to the three compounds on the basis of TCA concentrations alone, while PER concentrations unambiguously point to PER exposure.

Several studies yielded quantitative indications about the relation between exposure and biological parameters. Guberan and Fernandez (106) estimated the relationship between exhaled air and respiratory exposure with the aid of a mathematical model. Some of their results are presented in Table 10.

Pezzagno *et al*. (222) investigated the relationship between PER absorption and PER concentrations in urine in 15 volunteers. They found strong and significant linear correlations, which indicate that also PER concentrations in urine can be used for biological-monitoring purposes. Monster and Houtkoper (193) found in their study with human volunteers that concentrations of PER in the blood can best be used for monitoring purposes. They calculated coefficients of variation for the individual absorption during 4 hours exposure, as determined with the biological parameters (concentrations in blood). The lowest coefficients were

**Table 10.** Relationship between respiratory exposure to PER and PER concentrations in exhaled air (table reproduced from 106).

| Day of the week | Post exposure time[a] | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 1 hour | 2 hours | 15 hours |
| Monday | 0.60 | 0.32 | 0.28 | 0.23 | 0.06 |
| Wednesday | 0.62 | 0.34 | 0.30 | 0.24 | 0.08 |
| Friday | 0.63 | 0.35 | 0.31 | 0.25 | 0.08 |

[a] The table lists the concentrations in the exhaled air as fractions of the constant concentration in ambient air for different days of the week, after at least 4 weeks of exposure.

found for the PER concentrations in blood (23 and 22% at 2 and 20 hours after exposure has ended). The authors point out that higher coefficients may be expected in the real-life occupational situation. The results of Lauwerys *et al.* (162) suggest that if the blood concentration does not exceed 1 mg/kg, 16 hours after exposure, the time-weighted exposure over the working day of dry cleaners is likely to have been below 50 ppm (345 mg/m$^3$). Monster *et al.* (194) showed that the concentration of PER and TCA in blood measured 15-30 minutes after work at the end of the workweek are good indicators for the time-weighted exposure to PER over the preceding week, followed by the TCA concentrations in urine. They estimated values of 13.2 and 33 $\mu$mol per litre for PER and TCA in blood respectively, when time-weighted exposure was 50 ppm (345 mg/m$^3$). PER concentrations in exhaled air were estimated to be 920 $\mu$mol/m$^3$ at these exposure conditions. In their study, the lower 95%-confidence limits of the biological parameters determined after exposure to up to 50 ppm (345 mg/m$^3$) were estimated to be 8.3 $\mu$mol/l PER in blood, 20 $\mu$mol/l TCA in blood, 515 $\mu$mol/m$^3$ PER in exhaled air and 3 $\mu$mol TCA/mmol creatinine in urine.

Ohtsuki *et al.* (212) estimated the lower 95%-confidence limits for total trichlorocompounds in urine, at exposure to 50 (345 mg/m$^3$) and 100 ppm (690 mg/m$^3$) (TWA), to be 30 and 61 mg/l, respectively.

Jang *et al.* (135) found that 1.6 mg/l PER in blood and 2.9 mg/l TCA in urine correspond with a time-weighted exposure to 50 ppm (345 mg/m$^3$).

## 5.6 Summary

### *5.6.1 Respiratory absorption*

Virtually all PER to which humans are exposed via the respiratory tract at the alveolar level enters the circulation. However, upon increasing concentrations of the compound in the blood the absorption decreases concomitantly. Respiratory absorption percentages well above 90% have been observed immediately upon exposure to 72 or 144 ppm (496 or 992 mg/m$^3$), while this percentage may decrease to about 50%, hours after exposure has started. Determinations during steady state still revealed absorption percentages of 78 to 90% when exposure amounted from 50 to 92 ppm (345 to 634 mg/m$^3$).

*5.6.2 Dermal absorption*

Dermal absorption of liquid PER cannot be quantified using studies with humans. However, concentrations in exhaled air during and following dermal exposure permit semi-quantitative comparison with inhalation. This shows that immersion of the thumb in fluid PER corresponds with a steady-state respiratory exposure to about 0.2-0.6 ppm (1.4-4.1 mg/$m^3$). From the same data it can be calculated that immersion of hands- and forearms for 1 hour would correspond to a respiratory exposure of 124 ppm for 8 hours.

Exposure to vapour only leads to a marginal dermal absorption in comparison with the respiratory absorption after inhalation of the same vapour; dermal absorption accounted for 1.1% of total absorption in the study of Riihimäki and Pfäffli (234).

Dermal absorption rates of 24.4 and 0.067 nmoles/min/$cm^2$ have been found for fluid PER in mice *in vivo* and in an *in vitro* rat system.

*5.6.3 Oral absorption*

The concentrations of PER or its metabolites in human blood and excreta after accidental oral exposure, indicate that PER is readily absorbed by the human gastrointestinal tract. However, more quantitative conclusions cannot be reached based on these data. Animal studies indicate a rapid and virtually complete oral absorption.

*5.6.4 Distribution*

Due to its marginal elimination by biotransformation and its high fat/blood partition coefficient, PER accumulates in the adipose tissues. It is unknown how long exposure must last or must be repeated, for an equilibrium to be reached. However, an increase was still observed after 5 daily periods of 5-hour respiratory exposure.

Analyses of human tissues after fatal exposure have revealed high concentrations in brain, kidneys, liver and lungs (from 30 to 240 mg/kg).

Tissue analyses in animal studies show clear-cut accumulation of PER in the adipose tissues. A deviating distribution is shown for the mouse, which can be explained by the higher biotransformation rate of PER in this animal.

One study indicates that PER is either transported in chylomicrons or in the phospholipid membranes of the red blood cells. Premature destruction of these cells leads to phagocytosis in the spleen, and, thereby, accumulation of PER in this organ.

*5.6.5 Biotransformation*

In humans and most experimental animals, the major part of PER is not transformed by metabolism, but excreted unchanged via exhalation. For humans, the biotransformed part of PER is estimated to be only 1-2%.

The major metabolite in humans is TCA. Doubt exists as to TCE being a metabolite of PER. The presence of this compound in body fluids can also be explained by simultaneous exposure to trichloroethylene, either as impurity of

PER or as solvent used in the same room as PER. One study demonstrated the presence of *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-*L*-cysteine in human urine, albeit in very low concentrations (less than 0.001 times the concentrations of TCA/TCE).

Other metabolites detected in humans, experimental animals or *in vitro* systems are: oxalic acid, dichloroacetic acid, ethylene glycol, trichloroacetyl amide, trichloroacetylaminoethanol, thioethers and $CO_2$.

Two biotransformation pathways are discerned:

An oxidative one in the liver, the first step of which is the epoxidation by cytochrome P450 to oxirane, resulting in TCA as major metabolite.

A conjugation pathway in the liver, the first step being the conjugation of PER with glutathione. This reaction is catalysed by glutathione transferase. It leads to trichlorovinylcysteine, which can be cleaved in the kidneys by β-lyase into cytotoxic and genotoxic metabolites.

The first pathway is by far the most important one in quantitative terms.

*5.6.6 Elimination*

PER is primarily eliminated via exhaled air, independent of exposure route. Half-times for respiratory elimination vary from 1 to 72 hours. This large variation most probably reflects the differences in elimination rate between PER in the blood, the adipose tissues and other tissues. The longest half-times are measured when the PER originally (immediately after termination of exposure) present in blood and non-adipose tissues is depleted, and the elimination rate is limited by a slow transfer from fat tissues to blood, due to a high fat/blood partition coefficient and poor perfusion of the adipose tissues. Other pathways do not significantly contribute to the elimination.

*5.6.7 Biological monitoring*

Concentrations of PER itself or its major metabolite TCA in body fluids can be used for biological monitoring purposes, in addition to the concentration of PER in exhaled air. The available data indicate that PER concentrations in blood and exhaled air give a reliable impression of the TWA exposure over several days, in particular when the rapidly eliminating PER (present in blood immediately after termination of exposure) has been allowed to be eliminated first. TCA can be regarded to be a less suitable indicator compound, as it is also the major metabolite of trichloroethylene and trichloroethane, compounds which are often present as impurity in PER or can be used in the same room as PER. Furthermore, the use of TCA may be hampered by interindividual differences in PER biotransformation.

# 6. Effects

## 6.1 Observations in humans

*6.1.1 Irritation and sensitisation*

A few case reports show PER to be a clear-cut skin-irritant for humans (108, 185, 187, 205). Extensive erythema and blistering were reported for a worker who had lain unconscious in a pool of solvents for about 5 hours (166).

A worker who had been unconscious for half an hour with PER-soaked clothes showed the same symptoms. Considerable reduction of the symptoms occurred within 5 days, although after 4 months there was still dryness, staining and irritation of the injured areas (196).

Three case reports point to sensitising properties. Skin sensitisation in a worker is described by Vail (276); a closed patch test with PER and 1% of PER in oil produced a positive response. The second report is concerned with the induction of PER-dependent asthma in a woman. It is described in Table 11. Finally, PER sensitisation of the skin was confirmed with patch testing in a woman wearing garments contaminated with PER by dry cleaning.

*6.1.2 Case studies*

*Therapeutic oral exposure*

The anthelminthic properties of PER were propagated for the first time in the 1920s. Subsequently, the compound has been regarded for a long time as an effective anthelminthic drug with relatively mild and transient side effects (compared to other anthelmintics) (141). It has, in particular, been used in South-East Asia and the Pacific for treatment of hookworm infections. Large numbers of patients have been treated with the substance. Oral doses of up to several millilitres per patient have been prescribed. The UK Health and Safety Executive (HSE) (119) mentions some case studies, which however, do describe clear effects. Vertigo, inebriation, giddiness, nausea, and sleepiness at doses of 4.5 or 6 g/patient were reported by Kendrick (141). In two other studies, similar doses have caused loss of consciousness lasting approximately 2 hours (239, 294). Haerer and Udelmann (107) report a severe psychosis upon oral treatment with PER. Anaphylactic reactions have been observed in children treated with 0.25 ml/kg twice daily for two days (228). Two lethal therapeutic treatments are mentioned in the HSE review (96, 164).

*Occupational exposure: Acute exposure*

Several accidental occupational exposures to PER are summarised by the US Environmental Protection Agency (EPA) (74) and HSE (119). Some will be touched upon here, based on these reviews and a number of original publications.

Saland (238) reports the case of nine firemen exposed to high levels of PER fume for three minutes. All nine became lightheaded and uncoordinated. Liver-function changes were induced which persisted until 63 days after exposure.

A case of virtual whole-body exposure is described by Patel *et al*. (217). Exposure lasted 7 hours. Effects were: loss of consciousness, coma, acute pulmonary oedema, and hypotension. Kidney and liver function were reported as normal. Normal liver- and kidney-function tests have also been reported in a case of respiratory overexposure, which resulted in clear depression of the central nervous system (CNS), such as to make mechanical ventilation necessary (217).

Stewart *et al*. (259) found an impaired liver function arising 9 days after an acute narcotic exposure to PER, which they attributed to the exposure.

Stewart (256) found a transient increase in serum glutamic oxaloacetic transaminase (SGOT) and a delayed elevation of urinary urobilinogen in a subject acutely exposed via inhalation to an anaesthetic concentration of PER.

Hake and Stewart (108) present the case of a dry-cleaning operator who was exposed by lying in a "pool" of PER for 12 hours. Apart from unconsciousness, the exposure led to a mild seizure and some temporary liver and kidney damage. Liver and kidney tests were negative after 21 days.

Breath analyses were carried out in the three publications of R.D. Stewart. Therefore, these allow the observed effects to be related with a measure of exposure, as the concentrations in expired air are directly and linearly linked with the concentrations in blood (Section 5). In the Hake and Stewart (108) case, concentrations decreased from about 600 ppm (4 134 mg/m$^3$) a few hours after termination of exposure (the concentration immediately upon exposure was not measured, but must have been much higher) in a bimodal way to 100 ppm (690 mg/m$^3$) after about half a day, 10 ppm (68.9 mg/m$^3$) after about 15 days and 4 ppm (27.6 mg/m$^3$) after about 25 days (read from the published graph by the reviewer).

Two publications report fatalities due to acute exposure (165, 169). The first concerns exposure during repair in a dry-cleaning shop, the second exposure during recycling of dry-cleaning PER by distillation.

*Occupational exposure: Long-term exposure*

A number of cases concerning long-term occupational exposure are summarised by EPA (74) and by Eberhardt and Freundt (68). Key data of these studies are listed in Table 11.

### *6.1.3 Volunteer studies*

Four male volunteers were exposed to 472 and 911 ppm (3 252 and 6 277 mg/m$^3$) of PER vapour for 130 and 95 minutes, respectively (35). The 472/130 combination gave rise to the following effects: eye irritation, secretion from mucous membranes, sensory changes, slight feeling of elation. The neurological effects became more severe at the 911/95 combination, when lassitude, mental fogginess, and exhilaration were experienced/observed. Increase of the highest concentration to 1 450 ppm (9 991 mg/m$^3$) after 95 minutes lead to inebriation. Increase to 1 940 ppm (13 367 mg/m$^3$) forced the subjects to leave the exposure room after 7.5 minutes; they reported "ringing in the ears" at this concentration (35).

**Table 11.** Cases of long-term occupational exposure to PER, based on (74).

| Exposure | Effects and symptoms | Reference |
|---|---|---|
| Males cleaning metal parts for 3 weeks to 5 months. | Fatigue, inebriation, dizziness, headache, nausea, vomiting, lack of appetite, sleeplessness, irritability, irritation of the eyes. All symptoms disappeared when the workers left the work site. | (167) |
| Male exposed for approximately 3 months to high levels. | Nausea, jaundice, unspecified grossly abnormal liver tests. | (122) |
| 65-year old female cleaning metal parts for 2 months. | Numbness, dizziness and anorexia; recovery following an 8-day absence from work. | (68) |
| Female exposed for 2.5 months, 10 h/day. | Liver toxicity: increased alkaline phosphatase (ALP), SGOT, bilirubin, cephalin flocculation. Liver biopsy 2 weeks after exposure: degeneration of parenchymal cells, focal collections of mononuclear cells and exaggeration of liver sinusoids. | (183) |
| 23-year old male; 4 months working in about 250 ppm (1 723 mg/m$^3$). | Death. Autopsy: enlarged liver with hepatic cell necrosis, fatty degeneration of the myocardium, lung oedema and hemorrhagic pneumonia. | (271) |
| Female working in a dry-cleaning establishment. | Breast-fed baby showed jaundice and enlarged liver. Breast milk contained up to 10 mg/l PER. Symptoms disappeared upon discontinuation of breast feeding. | (57) |
| 7 male degreasing workers exposed to 230-385 ppm (1 585-2 653 mg/m$^3$) for 2-6 years. | Memory impairment, staggered gait, drunken-like state, lightheadedness, dizziness, tiredness. Three had impaired liver function as diagnosed by sulfobromophthalein retention; 4 had positive urobilinogen. | (74) |
| 47-year old male working in a dry-cleaning plant. | *Acute:* nausea, vomiting, dizziness, staggered gait, disorientation.<br>*Chronic:* memory impairment, mood lability, fatigue. It is reported that the clinical picture suggested both basal ganglia involvement and cerebral-cortical damage. | (94) |

**Table 11.** Cont.

| Exposure | Effects and symptoms | Reference |
|---|---|---|
| 14 males working in animal-cadaver destruction plant, involved in fat extraction with PER; no data about concentrations available; estimated to lie well above prevailing threshold limit value (TLV). | *Complaints:* Impairment of memory, concentration and affectivity, loss of interest and drive, social retreat, vertigo, arthralgia, hyperhidrosis, headache, tremor, muscular weakness, disturbances of minute motor activity, myalgia, impaired hearing and sleep disturbances.<br>*Internal check up:* high blood pressure, hepatomegaly, liver damage indicated by biochemical markers, abnormal blood triglyceride, blood sugar and partial thromboplastin time. No affected lung function and electrocardiogram.<br>*Neurological examination:* impairment of coordination. No effects on electroencephalogram (EEG), electromyogram, nerve conduction velocity, visual evoked potential and somato-sensory evoked potential.<br>*Psychometry:* psycho-organic syndrome with decrease in performance and personality disorders.<br>*Nuclear magnetic resonance tomography:* signs of cerebral atrophy. | (168) |
| 55-year old woman working for 2 years in a dry-cleaning establishment. | Asthma induced by two massive exposures; subsequently asthmatic attacks when in establishment. | (216) |
| Middle-aged male 2 years occupational exposure followed by 2 years of intermittent occupational exposure, due to metal cleaning. | Symptoms appeared in 4th year. Gastrointestinal disturbances, abdominal pain, severe headaches. 2 years after cessation of exposure, upon hospitalisation, neuroautonomic dystonia, virile climacterium, mental depression, followed by labyrinthine disorders, memory loss, somnolence, insomnia, slight motor incoordination and abnormal reflexes. | (167) |
| Male in his late 30's, 6 months of occupational exposure to trichloroethylene, followed by 1 year of occupational exposure to PER, due to metal degreasing. | Headaches, dizziness, fatigue, anorexia, nausea, loss of libido, intolerance to alcohol. Within 3 months after cessation of employment, gait problems, numbness, sweating, blanching and flushing of the fingertips (indicative of Raynaud's syndrome). Afterwards, progressing and persisting neurological symptoms. | (167) |

**Table 12**. Human-volunteer experiments carried out by Rowe *et al*. (236).

| Exposure regimen | Symptoms |
|---|---|
| 4 persons, 930-1 185 ppm (6 408-8 165 mg/m$^3$), 1-2 min | Marked irritation of the eyes and the upper respiratory tract. 2 minutes exposure (1 person) lead to considerable dizziness. Rapid and complete recovery. |
| 2 males, 513-690 ppm (3 535-4 745 mg/m$^3$), 10 min | Irritation of eyes and nose, dizziness, tightness and numbness about the mouth, some loss of inhibitions. Good motor co-ordination requires effort. Complete recovery within an hour. |
| 4 males, 206-356 ppm, (1 419-2 453 mg/m$^3$) up to 2 h | Lightheadedness, burning sensation in the eyes, congestion of the frontal sinuses, thickness of the tongue, irresponsibility, nausea, impaired motor co-ordination, effort required for motor co-ordination. Recovery within 1 hour, except one subject, who felt unwell for several hours. |
| 4 persons, 206-235 ppm (1 419-1 619 mg/m$^3$), 45-120 min | Eye irritation and congestion of nasal sinuses with discharge within 20-30 min, dizziness (inebriation), sleepiness. |
| 6 persons, 83-130 ppm (572-896 mg/m$^3$), 1 h | No adverse effects reported, except for eye irritation when peak concentrations occurred during dosage of liquid PER in the exposure room. |

Rowe *et al*. (236) subjected human volunteers to various exposure regimens. Only the effects of exposure to PER vapour were investigated. The experiments and results are concisely presented in Table 12.

This study confirms the finding of Carpenter (35) and suggests a no-observed-adverse-effect-level (NOAEL) of about 100 ppm (690 mg/m$^3$) or lower for acute and overt neurological effects and for eye irritation. The neurobehavioral findings of Rowe *et al*. (236) are based on a limited number of volunteers using rather crude qualitative observational methods (no neuropsychological tests, electro-encephalogram (EEG), etc.).

Stewart *et al*. subjected groups of 6 male volunteers to different exposure regimens (260). Three experiments were carried out. Concentrations of PER were measured in blood and expired air. The effects observed/experienced in these experiments are listed in Table 13. No effects were found on SGOT and serum glutamic pyruvic transaminase (SGPT) activities, as well as urobilinogen excretion (260). This study suggests a NOAEL of 100 ppm (690 mg/m$^3$) for neurological effects and exposures lasting longer than 30 minutes.

Stewart *et al*. (257) exposed 16 volunteers for 7 hours to 100 ppm (690 mg/m$^3$); 5 volunteers of the group received this treatment for 5 consecutive days, the other 11 only once. Effects investigated were: subjective symptoms reported by the exposed themselves; a neurological examination, including a modified Romberg test (test of balancing ability); haematological parameters and biochemical parameters measured in blood or serum, including the activities of SGOT, SGPT and serum alkaline phosphatase (ALP) and serum lactic acid dehydrogenase (SLDH); "complete" urine analysis, including urobilinogen, 17-ketosteroids,

**Table 13**. Effects of respiratory exposure to PER on human volunteers (260).

| Concentration (ppm and mg/m$^3$) | Time after start of exposure (min) | Symptoms |
|---|---|---|
| 75-80 (517-551) | 1-4 | Very slight eye irritation (a mild burning sensation); subjects became unaware of irritation after a few minutes of exposure. |
| 100-120 (689-827) | 4-6 | Slight soft palate irritation and dryness noticeable. |
| 200 (1 378) | 6-30 | Romberg test normal |
| 210-244 (1 447-1 681) | 30+ | Slight lightheadedness; increased effort necessary to maintain a normal Romberg test. |

17-hydroxycorticosteroids, catecholamines, and creatinine.

Most of the exposed reported one or more of the following subjective symptoms: mild eye, nose or throat irritation, frontal headache, flushing, sleepiness, difficulty in speaking. These effects decreased upon repeated exposure, which points to adaptation.

Three of the 16 subjects showed an abnormal Romberg test within the first 3 hours of exposure, while a number of other tests (Crawford manual dexterity, Flanagan co-ordination, arithmetic and inspection) were normal. A NOAEL lower than 100 ppm (690 mg/m$^3$) is suggested by this study on the basis of neurological endpoints.

In the study of Hake and Stewart (108), two to four female or male volunteers were subjected for 5 days (1, 3 or 7.5 hours/day) to 0, 20, 100 or 150 ppm (138, 689 and 1 034 mg/m$^3$). Effects investigated were: subjective effects reported by the volunteers themselves as well as neurological, behavioural, and physiological effects (no complete overview of all effects investigated is presented). EEG scanning by a neurologist suggested altered patterns indicative of cortical depression. No effects were found on visual evoked response, equilibrium, math skills (tested for males only), time discrimination, inspection, and reaction time. Co-ordination scores (Flanagan test) were significantly decreased in the 100 and 150 ppm group. Various subjective symptoms were reported (not specified); but these disappeared entirely as exposure progressed. No effects on physiological parameters (includeing pulmonary function; other parameters not specified) were found (108). A NOAEL of 20 ppm (138 mg/m$^3$) is indicated by this study on the basis of neurological endpoints. However, due to the very limited number of persons studied, the reliability of this NOAEL is rather low.

Six female and six male volunteers were exposed to ethanol (0, 0.75, 1.5 ml of 100-proof vodka per kg), diazepam (Valium$^R$; 0, 6 or 10 mg/day, once a day when appropriate), or PER (0, 25 or 100 ppm (172 or 690 mg/m$^3$) of PER; 5.5 hours/day for 11 weeks), or to combinations, this, to study the interactions between either ethanol ingestion or diazepam ingestion and respiratory exposure to PER (261, see also 108). Exposure was checked by analysis of biological samples. A series of

neurobehavioral and neuropsychological tests as well as EEG recordings were carried out at peak blood levels of the two drugs during PER exposure. Although the drugs alone did yield the expected effects on one or more of the investigated endpoints, no interactions with PER were observed, indicating that they do not aggravate possible effects of PER or that PER does not aggravate the effects of these drugs. A slight statistically significant effect of PER was only found in the Flanagan co-ordination test, on a limited number of days. However, this effect was considered to be inconsistent by the authors. No clear PER-exposure related effects on the EEGs were observed (261, see also 108).

Groups of 11 male volunteers (23-35 years) were exposed for 4 days, 4 hours/day, to 10 or 50 ppm (68.9 or 345 mg/m$^3$) of PER (4). Visual evoked potentials and brain-stem auditory evoked potentials (BAEP) were measured during exposure. In addition visual contrast sensitivity was determined psychophysically in some volunteers. The 50-ppm group showed increased visual evoked potential peak latencies during exposure (statistically significant from the 10 ppm group). No effects on BAEP were found. A tendency of increased visual contrast sensitivity threshold levels was observed. A significant ($p<0.03$; $r = -0.45$) blood-concentration dependence of one visual evoked potential peak latency (N150) was established. The study was undertaken to see whether the suggestions of two case reports for effects of PER on visual capabilities could be substantiated. The study indicates effects of PER on the visual system. For the investigated effects, 10 ppm (68.9 mg/m$^3$) can be regarded as a NOAEL in case of 4-hour exposure of young healthy males (4).

The committees decided that due to the poor presentation and statistical analysis of the data, the study has only a limited value for the evaluation of the human neurotoxicity of PER.

### *6.1.4 Epidemiological studies*

#### *Genotoxicity*

The occurrence of cytogenetic effects was investigated in lymphocytes of two groups of 5 factory workers, differing in supposed PER exposure (127). Exposure varied from 30 to 220 ppm (207-1 516 mg/m$^3$), with a geometric mean of 92 ppm (25 determinations) for the highly exposed group, and from 10 to 40 ppm (68.9-276 mg/m$^3$), for the low exposure group (no further details presented). The differences in exposure were confirmed by the urine analysis. The persons in the high exposure group were much longer employed than those in the low exposure group. The highly exposed group was involved in degreasing with PER; the other group was employed in the same workshop, without exposure that could directly be attributed to working with PER. Technical PER was the sole solvent used in the workshop. Personal air sampling and urine analysis were carried out for exposure assessment. Besides cytogenetic effects, a number of haematological and liver-function parameters were studied. The same endpoints were studied in a group of 9 non-exposed workers.

No significant increase of cytogenetic effects was observed. Also liver-function tests and haematology did not show a relation with PER exposure (127).

The value of the study is deemed limited by the ATSDR (3) because of the small number of subjects involved and the wide exposure range.

Fender investigated the occurrence of cytogenetic effects in lymphocytes of 9 women employed in dry cleaning shops in Berlin and 9 control women with office jobs (80). The dry-cleaning group was exposed to 144-348 mg/m$^3$ of PER. The exposed group had twice as many cells with aberrations than the control group when gaps were not taken into account. When the cells with gaps were included, this difference increased markedly. Dicentric chromosomes occurred 13 times as much in the cells of the exposed groups. It is reported that the PER was contaminated with 0.11-0.43% (volume) of trichloroethylene. The authors state further: "Technical PER is usually contaminated with trichloroethylene" (80).

In its report on PER, the German MAK Committee summarised two more studies on the genotoxic effects of PER exposure in humans. The first dealt with the frequency of sister chromatid changes in smoking and non-smoking, exposed workers and controls (250). No effect of the exposure was found within the groups of smokers and non-smokers. In the second study (32), no effects on the frequency of chromosome aberrations and sister chromatid exchanges in lymphocytes was found when 38 workers in dry-cleaning shops were compared with 45 controls. The possible effects of smoking were taken into account.

*Reproduction toxicity*

In a study by Bosco *et al*. (27), 67 women working in 53 dry-cleaning shops were interviewed on their obstetrical history. Exposure was indicated by elevated TCA concentrations in their urine. 102 pregnancies were reported, of which 46 occurred when the women were not involved in dry cleaning, and the remaining part when they were involved in dry cleaning. Endpoints considered were: live births, birth weight, spontaneous abortion, and birth defects. No significant differences were found between the two groups of pregnancies. The most marked difference was a more than four times as high frequency of spontaneous abortions (8.9% versus 2.2%) in the "PER-exposed" group ($p<0.10$). The low power of the study is demonstrated by the fact that such a difference did not reach significance. The authors conclude that "the study ... indicates the absence of overt reproductive pathology. Given the small sample size, however, these findings should be considered as merely tentative" (27).

A nested case-referent study (266) was carried out to investigate the effect of paternal exposure to solvents (among them PER) on pregnancy outcome (spontaneous abortion and malformation). "Cases" were spontaneous abortions or malformations selected from a medical register. Data on paternal pre-conception exposure and maternal post-conception exposure were obtained from "The register of biological exposure measurements" (workers ever monitored by the Finnish Institute for Occupational Health from 1965 to 1983; concentrations of PER in blood were taken as a measure for exposure to this compound) and questionnaires.

Odds ratios (ORs) pointed to a significant effect of spontaneous abortion due to paternal exposure to solvents in general (OR: 2.7, $p<0.01$), xylene (OR: 1.8,

$p<0.05$), "thinners" (OR: 1.7, $p<0.05$), dusts (OR: 2.3, $p<0.01$) and smoking (OR: 0.6, $p<0.05$). For PER an OR of 0.5 was found ($p>0.05$).

No effects of maternal exposure to solvents were found on spontaneous abortion. Matching for confounders and stratification according to intensity of exposure did not lead to an effect for halogenated hydrocarbons, while the effect for dusts disappeared; the effect increased for solvents in general.

No significant effects of the investigated exposures on congenital malformations were observed (266).

The power of this study is low, due to the small numbers of cases involved (spontaneous abortions: OR=2, 34%, $\alpha=0.05$). This holds in particular for the congenital malformations; so no firm conclusions may be drawn based on the absence of significant effects with respect to this endpoint.

However, the study suggests that a risk for spontaneous abortions due to paternal PER or halogenated-solvent exposure, if present, is relatively low compared to that of other paternal solvent exposures.

Stratification according to paternal occupation confirms this conclusion, as metal workers (supposed to be exposed to chlorinated solvents for degreasing) had an insignificant OR of 1.3, while that of painters was 3.3 ($p<0.001$) (266).

Olsen *et al*. present a series of nested case-reference studies on the obstetric history in women doing dry-cleaning work (213). The studies were carried out in the four Nordic countries, and to a certain extent common procedures were followed. It was attempted to identify all women who had worked for at least one month in a dry-cleaning shop during part of the 1970s and 1980s. Information on exposure during pregnancy were collected with interviews or questionnaires, in three countries the information was obtained from the women, in Norway it was obtained from employers. The following cases were selected: birth weight less than 1500 g, congenital malformations, perinatal death or spontaneous abortion. Total number of cases was 20. The small number of exposed cases limited the power of the study. The results suggest an increased risk of spontaneous abortions, in particular in Finland. The study is inconclusive to the extent that it cannot be explained why this effect is in particular manifest in Finland (213).

A case-referent study was carried out by Windham *et al*. to investigate the effects of maternal exposure to solvents on spontaneous abortion (292). "Cases (n=852) were women 18 years of age or older, who had a spontaneous abortion by 20 weeks gestation, for which a pathology specimen was submitted to one of the 11 hospital laboratories in Santa Clara County, California". For each case, two controls were selected. Exposure and confounders were assessed by telephone interviews. Five cases reported exposure to PER, versus two controls, which results in a crude OR of 4.7 (95% confidence interval: 1.1-21.1).

The OR for the any solvent (including PER) group was 1.2 (95% confidence interval: 0.87-1.6). Other solvents with high ORs were trichloroethylene (3.1; 0.92-10.4) and "thinners" (2.3; 1-5.1). The authors conclude that "this study and others suggest that exposure to certain solvents, particularly perchloroethylene, trichloroethylene and aliphatic hydrocarbons, may confer excess risk of spontaneous abortion." The committees feel that the word "suggest" should be

emphasised in this citation, as the conclusion is based on very small numbers (292).

Semen quality of 34 dry cleaners was compared to that of 48 laundry workers (76). Breath concentration of PER was used as a measure for PER exposure. Furthermore an exposure index was established on the basis of a questionnaire. The first measure gives a reliable impression of PER exposure in the week preceding sampling, while the second can be regarded as a more rough estimate, which covers, however, a larger part of spermatogenesis. The two groups clearly differed in exposure according both measures.

Although on the whole, the semen quality of both groups was lying within normal limits ("by standard clinical measurements"), the following differences between the two groups suggest an effect of PER exposure on semen quality: 1) higher number of round sperm ($p<0.002$); dose related; this effect is associated with infertility; 2) sperm tends to be less narrow ($p<0.02$); dose related; and 3) greater amplitude of lateral head displacement ($p<0.09$); dose related; this effect possibly reflects alterations in the functioning of the cell-plasma membrane.

The exposure measures are not presented in detail, but it was noted that 6 laundry workers had breath PER concentrations higher than 100 $\mu g/m^3$; for 4 dry cleaners these concentrations were lying below this concentration. Further, it was not clear whether the described effects are due to exposure to PER alone or to a mixture of compounds.

The authors conclude that "these results suggest that occupational exposure to PCE (PER) can have subtle effects on sperm quality. Additional analyses are required to determine whether these effects are associated with changes in fertility" (76).

The female partners of workers participating in the aforementioned study (76) were examined for reproductive outcome (77). 17 partners of dry cleaners and 32 partners of laundry workers participated in the study.

No differences were found for number of pregnancies and live births and rates of spontaneous abortion. Standardised fertility ratios were almost identical for the two groups.

However, the partners of the dry cleaners "were more than twice as likely to have a history of attempting to become pregnant for more than 12 months or to have sought care for an infertility problem." Furthermore, partners of laundry workers tended to be pregnant within the first two cycles for a larger percentage than partners of dry cleaners.

The authors regard these results too little outspoken and the sample size to inadequate, to allow for definitive conclusions (77).

*Nephrotoxicity*

In a study by Franchini *et al*. (84) 57, mostly female persons, working in 29 dry-cleaning shops, were compared with control groups consisting of 50 females and 30 males or 16 females and 65 males, for proteinuria, albuminuria, urine lysozyme activity and urine β-glucuronidase activity, as parameters of renal changes. The authors calculated the exposure concentration via biological monitoring to be 10

ppm (68.9 mg/$m^3$). Since, the latter two parameters were significantly enhanced, the authors conclude that "the damage seems to be very weak and tubular, rather than glomerular" (84).

Lauwerys *et al*. (162) compared 26 persons (24 females and 2 males) working in dry-cleaning shops with a non-exposed population of 31 females and 2 males, for several biochemical urine parameters (β2-microglobulin, albumin and retinol-binding protein). Exposure was determined by personal air monitoring, analysis of breath and blood for PER and analysis of urine for TCA. TWA exposure varied from 8.9 to 37.5 ppm (61.3-258.3 mg/$m^3$), with a mean for all samples of 20.8 ppm (143.3 mg/$m^3$). No effects on the parameters were found (162).

Biochemical markers of kidney damage were examined in 16 female workers from five dry-cleaning shops (283). Thirteen non-exposed females served as a control group. Exposure was investigated with personal passive diffusion samplers and personal active charcoal tube samplers. Urine was investigated for β2-microglobulin, creatinine, glucose, lysozyme, lactic acid dehydrogenase (LDH), and total proteins. The exposure levels varied between 9 and 799 mg/$m^3$, the mean level amounting to 157 mg/$m^3$ (all TWA).

The only significant effect of exposure was an increased lysozyme activity. No correlation with concentration was found, neither was there an effect of duration of exposure.

The authors conclude that "in view of these limitations and results of other authors, the existence of a chronic nephropathy at low exposure levels remains very hypothetical" (283).

Nine men and 41 women working in dry-cleaning shops were compared in a cross-sectional study with 50 sex- and age-matched controls for 23 biochemical or immunochemical parameters of renal damage, 20 in urine and 3 in serum (200). Exposure was determined by measuring PER concentrations in blood of the exposed, in air samples (environment and breathing zone). Air concentrations ranged from traces to 85 ppm, giving rise to blood concentrations of 9-900 mg/l; the median values were 14.8 ppm and 143 mg/l for air and blood, respectively. The authors found that, based on the values of these parameters, the memberships of the groups was correctly predicted for 93% of the examined persons. If only 13 instead of 23 parameters were used this percentage decreased to 87%. Comparison of means yielded significant ($p<0.05$) differences for albumin, transferrin, brush-border antigens (BB50, HF5 and BBA), tissue non-specific ALP and fibronectin in urine and anti-glomerular basement membrane antibodies and laminin fragments in serum. Like the Vyskocil study (283), no significant effects on β2-microglobulin were found. The results point to both, glomerular as well as tubular changes due to the exposure. The study does not suggest a correlation between duration or intensity of exposure and renal damage (200).

*Hepatotoxicity*

The study by Lauwerys *et al*. (see under "*Nephrotoxicity"*) did not reveal effects of exposure on the enzymes SGPT and γ-glutamyl transferase (γ-GT) in serum,

indicating the absence of effects on the liver by PER at the measured low exposure levels (162).

Gennari *et al*. studied iso-enzyme patterns in a group of 141 workers from 47 small laundries and dry-cleaning shops in Bologna, exposed to PER, and a control group of 130 subjects from among staff and students of the University of Bologna, Italy (91).

Both clinical examination and questionnaire results ascertained that all the study subjects showed a) no history or current signs of liver disease, b) no alcohol intake exceeding 8 g daily, c) no use of drugs known to affect enzyme activity, and d) no clinical condition or habit likely to affect enzyme activity.

Exposure to PER was assessed measuring TCA in urine at the end of the work shift, after at least 5 consecutive days of exposure. Environmental PER measurements showed that in all instances, the mean value for 8 working hours was lower than the ACGIH 1989/1990 threshold limit value (TLV)-TWA of 50 ppm. The TLV-short-term exposure limit (STEL) of 200 ppm was not exceeded in any of the measurements.

Total γ-GT activity was higher in the exposed group (12.36 ± 6.90 U/l versus 8.76 ± 4.94 U/l, $p < 0.01$). Also the γ-GT-2 isoenzyme fraction was higher in the exposed group (6.79 ± 5.74 U/l versus 3.48 ± 3.29 U/l, $p < 0.01$).

No correlation existed between serum γ-GT activity and PER exposure level or duration. No differences in enzyme activity of other enzymes measured (alanine aminotransferase (ALT), aspartate aminotransferase (ASP), ALP, LDH, 5'-nucleotidase (5'-NU) was mentioned by the authors (91).

The control group and the exposed group are likely to be different in lifestyle, especially nutrition, but also with respect to the consumption of alcohol (although this latter feature was controlled for). However, γ-GT is known to be a very sensitive marker of exposure to alcohol and also of various other clinical and non-clinical conditions, and the method of controlling for the consumption of alcohol used in this study, seems susceptible for introducing confounding. In addition, both a dose-effect-relationship and (probably) effects on other enzyme activities are absent. At this stage, therefore, it is considered premature to conclude that the finding of a slightly higher serum γ-GT (isoenzyme) activity within normal limits should be considered a biologically significant adverse health effect of PER exposure.

*Cancer*

This section is largely based on references (3, 12, 69, 74, 119). However, most of the original publications have been consulted for verification and for comparison of conclusions.

A number of studies on drinking-water for evaluating the carcinogenicity of PER have been published (11, 39, 133, 161, 279); all summarised in (124). These studies will not be described here in detail because there are sufficient occupational exposure studies in which the carcinogenicity of PER has been studied. Further, oral exposure of the general population is less relevant in the context of occupational exposure to PER. Moreover, these drinking-water studies did not

provide clear evidence for the carcinogenicity of PER due to the lack of clear differences in cancer incidences and the impossibility to differentiate between effects of PER and effects of other chlorated hydrocarbons present in drinking-water.

The publication by Kaplan (137) is concerned with a retrospective cohort mortality study involving 1 597 members (285 deaths) of four unions, who had been employed in dry-cleaning shops for at least one year before 1960. Comparison of standardised mortality ratios (SMRs) of the cohort with the overall mortality rates of the United States' population revealed significant excesses of urinary-tract cancers (observed 5, expected 2.84 and 2.92 for whites and blacks, respectively; SMRs: 203 and 198) and colon cancers (observed 11, expected 6.98 and 6.77; SMRs: 182 and 187). The author did not draw definitive conclusions because of the small numbers within the cohort (137).

In 1987 the cohort was expanded to 1 690 (493 deaths) workers with 42 267 person-years-at-risk (30). A subcohort was defined which consisted of 615 (137 deaths) workers exposed almost exclusively to PER. In the main cohort, a significant excess of urinary-tract cancer deaths was observed, i.e. 12 (8 bladder and 4 kidney) against 4.7 expected; SMR: 255, 95% confidence limits: 132-450; only the excess of bladder cancer was significant. However, no excess of urinary-tract cancer was found in the PER-only subcohort. When latency time and employment time were taken into account, the results indicated an occupational aetiology for bladder-cancer mortality.

Although the absence of an increased risk in the PER-only subcohort does not prove the absence of an association with urinary-tract cancer for this compound, it suggests a contribution by other factors (exposures). The authors hypothesise Stoddard solvent (a mixture of petroleum solvents similar to gasoline) to be a possible factor. Stoddard solvent has extensively been used before the introduction of PER (in the 1950s), and many years thereafter. Other solvents that have been used are tetrachloromethane and trichloroethylene, although subjects known to be exposed to the former were excluded from this study.

In view of the findings in experimental animals (Section 6.2), it is important to note that no cases of liver cancer were found in the main cohort, while cancers of the lymphatic and hemopoietic systems were significantly lower in the main cohort.

Smoking was not accounted for as a confounder in this study. The authors recognise this and mention smoking as an etiologic factor for urinary-tract cancer. However, based on the influence of smoking on urinary-tract cancer incidence and mortality, which is observed in other studies, they regard a substantial contribution of smoking to the urinary-tract SMR to be impossible.

The authors conclude: ".... the confounding exposure to petroleum solvents complicates any conclusions regarding the association between PER exposure and cancer of the urinary tract". The committees add that the absence of risk in the PER-only subcohort strongly suggests other factors to contribute to the risk observed in the main cohort.

The results of a second update of this study have been published in 1994 (237). In general, this update confirmed the conclusions of the first publication and the first update. Most notable was the increase of oesophagus cancer in both the main cohort and the PER-only subcohort. In the latter, an SMR of 7.17 (95% confidence limits: 1.92-19.82) was found for the decedents with more than 20 years of latency time who had been employed for at least 5 years in dry cleaning. A contribution of smoking and drinking to the SMR of oesophagus cancer could be excluded on the basis of the SMRs for lung cancer and liver cirrhosis. Again, the PER-only group did not show an elevated SMR for urinary-tract cancer. Neither was there an elevated liver-cancer SMR.

Blair *et al*. (22) determined SMRs for a cohort of 11 062 members of a dry-cleaners union, which were employed between 1945 and 1977. Incidences of laryngeal cancer, bladder cancer and lymphoma were elevated in workers employed before 1963 (SMRs: 256, 156 and 199, respectively). For black male subjects with medium or high PER exposure, oesophagus cancer was significantly elevated (SMR: 280); in a subcohort of black male workers with more than 15 years of union membership the SMR for this type of cancer increased to 463. The study clearly suggests an occupational cancer risk of dry cleaning. However, it does not indicate a risk of PER exposure, as the risk was partly associated with employment before 1963, when other solvents than PER (tetrachloromethane, trichloroethylene and Stoddard solvent) were predominantly used. Oesophagus cancer is strongly related with smoking and drinking. These confounding factors were not taken into account. However, if they had contributed to oesophagus-cancer incidence, an increase of lung-cancer mortality or liver-cirrhosis mortality would have been detected, which was not. Again, no enhanced liver-cancer risk was found (22).

Proportional mortality ratios (PMRs) were calculated for 279 trade-union decedents in the period 1957-1977, which had exclusively worked in dry-cleaning shops (21). Elevated PMRs were found for lung cancer (PMR: 170; $p<0.05$), cervix uteri (PMR: 208; $p<0.05$), skin cancer (PMR: 429; $p<0.05$), leukaemia (PMR: 227) and liver cancer (PMR: 235). Breast cancer showed a deficit (PMR: 69), which, together with the increased risk for cervix cancer, points to an influence of socio-economic status. Smoking histories were not taken into account (21). It is impossible to conclude a carcinogenic effect of PER from these findings, because the possible confounding effect of smoking and the exposure to other solvents in use during the occupational life of the decedents (tetrachloro-methane, trichloroethylene and Stoddard solvent). Another limitation of the study is related to the use of the PMR as a measure for risk. Eighty-seven deaths were due to cancer, while only 67.9 were expected. This difference is not necessarily the result of a "stronger carcinogenicity" of life style or occupation; it is probably caused by less mortality from other, non-cancer causes. If the latter suggestion is indeed valid, more cancer deaths have to occur within the group decedents. Consequently, some cancers will yield higher than expected PMRs, without necessarily being related to occupational exposure.

Another PMR study has been conducted by Katz and Jowett (139). The group of decedents consisted of 671 female white laundry and dry-cleaning workers, which had died in the period 1963-1977. PMRs were calculated for 25 causes of death. Two control populations were used: all occupations and lower-wage occupations, both taken from the same region. The distinction was made, to enable a correction for possible effects of the low socio-economic status of laundry and dry-cleaning workers.

Notwithstanding this correction, elevated PMRs were found for unspecified cancers of the genitalia (PMR: 467; 4 observed against 0.9 expected; $p \leq 0.01$) and kidney cancer (PMR: 253; 7 observed against 2.8 expected; $p < 0.05$). Furthermore non-significant increases of bladder cancer and skin cancer were found (bladder cancer: PMR: 190; 5 observed against 2.6 expected; skin cancer PMR: 263; 7 observed against 2.8 expected). A significant PMR for cervix uteri cancer (PMR: 195; 10 observed against 5.1 expected; $p < 0.05$), decreased to statistical insignificance (PMR 140; 10 observed against 7.1 expected).

Smoking was not corrected for, and this factor might thus explain the elevated kidney and bladder cancer risk. However, in that case an increased lung-cancer risk would have been observed, which was not the case (PMR: 95; 10 observed against 10.6 expected).

Laundry workers are not or much less exposed to organic solvents, let alone PER. The committees points out that the inclusion of these workers in the group of decedents will certainly have lead to a reduced sensitivity of the study as regards the detection of cancer caused by PER or other dry-cleaning solvents.

This study did reveal a PMR of 100 for all cancer deaths (141 observed against 141.5 expected).

The value of this study is limited by the fact that many of the decedents will not have been solely exposed to PER; exposure to other dry-cleaning solvents in earlier phases of their occupational life may very well have contributed to the elevation of the PMRs for some cancers (139).

Standardised mortality odds ratios (SMORs) were calculated for a group of 440 laundry and dry-cleaning workers deceased in the period 1975-1981, with stratification on sex, race and age of death (58). No excess death was found for all cancers, while the SMORs were statistically significantly increased for respiratory-tract cancer (39 observed against expected 23.8; SMOR: 1.8), cancer of the lungs (37 observed against 22.6 expected; SMOR: 1.7) and kidney cancer (7 observed against 1.9 expected; SMOR: 3.8). Breast, bladder and liver cancers were markedly less frequent than expected. Again, smoking habits were not accounted for. The inclusion of deceased laundry workers will have resulted in a reduced sensitivity.

In this case, the subjects had died later than in the other studies, which suggests more exclusive PER exposure for the dry cleaners. However, the authors point out that petroleum solvents account for over 50% of the solvents used, which affects the validity of the study as to the human carcinogenicity of PER (58).

Nakamura again, used death certificates as study material (201). It concerned 1 711 members of the “All-Japan Laundry and Dry-Cleaning Association”, who

died in the period 1971-1980. For 294 of the members information of smoking and drinking habits as well as exposure was obtained from the families by questionnaire. No excess of overall cancer mortality was observed. Standardised proportional mortality ratios (SPMRs) were found to be significantly increased for the small intestine (SPMR: 170; 18 observed against 10.6 expected; $p<0.05$). No clear (and significant) elevated SPMRs were observed for the urinary tract and kidneys, with the exception of bladder cancer in males who died at 75 years or older (SPMR: 455; 5 observed against 1.1 expected; $p<0.01$). The authors point out that prior to 1985 only 30% of the Japanese dry-cleaning shops used PER as solvent, which makes it impossible to draw conclusions on the human carcinogenicity of PER on the basis of this study. Furthermore, the sensitivity of the study was reduced by the inclusion of death certificates of laundry workers.

In a case-referent study (253) bladder-cancer cases were drawn from a national bladder-cancer study conducted by the US National Cancer Institute in 1978. The total investigated population consisted of 103 subjects who had been employed for at least 6 months in dry-cleaning or laundry shops, 5 776 subjects who had worked in other occupations which might lead to exposure to similar chemicals and 1 869 subjects who had not been exposed to such chemicals. A relative risk of 1.31 (95% confidence limits: 0.85-2.03) was found for non-smokers employed in dry-cleaning or laundry shops, which does not indicate an association of bladder cancer with this employment. Smoking habits were taken into account in this study. The authors calculated the power of their study to reject the null hypothesis in case of a real relative risk of 2. The outcome was 40% for non-smokers and former smokers and only 20% for smokers (253). Again, the sensitivity of the study for solvent-related cancers will have been limited by the inclusion of laundry workers.

The case-referent study by McLaughlin *et al*. (182) was concerned with renal cancers occurring in Swedish laundry and dry-cleaning establishments. 7 405 cases of renal cancer between 1960 and 1979 were identified in the Cancer-Environment Registry. Standard incidence ratios (SIRs) were 0.99 (18 cases) and 0.86 (25 cases) for male and female workers, which does not indicate an association between employment in laundry or dry-cleaning shops and the occurrence of renal cancer. As laundry workers were included, a dilution of dry-cleaning-related cancers may have occurred.

A retrospective cohort study was carried out by Spirtas *et al*. (254). The cohort consisted out of 14 457 aircraft maintenance workers, exposed to over 20 different solvents. For PER an elevated number of deaths in female workers was observed due to multiple myeloma or non-Hodgkin's lymphoma, who had been exposed to the solvent for more than one year during their work. The ATSDR concludes that "confidence in these data is low primarily because multiple and overlapping exposure to more than one chemical was considerable. In addition, the levels of tetrachloroethylene to which the workers were exposed were not provided, and lifestyle factors such as smoking and consumption of alcohol were not assessed" (3).

A Finnish cohort exposed to PER, trichloroethylene and trichloroethane and consisting of 2 050 male and 1 924 female workers was followed from 1967 to 1992 for the occurrence of cancer (7). Individual exposure was biologically monitored. In case of PER, this was done by measuring the concentrations of this compound in blood samples. PER monitoring data were available for the years 1974-1983.

The overall cancer incidence did not differ from that of the Finnish general population. Excess incidences were observed for cancers of the cervix uteri and lymphohaematopoietic tissues for the complete cohort. No excess of specific cancer incidences could be linked with PER exposure (7).

The epidemiological evidence for the induction of renal-cell cancer by PER exposure was reviewed by McLaughlin and Blot (181). The following is a citation of the summary of this review:

"There is little evidence of an increased risk of renal-cell cancer and exposure to TCE (trichloroethylene) or PCE (PER). The few studies with elevations in risk suffer from important methodological shortcomings. Although it is virtually impossible using epidemiology data to rule out conclusively a small increase in risk of renal-cell cancer, the totality of epidemiological evidence clearly does not support a causal association with TCE or PCE" (181).

A PMR study was carried out by Walker *et al*. (287). The causes of 8 163 deaths of people formerly employed as laundry and dry-cleaning workers in 28 states of the USA were analysed. Comparison with the PMR of the general population in the same states yielded the following results. Black men had higher PMRs for total cancer cases (PMR=131; 95% CI: 105-159) and for oesophagus cancer (PMR=215; 95% CI: 111-376). In the subpopulation of white men, cancer of the larynx yielded a higher PMR (318; 95% CI: 117-693). The value of this study is reduced by the fact that no separate dry-cleaning and laundry deaths were analysed. Moreover, increased PMRs are not necessarily the result of real increases of incidence. They may very well be associated with decreases in other causes of death. Nevertheless, the study again suggests an enhanced risk of oesophagus cancer in black males (287).

A case control study suggested an increased risk of oesophageal cancer upon working in dry cleaning (280). However, this result was only based on 2 cases of a total of 404 cases. The authors conclude: "These findings could easily be explained by chance; nevertheless, they are consistent with previous reports ...". Moreover, it is stated in the discussion section of the publication: "The main limitation of this study is the low prevalence in the population of a history of working in dry cleaning, with the consequent low statistical power to detect a true association" (280). The committees like to emphasise this statement.

The possible association between renal-cell cancer and exposure to trichloroethylene and PER was investigated in a case control study with 59 cases and 84 controls (278). Whereas a high odds ratio was found for trichloroethylene, no cases with PER exposure were identified. The control group included two individuals with a history of PER exposure.

*Neurotoxicity*

Volunteers who worked in various dry-cleaning or laundry jobs were subjected to the following tests: feeling tone checklist, Wechsler digit span, Wechsler digit symbol, Neisser letter search, critical flicker fusion, Santa Ana dexterity test, choice reaction time, simple reaction time (275).

The exposed group consisted of 18 dry-cleaners (9 males and 9 females), while 9 laundry workers participated as controls. Exposure was extensively investigated by analysing breath samples and environmental monitoring. TWAs were calculated for the subjects for the 5 days of testing. Mean TWA for the complete dry-cleaning group amounted to 18 ppm of PER (124 mg/m$^3$), while the males (who are often machine operators) had a mean TWA of 32 ppm (220 mg/m$^3$). Significant differences were found for two of the 11 measured endpoints (electric diagnostic rating score: $p<0.1$; total neurological score: $p<0.05$). Multiple regression analysis suggests these effects to be rather related with exposure to Stoddard solvent than to PER exposure (275). The power of this study is limited by the small number of subjects involved.

In a cross-sectional study (82) 60 female workers of dry-cleaning shops were compared with 30 female workers from a cleaning plant, which did not use solvents, as regards the dopaminergic control of prolactin secretion and the performance in neurobehavioral tests (finger tapping, simple reaction times, digit symbol and shape comparison tests). PER concentrations varied from 1 to 67 ppm (6.89-461.6 mg/m$^3$) (median: 15 ppm or 103.4 mg/m$^3$). During the menstrual cycle, PER-exposed workers showed increased basal levels of serum prolactin as compared to their matched controls. These endocrine changes suggest involvement of the CNS, since prolactin release is under control of pituitary dopamine. The PER-exposed workers also showed a poorer performance in the tests. Within the exposed group, no significant correlations with relevant exposure measures were found (82).

Nervous-system effects were investigated in a group of 65 dry-cleaners (70). On the basis of analysis of PER in breath and air, subjects were classified in a low-, moderate- or high-exposure zone, with respective mean air levels of 11.2, 23.3 and 40.8 ppm (77.2, 160.5, 281.1 mg/m$^3$) PER. In addition, an index of lifetime exposure to PER was determined for each participant. All subjects underwent extensive testing, using a broad set of neurophysiological and neurobehavioral tests, including the following endpoints: visual reproduction, pattern memory, pattern recognition, simple attention, psychomotor performance, executive function/complex-attention, test-taking effort, mood and verbal skills. Decreased performances were observed for visual reproduction, pattern memory, (number correct and latency) and pattern recognition in employees in the high exposure group (70).

The results suggest that 3 years of exposure to PER levels below 50 ppm (345 mg/m$^3$) may cause permanent nervous system effects. However, the committees agree that this single study cannot at the moment be used for the setting of an occupational exposure limit. However, it points to the need of further investigations.

*Miscellaneous*

Workers (n=106) exposed to PER in a railway repair shop were compared with 101 controls (78). The exposed had been involved in cleaning of machine and engine parts with PER for at least two years. Estimation of exposure was based on environmental monitoring. Environmental concentrations were often exceeding 400 ppm (2 756 mg/m$^3$), 74.3% of the analyses pointed to concentrations lying between 0.2 and 50 ppm (1.4-345 mg/m$^3$). The comparison of the two groups concerned: subjective symptoms, biochemical, and clinical symptoms of nephrotoxicity and hepatotoxicity, histology of liver biopsies, neurological symptoms (abnormal reflexes, sensory disturbances, motor disturbances).

No indications for PER-related liver or kidney damage or neurological effects could be obtained, this notwithstanding the fact that there was a clear elevated frequency of complaints (subjective symptoms) in the exposed group. In particular dizziness and dermal irritation were much more often complained about by the exposed (78).

The occurrence of subjective symptoms was compared for dry-cleaning workers and workers not exposed to PER (33). The exposed group consisted of 56 dry-cleaning workers with a mean TWA of 20 ppm (138 mg/m$^3$) (measured by passive personal-air sampling). The non-exposed were from the same "factories", but they were employed in workshops where no dry-cleaning took place. In addition, several biochemical liver- and kidney-function as well as haematological parameters were determined.

Statistically significant differences between the exposed and the non-exposed group were observed (33). Altogether, these differences suggest neurotoxicity and local irritation. However, the subjective character of the symptoms warrants caution in the interpretation of this result. No indications for haematological effects or impaired liver or kidney function were found.

### *6.1.5 Summary*

*Case studies*

- Application of PER as anthelminthic drug has revealed a relative low acute oral lethality of the compound for humans. Several grams of PER can be tolerated without serious acute toxicity.
- Only two lethal occupational PER intoxications have been described. Doses were not determined; exposure occurred largely via the respiratory tract.
- The following effects of acute respiratory occupational exposure (sometimes combined with dermal exposure) have been described: depression of the central nervous system (lightheadedness, dizziness, loss of consciousness, coma, seizures), pulmonary oedema, hypotension, temporary liver and kidney damage. The reports do not allow the establishment of effective dose levels.
- Two cases of skin irritation are described which can be attributed to PER exposure.
- One case of asthma has been described, which appears to be related with PER exposure.

- Case studies indicate that long-term respiratory exposure leads to a wide variety of neurological symptoms. In most cases, symptoms disappeared some time after cessation of exposure. However, some studies point to less reversible, even progressive symptoms. One study points to cerebral atrophy. When concentrations were measured, they were lying between 200 and 400 ppm (1 378 and 2 756 mg/m$^3$). Furthermore, the relationship with PER exposure is too uncertain to use these case reports as basis for evaluation.
- Long-term exposure case studies point to hepatotoxicity, expressed as liver enlargement, jaundice, biochemical effects and histopathological effects. These effects were also observed between 200 and 400 ppm (1 378-2 756 mg/m$^3$).
- One case indicates that PER is transferred to breast milk and may cause jaundice in breast-fed babies at high exposure levels.

*Volunteer studies*

- Respiratory exposure to concentrations of PER lower than 100 ppm (690 mg/m$^3$) leads almost immediately to transient eye, nose and throat irritation in human volunteers.
- Repeated respiratory exposure of human volunteers for several hours per day to concentrations of PER lower than 100 ppm (690 mg/m$^3$) gave rise to transient neurological symptoms, such as headache, sleepiness, difficulty of speaking and reduced co-ordination scores. The overall NOAEL (i.e. for all effects) of these studies was 20 ppm.
- One volunteer study suggests a NOAEL of 10 ppm (68.9 mg/m$^3$) based on increased latencies of visual evoked potentials.
- The group sizes were generally very small in the volunteer studies, which reduces the reliability of the NOAELs.

*Epidemiological studies*

- Two epidemiological studies show that persons exposed during their work (first study: 74.3% of environmental concentrations between 0.2 and 50 ppm (1.4-345 mg/m$^3$), many exceeding 400 ppm (2 756 mg/m$^3$); second study: mean TWA of 20 ppm (138 mg/m$^3$)) complain more often about subjective symptoms related with neurotoxicity than controls, although this appears not to be accompanied by effects on objective neurological parameters determined in one of the studies.
- Another study associates working in dry-cleaning shops with decreased performance in various neurological/psychomotor tests, although the effects correlated with previous exposure to another dry-cleaning solvent, i.e. Stoddard solvent and not with PER exposure.
- A study of female dry cleaners exposed to 1-67 ppm (6.89-461.6 mg/m$^3$) of PER indicated effects on neurobehavioral endpoints and prolactin secretion, suggesting involvement of the CNS.

- A study concerning chronic exposure of dry-cleaners for at least 3 years to levels of PER below 50 ppm (345 mg/m$^3$) suggested permanent alterations in neurobehavioral and neuropsychological tests.
- Significant elevated serum γ-GT activity was found in a group of dry-cleaners and laundry workers, as compared to university students and staff. In addition, a shift in the isoenzyme pattern of the enzyme was observed. However, the study does not allow these effects to be unambiguously attributed to PER exposure.
- Three epidemiological studies show effects of dry cleaning on renal function. The nature of the effects measured, points to glomerular as well as tubular impairment.
- Several epidemiological studies were focused on the cancer mortality of dry-cleaners and other workers exposed to PER. On the whole, these studies do not lead to definitive conclusions about the carcinogenicity of PER exposure. They suggest that dry cleaning or other occupations accompanied by solvent exposure, give rise to an elevated cancer risk, although no typical cancer pattern emerges.
- In most studies a substantial exposure of the exposed group to other solvents in addition to PER could not be excluded. In particular Stoddard solvent is of importance, because it was the main solvent used before PER was introduced and some time thereafter. In addition, trichloroethylene and tetrachloromethane can be mentioned as solvents, which have been used for dry cleaning and could have influenced the outcome of the studies. Furthermore, the value of most studies is affected by the fact that life-style-related factors, in particular smoking, were not or not adequately accounted for. Moreover, laundry workers are often included in the exposed groups, which leads to a dilution of possible effects, as these workers are not exposed to PER.
- One study defined a subcohort of PER-only subjects, which showed a statistically increased mortality due to oesophagus cancer. Incidences of lung cancer and liver cirrhosis suggest that this effect cannot be attributed to smoking and alcohol consumption. Oesophagus cancer was also found to cause excessive mortality in one other study, which did not comprise a PER-only cohort.
- In view of the outcome of animal experimental studies (Section 6.2), it is reassuring that no consistent increases of liver or kidney cancer were found.
- The committees conclude that the results warrant further investigation of the incidence of certain cancers, in particular oesophagus and bladder cancer, but that they do not allow the conclusion that working with PER leads to an enhanced cancer risk.
- Two epidemiological studies suggest an increased risk of spontaneous abortions among female dry cleaners. However, a possible contribution of PER cannot be distinguished from contributions of other solvents used in dry cleaning.
- Effects on semen quality have been observed for dry cleaners. As exposure to other solvents will be less important in this study, it suggests an important human reproduction toxic effect of PER. Furthermore, this study suggests a

fertility problem, as the female partners had more difficulty in becoming pregnant. Although the study does not allow a definite conclusion, it certainly warrants further investigation.

## 6.2 Animal experiments and in vitro systems

### *6.2.1 Irritation and sensitisation*

Using the official Draize method, PER turned out to cause severe skin irritation in rabbits (60), while no macroscopic changes of the skin were found in guinea pigs (152), although microscopic examination did indicate skin damage (degenerative changes in the epidermis, junctional separation and cellular infiltration in the dermis) in the second species.

An eye-irritation study with rabbits showed PER to be a mild irritant (60). The compound caused discharge with epithelial damage and epithelial keratosis. No details about the dose are presented in this study. In another study (101) PER was directly sprayed into rabbit eyes from a distance of one foot. This treatment resulted in the following effects: blepharospasm, a granular and optically irregular appearance of the corneal epithelium and loss of patches of epithelium. Complete recovery occurred within 2 days.

No dermal sensitising properties were observed in the guinea pig when the split adjuvant technique was applied (230).

Although PER has amply been investigated for toxic properties upon respiratory exposure, no indications for irritation or sensitisation of the respiratory tract have been obtained for experimental animals.

### *6.2.2 Toxicity due to acute exposure*

*Inhalation*

The results of acute inhalation studies will be treated separately for each of the effects caused by PER.

*Inhalation: Lethality*

$LC_{50}$ values have been determined for rats and mice. A number of them are listed in Table 14.

In the study of Holmberg *et al*. (116), the time required to kill half of the

**Table 14.** $LC_{50}$ values.

| Species | Duration (h) | $LC_{50}$ | | Reference |
|---|---|---|---|---|
| | | ppm (95% CI[a]) | $mg/m^3$ (95% CI[a]) | |
| Rat | 6 | 4 100 (3 899-4 387) | 28 250 (26 900-30 200) | (25) |
| Rat | 8 | 5 027 (3 334-7 571) | 34 600 (22 300-52 200) | (226) |
| Mouse | 2 | 5 800 | 40 000 | (87) |
| Mouse | 4 | 5 200 | 36 000 | (87) |
| Mouse | 6 | 2 978 (2 758-3 215) | 20 500 (19 000-22 200) | (59) |

[a] CI = confidence interval.

animals exposed to a defined dose ($LT_{50}$) was found to be 12.2 hours (95% confidence limits 11.8-12.5 hours) at 3 700 ppm (25 500 mg/m$^3$) for mice.

In NTP (208) a highest non-lethal dose of 2 445 ppm (16 850 mg/m$^3$) and lowest lethal doses of 2 613 and 3 786 ppm (26 100 mg/m$^3$) is mentioned for mice and rats, respectively, when these animals have been exposed for 4 hours.

Rowe *et al*. (236) report non-lethality for 10- or 14-hour exposures of rats to 2 000 ppm (13 800 mg/m$^3$) and 4-hour exposure of rats to 3 000 ppm (20 700 mg/m$^3$), while lethality was found when exposure to the latter dose lasted 5 hours or longer. 12 000 ppm (82 700 mg/m$^3$) did not give rise to death when exposure lasted 0.2 hour; complete survival was also found at 1 600 ppm (11 020 mg/m$^3$) for 5 hours.

Kennedy and Graepel (142) mention in their study an approximate lethal concentration, which is the concentration at which mortality was first observed, in rats of 6 000 ppm (41 340 mg/m$^3$) for a 4-hour exposure.

*Inhalation: Neurotoxicity*

Most notably are the short-term neurotoxic effects of respiratory exposure. PER has been used in the past for human anaesthesia (83), and it is, therefore, not surprising that effects related to this application are abundantly reported in the literature on acute respiratory toxicity. Various short-term neurobehavioral effects have been observed, among them hyperactivity (excitability), hypoactivity, hypotonia, loss of reflexes, lateral position, drowsiness, trembling, ataxia and "drunken" stupor (3, 119). At doses approaching the lethal dose, respiration is affected. Failure of respiration is the direct cause of death. Concise summaries of a number of original publications are presented in Table 15.

*Inhalation: Cardiac arrhythmia*

Epinephrine-induced cardiac arrhythmias have been observed in rabbits during respiratory exposure for 1 hour to 5 200 ppm (35 800 mg/m$^3$) PER (34). The author of the original publication considers the influence of PER on this endpoint to be weak (compared with trichloroethylene and methylchloroform, which were investigated for this effect by the same author, although the results were published elsewhere). Little more than half of the exposed rabbits showed an increased sensitivity. Furthermore, the arrhythmias did only occur early in the exposure period, and after 15-30 minutes of exposure the rabbits appeared to adapt and no longer responded to the same degree. In contrast to the effects observed with trichloroethylene and methylchloroform, the sensitivity of the animals did not increase after administration of Lilly 18947, an inhibitor of the oxidative metabolism to TCA and TCE. This is not surprising, in view of the marginal oxidative metabolism of PER in mammals.

No epinephrine-induced arrhythmias have been observed in beagle dogs exposed for 10 minutes to 5 000 or 10 000 ppm (34 500 or 68 900 mg/m$^3$) (the latter treatment was not well tolerated) (232).

**Table 15**. Overview of studies on acute neurotoxicity in experimental animals after respiratory exposure.

| Species | Exposure | Concise summary | Reference |
|---|---|---|---|
| Mouse | Several exposure times, 6 800 or 12 200 ppm (46 950 or 84 100 mg/m$^3$) | The anaesthetic effect of respiratory exposure was investigated by studying the mobility of mice placed in a rotating cylinder. Complete immobility was obtained after exposure to 6 800 or 12 200 ppm (46 950 or 84 100 mg/m$^3$) for 4 min. | (87) |
| Rat | 150 ppm (1 034 mg/m$^3$), 18 h | Exposure of rats to 5 times the Danish TLV (5 x 30 = 150 ppm) had no influence on the calcium uptake in brain synaptosomes, while such an effect was found for white spirit, toluene, turpentine and xylene at comparable doses. | (71) |
| Rat | 4 h, 2 300 ppm (15 800 mg/m$^3$) | Ataxia in a pole-climbing experiment. | (95) |
| Mouse | Several concentrations during 1 h, lowest: 90 ppm (620 mg/m$^3$) | A dose-related increase of motor activity upon the start of exposure. The lowest dose (90 ppm or 620 mg/m$^3$) still exerted an effect. In contrast with the other solvents tested, no clear exposure-related period of hypoactivity was observed after termination of exposure. | (143) |
| Mouse | 4 h, several concentrations | A clear dose-related decrease of immobility was found in a swimming test with mice. The $ID_{50}$ (concentration which brings about a 50% decrease of immobility when the animals have been exposed for 4 h) was 713 ppm (95% confidence limits: 665-804) (4 900 mg/m$^3$ (4 600-5 540)). | (46) |
| Rat | 4 h, several concentrations | The influence of PER inhalation (4 h) on EEG and electromyogram activity in rats following a light stimulus was investigated. The exposure resulted in an intensified motor reaction. 67 ppm (462 mg/m$^3$) was reported as NOAEL. | (53) |
| Mouse | Several exposure times, 3 700 ppm (25 500 mg/m$^3$) | $ET_{50}$ for anaesthesia in mice (i.e. the time required for 50% of a group of mice to become anaesthetised by a defined concentration) was 24 min at 3 700 ppm (95% confidence limits: 20.2-28.6 min). The ratio between the $ET_{50}$s for lethality and anaesthesia was over 30, showing that anaesthesia occurs at much lower doses than death. | (116) |
| Rat | Repeated acute exposures to 2 750 or 10 000 ppm (18 650 or 68 900 mg/m$^3$) | Tolerance to PER-induced anaesthesia can be developed by rats. A first exposure to 2 750 ppm caused anaesthesia, while the same rats could not be anaesthetised by doses above 10 000 ppm after six exposures to 2 750 ppm (18 950 mg/m$^3$). | (35) |
| Mouse | Exposure till effect emerged; 3 000 or 6 000 ppm (20 670 or 41 340 mg/m$^3$) | Anaesthesia of mice within a few minutes when exposed to 6000 ppm (41 340 mg/m$^3$), while several hours of exposure were necessary to achieve anaesthesia by a concentration of 3 000 ppm (20 670 mg/m$^3$). | (35) |
| Mouse | 2 328 ppm (16 040 mg/m$^3$), 4 h | Mice were anaesthetised by a 4-hour exposure to 2 328 ppm (16 040 mg/m$^3$). | (208) |

*Inhalation: Hepatotoxicity*

Several acute respiratory studies have revealed clear-cut hepatotoxicity, expressed in histopathological and biochemical changes. A number of studies are concisely summarised in Table 16.

**Table 16.** Overview of studies on the acute respiratory hepatoxicity of PER in experimental animals.

| Species | Exposure conditions | Concise summary | Reference |
|---|---|---|---|
| Rat | 24 h after termination of acute exposure, which causes high mortality. | Slight cloudy swelling and diffusely distributed fat globules in the livers. | (236) |
| Mouse | Acute lethal concentrations. | Cloudy swelling, anisokaryosis, anisocytosis and infiltration of Kupffer cells in livers. | |
| Rat | 24 h prior to, 1 h after, 24 h after and 48 h after a 1-h exposure to 500, 1 000 and 2 000 ppm (3 450, 6 890 and 13 780 mg/m$^3$). | Effects on rat serum enzymes indicating hepatotoxicity, viz. SGOT, SGPT, serum glucose-6-phosphatase (SG-6-P) and serum ornithine carbamyl transferase (SOCT). The highest concentration lead to marked increases of the activities of all four enzymes after 24 and 48 h. Less, but still substantial increases were found after exposure to 1 000 ppm (6 890 mg/m$^3$). Marginal increases were found when enzyme activity was measured within 1 h after exposure, or when the animals were exposed to 500 ppm (3 450 mg/m$^3$). | (56) |
| Mouse | 24 and 72 h after a 4-h exposure to 200, 400, 800 and 1 600 ppm (1 378, 2 756, 5 512 and 11 024 mg/m$^3$). | A dose-dependent fatty infiltration in the liver at all doses as well as a dose-dependent increase of extractable fat at 400 ppm (2 756 mg/m$^3$) and higher doses. No effects on SOCT. | (153) |
| Mouse | Till 20 h after a 3-h exposure to 800 ppm (5 512 mg/m$^3$). | Clear-cut decrease (to 45% of control) of liver adenosine triphosphate (ATP) content in mice, which lasted till observation was terminated (to 55% of control after 20 h). Furthermore, marked (up to 160%) and rather persistently increased liver lipid levels and liver triglyceride levels were found. | (211) |
| Mouse and rat | 4 h, 1 080 ppm (7 441 mg/m$^3$). | Centrilobular fatty degeneration and increases of lipids and triglycerides in livers of mice. No such changes occurred in rats. | (128) |

**Table 17**. Oral $LD_{50}$ values of PER.

| Species | $LD_{50}$ (mg/kg body weight) | Reference |
|---|---|---|
| Rat | 2 438 (1 415-4 215)[a] | (226) |
| Rat | 4 460 (3 810-5 210)[a] | (293) |
| Rat, male | 3 835 | (112) |
| Rat, female | 3 004 | |
| Mouse | 7 814 (pure PER) | (67) |
| | 9 607 (in herring oil) | |
| Mouse | 5 000 | (290) |
| Mouse | 4 700 | (145) |

[a] 95% confidence limits.

*Inhalation: Nephrotoxicity*

Histopathological effects of acute respiratory exposure on the kidneys are reported. Slight and irregular scattered necrotic and degenerative lesions were observed upon the exposure to 2 978 ppm (20 500 mg/$m^3$) for 6 hours (59, 100).

*Inhalation: Immunotoxicity*

Aranyi *et al*. (8) investigated the influence of PER inhalation (3 hours, 25 and 50 ppm) on mice mortality from *Streptococcal pneumonia* and mouse pulmonary bactericidal activity to inhaled *Klebsiella pneumonia*. Both endpoints were significantly affected; mortality increased and the bactericidal effect decreased.

*Oral*

The oral acute toxicity of PER has been amply investigated in rats, mice, dogs, cats, foxes, cows, horses, sheep and pigs. Most of these studies are rather old, some of them dating back to the period before and shortly after the second world war, when interest in the toxicity of PER was mainly due to its use as anthelmintic drug, see references in (35, 37, 74, 242).

Oral lethal doses for 50% of the exposed animals at single administration ($LD_{50}$) for rats and mice are listed in Table 17.

Although the other studies summarised by (3, 119) do not allow for the estimation of $LD_{50}$ values, they confirm that acute (within several days) death of the exposed animals will only occur when doses of several grams per kilogram or higher are administered.

The effects observed in oral acute toxicity studies are listed in Table 18. The contents of this table should be interpreted with caution, because most studies were carried out long ago and were of a rather limited nature (small numbers of animals). Furthermore, a high incidence of pre-existing infections restricts the value of some studies (119). However, in an overall sense, the table provides a qualitative impression about overt macroscopic and microscopic effects that can be expected in experimental animals shortly after the application of high oral doses. The table identifies liver, kidneys, and spleen as important target organs. Furthermore, indications for neurotoxicity are present.

**Table 18**. Effects observed in oral acute toxicity studies (3, 35, 37, 74, 119, 242).

| Dose[a] (mg/kg bw) | Species | Effects |
|---|---|---|
| 500 | Mouse | Irreversible binding in hepatocytes. |
| 500 | Rat | Increased relative weight of liver; increased enzyme-altered foci in liver (γ-glutamyltranspeptidase activity), irreversible binding in hepatocytes. |
| 286-557 | Dog | Depression of heart and ventilation rate; shrivelling and inflammation of small intestines; fatty infiltration of the liver; severe ataxia; depression. |
| 376-753 | Cat | Fatty infiltration of the liver; fatty changes (infiltration?) in kidneys; congestion in liver spleen and kidneys; cloudy swelling of hepatocytes; hepatic necrosis; restlessness, drowsiness and unsteadiness in the hind limbs. |
| 376-4 426 | Fox | Cloudy swelling of kidney tubule cells; fatty degeneration of liver. |
| 136-241 or 15 mg/animal | Cow | Cloudy swelling, reticulation, centrilobular necrosis, disassociation of hepatic cords and oedema in liver; hyaline casts in kidneys and tubules; anorexia; inability to stand. |
| 99-226 | Horse | Centrilobular oedema, necrosis, and cloudy swelling in liver; hemosiderosis in spleen; congestion, cloudy swelling and oedema in kidney. |
| 331-1 254 | Sheep | Cloudy swelling, reticulation and fatty changes in periportal regions of lobules (liver); cloudy swelling, reticulation and atrophy and vacuolisation of kidney tubules. |

[a] Rough indication of doses.

The study of Moslen *et al*. (198) deserves special attention, because it shows that the acute hepatotoxicity of PER increases markedly after pretreatment of rats with phenobarbitone or Aroclor-1254, a mixture of polychlorinated biphenyls (PCBs) which, like phenobarbitone, stimulates biotransformation by inducing mixed-function oxidases. Rats pretreated with Aroclor-1254 or phenobarbitone were treated once by gavage with 0.75 ml/kg in mineral oil. The influence on SGOT and SGPT activities, excretion of trichlorocompounds (as measure of oxidative metabolism), mixed-function oxidases, and liver histopathology was examined. Urinary excretion was increased 5-7 fold by the pretreatment with either phenobarbitone or Aroclor-1254. Aroclor-1254 pretreatment did also lead to a clear-cut increase of SGOT and histopathological effects, such as vacuolar degeneration and necrosis. The study thus points to metabolites instead of PER as the ultimate hepatotoxic agent. The absence of an increase of hepatotoxicity due to phenobarbitone treatment is difficult to explain.

*Intraperitoneal*

The $LD_{50}$ of intraperitoneal exposure has been determined for mice and dogs (74, 90, 119, 145, 146). For the first species values of 5 671 and 4 600 mg/kg have been reported, while a study with the second species yielded an $LD_{50}$ of 3 163 mg/kg. The dose at which a described effect is found in 50% of the exposed animals ($ED_{50}$) determined in the same studies for ALT activity were 3 980 and 5 300 mg/kg for mice and 1 114 mg/kg for dogs.

A number of acute intraperitoneal studies were aimed at specific effects (41, 52, 109, 119, 199, 224). Measurement of enzyme activities in serum pointed to liver damage in the rat and the guinea pig at doses between 452 and 3 011 mg/kg for the first, and 200 and 400 mg/kg for the second species.

Rats treated intraperitoneally with PER (1.3 ml/kg) show an altered bile duct pancreatic secretion (increased volume (5.8 times) and decreased protein concentration (8.6 times)). Indications were obtained that this effect is not caused by an interaction with the cholinergic system.

Renal damage was indicated in studies with mice and dogs by biochemical changes (proteinuria; reduced phenolsulphthalein excretion) and histopathological changes (swelling of tubules).

Effects on locomotor activity have been investigated and demonstrated for rats at doses of 500 and 1 000 mg/kg.

*Subcutaneous*

Plaa *et al*. found a subcutaneous $LD_{50}$ for mice of 5 g/kg (223). Histological examination of liver and kidneys revealed changes of the centrilobular area (only in the liver), including alterations of cell staining and cytoplasmic vacuolisations. A clear prolongation of phenobarbitone-induced sleeping time was observed, the $ED_{50}$ for this effect being 4 477 mg/kg (223).

A minimum lethal dose of 2.2 g/kg was found for the rabbit (119).

*Intravenous*

A minimum lethal intravenous dose of 85 mg/kg has been found for dogs (119).

The effects of intravenously administered PER on the vestibular-ocular reflex of rats was investigated by Tham *et al*. (267). An excitation of this reflex was found at blood concentrations equal to or higher than 0.9 mM (149 mg/l).

Rabbits, dogs and cats showed a clear increase of noradrenalin-induced cardiac arrhythmias upon intravenous application of PER (147). A mean lethal dose for cats of 81.4 mg/kg was found.

*Intratracheal*

The acute toxicity of PER after intratracheal instillation for rats has been investigated by McCarty *et al*. (180). An approximate lethal dose of 450 mg/kg was established. The same study yielded an oral $LD_{50}$ of 2 600 mg/kg.

*6.2.3 Toxicity due to short-term exposure*

*Inhalation: Multi-endpoint studies*

Rowe *et al*. investigated the effects of various subacute and semichronic respiratory exposure regimens on a number of endpoints in rats, rabbits, guinea pigs and rhesus monkeys (236). Table 19 presents their results in a summarised form. The following endpoints were investigated: mortality, behaviour, body weight, organ weight, histopathological examination of a wide range of organs, lipids in liver, free and esterified cholesterol in liver; estimated in blood were: urea-nitrogen, total non-protein nitrogen, serum phosphatase, prothrombin clotting time (236). It is not clear whether all these endpoints are included in the experiments presented in Table 19. They were certainly not investigated in all animals involved. The paper does not provide a complete and clear presentation of the experiments and the results, which means that the results should be interpreted with caution.

Notwithstanding this shortcoming, due to its extensive nature, the study provides a fairly global impression of the effects that can be expected upon the subacute or semichronic respiratory exposure. It identifies the CNS and the liver as targets for PER. Furthermore, it shows that in experimental animals concentrations as low as 100 ppm (690 mg/m$^3$) can induce adverse effects upon inhalation.

*Inhalation: Hepatotoxicity*

A number of studies showing hepatotoxicity are summarised in Table 20.

*Inhalation: Nephrotoxicity*

EPA (74) mentions a study of Brancaccio *et al*. (28; see also 119) in which the renal function was investigated in rabbits, which were exposed 45 days to 2 280 ppm (15 710 mg/m$^3$) (4 hours/day; 6 days/week). Concentrations of creatinine and para-aminohippuric acid in the urine lead the authors to conclude that tubular function was affected more than glomerular capacity. EPA (74) and HSE (119) criticise the study (28) for the lack of information on raw data and statistical methods.

In the study by Odum *et al*. (210, see under "Hepatotoxicity") the effect of PER in kidneys was investigated with light and electron microscopy. No histopathological changes were found. Slight indications for increased peroxisomal cyanide-insensitive palmitoyl CoA oxidation activity were obtained for female mice; catalase activity in the kidneys was unaffected in both species. The authors conclude that peroxisome proliferation does not significantly contribute to the nephrotoxicity of PER, even in the relatively efficient TCA-producing mice.

The NTP (208) describes a study in which mice and rats were exposed for 13 weeks to 100, 200, 400, 800 and 1 600 ppm (689, 1 378, 2 756, 5 512 and 11 024 mg/m$^3$). The mice showed renal tubular karyomegaly at all doses except the lowest one. Only the kidneys of the highest rat dose-group were microscopically investigated, and these did not show any lesions.

**Table 19**. Study of Rowe *et al*.; summary of results (236).

| Concentration ppm (mg/m$^3$) | Animal | Groups | Exposure regimen | Effects |
|---|---|---|---|---|
| 2 500 (17 225) | Rat | 5 of each sex | 7-h exposures, up to 13 in 18 days | Only 1 animal per group survived 13 treatments; severe depression of CNS, loss of consciousness, cloudy swelling in liver with few, diffusely distributed small fat vacuoles. |
| | Rabbit | 2 males | 28 ·7-h exposures in 39 days | Severe depression of CNS; parenchymatous degeneration in the liver. |
| | Guinea pig | 4 of each sex | 18 ·7-h exposures in 24 days | Severe depression of CNS; weight loss; increased weights of liver and kidneys; central fatty degeneration in liver; cloudy swelling in renal tubuli. |
| 1 600 (11 024) | Rat | 8 females | 18 ·7-h exposure in 25 days | Various marked effects on behaviour, which could be prevented by an i.p. treatment with atropine; decreased body weight; enlarged liver and kidneys; no histopathological changes in these organs. |
| | Guinea pig | 7 males | 8 ·7-h exposures in 8 days | Decreased body weight; increased weight of liver; moderate central fatty degeneration in liver; slight degenerative charges in germinal epithelium. |
| 400 (2 756) | Rat | 15 of each sex | 130 ·7-h exposures in 183 days | No adverse effects found. |
| | Guinea pig | 8 of each sex | 169 ·7-h exposures in 236 days | Depressed growth; increase of liver weight; increase of neutral fat and esterified cholesterol in liver; central fatty degeneration in liver with slight cirrhosis. |
| | Rabbits | 2 of each sex | 159 ·7-h exposures in 222 days | No adverse effects found. |
| | Rhesus monkey | 2 males | 179 ·7-h exposures in 250 days | No adverse effects found. |

**Table 19**. Cont.

| Concentration ppm (mg/m$^3$) | Animal | Groups | Exposure regimen | Effects |
|---|---|---|---|---|
| 200 (1 378) | Guinea pig | 8 of each sex | 158 ·7-h exposures in 220 days | Depressed growth; increased liver weight; increased total lipid and esterified cholesterol in liver; central fatty degeneration in liver. |
| | | 5 females, 15 males | 14 ·7-h exposures in 18 days | Depressed growth; increased liver weight; very slight fatty degeneration of the central area of the liver. |
| 100 (689) | Guinea pig | 4 females, 7 males | 132 ·7-h exposures in 185 days | Females: increased liver weight<br>Males: few small fat vacuoles in the liver. |
| | Guinea pig | 7 females | 13 ·7-h exposures in 17 days | No adverse effects. |

**Table 20.** Overview of short-term respiratory studies showing hepatotoxicity.

| Species | Exposure conditions | Results | Reference |
|---|---|---|---|
| Mouse | 4 h daily, 6 days/week for up to 8 weeks, 200 ppm (1 378 mg/m$^3$) | Increase of the number of 4-h exposures led to an increase of the severity of hepatotoxicological effects. After 8 weeks massive, central infiltration of about 80% of the liver with fat was observed. At least half of the lobules was effected. Liver fat content doubled during the first week of exposure, but did not increase any further afterwards. No cirrhosis or necrosis reported. | (154) |
| Rabbit | 4 h daily, 5 days/week for 9 weeks, 2 790 ppm (19 223 mg/m$^3$) | An increase of SGOT, SGPT and glutamic dehydrogenase (GDH) in serum. The increases of all activities were significant 45 days after the start of exposure. These biochemical effects were accompanied by damage to the cytoplasmic and mitochondrial structures of the liver parenchyma. | (178) |
| Mouse and rat | 6 h daily, 14, 21 or 28 days to 400 ppm (2 756 mg/m$^3$) and 6 h/day for 28 days to 200 ppm (1 378 mg/m$^3$) | Livers were examined for peroxisomes by electronmicroscopy; furthermore peroxisomal cyanide-insensitive palmitoyl CoA oxidation was determined. Clear increases were observed in the mouse livers[a] while the rat did hardly yield indications for an effect of PER on these endpoints. No effects on the peroxisomal enzyme catalase were observed in either species. In the mice small but significant increases of the liver/body weight ratios were found, an effect that was absent in the rats. The marked difference in effect between rat and mouse could be explained by the difference in PER metabolism between the two species. Concentrations of the known inducer of peroxisome proliferation TCA (73), the major metabolite of PER, were much higher in mouse blood than in rat blood. | (210) |
| Mouse | Continuous (24 h daily) for 30 days to 9 or 75 ppm (62 or 517 mg/m$^3$) | A significant increase of relative liver weight was observed when mice were continuously exposed to 9 ppm for 30 days, while the liver weight was doubled when the mice were exposed for the same period to 75 ppm. The increase was accompanied by clear-cut histopathological changes, viz. cell hypertrophy and vacuolisation. | (143, 144) |

[a] At the lowest dose (200 ppm, for 28 or 400 ppm for 14 days), a clear and significant doubling of the CoA oxidation was found in male mice; effects were somewhat less marked in females. Light microscopic examination revealed centrilobular eosinophilia and centrilobular fatty vacuolisation for the 400 ppm/14 or 28 day dose groups. Electron microscopy revealed effects at lower dose levels.

*Inhalation: Endocrinological effects*

Mazza and Brancaccio (179) observed in rabbits slight increases (not statistically significant) of adrenal hormones (both cortical and medullar) upon the exposure to 2 200 ppm (15 158 mg/$m^3$) of PER 1 hour/day, 6 days/week for 15 days (from reference 74). The enzyme levels increased gradually with number of exposures.

The studies of Kjellstrand *et al*. do not point to a clear interaction between PER and testosterone (144, 143), see under *"Inhalation: Effects on plasma butyryl-cholinesterase"*.

*Inhalation: Immunotoxicity*

EPA mentions a study in which 7 days of continuous exposure of rats to 185 ppm (1 275 mg/$m^3$) results in morphological changes in mast cells ("...increased vacuolisation of the cytoplasm and conformational changes in granules with some degranulisation" (74)). Furthermore, EPA (74) cites a study of Schmuter (243) in which exposure of chinchillas to 1.5 and 15 ppm (10.3 and 103.4 mg/$m^3$) (8-10 months, 6 days/week, 3 hours/day) results in significant changes in antibody production to *Salmonella typhosa*. However, EPA (74) regards the study as too poorly reported to enable the evaluation of these results.

*Inhalation: Electrophysiological effects*

An effect of respiratory exposure to PER on electroconductance and contraction of muscles (maximum reductions of 10 and 24% for these endpoints, respectively) has been described by Dmitrieva (54, cited in 74) for mice. The animals were exposed for 3 months, 5 hours/day, to 15 or 75 ppm (103 or 517 mg/$m^3$). Dmitrieva and Kulshov (54, cited in 74) found effects on the EEG in rats exposed to 15 ppm (103 mg/$m^3$) for 5 months, 5 hours/day. EPA (74) concludes about these studies that "these data should not be relied upon to develop a regulatory strategy", as "it is often difficult to interpret these results because of the investigators´ reporting methods".

*Inhalation: Effects on behaviour and related effects*

Clear effects on behaviour and related effects are reported in several subacute and semichronic studies. They do not differ essentially from the effects observed in acute studies in a quantitative or a qualitative sense. The tolerance observed by Carpenter (35) after repeated exposures points to changes in either the biotrans-formation of PER or the sensitivity of the neurological target to its effect.

*Inhalation: Neurochemical effects*

The overt neurological effects of PER have prompted a series of investigations into possible biochemical changes caused by this compound in the brain. The results of these studies are summarised in Table 21.

Taken together, the results presented in Table 21 suggest that long-term respiratory exposure to PER leads to structural damage of the brain as a loss of brain cells (possibly glial cells), as partly reversible changes in the composition of cerebral membranes and as interference in the metabolism of the structural

proteins of brain cells. It should be noted that the observed biochemical changes are small and that the relation with functional changes is unknown. Furthermore, much of the evidence has been obtained by a single research group using a rather uncommon experimental animal (Mongolian gerbil). Some of the data have been confirmed by the same research group in the rat model.

*Inhalation: Effects on plasma butyrylcholinesterase*

Kjellstrand *et al*. have investigated the effect of respiratory PER exposure of mice on plasma butyrylcholinesterase (143, 144). Two exposure regimens were applied: continuous exposure for 30 days and 16, 8, 4, 2 or 1 hour/day for 30 days. The first regimen resulted in a maximum increase of 1.7-fold for females and 2.5-fold for males, indicating a clear-cut effect of the PER inhalation on the activity of this enzyme. Significant effects were observed at 37 ppm (255 mg/$m^3$); the next lower concentration (9 ppm or 62 mg/$m^3$) did not result in an effect. The effect reached a maximum at concentrations little higher than 37 ppm (255 mg/$m^3$). The intermittent exposure showed that decrease of concentration can be compensated for by an increase of daily exposure time.

In a second study of the same authors (143), the question was addressed as to whether the effects of PER on butyrylcholinesterase were caused by hepatotoxicity or had an endocrinological background. Mice were exposed continuously to 150 ppm (1 034 mg/$m^3$) for one month. In addition, the influence of castration and testosterone administration was investigated. The results of this study indicate that the effect on butyrylcholinesterase activity is not directly correlated with the effects of testosterone levels nor with liver toxicity.

Changes in butyrylcholinesterase activity by PER are difficult to interpret in a functional sense, because the biochemical/biological role of this enzyme is largely unknown. In addition, a poor correlation has been found between butyryl cholinesterase activity and plasma acetyl cholinesterase activity.

*Inhalation: Haematological effects*

Seidel *et al*. exposed female mice 6 hours/day, 5 days/week for up to 7.5 weeks to 135 ppm (930 mg/$m^3$) of PER and for up to 11.5 weeks to 270 ppm (1 860 mg/$m^3$) of PER, followed by an exposure-free period of 3 weeks (249). They investigated the effects of this treatment on a number of haematological parameters. In the peripheral blood, reductions of the lymphocyte, monocyte and neutrophil counts were observed, followed by an almost complete regeneration during the exposure-free period. Reticulocytosis during and after exposure pointed to a compensatory reaction of the red blood cell system.

No effects on the bone-marrow pluripotent stem cells were seen. The number of erythroid-committed cells was suppressed and slight indications for a disturbance of the granulocyte cell series were found (249).

**Table 21.** Neurochemical effects of respiratory exposure to PER.

| Species | Concentration ppm (mg/m$^3$) | Exposure regimen | Results | Comments and details | Reference |
|---|---|---|---|---|---|
| Rat | 200, 400 and 800 (1 378, 2 756 and 5 512) | Continuous for 1 month | Marked dose-related decrease of acetylcholine in the striatum. Slight, but not significant changes observed of dopamine in the striatum, norepinephrine in the hypothalamus and serotonin in the cortex and hippocampus. | | (117) |
| Rat | 200, 400 and 800 (1 378, 2 756 and 5 512) | Continuous for 1 month | Marked and dose-related increase of glutamine, threonine and serine, while γ-butyroamino acid decreased. | | (118) |
| Mongolian gerbil[a] | 120 (827) | Continuous for 12 months | Small changes of fatty-acid pattern of phospholipids. Decrease of long-chain linolenic acid-derived 22-carbon fatty acids. No changes in content/concentrations of protein, lipid phosphorous and cholesterol in hippocampus and cerebral cortex. | It is concluded that small changes are induced in membrane fatty acids at doses well below those causing anaesthesia. | (155) |
| Mongolian gerbil | 60 (413) and 320 (2 204) | Continuous for 3 months, followed by 4 months without exposure. | Slightly increased concentrations of the astroglial protein S100 in hippocampus, cerebral occipital cortex and cerebellum. S100 as well as DNA concentrations were decreased in the frontal cerebral cortex. Effects on DNA concentrations were already observed at the lowest concentration (60 ppm). | The results point to astroglial hypertrophy in hippocampus, cerebral occipital cortex and cerebellum and atrophy, affecting the astroglial cells in the frontal cerebral cortex. | (235) |
| Mongolian gerbil | 60 (413) | Continuous for 3 months, followed by 4 months without exposure. | Slight decrease of DNA concentration in frontal cerebral cortex. | Indications of loss of neuronal/glial cells in the frontal cortex. The effect is not caused by metabolites but by PER itself. | (138) |
| Mongolian gerbil | 320 (2 204) | Continuous for 3 months | Minor decrease of brain weight. Shift in fatty-acids of ethanolamine phospholipids towards less saturated forms. | Indicates slight changes in composition of cerebral membranes. Confirms earlier findings. | (157) |

**Table 21.** Cont.

| Species | Conc. ppm ($mg/m^3$) | Exposure regimen | Results | Comments and details | Reference |
|---|---|---|---|---|---|
| Rat | 320 (2 204) | Continuous for 30 days | Slight reduction of cholesterol and phospholipids in the brain. Shift in fatty-acid composition of the brain. No such effects were observed for freon and 1,1,1-trichloroethane. | Indicates slight changes in composition of cerebral membranes. The effect is specific for PER. | (158) |
| Rat, Guinea pig (30 days pregnant) and Mongolian gerbil | 320 (2 204) and 160 (1 102) (Guinea pigs) | Continuous for 30 days. | "Tendency towards decreased brain weight". Shift in fatty-acid composition of the brain. No increased sensitivity during second half of gestation. | The absence of an increased sensitivity during gestation might indicate (according to the authors) that membranes are not particularly sensitive to the effects of PER during their synthesis. | (156) |
| Rat | 320 (2 204) | Continuous for 90 days, followed by a recovery of 30 days. | Slight shifts in fatty-acid composition of brain phospholipids. Most changes normalised during post-exposure period. Slight persistent changes in brain cholesterol content. | Indicates slight, partly reversible changes in composition of cerebral membranes | (159) |
| Rat | 300 (2 067) and 600 (4 134) | Continuous for 4 or 12 weeks | Slower increase in brain weight at 600 ppm after 4 and 12 weeks. At highest dose after 12 weeks decrease in DNA total protein and brain-region weights in frontal cerebral cortex and brain stem, but not in hippocampus. Glial and neuronal cytoskeletal proteins decreased in frontal cortex at the highest dose. Glial proteins were decreased in frontal cortex, brain stem and hippocampus (the three brain regions investigated) at the highest dose. No effects were found on neuronal enolase. | Indications for reduction in the number of brain cells, possibly glial cells, and interference with the metabolism of cytoskeletal elements in both glial and neuronal cells. | (288) |

[a] Meriones ungiculates.

*Oral: Multi-endpoint studies*

Kaemmerer *et al*. (136) applied various dose regimens to rats. Their results are summarised in Table 22.

Hayes *et al*. (112) administered PER via drinking water. Actually all PER was offered to the animals as emulsion droplets and not in the dissolved state. Theoretical doses applied were 14, 400 and 1 400 mg/kg/day for 90 days. The results of this study are summarised in Table 23.

Even these rather high doses did not result in clear-cut effects on liver and kidneys, important target organs, when administered via the drinking water.

*Oral: Hepatotoxicity*

No effects on histopathology and relative liver weight were seen in rats treated for 12 days by gavage with 500 mg/kg/day (245, 263).

In the study of Buben and O'Flaherty (31) PER dissolved in corn oil was administered once a day, for 5 days per week, over a 6 week period, to mice, in doses of 0, 20, 100, 200, 500, 1 000, 1 500 and 2 000 mg/kg. At 100 mg/kg and higher doses, a dose-related increase of liver weight (up to 75%) and accumulation of triglycerides (up to 8-fold) were observed. At higher doses liver glucose-6-phosphatase was less active (starting at 500 mg/kg), while SGPT activity was raised at 200 mg/kg and higher doses (up to 2.9-fold). The DNA content of the liver was determined at 200 and 1 000 mg/kg; it was decreased by 17% at the latter dose compared to the control. In addition, karyorrhexis, polyploidy, degeneration and necrosis were observed at both doses upon histological examination, although, with the exception of degeneration, these effects were minimal to slight, while degeneration was moderate to severe. The authors established a NOAEL of 20 mg/kg.

The study by Buben and O'Flaherty comprised a detailed comparison of metabolism (determined as urinary excretion of TCA and TCE) and hepatotoxicity (31). Good linear relationships were found between on one hand urinary

**Table 22.** Summary of the studies reported by Kaemmerer *et al*. (136).

| Dose regimen | Results |
|---|---|
| 25 or 500 ppm in diet for 7 days and/or 25 ppm in diet for 14 days | Increase of cytochrome P450 levels; at 25 ppm significant after 14 days; at 500 ppm significant after 7 days. |
| 25, 100, 200 or 500 ppm in diet for 7 days | No effect of doses on anaesthesia induced by hexabarbitone. |
| 25 or 400 ppm in diet for 1 day to 2 weeks | No effects on acetylation and elimination of 50 mg/kg sulphadioxide intramuscular. |
| 25 ppm in diet for 14 days or 500 ppm for 7 days | Effects on coagulation, i.e. increase of prothrombin time and thrombin time; no change in thrombocyte number. |
| 25, 200 or 500 ppm in diet for 7 days | No increase of mortality caused by Neguvon, 2,4-dinitrophenol or ouabain due to PER exposure. |

**Table 23.** Effects of PER emulsified in drinking water on rats (112).

| Endpoint | Effect | Effective doses (mg/kg/day) |
|---|---|---|
| Relative body weight | Decrease | Males: 1 400<br>Females: 400 and 1 400 |
| Kidney weight/body weight | Increase | Males: 400 and 1 400<br>Females: 1 400 |
| Liver weight/body weight | Increase | 1 400 |
| Serum parameters; LDH, SGPT, SGOT, ALP, 5'-NU, blood urea nitrogen, protein, glucose, cholesterol, bilirubin, creatinine, calcium, phosphorus, albumin, globulin, sodium, potassium | Not treatment related except an increase of 5'-NU. | Females: 400 and 1 400 |

excretion of metabolites and on the other hand SGPT, SG-6-P, triglycerides and liver weight, thus presenting clear-cut indications that the toxicity is caused by metabolites and not by PER itself, see also (198).

Schumann *et al*. (244) compared the hepatotoxicity of PER in mice and rats. The animals were exposed by gavage to 100, 250, 500 or 1 000 mg/kg each day of 11 days. All doses resulted in histopathological changes in the liver of mice (described as "accentuated lobular pattern with hepatocellular swelling in the centrilobular region"), while only marginal effects were found in the livers of rats treated with the highest dose (described as "altered staining ability of the hepatocytes in the centrilobular region"). Furthermore, all doses led to a decrease in the DNA concentration in the mouse livers, while such effect was not observed for the rats. At the same time, a marked increase of DNA synthesis ([3H]thymidine incorporation) was found in the mouse livers of all treated groups. Again, no such effect was observed in the rat. The differences between the two species are explained by the higher biotransformation capacity of the mouse compared to the rat, if it is assumed, that the effects are caused by metabolites instead of the parent compound.

*Oral: Nephrotoxicity*

The effects of PER (500 mg/kg, administered orally by gavage in corn oil, daily for 4 weeks) on renal function and renal histopathology in rats have been investigated by Bergamaschi *et al*. (17). The results of this study are summarised in Table 24.

At first observation, these results look inconsistent, as histopathology points to damage of the tubuli, while biochemistry points to glomerular damage. However, in the discussion section of the paper in which this study is described (17), the authors present a plausible explanation, based on a "tubular target selectivity" of PER (PER appears to affect tubular segment S2 in particular).

**Table 24**. Effects of oral PER treatment (500 mg/kg bw/day gavage during 4 weeks) on renal function and renal histopathology in rats (17).

| Endpoint | Effects | |
|---|---|---|
| | Female | Male |
| Albuminuria (marker for glomerular permeability) | Minor, but significant increase | Marked increase (up to 15 times of control) |
| Urinary excretion of $\alpha_{2u}$-globulin (marker for tubular dysfunction) | Marked increase (up to 4 times of control) | Transient increase |
| Urinary excretion of retinol-binding protein (marker for tubular dysfunction) | Slight increase | Increase (up to 2 times of control) |
| Urinary excretion of N-acetylglucosaminidase | Slight increase | Transient, but significant increase |
| Electrophoretic analysis of proteinuria | Increase in low-molecular-weight proteins | Increase in high-molecular weight proteins |
| Histopathology | Same effects as in the male but much less severe | "Progressive increase in number and size of phagolysosomes (hyaline droplets) in proximal convoluted epithelial cells" |

The nephrotoxicity of PER has also been investigated by Goldsworthy *et al*. (98). These authors exposed rats to PER by gavage (1 000 mg/kg/day) for 10 days. Endpoints examined were: renal $\alpha_{2u}$-globulin, protein-droplet accumulation (PDA) and cell replication. Only the male rats showed increases of PDA (including crystalloid accumulation) in the cytoplasm of the P2 segment of the proximal tubule. The P2 segment also showed an increased cell replication, which was again seen only in the male. $\alpha_{2u}$-Globulin correlated well with PDA. Comparable effects were observed in rats treated with pentachloroethane, but not in those treated with trichloroethylene.

*Oral: Peroxisome proliferation*

The induction of peroxisomal enzyme activity (cyanide-insensitive palmitoyl CoA oxidation, regarded to be a sensitive measure for peroxisome proliferation) by PER (1 000 mg/kg; gavage once a day, over a period of 10 days) was investigated by Goldsworthy and Popp (97) in liver and kidneys of rats and mice. A summary of their results is presented in Table 25.

**Table 25**. Effects on peroxisomal enzyme activity by oral gavage exposure to PER and some other compounds for 10 days.

| Compound | Dose (mg/kg bw/day) | Rat liver, % of corn oil | Mouse liver, % of corn oil | Rat kidney, % of corn oil | Mouse kidney % of corn oil |
|---|---|---|---|---|---|
| Corn oil | | 100 | 100 | 100 | 100[a] |
| Wy-14,643[b] | 50 | 561[a] | 1 223[a] | 958[a] | 689[a] |
| Trichloroethylene | 1 000 | 180 | 625[a] | 300[a] | 360[a] |
| PER | 1 000 | 142 | 428[a] | 174 | 232[a] |
| Pentachloroethane | 150 | 127 | 158 | 53 | 310[a] |
| TCA | 500 | 284[a] | 280[a] | 175 | 305[a] |

[a] Significantly different from corn-oil control, $p<0.05$, Newman-Keul's multicompare test.
[b] Wy-14,643: positive-control compound; identity not specified.

The table shows PER to induce peroxisome proliferation in mouse liver and mouse kidney, while no significant effects were found in the rat.

*Oral: Haematotoxicity*

The effects of very low oral doses of PER have been investigated by Marth *et al*. (176, 177) and Marth (175). These authors dissolved PER in drinking water of mice, resulting in doses of 0.05 and 0.1 mg/kg body weight/day for 7 weeks.

Histopathological examination revealed effects in the spleen at both dose levels; no effects were observed in other examined organs, i.e. brain, liver and kidneys. In the spleen the following changes were observed: pulpa cords rich in erythrocytes, many blood-formation centers in the red pulpa with megakaryocytes and hemosiderin storage in the macrophages of the red pulpa.

The authors assume the effects in the spleen to be the result of premature erythrocyte disintegration, which in its turn is caused by the interaction of the apolar PER with the erythrocyte membrane. The spleen macrophages remove the erythrocyte fragments from the bloodstream, and are thus subject to hemosiderin deposition.

The exposure led to a decrease in body weight and an increase in relative spleen weight and relative kidney weight. No weight changes were observed for the liver and the brain. Neither were swelling or enlargement of the liver observed.

Effects on the hemopoietic system were reflected by clear-cut increases of LDH activity, peripheral blood count and microscopic examination of the bone marrow. No increase of SGPT or changes of the proportion among serum proteins were found, indicating unaffected liver function. Lipoprotein electrophoresis showed a clear-cut change in the proportion among classes of lipoproteins (HDL, VLDL and LDL). Furthermore, a decrease of cholesterol was found, which is assumed by the authors to be the result of an inhibition of HMG CoA reductase.

*Oral: Tumour initiation and promotion*

Story *et al*. (262) investigated the initiating and promoting properties of PER in an oral rat model. The rats were partly hepatectomised and subjected to various exposure regimens. The investigated endpoint was the occurrence of enzyme-

altered foci in the liver. According to the authors, the foci are assumed to be potentially capable of developing into trabecular hepatocellular carcinomas (i.e. they were regarded as putative preneoplastic lesions). Initiating properties were investigated by scoring the number of foci after one intraperitoneal treatment with PER (maximum tolerated dose: 6 mmol/kg), followed by a long-term oral treatment with phenobarbitone in drinking water (7 weeks), while the number of foci occurring after one intraperitoneal treatment with diethylnitrosamine, followed by daily oral gavage (5 days/week for 7 weeks) of PER in corn oil (6 mmol/kg), was taken as an indicator for promoting activity. PER induced an increase of foci in rats subjected to the second exposure regimen. Thus, the outcome of this study suggests PER to have promoting and not initiating properties.

Comparable studies have been carried out by Lundberg *et al*. (170) and Holmberg *et al*. (115), although these were only concerned with promotion (rat, initiator: diethyl nitrosamine). They did not confirm the finding of the aforementioned study (262), in the sense that no indications for promoting properties were found. Moreover, Maronpot *et al*. did not find such indications in a pulmonary tumour promotion assay with mice (174).

*Intraperitoneal*

Bernard *et al*. (19) investigated the nephrotoxicity of 5 intraperitoneal PER injections per week for 2 weeks in rats (1 000 mg/kg body weight/injection). Endpoints were β-*N*-acetylglucosaminidase, $\beta_2$-microglobulin and albumin in urine. No treatment-related effects were observed. Under the same conditions cyclohexane exposure caused a clear-cut increase of $\beta_2$-microglobulin. Thus, this study does not indicate an effect of PER on the functioning of the tubuli or the glomeruli.

### *6.2.4 Toxicity due to long-term exposure and carcinogenicity*

*Inhalation*

Rampy *et al*. [(229), study is only published in the form of an abstract; unpublished details and results were available to the authors of the EPA publication (74); summary presented here is based on (74)] exposed rats (Sprague Dawley) to 0, 300 and 600 ppm (2 067 and 4 134 mg/m$^3$) of PER for 12 months (6 hours/day, 5 days/week; followed by an observation period of 19 months). An increase of mortality was found for male rats between the 5th and the 24th month in the 600-ppm group (characterised as "slight" in references 69, 119), while no exposure-related mortality was demonstrated in the female group. The mortality in the males is attributed to "spontaneous advanced chronic renal disease". Histopathology revealed increased numbers of inflammatory cells in the kidneys and focal progressive nephrosis in the exposed rats of both sexes at the highest exposure level. Other endpoints investigated were: body weight, mortality, haematology, urine analysis, clinical chemistry, cytogenetics, organ weights, gross histological tissue changes including tumour incidence.

Slight indications were obtained for haematological effects in females. However, these could not be confirmed by a second determination carried out some

days later. Other endpoints appeared to be unaffected by the exposure, including tumour incidence.

EPA (74) does not draw specific conclusions from this study as to the carcinogenicity of PER upon respiratory exposure. In the HSE review (119), it is concluded that evaluation of this property is not possible, due to the summarised presentation, which is lacking the necessary details, and the relatively short exposure period. However, the EPA publication (74) is based on unpublished and probably more detailed results, which would mean that one year of respiratory exposure to 600 ppm of PER does not have a carcinogenic effect in rats.

A study on the carcinogenicity of PER in B6C3F1 mice and F344 rats (50 of each sex per dose) after respiratory exposure was carried out within the framework of NTP (208). The rats were exposed to 0, 200 and 400 ppm (1 378 and 2 756 mg/m$^3$), the mice to 0, 100 and 200 ppm (689 and 1 378 mg/m$^3$). Exposure lasted 103 weeks; the animals were exposed 6 hours/day, 5 days/week.

The rats showed high incidences of karyomegaly and cytomegaly in tubular cells of the proximal convoluted tubules. Furthermore, the treatment induced thrombosis of the nasal cavity, squamous metaplasia (only in males), adrenal medullar hyperplasia (only in males), adrenal cortical hyperplasia (only in females), and forestomach ulcers (only in males). A not significant increase of tubular-cell adenoma and adenocarcinoma was found for the male rats (1/49, 3/49 and 4/50 for 0, 200 and 400 ppm respectively). Moreover, a significant increase of mononuclear cell leukaemia was found for both male and female rats (males: 28/50, 37/50 and 37/50 for 0, 200 and 400 ppm respectively; females: 18/50, 30/50 and 29/50 for 0, 200 and 400 ppm respectively). In view of the high and variable incidence of this form of cancer in F344 rats and the high control incidence in this study, it is doubtful whether the observed increase (although significant when considered within the limited scope of the experiment) does really point to a treatment-related effect, see also (69, 119). However, NTP's Board of Scientific Counselors considered the incidence of rat leukaemia to be a valid finding (3), because of the shorter time to the onset of the disease and its greater severity in the treated animals as compared to the control animals.

The treatment of mice induced liver degeneration and necrosis, kidney casts, tubular-cell karyomegaly, nephrosis (in females only) and acute passive lung congestion. The mice (both sexes) showed a statistically significant increase of hepatocellular carcinoma (see Table 26), part of which had metastasised to other organs.

So, these studies show PER to be a clear-cut liver carcinogen for mice, while indications were obtained for the compound to exert a carcinogenic effect in the kidneys of male rats.

The ATSDR (3) mentions the following limitations: "numerous instances of mice and rats loose from their cages within the exposure chambers, with the potential for aberrations in exposure and animal identification".

**Table 26.** Hepatocellular neoplasms in B6C3F1 mice exposed to PER via inhalation; reproduced from (3).

| | Control | | 100 ppm (690 mg/m$^3$) | | 200 ppm (1 378 mg/m$^3$) | |
|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female |
| Hepatocellular adenoma | 12/49 (24%) | 3/48 (6%) | 8/49 (16%) | 6/50 (12%) | 19/50 (38%) | 2/50 (4%) |
| Hepatocellular carcinoma | 7/49 (14%) | 1/48 (2%) | 25/49 (51%) | 13/50 (26%) | 26/50 (58%) | 36/50 (72%) |
| Hepatocellular adenoma or carcinoma | 17/49 (35%) | 4/48 (8%) | 31/49 (63%) | 17/50 (34%) | 41/50 (82%) | 38/50 (76%) |

*Dermal*

Van Duuren *et al*. (66) investigated the carcinogenic and tumour initiating activity of PER in ICR Swiss mice upon dermal application. Thirty female mice were treated 3 times/week with acetone solutions of the test compound (applied to the shaved skin) for at least 440 days. Doses were 54 and 18 mg/mouse/day. This treatment did not significantly alter the number of skin tumours.

In a second experiment 30 female mice were treated once with PER, followed by treatment with phorbol myristate acetate for at least 428 days. Also this regimen did not reveal a significant influence of PER on the tumour incidence.

*Oral*

The carcinogenic effects of oral treatment of PER were investigated by the National Cancer Institute (202; see also 3, 69, 119). Groups of 50 male and 50 female B6C3F1 mice and Osborne-Mendel rats were treated with PER in corn oil by gavage. The control groups and the vehicle-treated groups consisted of 20 animals of each sex.

The mice were treated with 536 and 1 072 mg/kg/day (males) and 386 and 772 mg/kg/day (females), for 5 days/week over a period of 78 weeks. The indicated values represent TWAs, because the actual dosage had to be adjusted during the study due to severe toxicity. The exposure period was followed by an observation period of 12 weeks.

The incidence of hepatocellular carcinoma increased from about 10% (2/20, 2/17, 0/20, 2/20, in females and males of controls and vehicle-treated groups respectively) to 40% (19/48) and 65% (32/49) in the low-dose group and 40% (19/48) and 56% (27/48) in the high-dose group, for females and males, respectively. In a number of animals the carcinomas metastasised to the lungs (1/49 of the low-dose females, 3/49 of the low-dose males and 1/48 of the high-dose females) or to the kidneys (1/18 of the untreated males). Tumours appeared much earlier in the PER treated groups than in the control or the vehicle-treated groups. A dose related increase of mortality was observed. 50% survival periods were 78, 43, 60 and 50 weeks for high and low-dose males and high and low-dose females respectively, while 50% survival periods were over 90 weeks in all control and

vehicle-treated groups. Nearly all treated mice showed nephropathy and the high mortality early in the study was probably caused by this effect. The nephropathy consisted of degenerative changes in the proximal convoluted tubuli at the junction of the cortex and the medulla, with cloudy swelling, fatty degeneration and necrosis of the tubular epithelium and hyaline intraluminal casts. No treatment-related liver lesions were reported, which is surprising in view of the clear-cut hepatotoxicity of PER for mice demonstrated in other studies.

Female and male rats were treated with 474 and 949 mg/kg/day (TWA) and 471 and 941 mg/kg/day (TWA) respectively, 5 days/week over a period of 78 weeks followed by a 32-week observation period. No tumour induction could be attributed to the treatment. A high mortality was observed in the early part of the study (50% survival periods in males: 88, 72 and 44 weeks for control, low dose and high dose respectively; 50% survival period in females: 102, 66 and 74 weeks for control, low dose and high dose respectively). No indications of hepatotoxicity were obtained. At autopsy 79% of the treated animals was affected by nephropathy.

The ATSDR (3) mentions the following shortcomings of these studies:
1) Smaller control groups than exposed groups 2) Dose adjustments because of nephropathy, which indicate that the maximum tolerated dose was exceeded
3) Pneumonia due to intercurrent infectious disease in both rats and mice.

Maltoni and Cotti (172; see also 69) treated 40 male and 40 female rats by gavage with 500 mg/kg/day (in olive oil), for 4 to 5 days/week over a period of 104 weeks. Observation lasted to death. Control groups consisted of 50 female and 50 male rats, which were treated with the vehiculum only. No increase in tumour incidence was observed. Only male rats (32%) showed renal damage: cytomegaly or karyomegaly in renal tubular cells.

Herren-Freund *et al.* (113) investigated the hepatocarcinogenicity in mice of the major metabolite of PER, viz. TCA. This compound was given to 22 mice in their drinking water at a concentration of 5 g/l for a period of 61 weeks. 7/22 mice developed hepatocellular carcinomas and 8/22 developed hepatocellular adenomas, while only 2 out of 22 control mice developed hepatocellular adenomas and no hepatocellular carcinomas were observed in the control group. This finding strongly suggest that the hepatocarcinogenicity of PER in mice is in fact caused by the major metabolite of this compound.

*Intraperitoneal*

Repeated intraperitoneal injection of PER in mice did not result in lung surface adenomas (268, see also 119); PER dissolved in tricaprylin; 3 injections/ week; 14 injections of 80 mg/kg or 24 injections of 400 mg/kg; animals killed 24 weeks after the first injection).

*6.2.5 Reproduction toxicity*

Carpenter (35) investigated the effects of respiratory PER exposure on the fertility and reproductive capacity of rats. The animals were exposed to 0, 70, 230 and 470 ppm (482, 1 585 and 3 238 mg/m$^3$) 8 hours/day, 5 days/week over a period of 28

weeks. No indications for a decreased fertility or reproductive capacity were obtained, based on an analysis of the number of litters in the different exposure groups.

In a two-generation reproduction study based on a standard protocol rats were exposed by inhalation to 0, 100, 300 and 1 000 ppm PER (690, 2 070 and 6 900 mg/m$^3$) 5 days/week, 6 hours/day for 11 weeks prior to mating for up to 21 days during which exposure was daily. Evidence of toxicity was seen at an exposure level of 1 000 ppm PER as shown by reduction in parental body weight gain during the pre-paring period and lactation in both generations and during pregnancy in the second generation. Decreases in litter size and survival during lactation were seen at 1 000 ppm but not at 300 ppm. This represents a toxic effect, which in part, may have been maternally related.

Schwetz *et al*. (247) exposed rats and mice to 0 and 300 ppm (2 067 mg/m$^3$) PER on days 6-15 of gestation (7 hours/day; 17 test animals and 30 control animals). For rats, exposure resulted in a significant decrease of maternal body weight; no significant effects on liver weight were found. No effects were found on the following endpoints: numbers of litters, corpora lutea, implantation sites, live foetuses, sex ratio, foetus weight, foetus length. A significant increase of the resorption rate was observed (4% in control versus 9% in exposed rats). Examination for soft-tissue anomalies and effects on skeleton did not reveal a significant treatment-related effect.

The same endpoints were investigated by Schwetz *et al*. for mice (247). The following difference between control and exposed were found: increase of maternal liver weight, decrease of foetus weight, delayed ossification of the skull and the sternebra, increase of the incidence of split sternebra, and increased subcutaneous oedema. The results point to the absence of teratogenic effects in both rats and mice, while for mice they indicate foetotoxicity.

Beliles *et al*. (15) and Hardin *et al*. (110) subjected pregnant rats to four exposure regimens (20 rats/regimen): exposure to I) 500 ppm (3 445 mg/m$^3$) on days 0-18 of gestation, II) 500 ppm 3 weeks before mating and on days 0-18 of gestation, III) 500 ppm 3 weeks before mating and on days 6-18 of gestation, IV) 0 ppm on days 0-18 of gestation and V) 0 ppm 3 weeks before mating and on days 6-18 of gestation.

Treatment-related maternal effects were restricted to elevated kidney weights (11%) in group II and elevated liver weights in group III (histological examination and necropsy). No indications of foetotoxicity or teratogenicity were obtained.

The same authors performed a strictly comparable study with rabbits, which did not yield clear significant treatment-related effects, except for an increase of placental abnormalities in the group exposed at days 7-21.

Nelson *et al*. (204) exposed groups of 13-21 rats to 900 ppm (6 200 mg/m$^3$) on days 7-13 or 14-20 of gestation or to 100 ppm (690 mg/m$^3$) on days 14-20 of gestation. A sham-exposed group was taken for each exposed group. The pups were examined for a series of behavioural and neurochemical effects and for effects on the histopathology of the brain. Furthermore, gross pathology and liver and kidney histopathology were examined in the dams.

The only maternal effects found were reduced feed intake and weight gain in the 900 ppm-days 7-13 group. Gross pathology and histopathology revealed no indications for treatment-related effects. No reference is made to the birth of deformed pups. The number of live-born pups was not affected by the treatment.

Behavioural tests pointed to a decreased neuromuscular function of the pups exposed during days 7-13 of gestation when they were between 10 and 14 days old. However, the pups exposed later during gestation performed better than the controls in another test for neuromuscular function. Neurochemistry revealed a decrease of acetylcholine in the brains of 21-days old animals of both 900 ppm groups, and of dopamine in the brains of 21-days old animals of the 7-13 days-900 ppm group. No neurochemical effects were observed in new-born rats. Exposure to 100 ppm did not result in behavioural effects. No effects on brain histopathology were observed.

The outcome of this study suggests that exposure during pregnancy may result in an affected function of the CNS.

Frederiksson *et al*. (86) treated mice orally to PER (5 and 320 mg/kg body weight/day) between days 10 and 16 postnatally. When they were 60 days old, the mice showed changes in spontaneous motor activity. The results suggest neurodevelopmental toxicity in mice resulting in persistent alterations in behaviour.

### *6.2.6 In vitro studies (except genotoxicity and cell transformation)*

*Effects on synaptosomal membranes*

The effect of PER on synaptosome membrane-bound enzymes has been investigated by Korpela (149). Synaptosomes isolated from rat brain were exposed to PER, and the activity of the following enzymes were determined: acetylcholinesterase, total ATPase and magnesium-activated ATPase (($Mg^{2+}$) ATPase). Slight but significant inhibitions were observed. Aromatic solvents and, in particular, 1,1,2,2-tetrachloroethane appeared to be stronger inhibitors.

Edelfors and Ravn-Jonsen (72) investigated the effects of PER on 1) the activity of the enzyme ($Ca^{2+}$/$Mg^{2+}$) ATPase in a preparation of synaptosomal membranes isolated from the rat brain and 2) the fluidity of the isolated membranes. PER exerted a biphasic effect on the enzyme activity. At lower concentrations an increase was found, while above a certain concentration, the activity tended to decrease again. Furthermore, a slight decrease of the membrane fluidity was observed.

*Effects on erythrocytes*

The results of Holmberg *et al*. (116) show that PER and other volatile anaesthetic compounds have a protecting effect on erythrocytes, which are subjected to hypotonic hemolysis. This effect appeared to be correlated with the octanol/water partition coefficient. It is hypothesised by the authors, that the effect is based on a solvent-induced increase of the membrane stability of the erythrocytes.

These effects have also been found by other authors, and a correlation with anaesthetic effect has been demonstrated (248).

Korpela and Tähti (150) determined the activity of cholinesterase in human erythrocyte membranes and the effect of PER thereon. A strong inhibition was found (remaining activity less than 40% at a dose of 4 000 ppm (27 650 mg/$m^3$); the dose unit is not clear).

Hidalgo *et al*. (114) investigated lipid peroxidation and haemoglobin breakdown in rat erythrocytes *in vitro* upon the exposure to a series of halo compounds, including PER. Oxyhaemoglobin was taken as an index for remaining haemoglobin, while lipid peroxidation was measured as the concentration of thiobarbituric-acid-reactive substances (TBARs). Both, haemoglobin breakdown and lipid peroxidation were found to depend strongly on the halo compound tested. PER induced a significant breakdown, while the increase (4 times compared with the control) of TBARs was not significant.

*Effects on hepatocytes*

Kefalas and Stacey (140) did not find effects of PER on lipid peroxidation (assayed as TBARs), enzyme leakage (LDH and ALT) and leakage of potassium ions in hepatocytes isolated from rats. However, on coincubation with carbon tetrachloride, the compounds had a potentiating effect on the leakage of the two enzymes caused by carbon tetrachloride. Indications of a decrease of ($Mg^{2+}$) ATPase were found upon exposure to PER alone.

*Intercellular communication*

PER and other chlorinated hydrocarbons with anaesthetic properties sensitise the heart to epinephrine-induced arrhythmias. Toraason *et al*. (270) tested the hypothesis that the underlying mechanism of this effect is the inhibition of intercellular communication between the cardiac myocytes. This they did by investigating the effect of the compounds on intercellular communication between cardiac myocytes *in vitro*. They found a clear inhibition for all compounds tested, including PER. The inhibition was strongly correlated with the octanol/water partition coefficient. PER was found to be an effective inhibitor, with an $EC_{50}$ value of 0.39 mM, compared with 21.05 found for methylene chloride (the least effective) and 0.2 for pentachloroethane (the most effective).

*6.2.7 Genotoxicity and cell transformation*

The genotoxicity and cell transformation studies carried out with PER have been extensively reviewed in the past (3, 69, 74, 119, 231, 277). Together, these reviews give a clear impression of the genotoxic properties of the compound. This section is largely based on these reviews. In addition, some more recent publications, as well as publications which are deemed of particular importance for interpretation and evaluation, will be treated in more detail.

*In vitro studies: Bacteria*

EPA (74) mentions one study, which indicates mutagenicity in bacteria (36). An induction of histidine prototrophic mutants was found in *Salmonella typhimurium* TA100 (spot test with undiluted compound). However, EPA (74) concludes that

the publication in question cannot be used for "risk characterisation", because it lacks information on the purity of the compound. In particular the presence of stabilisers might lead to a false positive.

Other studies on the mutagenicity in bacteria did in general not yield positive results (13, 40, 105, 111, 151, 173, 188, 255, 289, 291). Strains used in these studies were the usual set of Salmonella strains, the less commonly used Salmonella strains UTH 8413 and UTH 8414, and *Escherichia coli* K12. The influence of mammalian metabolism was investigated by using rat, hamster or mice liver homogenates from animals treated with either Aroclor-1254 or phenobarbital as inducers of hepatic biotransformation enzymes. PER was tested in the fluid or the vapour phase. In some of these studies indications for mutagenicity were obtained with commercial or technical samples. However, the same studies yielded negative results when highly purified PER was tested, which shows that the mutagenicity is caused by other compounds than PER. PER was negative in a SOS-repair assay with *Escherichia coli* (SOS-chromo test).

*In vitro studies: Yeasts*

PER has been extensively tested for genotoxicity in *Saccharomyces cerevisae* by Bronzetti *et al*. (29). The following endpoints were studied: point mutations, mitotic gene conversion, and mitotic gene recombination. The results do not indicate a significant genotoxic effect. Other studies yielded negative, equivocal or borderline results (severe toxicity, lack of adequate positive controls, unknown purity (69, 119).

*In vitro studies: Mammalian cells, genotoxicity*

PER failed to induce mutations in mouse lymphoma cells (L5178Y/+/-), sister chromatid exchanges in Chinese hamster ovary cells, and chromosomal aberrations in Chinese hamster ovary cells (88, 208).

Furthermore, negative results were obtained in tests for the induction of unscheduled DNA synthesis in human lymphocytes, W-38 human cells and rat and mouse hepatocytes (14, 43, 188, 291). However, a positive result at toxic doses is reported for hepatocytes (species not specified) in an abstract (251). The PER used in this study was stabilised.

*In vitro studies: Mammalian cells, cell transformation*

PER was found to be negative in a cell-transformation assay with BALB/c-3T3 cells (274). The same study yielded positive responses for other halogenated compounds, such as vinyl chloride and 1,1,1-trichloroethane, indicating that the cells were capable of metabolic activation of related compounds. Milman investigated PER with the same type of cell transformation assay, with the same negative result. Furthermore, negative results were obtained in an assay with BHK 21/C13 cells (188). A positive result was scored in an assay with rat embryo cells F1706p108 (227). The cells used in the latter assay were infected with Rauscher leukaemia virus.

*In vivo studies with insects*
No positive effects were obtained upon testing in the sex-linked recessive lethal test with *Drosophila melanogaster*. The animals were exposed to PER via inhalation, feeding or injection (14). Furthermore, no effects on the chromosomes were found in this test animal (14).

*In vivo studies with mammals: Host-mediated assay*
EPA (74) mentions one study, which shows positive results in a host-mediated assay employing the *Salmonella typhimurium* strains TA1950, TA1951, and TA1952 in mice (36). However, EPA (74) concludes that the publication in question cannot be used for "risk characterisation", because it lacks information on the purity of the compound. A positive result was also found in another host-mediated assay (14). The substance tested had a rather low purity (91.43%), which means that the effect can be caused by other compounds. (e.g. stabilisers).

Bronzetti *et al*. (29) performed a host-mediated assay with mice and yeast (unusual protocol, lack of positive control), without positive results.

*In vivo studies with mammals: Cytogenetic studies*
Chromosomal aberrations were studied in the bone marrow of rats exposed to up to 600 ppm (4 134 mg/m$^3$) for 12 months and in the bone marrow of mice exposed by single or repeated intraperitoneal injections (14, 36, 229). One study yielded an equivocal increase (the test substance had a low purity); the other were negative.

*In vivo studies with mammals: Sperm-head abnormalities*
Three sperm-head abnormality tests are described in the literature, carried out with Chinese hamsters, mice or rats (14, 184). A positive result was only found in mice. However, the test material used was of a low purity.

*In vivo studies with mammals: Dominant lethality*
A negative result was obtained in a dominant-lethal test with rats (100-500 ppm (689-3 445 mg/m$^3$), 7 hours/day, 5 days) (14).

*In vivo studies with mammals: Unscheduled DNA synthesis*
No unscheduled DNA synthesis was induced in rat kidney cells after oral exposure to 1 000 mg/kg (98).

*In vivo studies with mammals: Single-strand breaks*
Walles (286) found single strand breaks in the liver and kidneys, but not in the lungs of mice treated with PER (99.8%; one injection; 4-8 mmol/kg) by intraperitoneal injection. A remarkable finding was that PER showed a higher potency than trichloroethylene. The latter compound is oxidised to TCA more rapidly and to a greater extent than PER. This might imply that it is not oxidative biotransformation alone, which leads to the DNA damage.

*6.2.8 Summary*

*Irritation, sensitisation, and acute toxicity:*

- PER has been shown to possess skin-irritating and eye-irritating properties in studies with experimental animals.
- No indications for skin sensitisation were obtained in a guinea-pig split-adjuvant test.
- No indications for respiratory sensitisation were obtained in the multitude of respiratory toxicity studies carried out with PER, although these were not specifically aimed at the observation of this effect.
- The acute lethality of PER has been investigated for various mammalian species after oral, respiratory, intraperitoneal, intratracheal and subcutaneous exposures. $LD_{50}$ values, $LC_{50}$ values and minimum lethal doses show the acute toxicity of PER to be rather low. Several grams per kilogram have to be administered to cause lethality via the oral, subcutaneous or intraperitoneal route. For the intravenous route, these doses are considerably lower (minimum lethal dose for dogs: 85 mg/kg). An intratracheal study yielded an approximate lethal dose of 450 mg/kg. Respiratory $LC_{50}$ values varied between 3 000 and 6 000 ppm (20 700-41 340 mg/m$^3$) (duration: 2-8 hours).
- Acute lethality is due to respiratory failure following deep coma.
- Acute respiratory exposure of experimental animals may lead to various neurotoxic effects, among them hyperactivity, hypoactivity, hypotonia, loss of reflexes, lateral position, drowsiness, trembling, ataxia, "drunken" stupor, anaesthesia and coma. The lowest observed effective dose was 90 ppm (620 mg/m$^3$). Indications for neurotoxicity were also obtained after acute oral, intraperitoneal, and intravenous exposure. Complete anaesthesia requires exposure to several thousand ppm for at least a few minutes.
- A weak stimulating effect on epinephrine-induced cardiac arrhythmias has been observed in rabbits after respiratory exposure to 5 200 ppm (35 800 mg/m$^3$) for 1 hour. The effect was expressed more clearly after intravenous injection.
- Acute exposure may lead to clear-cut hepatotoxicity expressed in histopathological and biochemical changes, among them, increase of liver weight, cloudy swelling, fatty infiltration, congestion, infiltration of Kupffer cells, necrosis, fatty degeneration, increases of triglycerides and lipids, anisokaryosis, anisocytosis, increases of serum-enzyme activities. In general these effects were observed at doses approaching those causing anaesthesia or lethality.
- Acute exposure may lead to clear-cut nephrotoxicity expressed in histopathological and biochemical changes, among them, scattered necrotic and degenerative lesions, fatty changes, congestion, cloudy swelling and vacuolisation of tubule cells, hyaline casts, oedema, reticulation, atrophy and proteinuria. In general, these effects were observed at doses approaching those causing anaesthesia or lethality.
- Other effects observed after acute exposure are: affected respiratory immunity against bacterial infections (respiratory exposure of mice to 25 and 50 ppm

(172 and 345 mg/m$^3$)) and change of pancreatic secretion (intraperitoneal, 1.3 ml/kg, rat).

*Toxicity due to short-term exposure:*

- The effects on behaviour and related neurotoxicological effects which are observed in short-term studies, do not differ essentially in a qualitative or quantitative sense from the effects observed in acute respiratory toxicity studies. Lower concentrations can to a certain extent be compensated for by longer exposure. However, repeated exposure leads to tolerance, indicating changes on a toxicodynamic (sensitivity of the endpoint) or a toxicokinetic level (biotransformation).
- Short-term respiratory exposure[3] of experimental animals leads to various neurochemical effects in the brain, among them small, partly reversible changes in fatty-acid pattern of phospholipids (rats, gerbils and guinea pigs), slight decreases of the DNA concentration in specific parts of the brain (rat, gerbil), changes in concentrations of neural and glial cytoskeletal proteins (rat, gerbil) and decreases of the amount of acetyl choline in the rat striatum. The lowest observed effect level was 60 ppm (413 mg/m$^3$): 4 months of continuous exposure resulted in a decreased DNA concentration in gerbil brains.
- Respiratory exposure of mice to 37 ppm (255 mg/m$^3$) resulted in an increase of butyrylcholinesterase activity. The significance of this finding is unknown.
- Marked signs of hepatotoxicity, histopathological as well as biochemical, are observed after short-term exposure to PER. In addition to the effects observed in the acute studies, the following can be mentioned: damage to cytoplasmic and mitochondrial structures of parenchyma cells, peroxisome proliferation, decreases of cell glycogen content, deposition of glycoproteins in blood vessels, changes of the DNA and the RNA content, parenchymatous degeneration and increase of cytochrome P450 levels. As the severity of the effects seems to correlate with the rate of oxidative metabolism, they are most probably caused by products of this metabolic pathway. TCA has been identified as an important hepatotoxic metabolite of PER. In particular, this compound is held responsible for the peroxisome proliferation induced by PER in mouse liver. Because of its high rate of oxidative metabolism, the mouse is by far the most sensitive species investigated so far. A subacute oral study with mice and rats showed clear effects for the first species at 100 mg/kg body weight (the lowest dose investigated), while only marginal effects were found at the highest dose in rats (1 000 mg/kg body weight). Another subacute study yielded an oral NOAEL of 20 mg/kg body weight for mice, the next higher dose being 100 mg/kg. One study reveals for mice a significant increase of liver weight upon 30 days of continuous exposure to concentrations as low as 9 ppm (62 mg/m$^3$).

[3] In some of these studies the animals were continuously exposed, which makes it difficult to allocate these studies to the category ”short term” or ”long term”.

- Nephrotoxic effects due to short-term exposure to PER include increased weight and size, congestion, cloudy swelling, tubular karyomegaly, changes in urine concentrations of creatinine and para-aminohippuric acid, albuminuria, urinary excretion of $\alpha_{2u}$-globulin, electrophoretic shifts in composition of excreted proteins, PDA in tubule cells and peroxisome proliferation. In general it can be stated that doses causing nephrotoxic effects are higher than those causing hepatotoxic effects. Like hepatotoxicity, nephrotoxicity is characterised by a marked interspecies variation. Moreover, in rats the effects vary clearly with sex, males being more sensitive than females. Continuous exposure to 200 ppm (1 378 mg/m$^3$) for 13 weeks is the lowest effective respiratory dose in a short-term study. It was found with mice; the next lower dose of 100 ppm (690 mg/m$^3$) did not lead to renal toxicity; the critical effect was tubular karyomegaly. At least three mechanisms can be put forward to explain the nephrotoxicity of PER. The induction of peroxisome proliferation by PER is not restricted to the liver, but occurs in the kidneys as well. It is probably also in these organs caused by the most important oxidative metabolite, TCA. A possible second mechanism is related with the other biotransformation pathway: the conjugation with glutathione, which can be followed by the formation of cyto- and genotoxic products in the kidneys by β-lyase. Finally accumulation of $\alpha_{2u}$-globulin (PDA) in the cells of the proximal tubuli can be mentioned as a third mechanism, which has so far only been found to occur in male rats, and not in female ones, or in animals of either sex belonging to other animal species. One study suggests that peroxisomal proliferation (the first mechanism) does not contribute much to the nephrotoxicity upon respiratory exposure, while the strong male-rat specificity of the third mechanism is extensively documented. As toxicity is also observed in female rats and in both sexes of other species, it can thus be concluded that the second, or other, as yet unidentified, mechanisms play an important role.
- Very low concentrations in drinking water lead to histopathological effects in the spleen of mice, which are presumable caused by premature erythrocyte breakdown. Furthermore, respiratory exposure of mice (135 and 270 ppm, or 930 and 1 860 mg/m$^3$) caused reversible effects on lymphocyte, monocyte and neutrophile counts. Exposure to 185 ppm (1 275 mg/m$^3$) of rats resulted in effects on mast cells.
- One study points to a tumour-promoting effect of PER. The number of enzyme-altered foci in the liver of partly hepatectomised mice increased when a single treatment with an initiator was followed by repeated oral administration of PER.

*Toxicity due to long-term exposure and carcinogenicity:*

- Mice showed a statistically significant increase of hepatocellular carcinoma upon respiratory and oral exposure.

- TCA, the major metabolite of PER, induces hepatocellular carcinomas and adenomas in mice upon oral administration, which strongly suggests that the hepatocarcinogenicity of PER is actually caused by TCA.
- A not significant increase of tubular cell adenoma and adenocarcinoma was found for male rats upon respiratory exposure. Moreover both sexes of this species showed a significant increase of mononuclear cell leukaemia, a type of cancer with a high background incidence in the applied strain.
- PER did not induce skin tumours in a dermal topical carcinogenicity study and a dermal initiation/promotion study, both carried out with mice.
- Besides carcinogenicity, the chronic studies revealed overt hepatotoxicity and nephrotoxicity. In case of the rat, a clear sex dependence of nephrotoxicity was observed, males being more sensitive than females.
- The sensitivity of the mouse for the hepatocarcinogenic effects of PER can most probably be attributed to the higher rate of the oxidative biotransformation in this organism resulting in the peroxisome proliferator and carcinogen TCA.
- The sex-specificity of nephrotoxicity and the induction of kidney tumours can most probably be attributed to the male-specific formation of protein droplets in tubular cells.

*Teratogenicity and reproduction:*

- On the whole, teratogenicity studies do not point to overt teratogenic effects. In some, signs of foetotoxicity were found. One study points to foetal skeletal abnormalities. Another one shows neurological effects in pups of exposed dams. Dose levels were much higher than the lowest levels causing hepatotoxic, nephrotoxic or neurotoxic effects.
- A two-generation reproduction study showed effects on the number of pups born alive, number of pups per litter, pup survival and pup body weights. Some of these reproduction effects may, in part, be caused by maternal toxicity. However also these effect levels were higher than the lowest levels causing hepatotoxic, nephrotoxic or neurotoxic effects.

*In vitro studies:*

- PER inhibits ATPase, magnesium-activated ATPase and acetylcholinesterase and decreases membrane fluidity in synaptosomes isolated from rat brain. However the effects observed were only slight.
- Treatment with PER protects erythrocytes against hypotonic hemolysis, which is probably the result of an increase of membrane stability. Furthermore the compound inhibits erythrocyte cholinesterase and induces haemoglobin breakdown.
- Indications for a decrease of magnesium-activated ATPase in rat hepatocytes were found upon exposure to PER.
- PER appears to be an effective inhibitor of intercellular communication between cardiac myocytes, which might explain its potency to induce arrhythmias.

*Genotoxicity and cell transformation:*

- PER has been extensively tested for genotoxic effects with many combinations of endpoints and test organisms. This includes *in vivo* tests with mammals focused on one or more of the following endpoints: chromosomal aberrations in bone marrow, sperm-head abnormalities, dominant-lethal mutations, unscheduled DNA synthesis in kidney cells and DNA single-strand breaks in liver, kidneys and lungs.
- Most tests show clear-cut negative results. Positive results were reported for the following tests: two tests with bacteria (11 negative), an unscheduled DNA-synthesis test with hepatocytes (two tests negative), a sperm-head abnormality test (two negative), a test for DNA single-strand breaks in liver and kidneys.
- Most positive tests were poorly reported or were carried out with impure PER, while the effects scored were often equivocal. The importance of impurities is illustrated by testing pure PER alongside technical or otherwise impure PER, which yielded only positive effects for the latter.
- The induction of unscheduled DNA synthesis in hepatocytes and single-strand breaks in the liver may reflect local genotoxicity caused by the postulated first product of oxidative metabolism, the very reactive and mutagenic oxirane. Likewise, the single-strand breaks in the kidney could be caused by the local formation of mutagenic products from the conjugation pathway, see (23, 24).
- Nevertheless, in view of the great number, as well as the types of negative tests, the committees conclude that PER is virtually devoid of genotoxic properties in mammals.

# 7. Existing guidelines, standards and evaluations

## 7.1 General population

The ATSDR (3) has derived the following minimal risk levels (MRLs) for inhalatory and oral exposure of the general population to PER. Acute respiratory exposure: 0.6 ppm (4.1 mg/m$^3$); intermediate-duration respiratory exposure (subacute to semichronic): 0.0009 ppm (0.0062 mg/m$^3$); intermediate-duration oral exposure: 0.1 mg/kg/day.

The Integrated Risk Information System (IRIS) has established an oral reference dose of 0.01 mg/kg/day (75).

The Science Advisory Board of EPA regards the evidence for carcinogenicity strong enough to place PER between the categories “probable human carcinogen” and “possible human carcinogen” (3, 74). However, this classification is presently under review.

IARC classifies PER as “probable carcinogenic to humans”, i.e. the compound is placed in category 2A (124). The IARC concludes that there is “*limited evidence* in humans for the carcinogenicity of tetrachloroethylene”, while there is

"*sufficient evidence* in experimental animals for the carcinogenicity of tetrachloroethylene".

EPA's Office of Drinking Water has set a maximum contaminant level (MCL) for drinking water of 0.005 mg/l (3).

No acceptable daily intake (ADI) is allocated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (79).

## 7.2 Working population

### *7.2.1 Occupational exposure limits*

Occupational exposure limits established for PER in a number of countries are listed in Table 27.

**Table 27.** Limits for occupational exposure to PER in a number of countries.

| Country | Concentration | | Time relation | Note | Reference |
|---|---|---|---|---|---|
| | ppm | $mg/m^3$ | | | |
| The Netherlands | 35 | 240 | 8-h TWA | A | (265) |
| Germany | | | | A, B | (51) |
| USA, ACGIH | 25 | 170 | 8-h TWA | C | (1) |
| | 100 | 685 | 15-min TWA | | |
| USA, OSHA | 100 | | 8-h TWA | D | (3) |
| | 200 | | Ceiling | | (3) |
| | 300 | | | F | |
| USA, NIOSH | -/0.4 | - | REL/LOQ | E | (3) |
| Sweden | 10 | 70 | 8-h TWA | G | (264) |
| | 25 | 170 | 15-min TWA | | (264) |
| Denmark | 10 | 70 | 8-h TWA | A, G | (10) |
| United Kingdom | 50 | 345 | 8-h TWA | | (120) |
| | 100 | 689 | 15-min TWA | | |
| Finland | 10 | 70 | 8-h TWA | | (121) |
| Iceland | 10 | 70 | 8-h TWA | A | (282) |
| Norway | 6 | 40 | 8-h TWA | A, G | (9) |

A: Skin notation.
B: Kategorie III B, i.e., suspected carcinogen. No "MAK-Werte" was established because of the uncertainty about the formation of genotoxic metabolites in the kidneys due to glutathione conjugation at relatively low exposure levels. For the time being, it is advised to use the "BAT-Werte" (biological limit value; see Table 28) as an "Orientierunggrosse" (orientation value).
C: Animal carcinogen. The TLV is based on subjective symptoms and discomfort reported in human-volunteer studies, in particular eye irritation, dizziness and incoordination, at 100-200 ppm. Furthermore, it is considered that this TLV provides a large margin of safety with respect to possible liver injury.
D: Final-rule limit.
E: Potential occupational carcinogen. Minimise workplace exposure.
F: 5 minutes peak in any 3 hours.
G: In the list of substances considered to be carcinogenic.
REL/LOQ: Recommended exposure limit/limit of quantitation.

*7.2.2 Biological limit values*

Biological limit values have been defined in the USA and Germany (Table 28).

**Table 28.** Biological limit values in the USA and Germany.

| Country | Indicator compound | Matrix | Time of sampling | Concentration | Reference |
|---|---|---|---|---|---|
| USA | PER | Expired air | Prior to last shift of workweek | 5 ppm (34.5 mg/m$^3$) | (1) |
| USA | | Blood | Prior to last shift of workweek | 0.5 mg/l | |
| USA | TCA | Urine | End of workweek | 3.5 mg/l[a] | |
| Germany | PER | Blood | Prior to next shift | 1 mg/l | (51) |

[a] Regarded by the ACGIH as a non-specific value, the quantitative interpretation of which is ambiguous; it has a semi-quantitative value, and is, therefore, only suitable for screening purposes.

# 8. Hazard assessment

## 8.1 Assessment of health hazard

PER causes eye irritation in humans at concentrations of about 690 mg/m$^3$ (100 ppm), exposure concentrations that are found in occupational environments. Due to adaptation, possible complaints are expected to have a transient character at lower doses. Skin irritation is only reported after intensive skin contact with liquid PER, for instance through submersion or wearing PER-soaked clothes. No publications were found about irritation problems under normal occupational conditions.

The sensitising properties of PER are considered low.

In a few long-term human exposure studies, hepatotoxicity was observed between 1 378 and 2 756 mg/m$^3$ (200-400 ppm). Although experimental animal studies clearly demonstrate the hepatotoxicity of PER, a quantitative extrapolation from these studies to the human occupational situation is difficult. This is due to the high oxidative biotransformation capacity of PER in the liver of experimental animals, which leads to the formation of the proven hepatotoxic endproduct TCA. Furthermore, the rate of oxidative biotransformation is much slower in humans than in experimental animals, in particular mice. Thus, these animal studies are less relevant for the human situation.

Adequate human data on nephrotoxicity are lacking. Working in dry-cleaning facilities is only accompanied by biochemical changes, which suggest nephropathy. Due to the differences in biotransformation in case of nephrotoxicity, the results of experimental animal studies are inadequate for extrapolation to humans as well.

None of the epidemiological studies concerned with cancer mortality or incidence yielded conclusive results. Animal studies on carcinogenicity of PER have limited relevance for the human situation because of species susceptibility.

Furthermore, the hepatocarcinogenicity in mice and the possible nephrocarcinogenicity in male rats were clearly not confirmed by the extensive epidemiological data base. The occurrence of an apparently not life-style related increased incidence of oesophagus cancer in a PER-only cohort deserves further attention.

Although the outcome of a study on the semen quality of dry cleaners and their reproductive success in humans suggests an adverse effect, the available data on the effects of PER on reproductive capacity do not allow definitive conclusions since exposure to other solvents cannot be excluded. Moreover, such an effect is not confirmed by the only available animal experiment concerned with this endpoint.

Foetotoxicity (skeletal abnormalities in mice at 2 067 mg/m$^3$ or 300 ppm) and developmental toxicity (decreases in litter size and survival during lactation in rats at 6 900 mg/m$^3$ or 1 000 ppm) in animals occur at dose levels, which are well above those inducing other adverse effects.

The available data strongly suggest that human neurological functions are adversely affected at exposure levels of about 690 mg/m$^3$ (100 ppm) and higher. Most neurotoxicological effects (headache, dizziness, lightheadedness, flushing, difficulty in speaking, sleepiness, loss of inhibitions, exhilaration, feelings of elation, impaired motor coordination) observed in humans appear to be reversible. Furthermore, adaptation is reported. Some human studies and studies with experimental animals suggest that exposure to PER levels below 690 mg/m$^3$ (100 ppm) or even below 345 mg/m$^3$ (50 ppm) may lead to adverse neurological effects. In a volunteer study, at 345 mg/m$^3$ (50 ppm) PER, an increase in visual evoked potential and visual contrast sensitivity threshold was observed, whereas in another study increased serum prolactine levels and a poor neurotoxicological response were observed at median PER levels of about 100 mg/m$^3$ (15 ppm). However, both committees regard these studies as inconclusive, mainly because of methodological shortcomings as poor statistics, presentation of data, low number of exposed persons or the subjective character of reported symptoms. Still, they warrant extra caution in setting exposure limits.

### 8.2 Groups at extra risk

The available toxicological information on PER does not point to groups of humans which are at extra risk.

### 8.3 Scientific basis for an occupational exposure limit

Based on currently available toxicological information, the committees regard neurotoxicity as the most sensitive effect of human respiratory exposure to PER. An overview of the database reveals that neurotoxicological effects can be expected to occur in humans at exposure to concentrations of about 690 mg/m$^3$ (100 ppm), the LOAEL. At these concentrations also eye irritation is observed. From the available data the committees are of the opinion that a skin absorption may contribute significantly to the systemic effects of PER.

# 10. Summary

de Raat K. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and the Dutch Expert Committee on Occupational Standards. 133. Tetrachloroethylene (PER).* Arbete och Hälsa 2003;14:1-110.

At ambient temperatures, tetrachloroethylene (PER) is a colourless liquid with an ethereal odour. It is used as a solvent in dry cleaning, extraction and vapour degreasing of metals, as an intermediate in chemical synthesis, an anthelminthic, a heat-exchange fluid, and as a grain-fumigation agent.

A high respiratory absorption has been observed in humans. Dermal exposure of liquid PER can substantially contribute to the body burden. In humans, the major part of absorbed PER is exhaled unchanged, while only a small fraction is metabolised. The major metabolite trichloroacetic acid is excreted in the urine. PER accumulates in adipose tissue.

PER is a skin- and eye-irritating compound. Controlled exposure of volunteers to 75-80 ppm resulted in very slight irritation of the eyes, nose and throat during the first few minutes of exposure. Human case studies show that skin sensitisation may occasionally occur under occupational conditions. There are no clear indications for respiratory sensitisation.

Short-term inhalation exposure gives rise to a series of clear-cut neurotoxic effects in man. PER causes a reversible depression of the central nervous system. High doses lead to coma, followed by respiratory failure and death.

Overall, central nervous system effects can be expected in humans at short-term exposure to approximately 100 ppm. Reported effects include headache, dizziness, lightheadedness, flushing, difficulty in speaking, sleepiness, loss of inhibitions, exhilaration, feelings of elation, and impaired motor coordination. Some inadequately performed studies suggest neurological effects below 100 ppm (15 ppm median level).

Temporary liver and kidney damage has been reported in cases of acute poisoning of humans by inhalation and in a few long-term human exposure studies, hepatotoxicity was observed at 200-400 ppm.

No clear signs of teratogenicity were found in animal studies at high doses, however, some indication of foetotoxicity was observed.

The results from genotoxicity testing warrant the conclusion that exposure to PER, does not present a genotoxic risk to humans.

Several epidemiological studies deal with the effects of PER exposure on cancer incidence or cancer mortality but the results are inconclusive. Interpretation is often hampered by concomitant exposure to other solvents and limited by lack of control for lifestyle-related factors.

*Keywords:* cancer, hepatotoxicity, irritation, neurotoxicity, occupational exposure limit, perchloroethylene, review, risk assessment, tetrachloroethene, tetrachloroethylene

# 11. Summary in Swedish

de Raat K. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and the Dutch Expert Committee on Occupational Standards. 133. Tetrachloroethylene (PER).* Arbete och Hälsa 2003;14:1-110.

Vid rumstemperatur är tetrakloretylen (PER) en färglös vätska med eterisk lukt. PER används som lösningsmedel vid kemtvätt, vid extraktion och ångavfettning av metaller, som intermediär vid kemisk syntes, läkemedel vid maskinfektion, som köldmedium och vid rökning av säd.

Ett högt upptag via lungorna har observerats hos människa. Hudexponering för PER i vätskeform kan bidra väsentligt till kroppsdosen. Merparten av absorberad PER kommer hos människa att utandas i oförändrad form. Endast en liten del metaboliseras. Huvudmetaboliten triklorättiksyra utsöndras i urinen. PER ackumuleras i fettväv.

PER irriterar hud och ögon. Kontrollerad exponering av frivilliga försökspersoner för 75-80 ppm gav upphov till mycket lätt irritation i ögon, näsa och hals under de första minuterna av exponeringen. Fallrapporter visar att hudsensibilisering kan uppstå vid yrkesexponering. Det finns inga klara indikationer på respiratorisk sensibilisering.

Korttidsexponering via inandning ger upphov till flera klart neurotoxiska effekter hos människa. PER ger upphov till reversibel depression av centrala nervsystemet. Höga doser leder till koma och död.

Sammantaget kan centralnervösa effekter på människa förväntas vid korttidsexponering för cirka 100 ppm. Rapporterade effekter är bland annat huvudvärk, svindel, yrsel, rodnad, talsvårigheter, sömnlöshet, minskade hämningar, känslor av upprymdhet och glädje samt försämrad koordination. Några ofullständigt genomförda studier antyder neurologiska effekter under 100 ppm (15 ppm medianvärde).

Tillfälliga lever- och njurskador hos människa har rapporterats vid fall av akut förgiftning via inandning. I ett fåtal långtidsstudier på människa har levertoxicitet observerats vid 200-400 ppm.

Inga tydliga tecken på teratogenicitet har setts i djurförsök vid höga doser, men vissa indikationer på fostertoxicitet har observerats.

Resultaten från genotoxicitetsstudier tyder på att exponering för PER inte utgör någon genotoxisk risk för människa.

Flera epidemiologiska studier behandlar effekterna av PER-exponering på cancerincidens eller dödligheten i cancer, men resultaten är svårbedömda. Tolkningen försvåras ofta av samtidig exponering för andra lösningsmedel och bristande kontroll för livsstilsrelaterade faktorer.

*Nyckelord:* cancer, gränsvärden, hepatotoxicitet, irritation, neurotoxicitet, perkloretylen, riskvärdering, tetrakloreten, tetrakloretylen, översikt

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## 13. Data bases used in the search for literature

Literature was retrieved from the on-line databases: MEDLINE, TOXLINE, and Chemical Abstracts. An additional search has been carried out in February 1996. From the papers published between February 1996 and June 2002 only the ones influencing hazard evaluation have been included in the report.

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