
Tetrachloroethylene (PER)

Health-Based Recommended Occupational Exposure Limit



Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

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Mijnheer de staatssecretaris,

Bij brief van 3 december, nr DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid de Gezondheidsraad om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen.

In dat kader bied ik u hierbij een advies aan over tetrachloorethyleen (PER). Dit advies is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving. Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volksgezondheid, Welzijn en Sport en de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieu.

Hoogachtend,

prof. dr JA Knottnerus

Tetrachloroethylene (PER)

Health-Based Recommended Occupational Exposure Limit

Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands,
in cooperation with the Nordic Expert Group for criteria
Documentation of Health Risks from chemicals

to:

the Minister and State Secretary of Social Affairs and Employment

No. 2003/01OSH, The Hague, January 14, 2003

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues...” (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Preservation & Fisheries. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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Samenvatting en advieswaarde

1 Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid beveelt de Gezondheidsraad gezondheidskundige advieswaarden aan voor beroepsmatige blootstelling aan toxische stoffen in lucht op de werkplek. Deze aanbevelingen worden opgesteld door de Commissie WGD van de Raad, de opvolgster van de Werkgroep van Deskundigen. Zij vormen de eerste stap in een drietraps-procedure die moet leiden tot de wettelijke grenswaarden (MAC-waarden).

Het voorliggende rapport is tot stand gekomen door samenwerking van de Commissie WGD met de NEG (Nordic Expert Group). In dit rapport bespreken de commissies de gevolgen van blootstelling aan tetrachloorethyleen (PER) en presenteert de Commissie WGD een gezondheidskundige advieswaarde voor deze stof. De conclusies van de commissies zijn gebaseerd op wetenschappelijke publicaties die vóór juli 2002 zijn verschenen.

2 Fysische en chemische eigenschappen

Onder normale omstandigheden is PER (CAS 127-18-4) een kleurloze vloeistof met een etherische geur. PER is goed oplosbaar in ethanol en diethylether; in water kan PER worden opgelost tot 150 mg/l. Bij 100 kPa is het kookpunt 121°C en het vriespunt -22.4°C.

PER wordt gebruikt als oplosmiddel in stomerijen, bij extractie en ontvetting van metalen, als intermediair in chemische syntheses, als een anti-wormmiddel, als warmte uitwisselingsvloeistof en als een ontsmettingsmiddel voor granen.

3 Monitoring

Diverse gevalideerde methoden zijn beschikbaar voor de bepaling van PER in lucht en biologische monsters. Voor de bepaling van PER in lucht wordt actief kool of Tenax* gebruikt voor de absorptie. Het absorbens of geëxtraheerde materiaal wordt geanalyseerd door middel van gaschromatografie met 'electron-capture' detectie, vlam-ionisatie detectie of massaspectrometrie.

Gevalideerde methoden voor biologische monitoring zijn gebaseerd op PER analyses van uitgedemde lucht of bloed en geven een betrouwbaar beeld van een tijd-gewogen-gemiddelde blootstelling over diverse dagen. Uitscheiding via de urine van trichloorazijnzuur, de meeste belangrijke metaboliet van PER in kwantitatieve zin, zou ook gebruikt kunnen worden als parameter voor humane blootstelling. Echter de analyse van PER in uitgedemde lucht of bloed geeft een beter betrouwbare schatting van de blootstelling.

4 Huidige Grenswaarden

De huidige wettelijke grenswaarde (MAC waarde, TGG 8 uur) in Nederland is 240 mg/m³ (35 ppm). In Zweden is de grenswaarde 69 mg/m³ (10 ppm). Het Verenigd Koninkrijk heeft een grenswaarde van 345 mg/m³ (50 ppm; TGG 8 uur) en in de VS heeft de American Conference of Governmental Industrial Hygienists (ACGIH) een grenswaarde van 170 mg/m³ (25 ppm; TGG 8 uur) vastgesteld.

MAC-TGG 15 min waarden zijn vastgesteld in Zweden (170 mg/m³, 25 ppm, het Verenigd Koninkrijk (689 mg/m³, 100 ppm) en in de VS (ACGIH, 85 mg/m³, 100 ppm).

Biologische grenswaarden zijn vastgesteld in Duitsland (1 mg/l PER in bloed) en door de ACGIH (5 ppm PER in uitademingslucht en 0.5 mg/l bloed). In Nederland geldt een indicatie voor huidopname.

5 Kinetiek

In principe komt al het geïnhaleerde PER in de bloedbaan terecht. Afhankelijk van de geïnhaleerde concentratie aan PER wordt bij de mens een opname van 50-90%

* Een polymere stof voor de sorptie van gasvormige organische componenten van lucht. Desorptie wordt verkregen door verwarming; zodat de gesorbeerde componenten niet opgelost hoeven te worden in een oplosmiddel .

gevonden. Gegevens van huidopname zijn schaars. Huidopname van PER damp is verwaarloosbaar in vergelijking met de inhalatoire opname van PER damp. Uit dierexperimenten blijkt dat de orale opname vrijwel volledig is.

PER hoopt zich op in het vetweefsel. Het is onbekend hoe lang het duurt voordat een evenwicht is bereikt tussen ophoping in vetweefsel en vrijkomen uit het vetweefsel. Relatief hoge concentraties PER worden verwacht in hersenen, nieren, longen en lever.

In de mens wordt het merendeel van PER onveranderd uitgeademd, slechts een kleine fractie (schatting 1-2%) wordt omgezet. De voornaamste metaboliet is trichloorazijnzuur, een product van de oxidatieve omzettingroute, die in de urine wordt uitgescheiden. Halfwaardetijden voor inhalatoire uitscheiding variëren van 1-72 uur voor de mens, afhankelijk van de relatieve concentraties van PER in bloed, vet of ander weefsel.

6 Effecten

Irritatie en sensibilisatie

PER is voor mens en dier een huid- en oogirriterende stof. Studies met vrijwilligers laten zien dat blootstelling aan concentraties onder 690 mg/m^3 (100 ppm) resulteren in tijdelijke irritatie van ogen, neus en keel. Humane studies, in tegenstelling tot studies in dieren, laten zien dat op de werkplek zo nu en dan sensibilisatie via de huid op kan treden. Er is geen indicatie voor sensibilisatie via inhalatie.

Letaliteit

PER vertoont een lage acute letaliteit na inhalatoire of orale blootstelling. Letaliteit wordt veroorzaakt door ademhalingsstoornis, hetgeen veroorzaakt wordt door acute neurotoxiciteit.

Neurotoxiciteit

Acuut toxische inhalatoire blootstelling veroorzaakt duidelijke neurotoxische effecten in mens en dier. PER veroorzaakt een omkeerbare onderdrukking van het centrale zenuwstelsel, hetgeen in het verleden heeft geleid tot gebruik van PER als humaan anaestheticum. Hoge doses leiden tot coma, gevolgd door ademhalingsstoornissen en dood.

Patiënten rapporten suggereren dat langdurende inhalatoire blootstelling tussen 1378 mg/m^3 (200 ppm) en 2756 mg/m^3 (400 ppm) PER diverse reversibele neurologisch effecten kan geven in de mens. Ook in humane vrijwilliger studies

worden reversibele neurologische symptomen gevonden vanaf 690 mg/m³ (100 ppm). De neurotoxiciteit van PER wordt bevestigd door diverse epidemiologische studies. De gerapporteerde effecten zijn: hoofdpijn, duizeligheid, opvliegingen, licht in het hoofd, moeizaam spreken, slapeloosheid, ontremming, opgewektheid, opgetogenheid en een vertraagde motorische coördinatie.

In sommige studies worden neurotoxicologische effecten waargenomen bij PER concentraties lager dan 690 mg/m³ (100 ppm): in een vrijwilligersstudie zijn bij 100 mg/m³ PER verhoogde serum prolactine concentraties gevonden en in een andere studie zijn bij 345 mg/m³ verhoogde 'visual evoked potential' en 'visual sensitivity thresholds' waargenomen. Vanwege methodologische tekortkomingen en onduidelijke resultaten acht de DECOS deze studies onvoldoende voor beoordeling van de risico's voor de mens.

De neurotoxische effecten uit de kortdurende dierexperimentele inhalatiestudies verschillen vrijwel niet met die gevonden in de acute inhalatiestudies. Lage concentraties kunnen tot een bepaalde hoogte gecompenseerd worden door langere blootstellingsduur. Echter, na herhaalde blootstelling treedt adaptatie op.

Lever- en niertoxiciteit

Acute inhalatoire blootstelling van vrijwel anaesthetische of letale doseringen van dieren veroorzaakt effecten aan lever en nieren. Tijdelijke lever- en nierschade wordt ook gerapporteerd in gevallen van acute vergiftiging van mensen na inhalatie van PER.

In patiënten rapporten, zijn levereffecten beschreven na langdurende inhalatoire blootstelling tussen 1378 en 2756 mg/m³ (200 en 400 ppm).

Kortdurende inhalatoire of orale blootstelling van dieren veroorzaakt (histo)patologische en biochemische effecten in de lever, waaronder peroxisoom proliferatie. Omdat de ernst van deze effecten gecorreleerd is met de snelheid van oxidatieve PER biotransformatie, worden ze mogelijk veroorzaakt door producten van deze metabole route. Trichloorazijnzuur wordt gezien als een belangrijke metaboliet die levertoxiciteit veroorzaakt. Deze stof is een effectieve induceerder van peroxisoom proliferatie en wordt daarom verantwoordelijk gehouden voor dit effect in dieren na blootstelling aan PER.

Niertoxiciteit is ook gesignaleerd in studies na kortdurende inhalatoire of orale blootstelling. In de muis is een geen-waargenomen-nadelig-effect-niveau (NOAEL) gevonden van 690 mg/m³ (100 ppm), vanwege karyomegalie in de nierbuisjes. Peroxisoom proliferatie wordt gevonden in de nier van de muis (niet in de rattennier), na orale gavage blootstelling aan 1 g/kg bw/dag gedurende 10 dagen. In een andere studie waar ratten gedurende 28 dagen aan 500 mg/kg bw/dag werden blootgesteld zijn histopatologische en biochemische effecten gevonden die duiden op schade aan de

nierbuisjes. Ophoping van eiwitdruppels ('protein droplet formation/accumulation'; a-2-u-globuline) is specifiek gevonden in mannelijke ratten na 10 dagen orale gavage dosering van 1 g/kg bw/dag.

Langdurende studies bevestigen de lever- en niertoxiciteit van PER. Niereffecten zijn duidelijk sexe gerelateerd in de rat; mannetjes zijn veel gevoeliger dan vrouwtjes.

Hematologische effecten

Na blootstelling van muizen aan 930 mg/m³ (135 ppm) en 1860 mg/m³ (270 ppm) (6 uur/dag, 5 dagen/week, 7.5 weken) zijn reversibele effecten op het aantal witte bloedcellen gevonden.

Carcinogeniteit

Diverse epidemiologische studies hebben het effect van blootstelling aan PER op het voorkomen van en overlijden aan kanker bestudeerd. Geen kanker van de nier, lever of witte bloedcellen is waargenomen in de mens, in tegenstelling tot de gevonden nier-, leverkanker en leukemie bij proefdieren. Aangezien in de meeste epidemiologische studies blootstelling aan andere oplosmiddelen dan PER niet kon worden uitgesloten, kan de commissie uit deze studies geen definitieve conclusies trekken.

Sommige studies duiden op een toegenomen risico van slokdarmkanker, welke niet volledig kunnen worden toegeschreven aan levensstijl gerelateerde factoren als rook- en drinkgedrag. De commissie kan vooralsnog uit deze studies geen conclusie trekken en acht nader onderzoek noodzakelijk.

Na inhalatoire en orale blootstelling van muizen is een toename van de incidentie van levercelcarcinomas gevonden. Deze tumoren zijn ook gevonden in muizen na orale toediening van trichloorazijnzuur (TCA), de belangrijkste oxidatieve metabooliet van PER, wat aangeeft dat het carcinogene effect het resultaat kan zijn van de vorming van deze metabooliet. Omdat zowel PER als haar voornaamste metabooliet TCA peroxisoom proliferatie induceren, wordt algemeen verondersteld dat de lever carcinogeniteit afhankelijk is van dit effect.

In mannelijke ratten is een kleine toename van nierbuis-cel adenoma en carcinoma gevonden in een chronische inhalatie studie. Het is onduidelijk in hoeverre dit effect werkelijk PER gerelateerd is, mogelijk is het gecorreleerd met de eerder genoemde eiwitdruppelvorming. De studie geeft ook een toename van de incidentie van eenkernige cel leukemie aan. Echter, de achtergrond incidentie van dit effect in de gebruikte rattenstam is erg hoog.

Genotoxiciteit

PER is uitgebreid getest voor genotoxiciteit in veel verschillende combinaties van eindpunten en testorganismen. Over het geheel genomen mag geconcludeerd worden dat PER niet genotoxisch is voor de mens.

Reproductie toxicologie

Er is enig epidemiologisch bewijs voor effecten op de fertiliteit (zaad kwaliteit) en ontwikkeling (spontane abortussen) bij medewerkers in stomerijen. Het is echter niet duidelijk of deze effecten worden veroorzaakt door PER of door (een combinatie van) andere oplosmiddelen gebruikt in de stomerij.

Geen duidelijke signalen voor teratogeniteit zijn gevonden in dierstudies bij hoge concentraties PER, wel zijn er indicaties voor fetale toxiciteit. In een reproductie studie, is een afname in nest-grootte en overleving tijdens de lactatieperiode gevonden bij een concentratie van 6900 mg/m³ (1000 ppm), maar niet bij 2070 mg/m³ (300 ppm). Bij 6900 mg/m³ werd echter ook enige toxiciteit van het moederdier gevonden.

7 Evaluatie, advieswaarde en huidnotatie

PER kan huid- en oogirritatie veroorzaken en ook wordt incidenteel huid sensibilisatie waargenomen in de mens. De effecten gezien bij de laagste concentratie zijn voornamelijk van neurotoxische aard.

Ofschoon uit proefdierstudies duidelijke hepatotoxiciteit van PER blijkt, kunnen deze studies niet voor extrapolatie naar de humane arbeidssituatie gebruikt worden. Dit heeft te maken met de snelle oxidatieve PER biotransformatie in de lever van proefdieren (met name muis), die leidt tot de vorming van het hepatotoxische trichloorazijnzuur, dat mogelijk verantwoordelijk is voor de levereffecten. Daarom acht de commissie deze effecten als niet relevant voor de beoordeling van de risico's voor de mens.

Vanwege de speciesverschillen in PER biotransformatie in het geval van niertoxiciteit zijn de resultaten van proefdierstudies onvoldoende voor extrapolatie naar de mens.

De commissie concludeert dat neurotoxiciteit het kritische effect is. De beschikbare humane studies laten zien dat aantasting van neurologische functies begint bij een concentratie van 690 mg/m³ (100 ppm). Deze concentratie beschouwt de commissie als de laagst geobserveerde nadelig effect niveau (LOAEL). Hoewel in enkele studies neurologische effecten zijn waargenomen bij PER concentraties lager dan 690 mg/m³,

acht de Commissie WGD deze studies onvoldoende kwaliteit voor kwantitatieve risico evaluatie. Bij 690 mg/m³ is tevens oogirritatie gemeld.

Een relatieve hoge veiligheidsfactor van 5 wordt toegepast om te extrapoleren van een LOAEL naar een NAEL, waarbij tevens wordt gecompenseerd voor de onzekerheden in neurologische effecten bij lagere concentraties. Toepassing van deze factor geeft voor PER een gezondheidskundige advieswaarde van 138 mg/m³ (20 ppm), gemiddeld over een achturige werkdag.

Omdat dermale blootstelling een substantiële bijdrage kan leveren aan de lichamelijke belasting met PER, stelt de Commissie WGD tevens een huidnotatie voor.

8 Gezondheidskundige advieswaarde

De Commissie WGD van de Gezondheidsraad stelt voor PER een gezondheidskundige advieswaarde voor van 138 mg/m³ (20 ppm), gemiddeld over een achturige werkdag. Ook stelt zij een huidnotatie voor.

Executive summary

1 Scope

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands recommends health-based occupational exposure limits for the concentration of toxic substances in air at the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS). It constitutes the first step in a three-step procedure that leads to legally-binding exposure limits (MAC-values).

The present report is a co-production of DECOS and the Nordic Expert Group. In the report the committees discuss the consequences of occupational exposure to tetrachloroethylene (PER). Furthermore, DECOS recommends a health-based occupational exposure limit. The committees' conclusions are based on scientific publications obtained from data retrieval systems prior to July 2002.

2 Physical and chemical properties

At ambient temperatures, PER is a colourless liquid with an ethereal odour. The compound is readily soluble in ethanol and diethylether; in water it can be dissolved up to a concentration of 150 mg/l. At 100 kPa its boiling point is 121 °C and its freezing point -22.4 °C.

PER is used as a solvent in dry cleaning, extraction and vapour degreasing of metals, as an intermediate in chemical synthesis, as an anthelmintic, as a heat-change fluid, and as a grain-fumigation agent.

3 Monitoring

Various validated methods exist for the determination of PER in ambient air and biological samples. In case of ambient air, active coal or Tenax* are used for adsorption. PER can be collected from biological samples by extraction, or by warming the sample, followed by trapping of the evaporated PER with an adsorbent. The adsorbed or extracted material is analyzed by means of gas chromatography with electron-capture detection, flame-ionization detection, or mass spectrometry.

Validated biological-monitoring methods are available. They are based on the analysis of exhaled air or blood for PER, and give a reliable impression of the (time weighted) average exposure over several days. Urinary excretion of trichloroacetic acid, which is the most important metabolite of PER in quantitative terms, is also proposed as a measure for human exposure. However, the analysis of the parent compound in exhaled air or blood is regarded to yield more reliable exposure estimates.

4 Current limit values

An occupational exposure limit (OEL; 8-h TWA) of 240 mg/m³ (35 ppm) is presently effective in the Netherlands. Sweden has a lower OEL, i.e. 69 mg/m³ (10 ppm). The United Kingdom established an OEL of 345 mg/m³ (50 ppm), 8-h TWA, while in the USA the American Conference of Governmental Industrial Hygienists (ACGIH) recommended an OEL of 170 mg/m³ (25 ppm), 8-h TWA.

Short-term exposure limits are established in Sweden (170 mg/m³, 25 ppm, 15-min TWA), the United Kingdom (689 mg/m³, 100 ppm, 15-min TWA), and by the ACGIH (685 mg/m³, 100 ppm, 15-min TWA).

Biological limit values are established in Germany (1 mg/l PER in blood prior to next shift) and by the ACGIH (5 ppm PER in expired air and 0.5 mg/l PER in blood prior to last shift of workweek, and 3.5 mg/l trichloroacetic acid in urine at the end of the workweek). The compound received a skin notation in the Netherlands.

* 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.

5 Kinetics

Actually all inhaled PER enters the blood. Depending on concentration inhaled, absorption percentages of 50-90% have been observed in humans under steady-state conditions.

Data on dermal absorption are scanty. However, immersion of the thumb in PER has been found to correspond with a respiratory exposure to 1.4 - 4.1 mg/m³ (0.2-0.6 ppm). For mice an absorption rate of 24.4 nmol/min per cm² has been determined. Dermal absorption of PER vapour is negligible compared to the respiratory absorption of the same vapour. Based on animal experiments, the oral absorption can be expected to be actually complete.

PER accumulates in the fat tissues. It is unknown how long it takes before an equilibrium is reached between accumulation in the fat and elimination from the fat. Furthermore, relatively high concentrations of PER are to be expected in brain, kidneys, liver and lungs.

In humans, the major part of the absorbed PER is eliminated in unaltered form via exhalation, while only a small fraction is metabolized (estimated to be 1-2%). The major metabolite is trichloroacetic acid, a product of an oxidative biotransformation pathway, which is excreted in the urine. Very low concentrations of a product of glutathione conjugation have been detected in human urine. The importance of this finding lies in the possible association with the formation of genotoxic metabolites in the kidney as a result of glutathione conjugation.

Half lives for respiratory elimination vary from 1-72 h for humans, depending on the relative concentrations of PER in blood, fat and other tissues.

6 Effects

Irritation and sensitisation

PER is a skin- and eye-irritating compound. Human volunteer studies showed that exposure to concentrations below 690 mg/m³ (100 ppm) resulted in transient irritation of the eyes, nose and throat. Although negative in an animal test for skin sensitisation, human case studies show that skin sensitisation may occasionally occur under occupational conditions. There are no clear indications for respiratory sensitisation.

Lethality

The compound has a low acute lethality after respiratory or oral exposure. Respiratory LC₅₀ values have been found to vary between 3000 and 6000 ppm (20,700 and 41,340 mg/m³), depending on animal species and exposure time. Lethality is caused by respiratory failure, which, in its turn, is a result of acute neurotoxicity.

Neurotoxicity

Acute toxic respiratory exposure gives rise to a series of clear-cut neurotoxic effects in man and experimental animals. The compound causes a reversible depression of the central nervous system, which has led in the past to its use as a human anaestheticum. High doses lead to coma, followed by respiratory failure and death.

Case reports suggest that longer-term respiratory exposure between 1378 mg/m³ (200 ppm) and 2756 mg/m³ (400 ppm) of PER results in various reversible neurological effects in humans. Also in a human volunteer study reversible neurological symptoms have been found at about 690 mg/m³ (100 ppm). The neurotoxicity of PER is confirmed by several epidemiological studies. Overall, the available information makes clear that neurotoxic effects have to be expected in humans after exposures to concentrations of about 690 mg/m³ (100 ppm) and higher. Reported effects include: headache, dizziness, lightheadedness, flushing, difficulty in speaking, sleepiness, loss of inhibitions, exhilaration, feelings of elation, and impaired motor coordination.

Some studies suggest neurological effects below 690 mg/m³ (100 ppm): volunteers exposed to 345 mg/m³ PER (50 ppm) showed increased 'visual evoked potential' and 'visual contrast sensitivity thresholds', whereas in another study increased serum prolactin levels and poor neurological responses were observed at a concentration of as low as 100 mg/m³ (median level). However, due to methodological shortcomings, or inconclusive results, these studies were not used by DECOS for hazard-assessment purposes.

The neurotoxic effects observed in short-term inhalation studies with experimental animals, do not differ essentially from those observed in acute inhalation studies. Lower concentrations can to a certain extent be compensated for by longer exposure. However, adaptation develops upon repeated exposure.

Hepatotoxicity and nephrotoxicity

In experimental animals, acute respiratory toxicity comprises effects on the liver and the kidneys at doses approaching anaesthetic or lethal levels. Temporary liver and

kidney damage has also been reported in cases of acute poisoning of humans by inhalation.

In case reports, effects on the liver were described after longer-term respiratory exposure between 1378 and 2756 mg/m³ (200 and 400 ppm). A single case report indicated that PER can be transferred to breast milk and might cause jaundice in breast-fed babies.

Short-term respiratory and oral exposure of experimental animals resulted in (histo)pathological and biochemical effects in the liver, among them peroxisome proliferation. As the severity of these effects was correlated with the rate of oxidative PER biotransformation, they were most probably caused by products of this metabolic pathway. Trichloroacetic acid has been identified as an important hepatotoxic metabolite. This compound is an effective inducer of peroxisome proliferation, and is, therefore, held responsible for this effect in experimental animals after PER exposure.

Nephrotoxicity has also been observed in respiratory and oral short-term studies. For mice, a no-observed-adverse-effect-level (NOAEL) of 690 mg/m³ (100 ppm) is established, based on tubular karyomegaly. Peroxisome proliferation has been observed in mouse kidneys, but not in rat kidneys, upon oral exposure by gavage to 1 g/kg bw/day for 10 days. In another study, exposure by gavage of rats for 28 days to 500 mg/kg bw/day resulted in histopathological and biochemical effects pointing to tubular damage. Protein-droplet accumulation was found specifically in male rats after 10 daily gavage doses of 1 g/kg.

The chronic studies confirmed the hepato- and nephrotoxicity of PER. The latter effect showed a clear-cut sex dependence in rats, males being much more sensitive than females.

Haematological effects

Exposure by inhalation of mice to 930 mg/m³ (135 ppm) and 1860 mg/m³ (270 ppm) (6 h/day, 5 days/week, 7.5 weeks) resulted in reversible effects on white-blood-cell counts.

Carcinogenicity

Several epidemiological studies dealt with the effects of PER exposure on cancer incidence or cancer mortality. No effects were found on the endpoints indicated by animal studies (carcinoma in liver and kidneys and leukemia). Interpretation of many epidemiological studies is hampered by concomitant exposure to other solvents in industry and limited treatment of risks of lifestyle-related factors. Some studies point to

an increased risk of oesophagus cancer, which cannot (fully) be attributed to lifestyle-related factors as smoking and drinking.

Statistically significant increases of the incidence of hepatocellular carcinoma were found in mice upon respiratory and oral exposure. These tumours are also induced in mice when they are treated orally with trichloroacetic acid, the major oxidative metabolite of PER, which indicates the carcinogenic effect to be the result of the formation of this compound. As both PER and its major metabolite induce peroxisome proliferation, it is generally assumed that the liver carcinogenicity depends on this effect.

In male rats, a small and statistically insignificant increase of renal tubular-cell adenoma and carcinoma was found in a chronic inhalation study. In so far as this finding can really be attributed to the treatment, it is probably connected to protein-droplet formation. However, the results of the study in question do not allow for a definitive conclusion on this point. The study also revealed a statistically significant increase of the incidence of mononuclear cell leukemia. However, the background incidence of this effect in the rat strain used appeared to be very high.

Genotoxicity

PER was extensively tested for genotoxicity with many combinations of endpoint and test organism. On the whole, the results warrant the conclusion that exposure to PER does not present a genotoxic risk to humans.

Reproduction toxicity

There is some epidemiological evidence for adverse effects on fertility (sperm quality) and on developmental toxicity (increased risk of spontaneous abortion) in dry cleaners. However, a possible contribution of PER could not be distinguished from contribution of other solvents used in dry cleaning.

No clear signs for teratogenicity were found in animal studies at high doses, however, some indication of fetotoxicity was observed. In a multigeneration reproduction study, some decreases in litter size and survival during lactation were seen at 6900 mg/m³ (1000 ppm) but not at 2070 mg/m³ (300 ppm). At 6900 mg/m³ also some maternal toxicity was observed.

7 Hazard assessment, HBR-OEL and skin notation

PER has skin- and eye-irritating properties, and has incidentally been shown to induce skin sensitisation in man. The effects observed at the lowest concentrations are largely

neurological in origin. Taken together, the available human database shows that affected neurological functions start to become manifest at doses around 690 mg/m³ (100 ppm).

In a few long-term human exposure studies, hepatotoxicity was observed between 1378 and 2756 mg/m³ (200 and 400 ppm). It is not justifiable to use hepatotoxicity data in experimental animals as a starting point in quantitative hazard assessment. Experimental animals (especially mice) have a specifically high sensitivity to the hepatotoxic effects of PER which is most probably linked with its high rate of oxidative biotransformation of PER. The hepatotoxic effects are, therefore, regarded by the committees to be irrelevant to humans in terms of predicting health effects in humans caused by exposure to PER.

Adequate human data on nephrotoxicity are lacking. Due to the differences in biotransformation in case of nephrotoxicity, the results of experimental animals studies are inadequate for extrapolation to humans as well.

The committees regard neurotoxicity as the most sensitive effect of human exposure to PER. The adverse effects on neurological functions are observed at a concentration of as low as 690 mg/m³. Some studies suggest that exposure to PER below 690 mg/m³ may lead to adverse neurological effects. However, both committees regard these studies as inconclusive, because of methodological shortcomings.

For deriving a health based occupational exposure limit (HBR-OEL), DECOS considers the concentration of 690 mg/m³ as the lowest-observed adverse effect level (LOAEL). At this concentration also eye-irritation is observed. A relatively large safety factor of five is applied for extrapolation from the LOAEL to the NAEL, taken into account the existence of inconclusive evidence for some minor neurological effects at concentrations lower than 690 mg/m³. Applying this safety factor, DECOS recommends a HBR-OEL for PER of 138 mg/m³ (20 ppm), as an eight-hour time-weighted average concentration.

As dermal exposure can substantially contribute to the body burden with PER, the DECOS recommends a skin notation.

8 Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based Occupational Exposure Limit for PER of 138 mg/m³ (20 ppm), 8-h TWA, as well as a skin notation.

Scope

1.1 Background

In the Netherlands occupational exposure limits for chemical substances are set using a three-step procedure. In the first step a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, on request of the Minister of Social Affairs and Employment (Annex A).

This evaluation should lead to a health-based recommended exposure limit for the concentration of the substance in air. Such an exposure limit cannot be derived if sufficient data are not available or if the toxic action cannot be evaluated using a threshold model. In the latter case an exposure-response relationship is recommended for use in regulatory standard setting.

In the next phase of the three-step procedure the Social and Economic Council advises the Minister on the feasibility of using the health based value as a regulatory Occupational Exposure Limit (OEL) or recommends a different OEL. In the final step of the procedure the Minister of Social Affairs and Employment sets the official Occupational Exposure Limit.

1.2 Committees and procedure

This document is a co-production of the DECOS and the Nordic Expert Group (NEG), hereafter called the committees. It is a result of an agreement between both groups to prepare jointly criteria documents which can be used by the regulatory authorities in the Netherlands and in the Nordic countries. The members of the committees are listed in annex B.

The draft document has been prepared by dr WK de Raat, from TNO Food and Nutrition Research, Department of Occupational Toxicology, by contract with the Ministry of Social Affairs and Employment, and was first reviewed by the DECOS and thereafter by the NEG. In 1998, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in annex C. These comments were taken into account in deciding on the final version of the document.

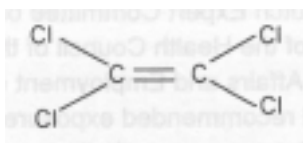
1.3 Data

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.

Identity, properties and monitoring

2.1 Identity

2.1.1 Structure



2.1.2 Chemical names and synonyms/registry numbers

Name	tetrachloroethylene
CAS registry number	127-18-4
RTECS	KX3850000
UN	1897.0000
EEC	602-028-00-4
EINECS	204-825-9

Synonyms	carbon dichloride	
	ethylene tetrachloride	
	per	
	perc	
	perchlor	
	perchloroethylene	
	perchloroethylen	
	perk	
	tetrachlorethylene	
	1,1,2,2-tetrachloroethylene	
	PCE	
	Abbreviation	PER (used in the present report)

2.2 Physical and chemical properties*

Molecular formula	:	C_2Cl_4
Molecular weight	:	166.0000
Boiling point (100 kPa)	:	121 °C
Freezing point (100 kPa)	:	-22.4 °C
Density (20°C)	:	1.62 g/ml
Vapor pressure (20°C/100 kPa)	:	1.9 kPa
Percentage of vapor in saturated air (20 °C/1 bar)	:	1.8
Vapor density (air=1; 100 kPa)	:	6.0000
Solubility in water	:	150 mg/l
Solubility in organic solvents	:	completely soluble in ethanol and diethylether
Physical form	:	liquid
Odor	:	ethereal
Odor threshold	:	5 ppm (34.5 mg/m ³)
Conversion factors 25°C, 1 atm.)	:	1 ppm = 6.89 mg/m ³ ; 1 mg/m ³ = 0.145 ppm

* Data obtained from WHO84, IARC95 and ACG02

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*, 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-ionization detection (FID) or mass spectrometry (MS), while the identification of the compound is based on retention time and mass spectra.

ISO method 9486:(E) (ISO91); 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 GS 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/m³ and 1000 mg/m³ (about 0.2 ml/m³ to 200 ml/m³) when 10 litres of air are sampled. Organic components which have the same or nearly the same retention time as PER in the gas chromatographic analysis will interfere. Proper selection of gas chromatographic columns and program conditions will minimise interference.

NEN method 2947/2964 (NEN99a, NEN00a); 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 2.5-1600 mg/m³ and has a detection limit of 238 µg/m³.

NEN method 2948/2965 (NEN99b, NEN00b); 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/m³ and has a detection limit of 0.1 µg/m³.

* 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 2950 (NEN99c); The sample is collected on a indicator tube and analysed by reading the colour change. The method has been validated over a range of 140-1150 mg/m³. The coefficient of variation was 25%.

NIOSH method S335 and S336 (NIO77); 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 655-2750 mg/m³, using a 3 litre 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.

IARC method 5 (IARC85a); 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 136-4060 mg/m³ using a 3 litre sample. The breakthrough volume is 21 litre at 2750 mg/m³. The limit of detection depends on the analyte and lies normally in the useful range.

IARC method 12 (IARC85b); 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 mass spectrometry. Component identification is normally accomplished by a library search routine, using GC retention times and mass-spectral characteristics. The limit of detection is generally of the order of 0.1-1.0 µg/m³.

BIA method 8690 (Sch89); The 'Berufsgenossenschaftliches Institut für Arbeitssicherheit' has published a method using Dräger active coal tubes, type B and gaschromatographic analysis using FID detection. The limit of detection limit is 1.2 mg/m³ for a air volume of 40 litre.

2.3.2 *Biological monitoring*

For biological-monitoring purposes, the concentrations of PER are determined in expired air, blood, or macerated tissues. 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 ECD (electron-capture detection); analysis can also be performed without concentration (head-space analysis). The solvent extracts are also analysed by GC/MS or GC with ECD.

IARC method 24 (IARC85c); This method can be used for the determination of PER concentrations 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 $0.33 \mu\text{g}/\text{m}^3$, and the linear range for the analysis depends mainly on the adsorption breakthrough-volume and on the sensitivity of the MS.

IARC method 25 (IARC85d); 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 x lower limits.

IARC method 27 (IARC85e); 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 $5 \mu\text{g}/\text{l}$.

DFG method 1 (Ang82); 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 $1.2 \mu\text{g}/\text{l}$.

Sources

3.1 Natural occurrence

PER is reported to be produced by algae and one micro-algae (IARC95).

3.2 Man-made sources

3.2.1 *Production (IARC79, WHO84, ATS97, IARC95)*

World production of PER amounted to 680 kilotonnes in 1972, and to 1000 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 UK 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 in IARC95. 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, U.S. 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 hydrolyzation. Thus stabilised, PER is transported in tanks and drums.

3.2.2 Use (WHO84, ATS97)

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 anthelmintic, as a heat-change fluid, in grain fumigation, and in the manufacture of fluorocarbons.

An overview of use-patterns, presented originally in IARC95, is reproduced here as table 3.1.

PER has been found in air, soil, surface water, sea water, 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 3.1 Use of PER in kilotonnes; reproduced from IARC95.

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

Exposure

4.1 Environmental levels

4.1.1 *Water (ATS97, IARC79, WHO84, IARC95)*

Rainwater has been found to contain up to 150 $\mu\text{g PER}/\text{m}^3$. Average and maximum concentrations in sea-water samples were 12 $\mu\text{g}/\text{m}^3$ and 2600 $\mu\text{g}/\text{m}^3$ respectively, while the maximum concentration in sediments was 4800 $\mu\text{g}/\text{m}^3$. Surface water from the Atlantic ocean contained 0.2-0.8 $\mu\text{g PER}/\text{m}^3$. In Western Europe, levels of 10-46,000 $\mu\text{g}/\text{m}^3$ were found in ground water. In the Netherlands, maximum levels of 22,000 $\mu\text{g}/\text{m}^3$ were measured. Concentrations up to 473 $\mu\text{g}/\text{m}^3$ 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 6200 $\mu\text{g}/\text{m}^3$; concentrations in the effluent of the plant before and after chlorination amounted to 3900 $\mu\text{g}/\text{m}^3$ and 4200 $\mu\text{g}/\text{m}^3$ 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 1300 μg of PER per m^3 , or less. The maximum concentration found in a drinking-water survey in 100 cities in Germany was 35,300 $\mu\text{g}/\text{m}^3$ in 1977, the average being 600 $\mu\text{g}/\text{m}^3$.

4.1.2 Food (WHO84, ATS97, IARC95)

An overview of PER concentrations in food is presented in table 4.1.

Table 4.1 Concentrations of PER in food products, adapted from WHO84, ATS97 and IARC95

country	food samples	concentration (µg/kg)
Switzerland	milk and meat products	3-3490
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
	olive oil (81 out of 98 samples)	<10
United Kingdom	olive oil (17 samples)	1-17
	tap water	0
United States/Pennsylvania, samples from a food-processing plant	chinese style sauce	0
	quince jelly	0
	crab apple jelly	0
	grape jelly	0
	chocolate sauce	0
	United States	93 out of 231 samples
United States	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
avocado's	14	

Table 4.1 Continued.

Country	Food samples	Concentration (mg/kg)
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 sherbert	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

4.1.3 Air

Indoor and ambient air

Median level of PER in about 400 Dutch homes was $4 \mu\text{g}/\text{m}^3$, while maximum levels varied between 49 and $205 \mu\text{g}/\text{m}^3$. A median outdoor level of $2 \mu\text{g}/\text{m}^3$ was measured in this study (Leb86).

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 $8.70 \mu\text{g}/\text{m}^3$ and $4.75 \mu\text{g}/\text{m}^3$, respectively. It was found that the indoor/outdoor concentration ratio was higher in winter than in summer, median concentration ratios being 2.15 and 1.38 respectively (Gil90).

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 are 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 (ATS97).

In Germany, annual mean levels of 6 ppb ($41 \mu\text{g}/\text{m}^3$) and 10 ppb ($69 \mu\text{g}/\text{m}^3$) were detected downwind of a chemical laundry and a rubber factory, respectively (WHO84).

According to the Toxics Release Inventory 1988 (an annual compilation of information on the release of toxic chemicals by manufacturing facilities in the US (TRI88)), 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 = $6.9 \text{ ng}/\text{m}^3$) in rural and remote areas, values in the higher-ppt and lower-ppb range (1 ppb = $6.9 \mu\text{g}/\text{m}^3$) are found in urban and industrial areas and areas near point sources of pollution (ATS97).

Surveys of the air in 9 cities in the USA showed concentrations between 0.2 and $52 \mu\text{g}/\text{m}^3$, with averages between 2 and $4 \mu\text{g}/\text{m}^3$. In 14 cities in Germany average concentrations were between 1.7 and $6.1 \mu\text{g}/\text{m}^3$ (WHO84).

Workplace air

Exposure levels for organic solvents at Dutch workplaces were measured by the Dutch Ministry of Social Affairs and Employment (Doo86). 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 ($352 \text{ mg}/\text{m}^3$), 39 ppm ($269 \text{ mg}/\text{m}^3$), and 16 ppm ($110 \text{ mg}/\text{m}^3$) were observed respectively.

A NIOSH survey of 44 dry cleaning facilities showed exposures for machine operators to range from 4.0 ppm ($28 \text{ mg}/\text{m}^3$) to 149 ppm ($1027 \text{ mg}/\text{m}^3$). 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 $21 \text{ mg}/\text{m}^3$), respectively. A study of the dry cleaning industry in the United Kingdom indicated exposure levels similar to those observed in American studies (ATS97).

An 8-h time-weighted average (8-h TWA) of up to $4000 \text{ mg}/\text{m}^3$ can occur in dry-cleaning establishments. In the United Kingdom, over 90% of 493 8-h measurements in 131 dry-cleaning establishments revealed concentrations below $680 \text{ mg}/\text{m}^3$, and over 50% of these samples revealed concentrations below $200 \text{ mg}/\text{m}^3$. Similar results were obtained in a survey of 46 dry-cleaning establishments in Germany (WHO, 1984).

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/m³, while the breath of residents, living adjacent to the shops, contained 1 mg/m³ (WHO84).

In Turin, Italy, blood samples of 30 volunteers (15 females, 15 males) contained a mean concentration of 1.33 µg/l (1330 µg/m³) and 0.46 µg/l (460 µg/m³) during winter and summer, respectively (Gil90).

In the USA the average daily intake by the inhalation route, assuming ambient concentrations of 0.3-2.5 ppb (2.1-17.3 µg/m³) and inhalation of 20 m³/day, is estimated to be 41-204 µg/day. The average daily intake from water, assuming concentrations of 0.3-3 µg/l and ingestion of 2 l water/day, is estimated to be 0.6-6 µg/day (ATS97).

In Switzerland and Germany total daily intakes via food were calculated to be 160 µg/day and 87 µg/day respectively (WHO, 1984).

4.2.2 Occupational population

The US National Institute for Occupational Safety and Health (NIOSH) estimated that nearly 500,000 US 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 States 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-h time weighted averages between 27 and 1010 mg/m³. Machine-operators in 9 plants had 8-h time-weighted average exposures exceeding 340 mg/m³, while in 7 plants, 15-min peak exposures exceeded 680 mg/m³. Other workers were exposed to a maximum 8-h time-weighted average of 251 mg/m³.

At a railway works, where PER was used as a cleaning agent, 94% of 104 8-h measurements exceeded 680 mg/m³ with peaks up to 1290 mg/m³ (WHO84).

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 µg/m³, while other sea-water concentrations may reach levels as high as several mg's per m³. Similar concentrations have been found for marine sediments. Ground water may reach even higher levels; in this matrix concentrations between 10 and 49,000 µg/m³ have been observed. Average drinking-water concentrations lie around 1000 µg/m³. However, much higher values (e.g. 35,500 µg/m³) have been measured.

Background ambient-air concentrations amount to a few ng/m³. 1000 times higher levels may be encountered in urban area's, in particular down wind 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 µg per 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 milligrams per cubic meter.

Kinetics

5.1 Absorption

5.1.1 Respiratory

Humans

Respiratory absorption of PER has been investigated in a number of human-volunteer studies (Ste61a, Ste70, Fer76, Hak77, Mon79a, Mon83a, Ben85, Opd86, Opd87, Pez88). Alveolar-retention was determined by Monster *et al.* (Mon79a) and Fernandez *et al.* (Fer76) by 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 h. Over a period of 4 h, lung clearance decreased from 6.7 to 3.8 l/min (Mon79a). These rather high absorption values are to be expected, in view of the high solubility of PER in blood and adipose tissues (Mon83a) and the partition coefficients of the compound (Gea93), which are listed in Table 5.1.

The decrease of retention with duration of exposure is caused by the concomitant increase of the PER blood concentrations.

Benoit *et al.* (Ben85) investigated retention during steady-state absorption (defined as the absorption when the concentration of PER in exhaled air in unit time is constant) in seven human volunteers. They found retention coefficients ranging from 0.78 to 0.93 when the subjects were exposed to concentrations of 50 to 92 ppm (345 to 634 mg/m³).

Table 5.1 Blood/air, tissue/blood, and tissue/air human partition coefficients for PER (from Gea93).

blood		air	
organ/tissue	coefficient	organ/tissue	coefficient
fat	125	fat	1.450
kidney	5	kidney	58.64
muscle	6	muscle	70.45
liver	5	liver	61
		blood	12

The actual absorption of PER by the respiratory tract equals the product of retention and ventilation volume (volume of breathed air). Monster *et al.* (Mon79a) calculated the human absorbed amounts which are listed in Table 5.2. based on their experimental observations.

The strong stimulating effect of exercise on absorption shown in this table was also found by Hake and Stewart (Hak77). These authors reported a fourfold increase in PER blood concentrations upon an exercise of 30 min.

Table 5.2 Estimated absorption of PER by 6 human volunteers exposed for 4 h to 72 or 144 ppm (469 or 992 mg/m³) PER at rest, or for 4 h to 142 ppm (978 mg/m³) PER combined with 2 half-hour periods of exercise equal to 100 W (from Mon79).

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

The influence of the fat content of the body on absorption has been investigated by Monster *et al.* (Mon79a). Their results show that the absorption depends stronger on lean body mass than on the amount of adipose tissue.

Experimental animals

Pegg *et al.* (Peg79) exposed rats for 6 h to 10 ppm (69 mg/m³) or 600 ppm (4134 mg/m³) of ¹⁴C-labeled PER and determined the radioactivity in exhaled air, urine and carcass. As in humans, excretion occurred largely via exhalation (see Section 4.4). 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 μmol PER eq. of radioactivity was recovered for the 10 ppm (69 mg/m^3) and 600 ppm (4134 mg/m^3) exposure group respectively, from exhaled air, faeces, urine and carcass together, during a period of 72 h after termination of exposure. Schumann et al. (Sch80) reported, that under comparable conditions, 2.44 μmol could be recovered over a period of 72 h after 6 h 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 (Ste64) investigated the dermal absorption of PER in five human volunteers. One of the thumbs of the subjects was kept immersed in the solvent for 40 min, 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 min after termination of exposure. The mean concentration decreased to 0.20 ppm (1.38 mg/m^3) over a period of 5 h; 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 (5.1.1).

Aitio *et al.* (Ait84) 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 h 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 (Ste64) 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 (see 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 (Rii78). The amount of PER exhaled by three volunteers after exposure to 600 ppm (4134 mg/m³) of the compound via inhalation and via the skin together and via the skin alone was compared. Exposure lasted 3.5 h and concentrations in exhaled air were followed up to 50 h after termination of exposure. It appeared that the amount of PER 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 (Tsu75) has investigated the dermal absorption of PER in mice. An amount of 0.5 ml of PER was applied to 2.9 cm² of clipped skin of the abdominal region for a period of 5 to 15 min. The compound was determined by gas chromatography 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 µg over a period of 15 min, 173 µg of which was eliminated via the exhaled air. This amount is equivalent to 24.4 nmoles/min/cm².

The same author (Tsu77) 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/cm²) in the *in vitro* system than in the *in vivo* system.

Jacobson *et al.* (Jac82) investigated the dermal absorption of PER with guinea pigs. These authors followed the concentration in the blood (carotid artery) of the anaesthetised animals during exposure. One, two or four 3.1 cm² areas of clipped skin were exposed to 1 ml of solvent for 6 to 12 h. A maximum blood concentration of 1.1

µg/ml was found after 0.5 h, followed by a slow decrease to 0.63 µg/ml after 6 h 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 based, 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 (Kop85, ATS97), most of them being the result of the use of the compound as an anthelmintic drug. The systemic nature of the effects observed (see Section 6.2.1) and the presence in blood and urine of PER or its metabolites TCA (trichloroaceticacid) and TCE (trichloroethanol), 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 for this purpose and could, therefore, not be used to establish a balance, while 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 (CIC90, ATS97, Mit85, Dan63, Peg79, Fra83b).

5.2 **Distribution**

5.2.1 *Respiratory*

Humans

Inhalation experiments with human volunteers by Stewart et al (Ste70) show that upon repeated exposure (101 ppm (696 mg/m³); periods of 7 h per day for 5 consecutive days), the PER concentration in the expired air increases compared to one exposure of 7 h. 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 (Ste70). After 10 days, the expired air still contained more than 1 ppm (6.89 mg/m³). 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 (Gub74, Fer76, Mon79).

The accumulation is predicted by kinetic modelling (Gub74). It is largely the result of the very high fat/blood partition coefficient, the much lower air/blood partition coefficient (Gea93; see Table 5.1) 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 Guberman and Fernandez (Gub74) predicts that half of the body burden of a 70-kg person after 8 h of respiratory exposure to 100 ppm (690 mg/m³) 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 Lukazewski (Luk79) with gas chromatography-mass spectrometry. The following concentrations were found: brains, 36 mg/100g; lungs, 0.3 mg/100 g; blood, 4.4 mg/100 ml; urine, below detection limit; stomach content, below detection limit. Levine et al. (Lev81) found 240 mg/kg in the liver, 71 mg/kg in the kidneys, 69 mg/kg in the brains and 30 mg/kg in the lungs of a deceased dry cleaner.

Experimental animals

Savolainen *et al.* (Sav77) studied the distribution of radioactivity in rats following respiratory exposure to 200 ppm (1378 mg/m³) of radiolabeled PER for 4 days, 6 h per day. PER appeared to be primarily distributed in the fat tissues, in particular the perirenal fat. A 145 times higher concentrations were found in the perirenal fat (4495 nmol/g after 6 h at day 4) than in the blood (31 nmol/ml after 6 h at day 4; corresponding concentrations for cerebrum, cerebellum, lungs and liver were 143, 92, 74 and 161 nmol/g respectively).

Pegg *et al.* (Peg79; see Section 5.1.3) found that respiratory exposure of rats to 10 or 600 ppm (69 or 4134 mg/m³) of ¹⁴C-labeled PER for 6 h resulted in 4.3 and 2.2 percent of the radioactivity being present in the carcass respectively, the distribution

being investigated 72 h following termination of exposure. The distribution over various tissues is presented in Table 5.3.

Table 5.3 Distribution of radioactivity (mmol-eq/g) over various rat tissues 72 h after respiratory exposure to ^{14}C -labeled PER (Peg79).

tissue	10 ppm (68.9 mg/m ³)	600 ppm (4134 mg/m ³)
liver	0	0,1
kidney	0	0,17
fat	0	0,08
brain	ND	ND
lung	0	0,07
heart	0	0,05
adrenal	ND	ND

ND - not determined

The fact that these authors did not find a predominant distribution towards the fat tissue, can most probably be attributed to the 72 h 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 (see Section 5.3).

Schumann *et al.* (Sch80) compared the distribution upon respiratory exposure in rats and mice (same exposure conditions as in Peg79): 10 ppm (68.9 mg/m³) resulted in 3% of the recovered radioactivity in the carcass 72 h 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 (see Section 5.3) in the mouse. Consequently less unmetabolized PER will be accumulated in the fat tissues and subsequently eliminated by expiration in this species.

Ghantous *et al.* (Gha86) studied the distribution of PER and trichloroethylene and metabolites in pregnant mice by means of whole-body autoradiography and gas chromatography, 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 trichloro acetic acid (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 min) non-volatile compounds were found in liver, kidneys, lungs, nasal mucosa and blood. A strong increase was observed within a period of 4 h, 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 (Ste64) 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.* (Rii78) after dermal exposure to a PER vapour concentration of 600 ppm (6134 mg/m³) during 3.5 h.

Experimental animals

Tsuruta (Tsu75) determined the presence of PER in the whole body and in expired air of mice which were dermally exposed to the compound for 15 min. They found 173 µg to be retained by the body; 3.95 µg was expired during the exposure period of 15 min.

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.* (Peg79; see Section 5.1.3) found retentions of 3.3 and 1.2 percent of recovered radioactivity in the carcasses of rats 72 h after oral administration (by gavage) to 1 and 500 mg/kg bw PER by rats respectively. The distribution obtained,

demonstrated the preference of PER for fat tissues for the 500 mg/kg bw group. Even 72 h after termination of exposure, the fat tissues showed the strongest radioactivity. In case of the 1mg/kg bw 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 (see Sections 5.2.1 and 5.3).

In the study of Schumann *et al.* (Sch80; see Section 5.1.3) 0.5 percent of the recovered radioactivity was found to be retained in the carcasses of mice, 72 h after a single gavage exposure to 500 mg/kg bw. 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, Franz and Watanabe (Fra83b) found a retention value for the carcass of 0.9 percent 72 h after a 12-h-long period in which rats received a saturated solution of PER as drinking water.

Vemmer *et al.* (Vem84) 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 (Mar87) investigated the transport of PER through the body with mice after oral application. 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 phagocytized 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.* (Dal94) investigated the time dependence of the PER concentrations in various organs in rats and dogs after a single bolus of 10 mg/kg bw, which was administered by gavage. The results of this study are summarised in Table 5.4.

The species differences which are revealed by Table 5.4 are attributed by the authors to a markedly higher rate and magnitude of exhalation and metabolism in the rat.

Table 5.4 Toxicokinetic parameters in rat and dog after a single oral gavage treatment with 10 mg/kg bw PER (from Dal94).

<i>dog</i>				
tissue	area under the curve (mg.min/ml)	half-life (min)	Cmax (mg/g)	Tmax (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
<i>rat</i>				
tissue	area under the curve (mg.min/ml)	half-life (min)	Cmax (mg/g)	Tmax (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

Cmax: maximum concentration; Tmax: time at which the maximum concentration is reached

5.3 Biotransformation

The biotransformation of PER has been reviewed by Dekant *et al.* (Dek86a), Clement International Corporation (CIC90), European Centre for Ecotoxicology and Toxicology of Chemicals (ECE90) and Agency for Toxic Substances and Disease Registry (ATS97).

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 (Ste61a, Ste64, Ste70, Gub74, Fer76, Hak77, Rii78 Mon79a, Mon79b, Ben85, Pez88). Compared to the amounts of PER absorbed, the metabolised amounts can justly be called small (Mon83b).

The major metabolite detected in human blood and human urine is trichloroacetic acid (TCA) (Fer76, Ike72, Ike77, Mon79a, Mon83a, Mon86, Zig85, Ske91, Pop92). The study of Ohtsuji et al. (Oht83) 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/m³). Exposure to 50 ppm (345 mg/m³) for 8 h resulted in the excretion of less than 2% of the compound via urine in metabolised form. Lauwerijs et al. (Lau83) did not find TCA in urine of laundry workers exposed to 8.9-37.5 ppm (61.3-258.3 mg/m³), while PER could be detected in expired air (0.2-10 ppm (1.4-68.9 mg/m³), 30 min after work) and blood (0.4-3.1 mg/l, 30 min after work).

Some authors reported the occurrence of other metabolites in human urine instead of, or next to TCA. Ikeda and Ohtsuji (Ike72) found trichloroethanol (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/m³) of PER, while increase of exposure to 200-400 ppm (1378-2756 mg/m³) resulted in a TCE/TCA ratio of 2/3.

Monster (Mon83a, Mon86) 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 (Mon86). 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%; Mon79b). Also the PER batches used in volunteer studies might be contaminated to a sufficient high level with trichloroethylene, to give rise to TCE in urine.

The study of Meuling and Ebens (Meu86) 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 (Zig85). Finally, it is doubted whether the presence of TCE can be deduced from results based on the Fujiwara reaction (Rei83), as is done in some studies.

LaFuente and Mallol (Laf86) 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.* investigated the biotransformation of PER in four humans occupationally exposed during dry cleaning to 50 ppm (345 mg/m³) (Bir96). GC/MS analysis of urine samples at the start and the end of the work week 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 1000-times lower than those of the first two (2.2-14.6 pmol/mg creatinine vs. 13.5-65 nmol/mg creatinine). Whereas the concentrations of TCA/TCE increased during the work week, 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 (see 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 (Sch80). According to Stott and Watanabe (Sto88) a 6 h respiratory exposure to 10 ppm (68.9 mg/m³) leads in the rat to a total metabolised amount of 10.5 µmol/kg liver protein, whereas in the mouse this amount is 89.5 µmol/kg liver protein.

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 5.5.

Very pure PER was used in the studies of Dekant *et al.* (Dek85), 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.* (Peg79) 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.* (Peg79).

Table 5.5 Metabolites of PER detected in body fluids and tissues of experimental animals upon exposure to PER.

refer.	species	exposure	blood	urine ^a	details
Yll61	Mouse	Inhalation, 0.5-1 g/kg in 2.5 h	TCA ^b	TCA (52%), oxalic acid (11%), dichloroacetic acid (traces)	20% excretion via urine 70% excretion via exhaled air Postulates the formation of an epoxide which is rearranged to trichloroacetyl chloride No monochloroacid, formic acid or TCE ^c were found
Dan63	Rat	Oral gavage labelled PER		TCA, chloride	
Dan67	Rat	Inhalation, 740 ppm (5099 mg/m ³) for 5 h/day, for 3 days		TCA (0.08-0.11 mg/ml), oxalic acid (1.08-1.62 mg/ml), ethylene glycol (0.52-0.83 mg/ml)	
Mos77	Rat	Oral, 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.
Peg79	Rat	Oral, 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.
		Inhalation, 10 and 600 ppm (69 and 4134 mg/m ³) for 6 h		Oxalic acid (0.27 and 2.3 mmol)	No TCA found.
Mit85	Rat	Oral, 6.0 mmol/kg bw 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.
	Mouse	Oral, 5.4 mmol/kg bw, chronic exposure			

^a Does not exclude the presence of other metabolites, unless indicated otherwise in the last column (Details) of the table

^b TCA = trichloroacetic acid

^c TCE = trichloroethylene

Table 5.5 Continued.

refer.	species	exposure	blood	urine ^a	details
Bub85	Mouse	Oral, up to 2 g/kg bw for 6 weeks		TCA ^b (up to 100 mg/kg/day)	Oxalic acid, ethylene glycol and TCE ^c could be excluded. Saturation of metabolism was observed.
Dek85	Rat	Oral, 800 mg/kg bw, [14C]-PER		Percentage of urinary radioactivity Oxalic acid (8%) Dichloroacetic acid (5.1%)TCA (54%) trichloroacetyl aminoethanol(5.4%) trichloroethanol (8.7%) trichlorovinylacetylcysteine (1.6%) conjugate of TCA (1.8)	
	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%) trichloroethanol (5.7%) trichlorovinylacetylcysteine (0.5%) conjugate of TCA (1.3%)	
Odu88	Rat	Inhalation, 400 ppm (2756 mg/m ³) for 6 h	TCA (max of approx. 7 mg/ml after 3 h)		
	Mouse	Inhalation, 400 ppm (2756 mg/m ³) for 6 h	TCA (max of about 130 mg/ml after 4 h)		
Gha86	Mouse	Inhalation, 5 mmol/4 mice in 10 min		TCA, traces of TCE	TCA was also found in the amniotic fluid, maternal plasma and foetuses.
		Intraperitoneal, 0.6 mmol/mouse		TCA, traces of TCE	

^a Does not exclude the presence of other metabolites, unless indicated otherwise in the last column (Details) of the table

^b TCA = trichloroacetic acid

^c TCE = trichloroethylene

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 5.6.

Table 5.6 Metabolites of PER detected in vitro.

refer.	species/organ/ tissue/cell	experimental setup	metabolites detected	details
Bon75	Rat/liver/ homogenate	PER was added as vapor to the carbogen (to the saturation level) used for oxygenation of the blood by which the liver was perfused; perfusate and homogenate were analyzed for metabolites.	TCA ^a , 10-15% (of absorbed PER) in perfusate and 3-5% bound to liver.	Not detected were dichloroacetic acid, choral, glucuronides and TCE* (gas chromatography).
Cos80	Rat/liver/ microsomes	Microsomal fraction of rat liver was incubated with PER (3.3 mMol); the incubation mixture was analyzed 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 gas chromatography)
Cos84	Rat/liver/ hepatocytes	Rat hepatocytes were incubated with PER; the incubation mixture was analyzed 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 gas chromatography)

^a TCA = trichloroacetic acid; TCE - trichloroethylene

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 (Yll61)). 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-life 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 (Cos80 and Cos84), who demonstrated a clear-cut stimulation of biotransformation by pretreatment of animals with inducers of this enzyme.

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.* (Dek86a) identified trichloroacetyl amide, 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 oxalylethanolamide, 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 5.1.

Conjugation

Originally it was believed that PER is only metabolised in mammals via epoxidation (Ike73).

More recently, however, it became clear that direct conjugation of PER with glutathione plays a role as well (Dek85, Dek86a, Dek86b). This is not to say that this pathway is important in quantitative terms. The amount conjugated is rather small compared to the amount epoxidized (Gre90a and Gre90b). 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 a-2-u globulin.

Conjugation of PER takes place in the liver (Dek86a, Odu86, Gre90a, Gre90b). The conjugate is converted to 1,1,2-trichlorovinylcysteine by glutamylpeptidase and cysteineglycinase, followed by transformation to the mercapturic acid 1,1,2-trichlorovinyl-N-acetylcysteine by the enzyme N-acetyltransferase (Dek85). 1,1,2-trichlorovinylcysteine may however also be converted by b-lyase in the kidneys (Gre85, Dek86b, Dek88) to dichlorothioketene and dichlorothioacetyl chloride (Dek88) and dichloroacetic acid (ECE90, Gre90a). It is assumed that the first two of these products are cytotoxic and bind to the DNA (Gre90a, And90, Dek86b).

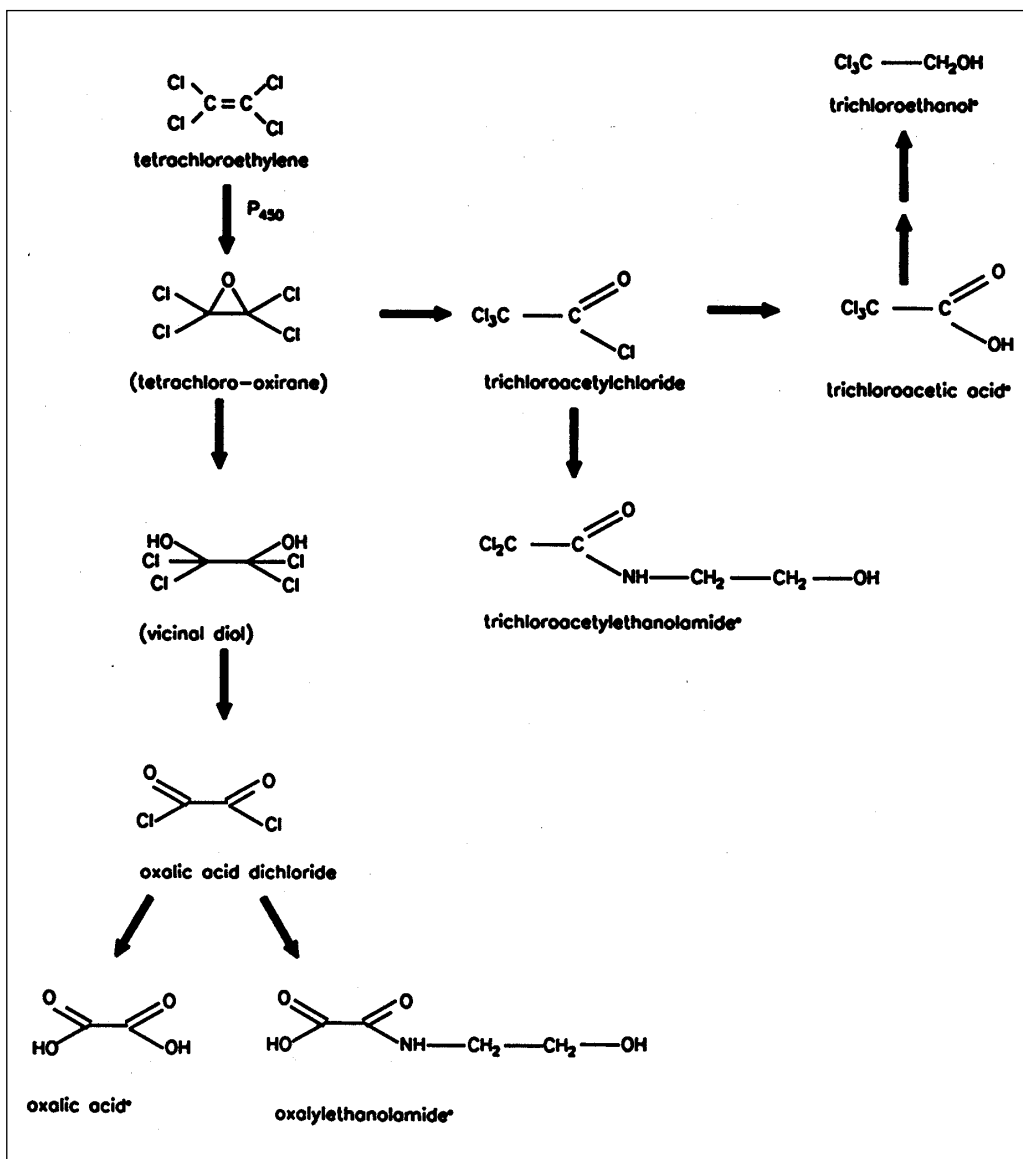


Figure 5.1 Oxidative biotransformation pathway of PER, adapted after ATS97.

Sofar, 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 (ECE90, Gre90a, Gre90b).

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 (Laf86). The Bir96 study is the only one providing direct evidence for glutathion conjugation products in human urine.

The conjugation pathway is depicted in Figure 5.2.

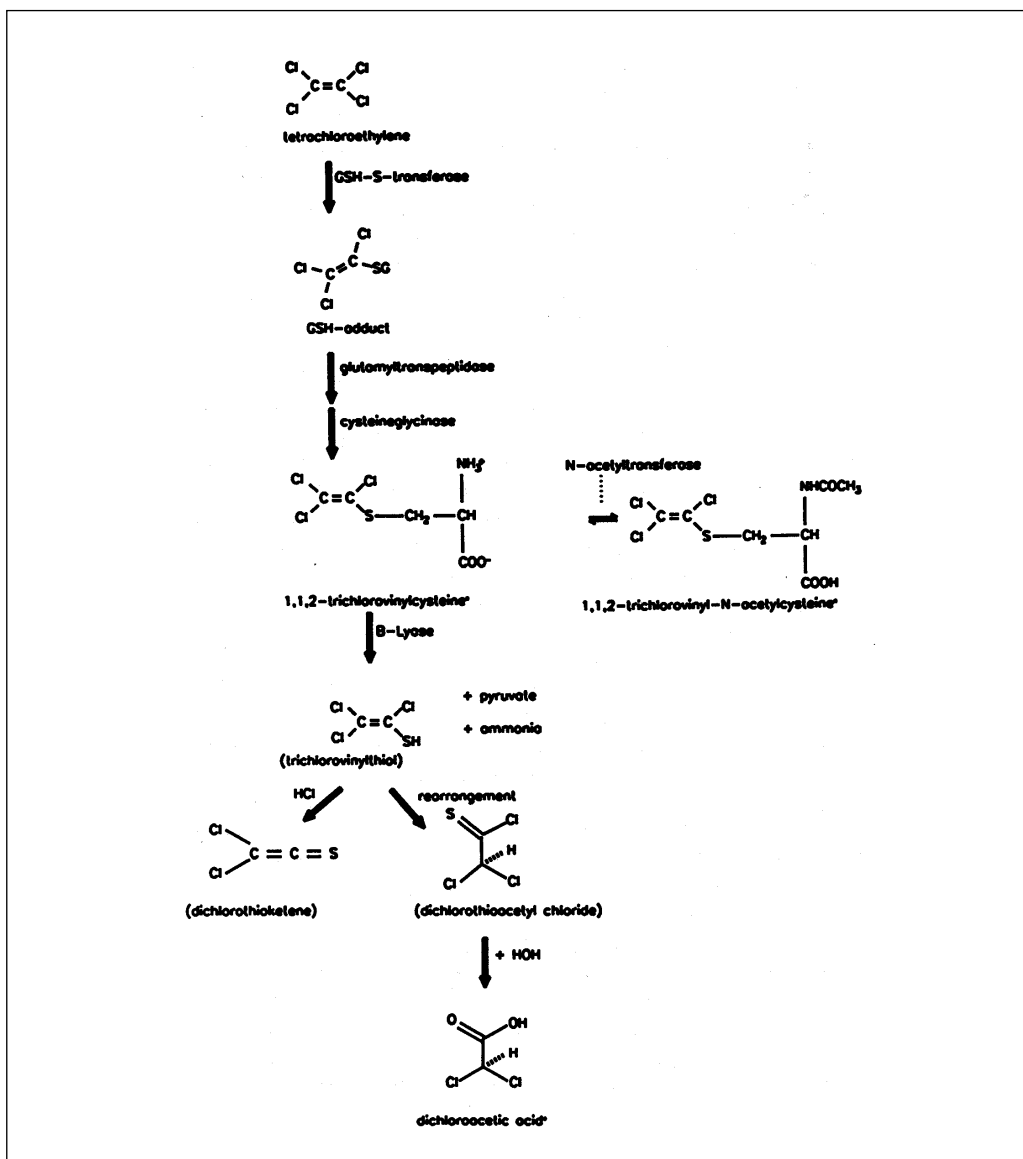


Figure 5.2 Conjugative biotransformation pathway of PER, adapted after ATS92.

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 CO₂. 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 5.7. It should be emphasised, that in most experiments listed in Table 5.7, 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 (Peg79, Mit85), carbondioxide may be one of the exhaled metabolites. The study of Morgan *et al.* (Mor79) is the only one which provides a complete balance, as ³⁸Cl-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. Mon79a, Mon79c, Fer76, Wal83, Wal86) 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 (see section 5.3), together with its air/blood and blood/fat partition coefficients (Mon79b, Gea93) and the poor perfusion of the adipose tissue (Mon86). Generally, two elimination phases are discerned for solvents like PER (Ber80). 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.

Table 5.7 Elimination of PER and its metabolites by humans.

ref.	exp. route	elim. route	eliminated compounds	experimental conditions	T 1/2 (h) ^a	details
Ste61a	Inhalation	Exhalation Blood Urine	PER	Volunteers exposed to 194 ppm (1337 mg/m ³) for 83 or 187 min, to 101 ppm (696 mg/m ³) for 183 min or to 393 ppm (2708 mg/m ³) for 210 min		No T1/2 estimated; within 30 min no detectable levels in blood or urine; total clearance from exhaled air took up to 400 h
Ste64	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 EPA85)
Mor72	Inhalation	Exhalation Urine	³⁸ Cl-compound(s) ³⁸ Cl-compound(s)	Volunteers inhaled approximately 5 mg in one breath	1.2 and 8.4 (see next column; estimation by author)	No T1/2 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 Excretion rate was less than 0.01%/min
Ste70/ Ike73	Inhalation	Exhalation	PER	7 h experimental exposure to 100 ppm (690 mg/m ³) per day for five days; 17 volunteers	65	Ike73 estimated T1/2 from elimination curves obtained by Ste70
Ike73	Inhalation	Urine	Trichloro compounds determined with Fujiwara reaction	Occupational exposure; 26 workers	144	
Rii78	Dermal	Exhalation	PER	Dermal exposure of volunteers to 600 ppm (4134 mg/m ³) vapor of PER for 3.5 h ; respiratory exposure precluded	1, 6 and 72	Three T1/2s were determined for the first 2 h post exposure, 3-8 h post exposure and 72 h post exposure respectively
Mon79a	Inhalation	Exhalation Blood	PER TCA	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 75-80	The T1/2s were observed 20, 50 and 100 h after termination of exposure respectively; the three T1/2s were assumed to represent the vessel-rich compartment of the body (blood), the muscles and the adipose tissue.

^a The long half life of TCA (trichloroacetic acid) may be related to the strong protein binding of this compound.

Table 5.7 Continued.

ref.	exp. route	elim. route	eliminated compounds	experimental conditions	T 1/2 (h) ^a	details
Mon83a	Inhalation	Exhalation	PER	Occupational exposure; 32 workers 340 mg/m ³ (50 ppm)		No T1/2 could be estimated four respiratory elimination; however, during the weekend the concentration in the exhaled air decreased to about 15% of the mean inhaled concentration in the previous workweek
		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	
Ben85	Inhalation	Exhalation	PER	5 volunteers exposed to 55 ppm (379 mg/m ³) 90 min	1	The authors explain the short T1/2 found by assuming that they only observed the clearance of the compound from the blood and not from body tissues
Gor88	Inhalation	Exhalation	PER	1 volunteer exposed to air in a dry-cleaning shop; subsequent determination of concentrations in exhaled air in a clean-air chamber for 10 h	21	
Wal83/ Wal85	Inhalation	Exhalation	PER	340 mg/m ³ (50 ppm)	21	

^a The long half life of TCA (trichloroacetic acid) may be related to the strong protein binding of this compound.

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 (Mon86).

Monster *et al.* (Mon79a) discern a phase between the two mentioned here above. Analysis of their results lead them to estimate a separate T1/2 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.

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 (see section 5.2). Species differences can be attributed to biotransformation, the most notable example being the mouse (Sch80). This species has a much more rapid oxidative biotransformation than other species investigated, including man (see section 5.3), leading to a large part of the absorbed PER being eliminated as TCA.

5.5 **Possibilities for biological monitoring (BM)**

The feasibility of biological monitoring (BM) of human exposure to PER has been investigated by several authors (Ima73, Gub74, Mon79c, Mon83a, Mon86, Lau83, Jan93). Four biological exposure parameters may in principle be used: the concentrations of PER in the exhaled air and blood, and the concentrations of TCA in blood and urine.

In his review paper Monster (Mon86) 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 (Ske91, Kez94).

The PER concentrations in exhaled air show a strong linear correlation with the concentrations in blood (Mon79a*) 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 (Mon79a). 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.

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

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 (Ste70, Fer76, Sav81), makes it possible to use the PER concentrations in exhaled air and blood as parameters for a time-weighted average (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 BM-studies which are only based on PER concentrations in blood and exhaled air, as the amount metabolised is only marginal (Mon79a, Mon79c). 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% (Mon79a, Fer76, Hake77).

However, the slow biotransformation of PER does not imply that the concentrations of metabolites cannot be used for BM 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, history of exposure.

Nevertheless, there are four reasons to use PER concentrations for biomonitoring instead of metabolite concentrations) 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/m³)) the metabolic capacity seems to reach saturation (Oht83). 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 (Mon79a); 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 (Ske91, Kez94). 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 (Mon79b). 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. Guberman and Fernandez (Gub74) estimated the

relationship between exhaled air and respiratory exposure with the aid of a mathematical model. Some of their results are presented in Table 5.8. Pezzagno *et al.* (Pez88) 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 (Mon79c) 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 hour exposure, as determined with the biological parameters (concentrations in blood). The lowest coefficients were found for the PER concentrations in blood (23 and 22% for 2 and 20 h after exposure has ended). The authors point out that higher coefficients may be expected in the real-life occupational situation. The results of Lauwerijs *et al.* (Lau83) suggest that if the blood concentration does not exceed 1 mg/kg, 16 h after exposure, the time-weighted exposure over the working day of dry cleaners is likely to have been below 50 ppm (345 mg/m³). Monster *et al.* (Mon83a) showed that the concentration of PER and TCA in blood measured 15-30 min 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 µmol per litre for PER and TCA in blood respectively, when time-weighted exposure was 50 ppm (345 mg/m³). PER concentrations in exhaled air were estimated to be 920 µmol/m³ at these exposure conditions. In their study, the 95%-confidence limits of the biological parameters determined after exposure to less than 50 ppm (345 mg/m³) were estimated to be 8.3 µmol/l PER in blood, 20 µmol/l TCA in blood, 515 µmol/m³ PER in exhaled air and 3 µmol/l TCA in urine.

Table 5.8 Relationship between respiratory exposure to PER and PER concentrations in exhaled air (table reproduced from Gub74).

day of the week	postexposure time				
	0 min	30 min	1 h	2 h	15 h
monday	1	0	0	0	0
wednesday	1	0	0	0	0
friday	1	0	0	0	0

Ohtsuki *et al.* (Oht83) estimated the 95%-confidence limits for total trichlorocompounds in urine which indicate 50 (345 mg/m³) and 100 ppm (690 mg/m³) (time-weighted average) to be 30 and 61 mg/l respectively. Jang *et al.* (Jan93) 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/m³).

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.

5.6 Summary

Respiratory absorption

Actually 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/m³), 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/m³).

Dermal absorption

Dermal absorption of liquid PER can not 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/m³).

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 (Rii78).

Dermal absorption rates of 24.4 and 0.067 nmoles/min/cm² have been found for fluid PER in mice *in vivo* and in an *in vitro* rat system.

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 virtual complete oral absorption.

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-h respiratory exposure.

Analyses of human tissues after fatal exposure have revealed high concentrations in brains, 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.

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 trichloroacetic acid (TCA). Doubt exists as to trichloroethanol 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, trichloroacetyl-aminoethanol, thioethers and CO₂.

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.

Elimination

PER is primarily eliminated via exhaled air, independent of exposure route. Half-lives for respiratory elimination vary from 1 to 72 h. 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-lives 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.

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 time-weighted average 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.

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 (Hak77, Met82, Mey73 and Nic73). Extensive erythema and blistering were reported for a worker who had lain unconscious in a pool of solvents for about 5 h (Lin71).

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 (Mor69).

Three case reports point to sensitising properties. Skin sensitisation in a worker is described by Vail (Vai74; 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 6.1. 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 anthelmintic properties of PER have been propagated for the first time in the 1920's. Subsequently, the compound has been regarded for a long time as an effective anthelmintic drug with relatively mild and transient side effects (compared to other anthelmintics) (Ken29). 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. Health and Safety Executive (HSE87) 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 (Ken29). In two other studies, similar doses have caused loss of consciousness lasting approximately 2 h (Wri37, San41). Haerer and Udelmann (Hae64) 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 (Rab85). Two lethal therapeutic treatments are mentioned in HSE87 (Gol54, Lem79).

Occupational exposure

Acute exposure

Several accidental occupational exposures to PER are summarised in Environmental Protection Agency (EPA85) and the Health and Safety Executive (HSE87). Some will be touched upon here, based on these reviews and a number of original publications.

Saland (Sal67) 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 in Patel (Pat77). Exposure lasted 7 h. Effects were: loss of consciousness, coma, acute pulmonary edema 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 CNS, such as to make mechanical ventilation necessary (Pat77).

Stewart et al. (Ste61b) found an impaired liver function arising 9 days after an acute narcotic exposure to PER, which they attributed to the exposure.

Stewart (Ste69) 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 (Hak77) present the case of a dry-cleaning operator who was exposed by lying in a 'pool' of PER for 12 h. 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. So, 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 (see chapter 4). In the Hake and Stewart (Hak77)-case, concentrations decrease from about 600 ppm (4134 mg/m³) 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/m³) after about half a day, 10 ppm (68.9 mg/m³) after about 15 days and 4 ppm (27.6 mg/m³) after about 25 days (read from the published graph by the reviewer).

Two publications report fatalities due to acute exposure (Luk79, Lev81). The first concerns exposure during repair in a dry-cleaning shop, the second exposure during recycling of dry-cleaning PER by distillation.

Long-term exposure

A number of cases concerning long-term occupational exposure are summarised by the Environmental Protection Agency (EPA85) and by Eberhardt and Freundt (Ebe66). Key data of these studies are listed in Table 6.1.

6.1.3 *Volunteer studies*

Carpenter (Car37); Four male volunteers were exposed to 472 and 911 ppm (3252 and 6277 mg/m³) of PER vapour for 130 and 95 minutes respectively. The 472/130 combination gave rise to the following effects: eye irritation, secretion of mucous membranes, sensory changes, slight feeling of elation. The neurological effects became more severe at the 911/95 combination, when lassitude, mental foginess and exhilaration were experienced/observed. Increase of the highest concentration to 1450 ppm (9991 mg/m³) after 95 min. led to inebriation. Increase to 1940 ppm (13,367 mg/m³) forced the subjects to leave the exposure room after 7.5 min; they reported 'ringing in the ears' at this concentration.

Table 6.1 Cases of long-term occupational exposure to PER (based on EPA85).

ref.	exposure	effects and symptoms
Lob57	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.
Hug54	Male exposed for approximately 3 months to high levels.	Nausea, jaundice, unspecified grossly abnormal liver tests.
Ebe66	65-year old female cleaning metal parts for two months.	Numbness, dizziness and anorexia; recovery following a 8-day absence from work.
Mec66	Female exposed for 2.5 months, 10 h/day.	Liver toxicity: increased alkaline phosphatase, serum glutamic oxaloacetic transferase (SGOT), bilirubin, cephalin flocculation. Liver biopsy two weeks after exposure: degeneration of parenchymal cells, focal collections of mononuclear cells and exaggeration of liver sinusoids.
Tre69	23-year old male; four months working in about 250 ppm (1723 mg/m ³).	Death. Autopsy: enlarged liver with hepatic cell necrosis, fatty degeneration of the myocardium, lung edema and hemorrhagic pneumonia.
Ano78	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.
Col53	7 male degreasing workers exposed to 230-385 ppm (1585-2653 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; four had positive urobilinogen.
Gol69	47-year old male working in a dry-cleaning plant.	Acute: nausea, vomiting, dizziness, staggered gait, disorientation. Chronic: memory impairment, mood lability, fatigue. It is reported that the clinical picture suggested both basal ganglia involvement and cerebral-cortical damage.
Lor90	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 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,. 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 ECG. Neurological examination: impairment of coordination. No effects on EEG, EMG, nerve conduction velocity, VEP and SSEP. Psychometry: psycho-organic syndrome with decrease in performance and personality disorders. NMR tomography: signs of cerebral atrophy.
Pal70	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.
Lob57	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 hospitalization, neuroautonomic dystonia, virile climacterium, mental depression, followed by labyrinthine disorders, memory loss, somnolence, insomnia, slight motor incoordination and abnormal reflexes.
Lob57	Male in his late 30's, 6 months of occup. exposure to trichloroethylene, followed by 1 year of occup. exposure to PER, due to metal degreasing.	Headaches, dizziness, fatigue, anorexia, nausea, loss of libido, intolerance to alcohol. Within three 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.

Rowe *et al.* (Row52); Rowe *et al.* (Row52) 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 6.2.

Table 6.2 Human-volunteer experiments carried out by Rowe *et al.* (Row52).

exposure regimen	symtoms
4 persons, 930-1185 ppm (6408-8165 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 (3535-4745 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, (1419-2453 mg/m ³) up to 2 hours.	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 (1419-1619 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 hour	No adverse effects reported, except for eye irritation when peak concentrations occurred during dosage of liquid PER in the exposure room.

This study confirms the finding of Carpenter (Car37) and suggests a no-observed-adverse-effect-level (NOAEL) of about 100 ppm (690 mg/m³) or lower for acute and overt neurological effects and for eye irritation. The neurobehavioral findings of Rowe *et al.* are based on a limited number of volunteers using rather crude qualitative observational methods (no neuropsychological tests, EEG, etc.).

Stewart *et al.* (Ste61a); Stewart *et al.* (Ste61a) subjected groups of 6 male volunteers to different exposure regimens. 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 6.3. No effects were found on serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transferase (SGPT) activities, as well as urobilinogen excretion.

This study suggests a NOAEL 100 ppm (690 mg/m³) for neurological effects and exposures lasting longer than 30 min.

Stewart *et al.* (Ste70); In this study, 16 volunteers were exposed for 7 h to 100 ppm (690 mg/m³), 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

Table 6.3 Effects of respiratory exposure to PER on human volunteers (Ste61a).

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 min. of exposure.
100-120 (689-827)	4-6	Slight soft palate irritation and dryness noticeable.
200 (1378)	6-30	Romberg test normal
210-244 (1447-1681)	30+	Slight lightheadedness; increased effort necessary to maintain a normal Romberg test.

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 and serum lactic dehydrogenase (SLDH); 'Complete' urinalysis, including urobilinogen, 17-ketosteroids, 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 h 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/m³) is suggested by this study on the basis of neurological endpoints.

Hake and Stewart, (Hak77); Two to four female or male volunteers were subjected for 5 days (1, 3 or 7.5 h/day) to 0, 20, 100 or 150 ppm (138, 689 and 1034 mg/m³). 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 (incl. pulmonary function; other parameters not specified) were found. A NOAEL 20 ppm (138 mg/m³) 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.

Stewart *et al.* (Ste77, see also Hak77); 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/m³) of PER; 5.5 h/day for 11 weeks), or to combinations, this, to study the interactions between either ethanol ingestion or diazepam ingestion and respiratory exposure to PER. Exposure was checked by analysis of biological samples. A series of neurobehavioural 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.

Altmann *et al.* (Alt90); Groups of 11 male volunteers (23 to 35 years) were exposed for 4 days, 4 h/day, to 10 or 50 ppm (68.9 or 345 mg/m³) of PER. Visual evoked potentials (VEP) and brain-stem auditory evoked potentials (BAEP) were measured during exposure. In addition visual contrast sensitivity (VCS) was determined psychophysically in some volunteers. The 50-ppm group showed increased VEP peak latencies during exposure (statistically significant from the 10 ppm group). No effects on BAEP were found. A tendency of increased VCS threshold levels was observed. A significant ($p < 0.03$; $r = -0.45$) blood-concentration dependence of one VEP 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/m³) can be regarded as a NOAEL in case of 4-h exposure of young healthy males.

The commissions 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

Ikeda and Koizumi (Ike80); The occurrence of cytogenetic effects was investigated in lymphocytes of two groups of five factory workers, differing in supposed PER exposure. Exposure varied from 30 to 220 ppm (207-1516 mg/m³), with a geometric

mean of 92 ppm (25 determinations) for the highly exposed group, and from 10 to 40 ppm (68.9-276 mg/m³), for the low exposure group (no further details presented). The differences in exposure were confirmed by the urinalysis. 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 urinalysis 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 nine 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.

The value of the study is deemed limited by ATS97 because of the small number of subjects involved and the wide exposure range.

Fender (Fen93); 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. The dry-cleaning group was exposed to 144-348 mg/m³ 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".

MAK Committee (Sei90 and Bot89); 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 (Sei90). No effect of the exposure was found within the of smokers and non smokers. In the second study (Bot89), 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

Bosco *et al.* (Bos87); 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.'

Taskinen *et al.* (Tas89); A nested case-referent study 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.

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 ($p < 0.001$).

Olsen *et al.* (Ols90); The publication presents a series of nested case-reference studies on the obstetric history in women doing dry-cleaning work. 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 1970's and 1980's. 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.

Windham *et al.* (Win91); A case-referent study was carried out to investigate the effects of maternal exposure to solvents on spontaneous abortion. 'Cases (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 (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.

Eskenazi *et al.* (Esk91a); Semen quality of 34 dry cleaners was compared to that of 48 laundry workers. 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 six laundry workers had breath PER concentrations higher than $100 \mu\text{g}/\text{m}^3$; for four dry cleaners these concentrations were lying below this concentration. Further, it was not clear whether the described effects are due 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.'

Eskenazi *et al.* (Esk91b); The female partners of workers participating in the Esk91a study were examined for reproductive outcome. 17 partners of dry cleaners and 32 partners of laundry workers participated in the Esk91b 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.

Aschengrau *et al.* (Asc93), Isacson P *et al.* (Isa85), Lagakos *et al.* (Lag86), Cohn *et al.* (Coh94), Vartiainen *et al.* (Var93); A number of studies on drinking-water for evaluating the carcinogenicity of PER have been published (Asc93, Isa85, Lag86, Coh94, Var93; all summarised in IAR95). 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.

Nephrotoxicity

Franchini *et al.* (Fra83a); In this study 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 b-glucuronidase activity, as parameters of renal changes. The authors calculated the exposure concentration via biological monitoring to be 10 ppm (68.9 mg/m³). Since, the latter two parameters were significantly enhanced, the authors conclude that 'the damage seems to be very weak and tubular, rather than glomerular.'

Lauwerijs *et al.* (Lau83); 26 persons (24 females and two males) working in dry-cleaning shops were compared with a non-exposed population of 31 females and two males, for several biochemical urine parameters (b2-microglobuline, 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 to 258.3 mg/m³), with a mean for all samples of 20.8 ppm (143.3 mg/m³). No effects on the parameters were found.

Vyskocil *et al.* (Vys90); Biochemical markers of kidney damage were examined in 16 female workers from five dry-cleaning shops. 13 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, LDH and total proteins. The exposure levels varied between 9 and 799 mg/m³, the mean level amounting to 157 mg/m³ (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.'

Mutti *et al.* (Mut92); 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 a 23 biochemical or immunochemical parameters of renal damage, 20 in urine and three in serum. 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 alkaline phosphatase and fibronectin in urine and anti-glomerular basement membrane antibodies and laminin fragments in serum. Like the Vys90 study, no significant effects on b2-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.

Hepatotoxicity

Lauwerijs *et al.* (Lau83); This study (see under 'Nephrotoxicity') did not reveal effects of exposure on the enzymes SGPT and g-glutamyl transferase (γ -GT) in serum, indicating the absence of effects on the liver by PER at the measured low exposure levels.

Gennari *et al.* (Gen92); 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.

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 trichloroacetic acid 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 TLV-TWA of 50 ppm. The TLV-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 vs 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 vs. 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), alkaline phosphatase (ALP), lactic acid dehydrogenase (LDH), 5'-nucleotidase (5'-NU)) was mentioned by the authors.

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 EPA85, Axe86, HSE87, ECE90 and ATS97. However, most of the original publications have been consulted for verification and for comparison of conclusions.

Kaplan (Kap80), Brown and Kaplan (Bro87) and Ruder *et al.* (Rud94); The Kaplan-publication (Kap80) is concerned with a retrospective cohort mortality study involving 1597 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 Ratio's (SMR) of the cohort with the overall mortality rates of the US 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.

In 1987 the cohort was expanded to 1690 (493 deaths) workers with 42,267 person-years-at-risk (Bro87). 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 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

1950's), 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 (see 6.5), 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 (Rud94). In general, this update confirmed the conclusions of the first publication and the first update. Most notable was the increase of oesophagous 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.* (Bla85); SMRs were determined 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.

Blair *et al.* (Bla79); Proportional Mortality Ratios (PMRs) were calculated for 279 trade-union decedents in the period 1957-1977, which had exclusively worked in dry-cleaning shops. Elevated PMRs were found for lung cancer (PMR: 170; $p < 0.05$), cervix uteri (PMR: 208; $p < 0.05$), skin cancer (PMR: 429; $p < 0.05$), leukemia (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. 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 (tetrachloromethane, 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.

Katz and Jowett, 1981 (Kat81): Another PMR study has been conducted by Katz and Jowett (Kat81). 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; $p > 0.01$) 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 committee points out that the inclusion of these workers in the group of decedents will certainly have led 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.

Duh and Asal (Duh84); 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. 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.

Nakamura (Nak85); Again, death certificates were used as study material. It concerned 1711 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.

Smith *et al.* (Smi85); A case-referent study is described in Smi85. 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, 5776 subjects who had worked in other occupations which might lead to exposure to similar chemicals and 1869 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. Again, the sensitivity of the study for solvent-related cancers will have been limited by the inclusion of laundry workers.

McLaughlin *et al.* (McL87); This case-referent study was concerned with renal cancers occurring in Swedish laundry and dry-cleaning establishments. 7405 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.

Spirtas *et al.* (Spi92); A retrospective cohort study was carried out. The cohort consisted out of 14457 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. AT97 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.'

Anttila *et al.* (Ant95); A Finnish cohort exposed to PER, trichloroethylene and trichloroethane and consisting of 2050 male and 1924 female workers was followed from 1967 to 1992 for the occurrence of cancer. 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 to 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.

McLaughlin and Blot (McL97); The epidemiological evidence for the induction of renal-cell cancer by PER exposure was reviewed. 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 methodologic 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.’

Walker *et al.* (Wal97); A proportionate mortality ratio (PMR) study was carried out. 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 PMR s 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.

Vaughan *et al.* (Vau97); A case control study suggested an increased risk of oesophageal cancer upon working in dry cleaning. 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.’ The Committees like to emphasise this statement.

Vamvakas *et al.* (Vam98); 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 control. Whereas a high odds ration was found for trichloroethylene, no cases with PER exposure were identified. The control group included two individuals with a history of PER exposure.

Neurotoxicity

Tuttle *et al.* (Tut77); 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.

The exposed group consisted of 18 dry-cleaners (nine males and nine females), while nine laundry workers participated as controls. Exposure was extensively investigated by analysing breath samples and environmental monitoring. Time weighted averages (TWA) 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/m³), while the males (who are often machine operators) had a mean TWA of 32 ppm (220 mg/m³). 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. The power of this study is limited by the small number of subjects involved.

Ferroni *et al.* (Fer92); In this cross-sectional study 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 a neurobehavioral tests (finger tapping, simple reaction times, digit symbol and shape comparison tests). PER concentrations varied from 1 to 67 ppm (6.89 to 461.6 mg/m³) (median: 15 ppm or 103.4 mg/m³). 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 central nervous system, since prolactin release is under control of pituitary dopamine. The PER-exposed workers also showed a poorer performances in the tests. Within the exposed group, no significant correlations with relevant exposure measures were found.

DECOS concludes that the outcome of this study is too weak to be used for risk-assessment purposes.

Echeverria *et al.* (Ech95); Nervous-system effects were investigated in a group of 65 dry-cleaners. 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/m³) 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.

The results suggest that 3 years of exposure to PER levels below 50 ppm (345 mg/m³) may cause permanent nervous system effects. However, DECOS agrees with Echeverria *et al.* that this outcome does only point to the need of further investigations, but, at the moment, cannot be used as a starting point for the establishment of a health-based occupational exposure limit (HBR-OEL).

Miscellaneous

Essing (Ess75); 106 workers exposed to PER in a railway repair shop were compared with 101 controls. 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 (2756 mg/m³), 74.3% of the analyses pointed to concentrations lying between 0.2 and 50 ppm (1.4 and 345 mg/m³). 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.

The subjective nature of the effects found as well as the broad range of concentrations to which the workers had been exposed, precludes the use of this study as a starting point for the establishment of a HBR-OEL.

Cai *et al.* (Cai91); The occurrence of subjective symptoms was compared for dry-cleaning workers and workers not exposed to PER. The exposed group consisted of

56 dry-cleaning workers with a mean TWA of 20 ppm (138 mg/m³) (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. Altogether, these differences suggest neurotoxicity and local irritation. However, the subjective character of the symptoms warrants caution in the interpretation of this result, and DECOS does not find it justified to use it as a starting point for the assessment of a HBR-OEL.

No indications for haematological effects or impaired liver or kidney function were found.

6.1.5 *Summary*

Case studies:

- Application of PER as anthelmintic 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 (1378 and 2756 mg/m³). 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 (1378 and 2756 mg/m³).
-

- 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/m³) lead 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/m³) 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/m³) 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 and 345 mg/m³), many exceeding 400 ppm (2756 mg/m³); second study: mean TWA of 20 ppm (138 mg/m³)) 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/m³) of PER indicated effects on neurobehavioral endpoints and prolactin secretion, suggesting involvement of the central nervous system.
 - A study concerning chronic exposure of dry-cleaners for at least 3 years to levels of PER below 50 ppm (345 mg/m³) suggested permanent alterations in neurobehavioral and neuropsychological tests.
 - Significant elevated serum g-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 effect to be unambiguously attributed to PER exposure.
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- 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 experimental studies (see 6.5), 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 an 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.
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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 (Dup76), while no macroscopic changes of the skin were found in guinea pigs (Kro81), 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 (Dup76). The compound caused discharge with epithelial damage and epithelial keratosis. No details about the dose are presented in this study. In another study (Gra62) 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 (Rao81).

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.

Lethality

LC₅₀ values have been determined for rats and mice. A number of them are listed in Table 6.4.

In the study of Holmberg *et al.* (Hol74), the LT₅₀ (the time required to kill half of the animals exposed to a defined dose) was found to be 12.2 h (95% confidence limits 11.8-12.5 h) at 3700 ppm (25,500 mg/m³) for mice.

Table 6.4 LC₅₀ values.

species	duration (h)	LC ₅₀ (ppm; (mg/m ³)) ^a	ref
rat	6	4100 (3899-4387); (28,250 (26,900-30,200))	Bon80
rat	8	5027 (3334-7571); (34,600 (22,300-52,200))	Poz59
mouse	2	5800; (40,000)	Fri53
mouse	4	5200; (36,000)	Fri53
mouse	6	2978 (2758-3215); (20,500 (19,000-22,200))	Dup79

^a 95% confidence limits

In NTP86 a highest non-lethal dose of 2445 ppm (16,850 mg/m³) and lowest lethal doses of 2613 and 3786 ppm (26,100 mg/m³) are mentioned for mice and rats, respectively, when these animals have been exposed for 4 h.

Rowe *et al.* (Row52) report non-lethality for 10- or 14-hour exposures of rats to 2000 ppm (13,800 mg/m³) and 4-hour exposure of rats to 3000 ppm (20,700 mg/m³), while lethality was found when exposure to the latter dose lasted 5 h or longer. 12000 ppm (82,700 mg/m³) did not give rise to death when exposure lasted 0.2 h; complete survival was also found at 1600 ppm (11,020 mg/m³) for 5 h.

Kennedy and Graepel (Ken91) mention in their study an ALC (the approximate lethal concentration, which is the concentration at which mortality was first observed) in rats of 6000 ppm (41,340 mg/m³) for a 4-hour exposure.

Neurotoxicity

Most notably are the short-term neurotoxic effects of respiratory exposure. PER has been used in the past for human anaesthesia (Foo43), 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 (HSE87, ATS97). 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 6.5.

Cardiac arrhythmia

Epinephrine-induced cardiac arrhythmias have been observed in rabbits during respiratory exposure for 1 h to 5200 ppm (35,800 mg/m³) PER (Car75). The author of

Table 6.5 Overview of studies on acute neurotoxicity in experimental animals after respiratory exposure.

species	exposure	concise summary	ref.
mouse	Several exposure times, 6800 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 6800 or 12,200 ppm (46,950 or 84,100 mg/m ³) for 4 min.	Fri53
rat	150 ppm (1034 mg/m ³) during 18 hours	Exposure of rats to 5 times the Danish TLV (5 x 30=150 ppm (1034 mg/m ³)) 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.	Ede85
rat	4 h, 2300 ppm (15,800 mg/m ³)	Ataxia in a pole-climbing experiment.	Gol64
mouse	Several concentrations during 1 hour, lowest: 90 ppm (620 mg/m ³)	A dose-related increase of motor activity upon the start of exposure. The lowest dose (90 ppm (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.	Kje85
mouse	4 h, several concentrations	A clear dose-related decrease of immobility was found in a swimming test with mice. The ID50 (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) (4900 mg/m ³ (4600-5540)).	DeC83
rat	4 h, several concentrations	The influence of PER inhalation (4 hours) 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 no-adverse-effect level.	Dmi66
mouse	Several exposure times, 3700 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 3700 ppm (95% confidence limits: 20.2-28.6 min). The ratio between the ET50s for lethality and anaesthesia was over 30, showing that anaesthesia occurs at much lower doses than death.	Hol74
rat	Repeated acute exposures to 2750 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 2750 ppm caused anaesthesia, while the same rats could not be anaesthetized by doses above 10,000 ppm after six exposures to 2750 ppm (18,950 mg/m ³).	Car37
mouse	Exposure till effect emerged; 3000 or 6000 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 3000 ppm (20,670 mg/m ³).	
mouse	4 h, 2328 ppm (16,040 mg/m ³)	Mice were anaesthetized by a 4-hour exposure to 2328 ppm (16,040 mg/m ³).	NTP86

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 to 30 min 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 5000 or 10,000 ppm (34,500 or 68,900 mg/m³) (the latter treatment was not well tolerated) (Rei73).

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 6.6.

Nephrotoxicity

Histopathological effects of acute respiratory exposure on the kidneys are reported in Dup79 and Gra78. Slight and irregular scattered necrotic and degenerative lesions were observed upon the exposure to 2978 ppm (2050 mg/m³) for 6 h.

Immunotoxicity

Aranyi *et al.* (Ara86) investigated the influence of PER inhalation (3 h, 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

Table 6.6 Overview of studies on the acute respiratory hepatotoxicity of PER in experimental animals.

species	exposure conditions	concise summary	ref.
Rat	24 h after termination of acute exposure which causes high mortality.	Slight cloudy swelling and diffusely distributed fat globules in the livers.	Row52
Mouse	Acute lethal concentrations	Cloudy swelling, anisokaryosis, anisicytosis 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, 1000 and 2000 ppm (3450, 6890 and 13,780 mg/m ³).	Effects on rat serum enzymes indicating hepatotoxicity, viz. SGOT (serum glutamic oxalacetic acid transaminase), SGPT (serum glutamic pyruvic transaminase), SG-6-P (serum glucose-6-phosphatase) and SOCT (serum omithine carbamyl transferase). 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 1000 ppm (6890 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 (3450 mg/m ³).	Dre78
Mouse	24 and 72 h after a 4-h exposure to 200, 400, 800 and 1600 ppm (1378, 2756, 5512 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 (2756 mg/m ³) and higher doses. No effects on SOCT.	Kyl63
Mouse	Till 20 h after a 3-h exposure to 800 ppm (5512 mg/m ³).	Clear-cut decrease (to 45% of control) of liver-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.	Oga68
Mouse and rat	4 h, 1080 ppm (7441 mg/m ³).	Centrilobular fatty degeneration and increases of lipids and triglycerides in livers of mice. No such changes occurred in rats.	Ike69

in the toxicity of PER was mainly due to its use as anthelmintic drug (see references in EPA85; Sch27, Chr33, Car37).

Oral LD₅₀ values for rats and mice are listed in Table 6.7.

Table 6.7 Oral LD₅₀ values of PER.

species	LD ₅₀ (mg/kg body weight)	ref.
rat	2438 (1415-4215) ^a	Poz59
rat	4460 (3810-5210) ^a	Wit75
rat, male	3835	Hay86
rat, female	3004	
mouse	7814 (pure PER) 9607 (in herring oil)	Dyb46
mouse	5000	Wen51
mouse	4700	Kla66

^a 95% confidence limits

Although the other studies summarised by HSE87 and ATS97 do not allow for the estimation of LD₅₀ 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 6.8. 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 (HSE87). 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 6.8 Effects observed in oral acute toxicity studies (EPA85, Sch27, Chr33, Car37, HSE87, ATS97).

species	effects	dose ^a
mouse	Irreversible binding hepatocytes	500 mg/kg bw
rat	Increase relative weight of liver; increased enzyme-altered foci in liver (g-glutamyltranspeptidase activity), irreversible binding in hepatocytes	500 mg/kg bw
dog	Depression of heart and ventilation rate; shrivelling and inflammation of small intestines; fatty infiltration of the liver; severe ataxia; depression	286-557 mg/kg bw
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	376-753 mg/kg bw
fox	Cloudy swelling of kidney tubule cells; fatty degeneration of liver	376-4426 mg/kg bw
cow	Cloudy swelling, reticulation, centrilobular necrosis, disassociation of hepatic cords and edema in liver; hyaline casts in kidneys and tubules; anorexia; inability to stand	136-241 mg/kg bw or 15 mg/cow
horse	Centrilobular edema, necrosis, and cloudy swelling in liver; hemosiderosis in spleen; congestion, cloudy swelling and edema in kidney	99-226 mg/kg bw
sheep	Cloudy swelling, reticulation and fatty changes in periportal regions of lobules (liver); cloudy swelling, reticulation and atrophy and vacuolization of kidney tubules	331-1254 mg/kg bw

^a Rough indication of doses, above effects mentioned in the table have been observed. observed.

The study of Moslen *et al.* (Mos77) 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 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 to 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 LD₅₀ of intraperitoneal exposure has been determined for mice and dogs (HSE87, EPA85 and Geh68, Kla66, Kla67). For the first species values of 5671 and 4600 mg/kg have been reported, while a study with the second species yielded an LD₅₀ of 3163 mg/kg. ED50 values determined in the same studies for alanineaminotransferase (ALT) activity were 3980 and 5300 mg/kg for mice and 1114 mg/kg for dogs.

A number of acute intraperitoneal studies were aimed at specific effects (HSE87 and Cor73, DiV74, Pla65, Ham77, Mot93). Measurement of enzyme activities in serum pointed to liver damage in the rat and the guinea pig at doses between 452 and 3011 mg/kg for the first, and 200 and 400 mg/kg for the second species.

Rats treated i.p. 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 1000 mg/kg.

Subcutaneous

Plaa *et al.* (Pla58) found an subcutaneous LD50 for mice of 5 g/kg. Histological examination of liver and kidneys revealed changes of the centrilobular area (only in the

liver), including alterations of cell staining and cytoplasmic vacuolizations. A clear prolongation of phenobarbitone-induced sleeping time was observed, the ED50 for this effect being 4477 mg/kg.

A minimum lethal dose of 2.2 g/kg was found for the rabbit (HSE87).

Intravenous

A minimum lethal intravenous dose of 85 mg/kg has been found for dogs (HSE87).

The effects of intravenously administered PER on the vestibular-ocular reflex of rats was investigated by Tham *et al.* (Tha84). 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 (Kob82). 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.* (McC92). An approximate lethal dose of 450 mg/kg was established. The same study yielded an oral LD50 of 2600 mg/kg.

6.2.3 Toxicity due to short-term exposure

Inhalation

Multi-endpoint studies

Rowe *et al.* (Row52) investigated the effects of various subacute and semichronic respiratory exposure regimens on a number of endpoints in rats, rabbits, guinea pigs and rhesus monkeys. Table 6.9 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. It is not clear whether all these endpoints are included in the experiments presented in Table 6.9. 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.

Table 6.9 Study of Rowe *et al.* (Row52); summary of results.

concentration ppm (mg/m ³)	animal	groups	exposure regimen	effects
2500 (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	28x7-h exposures in 39 days	Severe depression of CNS; parenchymatous degeneration in the liver
	Guinea pig	4 of each sex	18x7-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
1600 (11,024)	rat	8 females	18x7-h exposure in 25 days	Various marked effects on behavior, 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	8x7-h exposures in 8 days	Decreased body weight; increased weight of liver; moderate central fatty degeneration in liver; slight degenerative changes in germinal epithelium
400 (2756)	rat	15 of each sex	130x7-h exposures in 183 days	No adverse effects found
	Guinea pig	8 of each sex	169x7-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	159x7-h exposures in 222 days	No adverse effects found
	rhesus monkey	2 males	179x7-h exposures in 250 days	No adverse effects found
200 (1378)	Guinea pig	8 of each sex	158x7-h exposures in 220 days	Depressed growth; increased liver weight; increase total lipid and esterified cholesterol in liver; central fatty degeneration in liver
		5 females and 15 males	14 x7-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 and 7 males	132x7-h exposures in 185 days	Females: increased liver weight Males: few small fat vacuoles in the liver
	Guinea pig	7 females	13x7-h exposures in 17 days	No adverse effects

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 central nervous system (CNS) and the liver as targets for PER. Furthermore, it shows that in experimental animals concentrations as low as 100 ppm (690 mg/m³) can induce adverse effects upon inhalation.

Hepatotoxicity

A number of studies showing hepatotoxicity are summarised in Table 6.10.

Table 6.10 Overview of short-term respiratory studies showing hepatotoxicity.

species	exposure conditions	results	ref.
mouse	4 h daily, 6 days per week for up to 8 weeks to 200 ppm (1378 mg/m ³)	Increase of the number of 4-h exposures exposure lead 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.	Kyl65
rabbit	4 h daily, 5 days per week for 9 weeks, 2790 ppm (19223 mg/m ³)	An increase of GOT (glutamic-oxalacetic transaminase), GPT (glutamic-pyruvic transaminase) and GDH (glutamic dehydrogenase) 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.	Maz72
mouse and rat	6 h daily, 14, 21 or 28 days to 400 ppm (2756 mg/m ³) and 6 h/day for 28 days to 200 ppm (1378 g/m ³)	Livers were examined for peroxisomes by electronmicroscopy; furthermore peroxisomal cyanide-insensitive palmitoyl CoA oxidation (PCAO) 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 (Elc85), the major metabolite of PER, were much higher in mouse blood than in rat blood.	Odu88
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 are 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 vacuolization.	Kje84, Kje85

^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 vacuolization for the 400 ppm/14 or 28 day dose groups. Electron microscopic revealed effects at lower dose levels.

Nephrotoxicity

EPA85 mentions a study of Brancaccio *et al.* (Bra71; see also HSE87) in which the renal function was investigated in rabbits which were exposed 45 days to 2280 ppm (15,710 mg/m³) (4 h/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. EPA85 and HSE87 criticize Bra71 for the lack of information on raw data and statistical methods.

In the Odu88 study (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 (PCAO) 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.

NTP86 describes a study in which mice and rats were exposed for 13 weeks to 100, 200, 400, 800 and 1600 ppm (689, 1378, 2756, 5512 and 11,024 mg/m³). 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.

Endocrinological effects

Mazza and Brancaccio (Maz71) observed in rabbits slight increases (not statistically significant) of adrenal hormones (both cortical and medullar) upon the exposure to 2200 ppm (15,158 mg/m³) of PER 1 h/day, 6 days/week for 15 days (from EPA85). The enzyme levels increased gradually with number of exposures.

The studies of Kjellstrand *et al.* (Kje84 and Kje85, see under 'Effects on butyrylcholinesterase') do not point to a clear interaction between PER and testosterone.

Immunotoxicity

EPA85 mentions a study in which 7 days of continuous exposure of rats to 185 ppm (1275 mg/m³) results in morphological changes in mast cells ('... increased vacuolisation of the cytoplasm and conformational changes in granules with some degranulization.' (EPA85)). Furthermore, EPA85 cites a study of Schmuter (Sch72) in which exposure of chinchillas to 1.5 and 15 ppm (10.3 and 103.4 mg/m³) (8-10 months, 6 days/week, 3 h/day) results in significant changes in antibody production to

Salmonella typhosa. However, EPA85 regards the study as too poorly reported to enable the evaluation of these results.

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 (Dmi68, cited in EPA85) for mice. The animals were exposed for 3 months, 5 h/day, to 15 or 75 ppm (103 or 517 mg/m³). Dmitrieva and Kulshov (Dmi68, cited by EPA85) found effects on the electro encephalograms (EEG) in rats exposed to 15 ppm (103 mg/m³) for 5 months, 5 h/day. EPA85 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'.

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 (Car37) after repeated exposures points to changes in either the biotransformation of PER or the sensitivity of the neurological target to its effect.

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 6.11.

Taken together, the results presented in Table 6.11 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.

Table 6.11 Neurochemical effects of respiratory exposure to PER.

species	conc. ppm (mg/m ³)	exposure regimen	results	comments and details	ref.
rat	200, 400 and 800 (1378, 2756 and 5512)	Continuous exposure 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		Hon80a
rat	200, 400 and 800 (1378, 2756 and 5512)	Continuous exposure for 1 month	Marked and dose-related increase of glutamine, threonine and serine, while GABA decreased.		Hon80b
Mongolian gerbil ^a	120 (827)	Continuous exposure 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.	Kyr84
Mongolian gerbil	60 and 320 (413 and 2204)	Continuous exposure 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.	Ros84
Mongolian gerbil	60 (413)	Continuous exposure for 3 months, followed by 4 months without exposure.	Slight decrease of DNA concentration in frontal cerebral cortex.	Indications for loss of neuronal/glia cells in the frontal cortex. The effect is not caused by metabolites but by PER itself.	Kar87
Mongolian gerbil	320 (2204)	Continuous exposure 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.	Kyr87
rat	320 (2204)	Continuous exposure 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.	Kyr88

^a Meriones unguiculatus

Table 6.11 Continued.

species	conc. ppm (mg/m ³)	exposure regimen	results	comments and details	ref.
rat, Guinea pig (30 days pregnant) and Mongolian gerbil	320 (2204) and 160 (1102) (Guinea pigs)	Continuous exposure 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.	Kyr90a
rat	320 (2204)	Continuous exposure for 90 days, followed by a recovery of 30 days.	Slight shifts in fatty-acid composition of brain phospholipids. Most changes normalized during post-exposure period. Slight persistent changes in brain cholesterol content.	Indicates slight, partly reversible changes in composition of cerebral membranes.	Kyr90b
rat	300 and 600 (2067 and 4134)	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.	Wan93

Effects on plasma butyrylcholinesterase

Kjellstrand et al. (Kje84 and Kje85) have investigated the effect of respiratory PER exposure of mice on plasma butyrylcholinesterase. Two exposure regimens were applied: continuous exposure for 30 days and 16, 8, 4, 2 or 1 h/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/m³); the next lower concentration (9 ppm or 62 mg/m³) did not result in an effect. The effect reached a maximum at concentrations little higher than 37 ppm (255 mg/m³). 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 (Kje85), 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 (1034 mg/m³) 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 (AChE) activity.

Haematological effects

Seidel *et al.* (Sei92) exposed female mice 6h/day, 5 days/week for up to 7.5 weeks to 135 ppm (930 mg/m³) of PER and for up to 11.5 weeks to 270 ppm (180 mg/m³) of PER, followed by an exposure-free period of 3 weeks. 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.

Oral

Multi-endpoint studies

Kaemmerer *et al.* (Kae82) applied various dose regimens to rats. Their results are summarised in Table 6.12.

Hayes *et al.* (Hay86) 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 1400 mg/kg/day for 90 days. The results of this study are summarised in Table 6.13.

Table 6.12 Summary of the studies reported by Kaemmerer et al. (Kae82)

Dose regime	Results
25 or 500 ppm in diet for 7 days / or 25 ppm in diet for 14 days	Increase of cytochrome P450 levels; at 25 ppm sign. 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 hexobarbitone
25 or 400 ppm in diet for 1 day to 2 weeks	No effects on acetylation and elimination of 50 mg/kg sulphadiazide 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 6.13 Effects of PER emulsified in drinking water on rats (Hay86).

endpoint	effect	effective doses (mg/kg/day)
relative body weight	decrease	males: 1400 females: 400 and 1400
kidney weight/body weight	increase	males: 400 and 1400 females: 1400
liver weight/body weight	increase	1400
serum parameters ^a (LDH, SGPT, SGOT, ALP, 5'-nucleotidase, BUN, protein, glucose, cholesterol, bilirubin, creatinine, calcium, phosphorus, albumin, globulin, sodium, potassium)	not treatment related except an increase of 5'-nucleotidase	females: 400 and 1400

^a LDH = lactase dehydrogenase; SGOT = serum glutamic oxaloacetic transaminase; LGPT = serum glutamic pyruvic transaminase, ALP = alkaline phosphatase

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.

Hepatotoxicity

No effects on histopathology and relative liver weight were seen in rats treated for 12 days by gavage with 500 mg/kg/day (Sch82, Sto82).

In the study of Buben and O'Flaherty (Bub85) 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, 1000, 1500 and 2000 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 1000 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 no-observed-adverse-effect level (NOAEL) of 20 mg/kg.

The Bub85 study comprised a detailed comparison of metabolism (determined as urinary excretion of TCA and TCE) and hepatotoxicity. Good linear relationships were found between on one hand urinary excretion of metabolites and on the other hand SGPT, G-6-Phosphatase, triglycerides and liver weight, thus presenting clear-cut indications that the toxicity is caused by metabolites and not by PER itself (see also Mos77).

Schumann *et al.* (Sch80) compared the hepatotoxicity of PER in mice and rats. The animals were exposed by gavage to 100, 250, 500 or 1000 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.

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.* (Ber92). The results of this study are summarised in Table 6.14.

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 (Ber92), the authors present a plausible explanation, based on a ‘tubular target selectivity’ of PER (PER appears to affect tubular segment S2 in particular).

Table 6.14 Effects of oral PER treatment (500 mg/kg bw/day gavage during 4 weeks) on renal function and renal histopathology in rats (Ber92).

endpoint	effects	
	female	male
Albuminuria (marker for glomerular permeability)	Minor, but significant increase	Marked increase (up to 15 times of control)
Urinary excretion of a-2-u-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 of 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.* (Gol88b). These authors exposed rats to PER by gavage (1000 mg/kg/day) for 10 days. Endpoints examined were: renal a-2-u-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 effect was again seen only in the male. a-2-u-globulin correlated well with PDA. Comparable effects were observed in rats treated with pentachloroethane, but not in those treated with trichloroethylene.

Peroxisome proliferation

The induction of peroxisomal enzyme activity (cyanide-insensitive palmitoyl CoA oxidation, regarded to be a sensitive measure for peroxisome proliferation) by PER (1000 mg/kg; gavage once a day, over a period of 10 days) was investigated by Goldsworthy and Popp (Gol87) in liver and kidneys of rats and mice. A summary of their results is presented in Table 6.15.

Table 6.15 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	50	561 ^a	1223 ^a	958 ^a	689 ^a
Trichloroethylene	1000	180	625 ^a	300 ^a	360 ^a
PER	1000	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-Keuls multicompare test.
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.

Haematotoxicity

The effects of very low oral doses of PER have been investigated by Marth *et al.* (Mar85a and Mar85b) and Marth (Mar87). These authors dissolved PER in drinking water of mice, resulting in doses of 0.05 and 0.1 mg/kg bw/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 lactate dehydrogenase activity, peripheral blood count and microscopic examination of the bone marrow. No increase of serum glutamic pyruvic transaminase (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.

Tumour initiation and promotion

Story *et al.* (Sto86) investigated the initiating and promoting properties of PER in an oral rat model. The rats were partly hepatectomized 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 carcinoma's (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 (MTD: 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 suggest PER to have promoting and not initiating properties.

Comparable studies have been carried out by Lundberg *et al.* and Holmberg *et al.* (Lun87, Hol86), although these were only concerned with promotion (rat; initiator: diethyl nitrosamine). They did not confirm the finding of Sto86, in the sense that no indications for promoting properties were found. Moreover, Maronpot *et al.* (Mar86) did not find such indications in a pulmonary tumour promotion assay with mice.

Intraperitoneal

Bernard *et al.* (Ber89) investigated the nephrotoxicity of 5 i.p. PER injections per week for 2 weeks in rats (1000 mg/kg bw/injection). Endpoints were b-N-acetylglucosaminidase, b2-microglobulin and albumin in urine. No treatment-related effects were observed. Under the same conditions cyclohexane exposure caused a clear-cut increase of b2-microglobulin. Thus, this study does not indicate an effect of PER on the functioning of the tubuli or the glomerula.

6.2.4 Toxicity due to long-term exposure and carcinogenicity

Inhalation

Rampy *et al.* (Ram78; study is only published in the form of an abstract; unpublished details and results were available to the authors of EPA85; summary presented here is based on EPA85) exposed rats (Sprague Dawley) to 0, 300 and 600 ppm (2067 and 4134 mg/m³) of PER for 12 months (6 h/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 HSE87 and ECE90), 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, urinalysis, 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.

The Environmental Protection Agency (EPA85) does not draw specific conclusions from this study as to the carcinogenicity of PER upon respiratory exposure. In Health and Safety executive (HSE87), 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, EPA85 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 the US National Toxicology Program (NTP86). The rats were exposed to 0, 200 and 400 ppm (1378 and 2756 mg/m³), the mice to 0, 100 and 200 ppm (689 and 1378 mg/m³). Exposure lasted 103 weeks; the animals were exposed 6 h/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), 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 leukemia 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 HSE87 and ECE90). However, NTP's Board of Scientific Counselors considered the incidence of rat leukaemia to be a valid finding (ATS97), 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 6.16), part of which had metastasised to other organs.

Table 6.16 Hepatocellular neoplasms in B6C3F1 mice exposed to PER via inhalation (reproduced from ATS97).

	control		100 ppm (690 mg/m ³)		200 ppm (1378 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%)

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 Agency for Toxic Substances and Diseases Registry (ATS97) 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'.

Dermal

Van Duuren *et al.* (Duu79) investigated the carcinogenic and tumour initiating activity of PER in ICR Swiss mice upon dermal application. Thirty female mice were treated three 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 (NCI77; see also ECE90, ATS97 and HSE87). 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 1072 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 time weighted averages (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 an 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 for hepatotoxicity were obtained. At autopsy 79% of the treated animals was affected by nephropathy.

The Agency of Toxic Substances and Diseases Registry (ATS97) 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 (Mal86; see also ECE90) 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.* (Her87) 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 (The77, see also HSE87; PER dissolved in tricapylin; 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 (Car37) 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, 1585 and 3238 mg/m³) 8 h/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 1000 ppm PER (690, 2070 and 6900 mg/m³) 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 1000 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 1000 ppm but not at 300 ppm. This represents a toxic effect which, in part, may have been maternally related.

Schwetz *et al.* (Sch75) exposed rats and mice to 0 and 300 ppm (2067 mg/m³) PER on days 6-15 of gestation (7 h/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. 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 fetotoxicity.

Beliles *et al.* (Bel82) and Harding *et al.* (Har81) subjected pregnant rats to four exposure regimens (20 rats/regimen): exposure to I) 500 ppm (3445 mg/m³) 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 fetotoxicity 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.* (Nel80) exposed groups of 13 to 21 rats to 900 ppm (6200 mg/m³) on days 7-13 or 14-20 of gestation or to 100 ppm (690 mg/m³) 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 central nervous system.

Frederiksson *et al.* (Fre93) treated mice orally to PER (5 and 320 mg/kg bw/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 (Kor89). Synaptosomes isolated from rat brain were exposed to PER, and the activity of the following enzymes were determined: acetylcholinesterase, total adenosinetriphosphatase (ATPase) and magnesium-activated ATPase ((Mg²⁺)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 (Ede92) investigated the effects of PER on I) the activity of the enzyme ($\text{Ca}_2^+/\text{Mg}_2^+$)ATPase in a preparation of synaptosomal membranes isolated from the rat brain and II) 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.* (Hol74) 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 (See72).

Korpela and Tähti (Kor85) 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 4000 ppm ($27,650 \text{ mg/m}^3$); the dose unit is not clear).

Hidalgo *et al.* (Hid90) 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 lipidperoxidation were found to depend strongly on the halocompound 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 (Kef91) did not find effects of PER on lipid peroxidation (assayed as TBARS), enzyme leakage (lactate dehydrogenase and alanine aminotransferase) and leakage of potassium ions in hepatocytes isolated from rats. However, the compounds did effect the leakage of the two enzymes caused by carbontetrachloride. 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.* (Tor92) 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 EC₅₀ 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 (Rei83, Vai85, EPA85, HSE87, ECE90, ATS97). 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

The Environmental Protection Agency (EPA85) mentions one study which indicates mutagenicity in bacteria (Cer77). An induction of histidine prototrophic mutants was found in *Salmonella typhimurium* TA100 (spot test with undiluted compound). However, the Environmental Protection Agency (EPA85) 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 (Gre75, Mar78, Bar79, Kri81, SRI83, Wil83, Haw83, Con85, Mil88, War88). 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).

Yeasts

PER has been extensively tested for genotoxicity in *Saccharomyces cerevisiae* by Bronzetti *et al.* (Bro83). 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 (HSE87 and ECE90)).

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 (NTP86, Gal87).

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 (Cos84, Bel80, Mil88, Wil83). However, a positive result at toxic doses is reported for hepatocytes (species not specified) in an abstract (Shi83). The PER used in this study was stabilised.

Mammalian cells, cell transformation

PER was found to be negative in a cell-transformation assay with BALB/c-3T3 cells (Tu85). 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 (Mil88). A positive result was scored in an assay with rat embryo cells F1706p108 (Pri79). 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 (Bel80). Furthermore, no effects on the chromosomes were found in this test animal (Bel80).

In vivo studies with mammals

Host-mediated assay

The Environmental Protection Agency (EPA85) mentions one study which shows positive results in a host-mediated assay employing the *Salmonella typhimurium* strains TA1950, TA1951 and TA1952 in mice (Cer77). However, the Environmental Protection Agency (EPA85) 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 (Bel80). 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. (Bro83) performed a host-mediated assay with mice and yeast (unusual protocol, lack of positive control), without positive results.

Cytogenetic studies

Chromosomal aberrations were studied in the bone marrow of rats exposed to up to 600 ppm (4134 mg/m³) for 12 months and in the bone marrow of mice exposed by single or repeated intraperitoneal injections (Cer77, Ram78, Bel80). One study yielded an equivocal increase (the test substance had a low purity); the other were negative.

Sperm-head abnormalities

Three sperm-head abnormality tests are described in the literature, carried with Chinese hamsters, mice or rats (Men85, Bel80). A positive result was only found in mice. However, the test material used was of a low purity.

Dominant lethality

A negative result was obtained in a dominant-lethal test with rats (100-500 ppm (689-3445 mg/m³), 7 h/day, 5 days) (Bel80).

Unscheduled DNA synthesis

No unscheduled DNA synthesis was induced in rat kidney cells after oral exposure to 1000 mg/kg (Gol88b).

Single-strand breaks

Walles (Wal86) 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. LD₅₀ values, LC₅₀ 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 LC₅₀ values varied between 3000 and 6000 ppm (20,700 and 41,340 mg/m³) (duration: 2-8 h).
- 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/m³). 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 5200 ppm (35,800 mg/m³) for 1 h. 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/m³)) 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* 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/m³): 4 months of continuous exposure resulted in a decreased DNA concentration in gerbil brains.
- Respiratory exposure of mice to 37 ppm (255 mg/m³) 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

* 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'.

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 (1000 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/m³).

- 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 a-2-u-globulin, electrophoretic shifts in composition of excreted proteins, protein-droplet accumulation 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 (1378 mg/m³) 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/m³) 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 b-lyase. Finally accumulation of a-2-u-globulin (protein-droplet accumulation) 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.
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- 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 (930 and 1860 mg/m³)) caused reversible effects on lymphocyte, monocyte and neutrophile counts. Exposure to 185 ppm (1275 mg/m³) of rats resulted in effects on mast cells.
- One study points to a tumor-promoting effect of PER. The number of enzyme-altered foci in the liver of partly hepatectomized 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 fetotoxicity were found. One study points to fetal 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 compounds 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
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formation of mutagenic products from the conjugation pathway. See Bol82 and Bol87.

- Nevertheless, in view of the great number, as well as the types of negative tests, the committee concludes that PER is virtually devoid of genotoxic properties in mammals.

Existing guidelines, standards and evaluations

7.1 General population

ATSDR (ATS97) 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/m³); intermediate-duration respiratory exposure (subacute to semichronic): 0.0009 ppm (0.0062 mg/m³); 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 (IRI90).

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' (EPA91, ATS97). However, this classification is presently under review.

IARC classifies PER as 'probable carcinogenic to humans', i.e. the compound is placed in category 2A (IARC95). 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 (ATS97).

No acceptable daily intake (ADI) is allocated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO94).

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 7.1.

Table 7.1 Limits for occupational exposure to PER in a number of countries.

country	concentration		time relation	note	ref.
	ppm	mg/m ³			
The Netherlands	35.0000	240.0000	8-h TWA	A	SZW02
Germany				A, B	DFG01
USA, ACGIH	25.0000	170.0000	8-h TWA	C	ACG02
	100.0000	685.0000	15-min TWA		
USA, OSHA	100.0000		8-h TWA	D	OSH02
	200.0000	300.0000		F	
USA, NIOSH	0.4000		REL/LOQ	E	ATS97
Sweden	10	70.0000	8-h TWA	G	ASF02
	25.0000	170.0000	15-min TWA		ASF02
Denmark	10.0000	70.0000	8-h TWA	AG	Arb96
United Kingdom	50	345.0000	8-h TWA		HSE02
	100.0000	689.0000	15-min TWA		

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 7.2) as an "Orientierungsgrosse" (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.

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. They are listed in Table 7.2.

Table 7.2 Biological limit values in the USA and Germany.

country	indicator compound	matrix	time of sampling	concentration	ref.
USA	PER	expired air	prior to last shift of workweek	5 ppm (34.5 mg/m ³)	ACG02
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	DFG01

^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.

Hazard assessment*

8.1 Assessment of health hazard

PER causes eye irritation in humans at concentrations of about 690 mg/m³ (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 1378 and 2756 mg/m³ (200 and 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 trichloroacetic acid (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

* For the recommendation of a health-based occupational exposure limit only DECOS (and not NEG) takes responsibility.

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 it can not serve as a starting point for evaluation.

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.

Fetotoxicity (skeletal abnormalities in mice at 2067 mg/m³ (300 ppm)) and developmental toxicity (decreases in litter size and survival during lactation in rats at 6900 mg/m³ (1000 ppm)) in animals, occur at dose levels which are lying 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/m³ (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/m³ (100 ppm) may lead to adverse neurological effects. In a volunteer study, at 345 mg/m³ (50 ppm) PER, an increase in visual evoked potential and visual contrast sensitivity threshold was observed, whereas in another study increased serum prolactin levels and a poor neurotoxicological response were observed at median PER levels of about 100 mg/m³ (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. Therefore, the DECOS is of the opinion that these studies cannot be used as a starting point for the HBR-OEL. However, they warrant extra caution in setting exposure limits.

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/m³ (100 ppm), the LOAEL. At these concentrations also eye irritation is observed.

DECOS uses this exposure level (LOAEL) as a starting point for the establishment of the health-based recommended occupational exposure level (HBR-OEL). A relatively large safety factor of five is applied for extrapolation from the LOAEL to the NAEL, taken into account the existence of inconclusive evidence for some minor neurological effects (described above) at concentrations lower than 690 mg/m³. Application of this factor results in a HBR-OEL of 138 mg/m³ (20 ppm).

Skin notation

From the available data the committee is of the opinion that a problem with skin contact might occur. Therefore the committee requests a skin notation calculation. It is assumed that a skin notation has to be added when exposure of 2000 cm² of the skin (i.e. hands + fore-arms) to PER during 1 h leads to an additional uptake of 10% of the maximum allowed uptake by inhalation according to the HBR-OEL. The committee made the calculation (see Annex D) and concluded that a skin notation should be applied for PER.

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 Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit for tetrachloroethene (PER) of 138 mg/m³ (20 ppm), 8-hours TWA. A skin notation is also recommended.

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A	Request for advice
B	The committees
C	Comments on the public review draft
D	Calculation skin notation
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Annexes

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advise the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in
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the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

The committees

Dutch expert committee on occupational standards

- GJ Mulder, *chairman*
professor of toxicology; Leiden University, Leiden
 - RB Beems
toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
 - PJ Boogaard
toxicologist; Shell International Petroleum Company, The Hague
 - PJ Borm
toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
 - JJAM Brokamp, *advisor*
Social and Economic Council, The Hague
 - DJJ Heederik
epidemiologist; IRAS, Utrecht University, Utrecht
 - AAJP Mulder, *advisor*
Ministry of Social Affairs and Employment, The Hague
 - TM Pal
occupational physician; Netherlands Center for Occupational Diseases, Amsterdam
 - IM Rietjens
professor of toxicology; Wageningen University, Wageningen.
 - H Roelfzema, *advisor*
Ministry of Health, Welfare and Sport, The Hague
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- T Smid
occupational hygienist; KLM Health Safety & Environment, Schiphol and
professor of working conditions, Free University, Amsterdam
- GMH Swaen
epidemiologist; Maastricht University, Maastricht
- RA Woutersen
toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
Health Council of the Netherlands, The Hague
- JM Rijnkels, *scientific secretary*
Health Council of the Netherlands, The Hague

Nordic Expert Group

- G Johanson, chairman
Professor of occupational toxicology; National Institute for Working Life,
Sweden
- V Kristjansson
Organic chemist; Administration of Occupational Safety and Health, Iceland
- K Savolainen
Toxicologist; Professor, Finnish Institute of Occupational Health, Finland
- V Skaug
Toxicologist; Occupational physician, National Institute of Occupational
Health, Norway
- K Sørig Hougaard
Toxicologist; National Institute of Occupational Health, Denmark
- J Järnberg, *scientific secretary*
National Institute for Working Life, Sweden

The first draft of the present advisory report was prepared by Dr WK de Raat, from TNO Food and Nutrition Research, Department of Occupational Toxicology, Zeist, Amsterdam, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: A Aksel.

Lay-out: J van Kan/M Javanmardi.

Comments on the public review draft

A draft of the present report was released in 1998 for public review. The following organisations and persons have commented on the draft document:

- dr P Evans
Health & Safety Executive, Great Britain
- prof.dr H Greim
Senatskommission der Deutschen Forschungsgemeinschaft

Calculation skin notation

Assuming a skin notation has to be added when exposure of 2000 cm² of the skin (i.e. hands + fore-arms) to PER during 1 h leads to an additional uptake of 10% of the maximum allowed uptake by inhalation according to the HBR-OEL, the following calculation can be applied.

A thumb-immersion (thumb surface 15 cm²) study with human volunteers showed that an exposure of 40 min leads to a maximum alveolar concentration of 2.14 mg/m³ (0.31 ppm). Based on a respiratory resorption under steady-state conditions of 50%, this value is equivalent to a steady-state respiratory exposure to 4.27 mg/m³ (0.62 ppm) (see Section 5.1.2), if it is assumed that 1) the respiratory resorption is not concentration dependent and 2) metabolism, distribution and elimination is not exposure-route dependent. It is further assumed, that dermal absorption is linearly related to surface and exposure time. The alveolar air concentrations occurring when 2000 cm² of skin is exposed for 1 h can then be calculated as $(2000/15) \times (60/40) \times 0.31 = 62$ ppm (428 mg/m³), in which calculation the skin surface of the thumb is assumed to be 15 cm². This corresponds with a respiratory exposure of 854 mg/m³ (124 ppm). This value exceeds the proposed HBR-OEL by a factor of 6, which means that a skin notation should be applied for PER.

A study with guinea pigs resulted in a dermal-absorption rate of 24.4 nmoles/min/cm², which (assuming linear dependencies) means that 1-h exposure of 2000 cm² would lead to an absorption of $60 \times 2000 \times 24.4$ nmol = 2928 μmol = 485.8 mg. One hour exposure to 138 mg/m³ (20 ppm), corresponds (assuming an absorption of

50%) with an absorbed amount of $1.25 \times 138 = 172.5$ mg (ventilation rate: $1.25 \text{ m}^3/\text{h}$). So, in this case the dermal absorption exceeds the respiratory absorption by a factor of 2.8. Thus, the need for a skin notation is confirmed by this independent animal experiment.

The value of the first calculation is affected by the uncertainty about the relation between concentration and respiratory absorption as well as the slow distribution of PER after dermal exposure (see Section 5.1.2).

The value of the second depends very much on the strong species-dependence of dermal absorption. However, the height of the factors induce DECOS to conclude that a skin notation for PER is fully justified.

Abbreviations

<i>bp</i>	boiling point
<i>EC₅₀</i>	concentration at which a described effect is found in 50% of the exposed animals or at which the effect is decreased up to 50% of the control value
<i>HBR-OEL</i>	health based recommended occupational exposure limit
<i>h</i>	hour
<i>IC₅₀</i>	concentration at which inhibition of a certain function is found up to 50% of the control value
<i>LC₅₀</i>	lethal concentration for 50% of the exposed animals
<i>LC₁₀</i>	lowest lethal concentration
<i>LD₅₀</i>	lethal dose for 50% of the exposed animals
<i>LD₁₀</i>	lowest lethal dose
<i>LOAEL</i>	lowest observed adverse effect level
<i>MAC</i>	maximaal aanvaarde concentratie (maximal accepted concentration)
<i>MAEL</i>	minimal adverse effect level
<i>MAK</i>	Maximale Arbeitsplatz Konzentration
<i>MOAEL</i>	minimal observed adverse effect level
<i>MTD</i>	maximum tolerated dose
<i>NAEL</i>	no adverse effect level

<i>NEL</i>	no effect level
<i>NOAEL</i>	no observed adverse effect level
<i>OEL</i>	occupational exposure limit
<i>PEL</i>	permissible exposure limit
<i>ppb</i>	parts per billion (v/v)10 ⁻⁹
<i>ppm</i>	parts per million (v/v)10 ⁻⁶
<i>RD₅₀</i>	dose at which a 50% decrease of respiratory rate is observed
<i>REL</i>	recommended exposure limit
<i>STEL</i>	short term exposure limit
<i>tgg</i>	tijd gewogen gemiddelde
<i>TLV</i>	threshold limit value
<i>TWA</i>	time weighted average
<i>V_{max}</i>	maximal reaction velocity of an enzyme

Organisations

<i>ACGIH</i>	American Conference of Governmental Industrial Hygienists
<i>CEC</i>	Commission of the European Communities
<i>DECOS</i>	Dutch Expert Committee on Occupational Standards
<i>DFG</i>	Deutsche Forschungsgemeinschaft
<i>EPA</i>	Environmental Protection Agency (USA)
<i>FDA</i>	Food and Drug Administration (USA)
<i>HSE</i>	Health and Safety Executive (UK)
<i>IARC</i>	International Agency for Research on Cancer (WHO)
<i>INRS</i>	Institut National de Recherche et de Sécurité (France)
<i>NIOSH</i>	National Institute for Occupational Safety and Health (USA)
<i>NTP</i>	National Toxicology Programme (USA)
<i>OECD</i>	Organisation for Economic Cooperation and Development
<i>OSHA</i>	Occupational Safety and Health Association (USA)
<i>RTECS</i>	Registry of Toxic Effects of Chemical Substances
<i>SER</i>	Social and Economic Council (Sociaal-Economische Raad NL)
<i>WATCH</i>	Working Group on the Assessment of Toxic Chemicals (UK)
<i>WHO</i>	World Health Organisation

Toxicological terms

<i>bid</i>	<i>bis in diem</i> (twice per day)
<i>bw</i>	body weight
<i>CARA</i>	chronic non-specific respiratory diseases
<i>CHD</i>	coronary heart disease
<i>CNS</i>	central nervous system
<i>ECG</i>	electrocardiogram
<i>EEG</i>	electro encephalogram
<i>FCA</i>	Freunds Complete Adjuvans
<i>FEV</i>	forced expiratory volume
<i>FSH</i>	follicle stimulating hormone
<i>GD</i>	gestation day(s)
<i>GPMT</i>	guinea pig maximisation test
<i>GSH</i>	glutathione
<i>HLiA</i>	hamster liver activated
<i>IHD</i>	ischaemic heart disease
<i>im</i>	intramuscular
<i>ip</i>	intraperitoneal
<i>ipl</i>	intrapleural
<i>it</i>	intratracheal
<i>iv</i>	intravenous
<i>LH</i>	lutheising hormone
<i>MAC</i>	minimal alveolar concentration
<i>MFO</i>	mixed function oxidase
<i>NA</i>	not activated
<i>PNS</i>	peripheral nervous system
<i>po</i>	<i>per os</i> (= oral)
<i>RBC</i>	red blood cells
<i>RLiA</i>	rat liver activated
<i>SCE</i>	sister chromatid exchange
<i>sc</i>	subcutaneous
<i>UDS</i>	unscheduled DNA-synthesis

Statistical terms

<i>GM</i>	geometric mean
<i>OR</i>	Odds Ratio
<i>RR</i>	relative risk
<i>SD</i>	standard deviation
<i>SEM</i>	standard error of mean
<i>SMR</i>	standard mortality ratio

Analytical methods

<i>AAS</i>	atomic absorption spectroscopy
<i>BEEL</i>	biological equivalent exposure limit
<i>BEI</i>	biological exposure index
<i>BEM</i>	biological effect monitoring
<i>BM</i>	biological monitoring
<i>ECD</i>	electron capture detector
<i>BM</i>	<i>biological monitoring</i>
<i>EM</i>	environmental monitoring
<i>FID</i>	flame ionisation detector
<i>GC</i>	gas chromatography
<i>GLC</i>	gas liquid chromatography
<i>GSC</i>	gas solid chromatography
<i>HPLC</i>	high performance liquid chromatography
<i>IR</i>	infrared
<i>MS</i>	mass spectrometry
<i>NMR</i>	nuclear magnetic resonance
<i>PAS</i>	personal air sampling
<i>TLC</i>	thin layer chromatography
<i>UV</i>	ultraviolet

Additional abbreviations in the present report

<i>ATP</i>	adenosine triphosphatase
<i>BAEP</i>	brain-stem evoked potentials
<i>CytP450</i>	cytochrome P450
<i>g-GT</i>	gamma-glutamyl transferase
<i>PER</i>	tetrachloroethene

<i>PMR</i>	proportional mortality ratio
<i>SGPT</i>	serum glutamic pyruvic transaminase
<i>SG-6-P</i>	serum glucose-6-phosphatase
<i>SIR</i>	standardized incidence ratio
<i>SMOR</i>	standardized mortality odds ratio
<i>SOCT</i>	serum omithine carbamyl transferase
<i>SPMR</i>	standardized proportional mortality ratio
<i>TBAR</i>	thiobarbituric acid-reactive substances
<i>TCA</i>	trichloroacetic acid
<i>TCE</i>	trichloroethanol
<i>VCS</i>	visual contrast sensitivity
<i>VEP</i>	visual evoked potential

DECOS-documents

DECOS has produced documents on the following substances. To be ordered from the Health Council of the Netherlands:

Aanpassing van grenswaarden bij flexibele werktijden	2001/06OSH
Acetone cyanohydrin	1995/05WGD
p-Aramid fibres	1997/07WGD
Azathioprine	1999/04OSH
Aziridine (ethyl imine)	2000/13OSH
Azobisisobutyronitril	2002/01OSH
1,2,3-Benzotriazole	2000/14OSH
Bisphenol A and its diglycidylether	1996/02WGD
Bromoethane	1998/10WGD
1,2-and t-Butanol	1994/10WGD
n-, iso-, sec-, tert-Butylacetaten	2001/03OSH
β -Butyrolactone	1999/05OSH
Cadmium and inorganic cadmium compounds	1995/04WGD
Calculating cancer risk	1995/06WGD
Carbadox	1999/06OSH
Carbon disulphide	1994/08
Chlorine dioxide	1995/07WGD
p-Chloroaniline	1998/09WGD
4-Chloro-o-toluidine	1998/08WGD
Chlorotrimethylilane	2001/05OSH
Chromium and its inorganic compounds	1998/01WGD
Chromium VI and its compounds	2001/01OSH
Cresols	1998/15WGD

Copper sulphate	1999/01OSH
1996-1997 WGD-rapporten/1996-1997 DECOS reports	1999/01WGD
1,2-Dibromoethane	1999/07OSH
1,2-Dichloroethane	1997/01WGD
Diethylsulphate	1999/08/OSH
Diglycidyl resorcinol ether	1999/09OSH
Diphenylamine	1997/05WGD
<Titeladv>	1998/03WGD
Epichlorohydrin (1-Chloro-2,3-epoxypropane)	2000/10OSH
1,2-Epoxybutane	1998/11WGD
1,2-Ethanediamine	1996/03WGD
Ethyleneglycol ethers	1996/01WGD
Ejthylene oxide	2001/11OSH
Ethylene thiourea	1999/03OSH
Formamide and dimethylformamide	1995/08WGD
Hydrazinoethanol, phenylhydrazine, isoniazid, maleic hydrazide	1997/03WGD
Isopropyl acetate	1997/04WGD
Lactate esters	2001/04OSH
Lindane	2001/07OSH
Man made mineral fibers	1995/02WGD
Manganese and its compounds	2001/02OSH
2-Methylaziridine (propylene imine)	1999/10OSH
Methyl Methacrylate	1994/09
Methacrylates. Ethyl methacrylate, n-butyl methacrylate and isobutyl methacrylate	1994/11
Methyl-t-butylether	1994/23
Methyl chloride	1995/01WGD
4,4'-Methylene bis (2-Chloroaniline)	2000/09OSH
4,4'-Methylene dianiline	2000/11OSH
Metronidazole	1999/11OSH
2-Nitropropane	1999/13OSH
N-Nitrosodimethylamine (NDMA)	1999/12OSH
2-Nitrotoluene	1998/12WGD
Pentaerythritol	1997/06WGD
Phenol	1996/04WGD
o-Phenylenediamine	1998/06WGD
Piperidine	1997/08WGD
Procarbazine hydrochloride	1999/14OSH
1- and 2-Propanol	1994/24
Propylene oxide	1997/02WGD
Ronidazole	1998/05WGD
Styrene	1998/07WGD
Styrene	2001/08OSH
Quartz	1998/02WGD
Toluene	2001/09OSH
1,1,1-Trichloroethane	1995/03WGD
1,2,3-Trichloropropane	1994/25
1,2,3-Trichloropropane	1998/14WGD
Urethane (ethyl carbamate)	200012OSH

Vinylbromide
Xylene
Wood dust

1999/15OSH
2001/10OSH
1998/13WGD