Occupational exposure to organic solvents: effects on human reproduction

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Occupational exposure to organic solvents: Effects on human reproduction*
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Geachte minister,

Graag bied ik u hierbij het advies *Occupational exposure to organic solvents: Effects on human reproduction* aan. In dit advies heeft een speciaal ingestelde commissie onderzocht of beroepsmatige blootstelling aan organische oplosmiddelen schadelijke gevolgen heeft voor de vruchtbaarheid van mannen en vrouwen of voor de ontwikkeling van het nageslacht. Het advies is binnen de Gezondheidsraad getoetst door een vast college van deskundigen: de beraadsgroep Gezondheid en omgeving.

De beantwoording van uw vragen heeft op zich laten wachten. De reden hiervoor is dat er veel wetenschappelijk epidemiologisch onderzoek beschikbaar is over de gevolgen van blootstelling aan organische oplosmiddelen voor de reproductie. De kwaliteit van het beschikbare onderzoek is echter zeer uiteenlopend. De commissie achtte het daarom van groot belang de kwaliteit van alle studies systematisch te beoordelen. Dit was een tijdrovende exercitie. Bij het beantwoorden van uw vragen heeft de commissie vervolgens de studies van onvoldoende kwaliteit buiten beschouwing gelaten.

Ondanks de hoeveelheid gegevens bleek het toch lastig te bepalen of, en zo ja, welke oplosmiddelen reproductietoxische effecten veroorzaken. Een van de grootste problemen was de afwezigheid van een goede beschrijving van de blootstelling in veel studies. Alleen blootstelling aan tolueen, tetrachloorethyleen, xyleen en enkele ethyleenglycolethers kan in verband worden gebracht met effecten op de reproductie. Ik onderschrijf dan ook de aanbeveling van de commissie om een nationale databank op te zetten met informatie over blootstellingen op de werkplek. Het is te overwegen deze databank niet te beperken tot alleen de organische oplosmiddelen.

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Van dit advies heb ik tevens een exemplaar aangeboden aan de minister van Volksgezondheid, Welzijn en Sport en de minister van Ruimte en Milieu.

Met vriendelijke groet,

prof. dr. J.A. Knottnerus

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Occupational exposure to organic solvents: effects on human reproduction

to:

the Minister of Social Affairs and Employment

the Minister of Health, Welfare and Sport

the Minister of Spatial Planning and the Environment

No. 2008/11OSH, The Hague, June 12, 2008

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Samenvatting

Nederlands onderzoek aanleiding voor vragen aan Gezondheidsraad

In Nederland wordt naar schatting een half miljoen werknemers met enige regelmaat blootgesteld aan organische oplosmiddelen. Enkele bekende oplosmiddelen zijn tolueen, styreen, xyleen, benzeen en terpentine. Ze worden op grote schaal toegepast om te ontvetten en te verdunnen en zijn bijvoorbeeld te vinden in verven, beitsen, lijmen en autolakken. Omdat oplosmiddelen verdampen, kunnen mensen die met deze producten werken deze inademen.

Al langere tijd is bekend dat inademing van organische oplosmiddelen de gezondheid kan schaden. Beroepsmatige blootstelling aan deze oplosmiddelen is de afgelopen decennia in verband gebracht met diverse effecten. Het bekendste en best gedocumenteerde effect is chronische toxische encefalopathie (CTE), ook wel organisch psychosyndroom (OPS) genoemd. Dit is een ernstige vorm van schade aan het zenuwstelsel, met als gevolg geheugenstoornissen, een verminderd concentratievermogen, mentale traagheid, vermoeidheid, hoofdpijn, depressiviteit en prikkelbaarheid. Ook is bekend dat blootstelling aan bepaalde organische oplosmiddelen (zoals benzeen) kanker kan veroorzaken.

Minder is bekend over een mogelijk effect op de voortplanting. In 1999 ontstond over dat onderwerp commotie na publicatie van een Nederlandse studie onder mannen die bij een fertiliteitskliniek waren onderzocht. Een verminderde spermakwaliteit leek samen te hangen met de blootstelling aan organische oplos-

middelen. De resultaten van dit onderzoek leidden tot Kamervragen. In 2005 kwamen de resultaten van een ander Nederlands onderzoek beschikbaar. Die suggereerden dat blootstelling aan oplosmiddelen de oorzaak zou zijn van aangeboren afwijkingen en ontwikkelingstoornissen bij kinderen van schilders.

Deze twee Nederlandse studies waren voor de Staatssecretaris van Sociale Zaken en Werkgelegenheid aanleiding om de Gezondheidsraad om advies te vragen over de mogelijke gevolgen van beroepsmatige blootstelling aan organische oplosmiddelen voor de voortplanting. Een speciaal ingestelde commissie zijn de volgende vragen voorgelegd:

- Zijn er aanwijzingen dat beroepsmatige blootstelling aan organische oplosmiddelen kan leiden tot effecten op de voortplanting?
- Is er een werkingsmechanisme bekend waarmee de mogelijke effecten te verklaren zijn?
- Beschermen de grenswaarden* die momenteel gelden voor diverse organische oplosmiddelen tegen effecten op de voortplanting?

Onder reproductiestoornissen verstaat de commissie alle problemen die kunnen ontstaan rond de vruchtbaarheid, de zwangerschap en de ontwikkeling van het nageslacht.

De helft van het beschikbare onderzoek is bruikbaar

De adviesvragen worden beantwoord door de resultaten uit wetenschappelijk onderzoek te beoordelen en te wegen. Een belangrijke bevinding is dat er weliswaar veel publicaties zijn die de gevolgen van beroepsmatige blootstelling aan oplosmiddelen voor de voortplanting bestuderen, maar dat die lang niet allemaal bruikbaar zijn om de vragen van de commissie te beantwoorden. Zo is blootstelling aan oplosmiddelen vaak lastig in kaart te brengen. Een van de grote problemen is dat werknemers in bijna alle gevallen zijn blootgesteld aan mengsels van verschillende oplosmiddelen. Daarnaast komen werknemers in veel bedrijfstakken ook nog in aanraking met andere stoffen (bijvoorbeeld metalen), die soms reproductiestoornissen kunnen veroorzaken. Relaties tussen blootstelling aan specifieke oplosmiddelen en reproductiestoornissen zijn in die gevallen niet meer vast te stellen. Er is maar weinig bekend over de samenstelling van de mengsels waarmee mensen werken, en over de concentraties van de individuele

* In de adviesvraag uit 2005 wordt verwezen naar de MAC-waarden (Maximaal aanvaarde concentraties). Deze zijn inmiddels vervangen door publieke (wettelijke) en private grenswaarden.

oplosmiddelen waaraan zij blootstaan – ondanks de inspanningen die de laatste jaren op dat gebied zijn geleverd.

Een ander punt is dat effecten in allerlei stadia in de voortplantingscyclus kunnen optreden. Het kan gaan om gevolgen voor sperma, eicellen, eierstokken en menstruatie, maar ook om het optreden van miskramen, geboorteafwijkingen en problemen in de psychomotorische ontwikkeling van het kind. Bij deze effecten kunnen verschillende mechanismen betrokken zijn, en ook de oorzaken zijn waarschijnlijk verschillend. Het is dan ook belangrijk dat in studies onderscheid gemaakt wordt tussen de verschillende effecten en dat ieder effect goed is gedefinieerd. Dat is in veel studies niet het geval.

Tot slot is er vaak geen informatie beschikbaar over het moment van blootstelling, terwijl dat bij het veroorzaken van reproductietoxische effecten juist een kritische rol speelt. Zo kan blootstelling in de vroege fase van de zwangerschap andere effecten veroorzaken dan blootstelling in een latere fase.

Door deze methodologische tekortkomingen is de kwaliteit van veel epidemiologisch onderzoek niet voldoende om uitspraken te kunnen doen over de gerapporteerde verbanden tussen blootstellingen en reproductiestoornissen. De commissie heeft de beschikbare studies beoordeeld en meer dan de helft van de beschikbare studies afgewezen. Er bleven ruim 80 studies over die wel aan de gestelde kwaliteitscriteria voldeden.

Voor een aantal oplosmiddelen en situaties zijn effecten op de voortplanting gevonden

Blootstelling aan ethyleen glycolethers vermindert de vruchtbaarheid van mannen

Voor enkele ethyleenglycolethers (EGEE* en EGME**) heeft de commissie in onderzoek bij mensen aanwijzingen gevonden dat blootstelling aan deze oplosmiddelen de vruchtbaarheid van mannen kan verminderen. Onderzoek in proefdieren bevestigt dit.

Werknemers kunnen aan ethyleenglycolethers blootgesteld worden tijdens verf- en onderhoudswerkzaamheden en in de halfgeleiderindustrie. Onderzoek onder werknemers in schilders- en onderhoudsbedrijven geeft zwakke aanwijzingen dat de kans op vruchtbaarheidsproblemen bij mannen is verhoogd, afhanke-

* EGEE: Ethyleenglycolethylether ofwel 2-ethoxyethanol.
** EGME: Ethyleenglycolmethylether ofwel 2-methoxyethanol.

lijk van de hoogte van de blootstelling aan waarschijnlijk ethyleenglycolethers. De afgelopen jaren zijn de blootstellingniveaus in Nederlandse bedrijven sterk gedaald als gevolg van regelgeving. Daarnaast is het gebruik van een aantal ethyleenglycolethers (waaronder EGEE en EGME) vervangen door andere (minder schadelijke) glycolethers. Recente blootstellinggegevens zijn echter niet beschikbaar. Daarom kan de commissie niet uitsluiten dat er nog steeds bedrijfstakken zijn waar schadelijke effecten op de voortplanting optreden als gevolg van blootstelling aan deze ethyleenglycolethers.

Blootstelling van zwangere vrouwen aan ethyleen glycolethers verhoogt de kans op een miskraam en geboorteafwijkingen

Als zwangere vrouwen aan ethyleenglycolethers worden blootgesteld kunnen er reproductietoxische effecten optreden. Er zijn namelijk aanwijzingen uit epidemiologisch onderzoek dat de kans op miskramen en geboorteafwijkingen na blootstelling aan EGEE en EGME verhoogd is. Daarnaast laat dierexperimenteel onderzoek overtuigend zien dat blootstelling aan ethyleenglycolethers schadelijke effecten veroorzaakt tijdens en na de zwangerschap (van vruchtdood tot geboorteafwijkingen).

Er wordt voornamelijk in de halfgeleiderindustrie met ethyleenglycolethers gewerkt. Studies in deze industrie geven ook aanwijzingen voor een verhoogde kans op deze effecten. Ook hier geldt dat de blootstelling intussen sterk is gedaald, maar dat de commissie niet kan uitsluiten dat er nog steeds bedrijven zijn waar concentraties in de lucht een effect zouden kunnen hebben de ontwikkeling van het nageslacht.

Blootstelling van zwangere vrouwen aan tetrachloorethyleen (PER) en xyleen verhoogt mogelijk de kans op een miskraam

De commissie concludeert dat er zwakke aanwijzingen zijn dat blootstelling aan tetrachloorethyleen (PER) en xyleen bij zwangere vrouwen de kans op een miskraam verhoogt. Ook dierexperimenteel onderzoek laat zien dat blootstelling aan deze oplosmiddelen de ongeboren vrucht kan schaden. De aanwijzingen zijn echter niet sterk genoeg als bevestiging van de epidemiologische gegevens.

Blootstelling aan PER komt voornamelijk voor in stomerijen (chemische wasserijen). Studies uit de jaren 80 van de vorige eeuw onder medewerkers van stomerijen en wasserijen bevestigen het beeld dat de kans op een miskraam is verhoogd, waarschijnlijk als gevolg van blootstelling aan hoge concentraties PER. De laatste jaren zijn de concentraties PER echter zo sterk gedaald dat de

commissie niet verwacht dat de huidige blootstellingniveaus tot effecten op de voortplanting zullen leiden.

Blootstelling aan xyleen wordt voornamelijk waargenomen in drukkerijen en in de petrochemische industrie. Omdat er in de beschikbare studies gelijktijdig blootstelling aan andere oplosmiddelen voorkomt, kan de commissie niet vaststellen of er op dit moment in deze bedrijfstakken een daadwerkelijk verhoogd risico is.

De kans op een miskraam is verhoogd na blootstelling van mannen en zwangere vrouwen aan tolueen

Volgens de commissie zijn er aanwijzingen uit onderzoek bij mensen dat blootstelling van zwangere vrouwen aan tolueen de kans op een miskraam verhoogt. Ook de kans op kinderleukemie is verhoogd, al zijn de aanwijzingen hiervoor zwak. De dierexperimentele gegevens bevestigen een mogelijk verband tussen blootstelling aan tolueen en het optreden van miskramen niet. De bewijskracht van deze laatste studies is echter beperkt.

In drukkerijen en de petrochemische industrie kan behalve blootstelling aan xyleen ook blootstelling aan tolueen plaatsvinden. De commissie concludeert dat er zwakke aanwijzingen zijn dat zwangere vrouwen die in drukkerijen of in petrochemische bedrijven blootstaan aan onder meer tolueen een verhoogde kans hebben op reproductiestoornissen. Omdat er ook blootstelling aan andere oplosmiddelen (bv xyleen en styreen) plaatsvindt, valt echter niet vast te stellen wat het aandeel van tolueen in dat risico is.

Niet alleen de blootstelling van zwangere vrouwen kan gevolgen hebben voor het nageslacht. Er zijn ook zwakke aanwijzingen dat als mannen vóór de bevruchting worden blootgesteld aan tolueen (bijvoorbeeld tijdens verf- en onderhoudswerkzaamheden) de kans op een miskraam bij hun partner eveneens verhoogd is.

Gegevens over de huidige concentraties tolueen zijn niet beschikbaar. De commissie kan daarom niet vaststellen of er momenteel nog een verhoogd risico is op miskramen in deze bedrijfstakken.

Van veel oplosmiddelen is weinig bekend

Informatie over de gevolgen van blootstelling voor de voortplanting is slechts voor een klein deel van de totale groep organische oplosmiddelen beschikbaar (tolueen, xyleen, styreen, aceton, ethyleenglycolethers, tetrachloorethyleen, benzeen en methyleen chloride). Voor een grote groep van organische oplosmidde-

len (zoals N-methylpyrrolidon, butanol, isopropanol, 2-butoxyethanol en vele andere) geldt dat er geen (epidemiologisch) onderzoek is gedaan naar de effecten op de voortplanting. Hoewel in de praktijk blootstelling aan deze oplosmiddelen regelmatig voorkomt, kan de commissie geen uitspraken doen over de gevolgen van beroepsmatige blootstelling voor de voortplanting.

Maar ook over oplosmiddelen waarvoor wel gegevens beschikbaar zijn is het laatste woord nog niet gesproken. De resultaten zijn soms zeer beperkt of tegenstrijdig. Daardoor zijn ook voor deze oplosmiddelen vaak geen conclusies te trekken.

Beoordelen van de huidige grenswaarden is niet mogelijk

Beschermen de grenswaarden voor de werkplek die momenteel van kracht zijn voor diverse organische oplosmiddelen ook tegen effecten op de voortplanting? Deze vraag van de staatssecretaris is volgens de commissie met de beschikbare gegevens niet te beantwoorden. Een gezondheidskundige grenswaarde beschermt, per definitie, werknemers (en hun nageslacht) tegen *alle* gezondheidseffecten als gevolg van beroepsmatige blootstelling aan stoffen. Bij het vaststellen van de gezondheidskundige advieswaarden moet dus ook rekening worden gehouden met de effecten op reproductie. Maar dan moet er wel onderzoek naar gedaan zijn en dat is vaak niet het geval.

Sinds de invoering van de nieuwe Arbowet op 1 januari 2007 moeten zowel de publieke als private grenswaarden op het niveau van de gezondheidskundige advieswaarde liggen. Voor enkele oplosmiddelen is een wettelijke grenswaarde beschikbaar: tolueen, aceton, xyleen, benzeen en enkele glycolethers. Voor de meeste andere oplosmiddelen geldt dat de sociale partners verantwoordelijk zijn voor het vaststellen van een veilige grenswaarde.

De commissie is dan ook van mening dat, als er in een epidemiologische studie effecten op de voortplanting worden gevonden, het belangrijk is vast te stellen aan welke oplosmiddelen blootstelling heeft plaatsgevonden en wat het niveau van blootstelling is geweest. Indien deze gegevens niet voorhanden zijn, is het niet mogelijk vast te stellen of de gevonden effecten op de reproductie het gevolg zijn van blootstelling aan een niveau boven de bestaande grenswaarden, of dat de bestaande grenswaarden niet voldoende beschermen.

Betere blootstellinggegevens zijn nodig om risico's in kaart te brengen

Voor een aantal oplosmiddelen (ethyleenglycolethers, tolueen, xyleen en tetrachloorethyleen) zijn er (zwakke) aanwijzingen dat blootstelling reproductiestoornissen kan veroorzaken. Actuele gegevens over de blootstelling in verschillende bedrijfstakken zijn echter niet publiek beschikbaar. Daarom adviseert de commissie voor deze stoffen het volgende:

- onderzoek allereerst in welke bedrijfstakken momenteel nog blootstelling aan deze oplosmiddelen plaatsvindt
- kwantificeer vervolgens voor die bedrijfstakken waar blootstelling waarschijnlijk is en wat de blootstellingconcentraties van de individuele oplosmiddelen zijn.

Beter ontsluiten van gegevens is ook noodzakelijk

Voor de meeste oplosmiddelen zijn geen of slechts beperkt gegevens over de blootstelling beschikbaar. In Nederland worden de meeste blootstellingsmetingen gedaan in opdracht van individuele bedrijven, waardoor de gegevens vaak niet openbaar toegankelijk zijn. De commissie adviseert daarom een nationale databank op te zetten, waarin alle blootstellinggegevens kunnen worden opgenomen. Het systeem in Groot-Brittannië of Duitsland kan hiervoor als voorbeeld dienen.

Daarnaast heeft de commissie geconstateerd dat de huidige registratiesystemen voor geboorteafwijkingen ook hun beperkingen kennen. Daarom adviseert zij ook één nationaal register op te zetten, waarin alle bestaande registers geïntegreerd worden.

Als beide databestanden (voor blootstelling en voor effecten) dan ook nog gekoppeld zouden kunnen worden, zou dat veel mogelijkheden bieden om risico's op de werkplek beter te onderzoeken dan op dit moment mogelijk is.

Executive summary

Dutch study poses questions for Health Council

It is estimated that half a million workers in the Netherlands are regularly exposed to organic solvents. Some examples of well-known solvents are toluene, styrene, xylene, benzene and turpentine. These are widely used for degreasing and diluting, and are found in such products as ordinary paints, car paints, stains, and glues. Solvents evaporate, so those who work with these products tend to inhale them.

It is known for a long time that inhaling organic solvent fumes may impair people's health. In recent years, occupational exposure to these solvents has been linked to various effects. The most well-known and best documented effect is the occurrence of chronic toxic encephalopathy (CTE), also known as organic psycho syndrome (OPS). This involves serious damage to the nervous system, resulting in memory disorders, impaired concentration, mental inertia, fatigue, headache, irritability and depression. In addition, it is known that exposure to certain organic solvents (such as benzene) can cause cancer.

Less is known about possible effects on reproduction. In 1999, that topic was at the centre of a commotion, following the publication of a Dutch study in men who had been examined at a fertility clinic. This study found that exposure to organic solvents appeared to be linked to reduced sperm quality. The results of this study led to questions being asked in the Dutch parliament. The results of another Dutch study were published in 2005. These results suggested that expo-

sure to solvents might cause birth defects among the children of professional painters.

In 2005, these two Dutch studies caused the State Secretary for Social Affairs and Employment to seek the Health Council's advice about the possible effects on reproduction of occupational exposure to organic solvents. The following questions were submitted to a specially appointed committee:

- Is there any evidence that occupational exposure to organic solvents can produce effects on reproduction?
- Is there a known mechanism of action that could explain these possible effects?
- Do the limit values* that currently apply to various organic solvents provide protection against effects on reproduction?

The Committee uses the term "reproductive disorders" to refer to any problems that may arise in connection with fertility, pregnancy, and development of the offspring.

Half of the available research is useable

Requests for advice are answered by assessing and weighing up the results of scientific research. One key finding is that, while there are many publications dealing with the effects of occupational exposure to solvents on reproduction, only a few of them can be used for answering the committee's questions. For example, exposure to solvents is often difficult to quantify. One of the major problems is that, in nearly every case, the employees in question are exposed to mixtures of different solvents. Furthermore, workers in many industries also come into contact with other substances (such as metals), which can sometimes cause reproductive disorders. In such cases, it is no longer possible to identify the relation between exposure to a given solvent and the occurrence of reproductive disorders. In spite of the efforts of research workers in recent years, very little is known about the composition of the mixtures used by people in the course of their work, and about the concentrations of the individual solvents to which they are exposed.

A second problem is that effects can occur at many different phases in the reproductive cycle. This may involve effects on sperm, ova, ovaries, and menstruation, as well as the occurrence of miscarriages, birth defects, and problems

* The 2005 request for advice refers to the MAC values (Maximum Allowed Concentrations). In 2007, these values were substituted by public and private occupational exposure limits.

in the child's psychomotor development. These effects all involve different mechanisms and, in all probability, a range of different causes. Therefore, for all studies it is essential to drew a distinction between the different effects and to define each effect accurately.

Finally, there is often no information available about the timing of exposure, even though this is thought to be one of the critical factors in causing toxic effects on the reproductive system. For example, effects of exposure at an early stage of pregnancy can differ from those resulting from exposure at a later stage.

As a result of these weaknesses, the quality of much epidemiological research is too poor to enable judgments about the reported links between exposures and reproductive disorders. After evaluating the available studies, the committee has rejected more than half of them. This still left well over 80 studies which did meet the quality criteria.

Effects on reproduction were identified for a number of solvents and situations

Exposure to some ethylene glycol ethers impairs male fertility

After examining studies in human subjects, the committee found indications that exposure to various ethylene glycol ethers (EGEE* and EGME**) may impair male fertility. This has been confirmed by experimental animal studies.

Workers exposed to ethylene glycol ethers include those carrying out painting or maintenance work, and those employed in the semiconductor industry. Studies of employees in the painting and maintenance trade show weak indications for an increased risk of fertility problems in men, depending on the level of exposure (probably to ethylene glycol ethers). The introduction of appropriate regulations caused exposure levels to these glycol ethers in Dutch companies to fall markedly in recent years. In addition, a switch was made from using ethylene glycol ethers (such as EGEE and EGME) to using other less harmful glycol ethers. However, recent exposure data aare not available. For this reason, the committee cannot exclude the possibility that employees in some sectors may still be at risk for harmful effects on reproduction as a result of exposure to these ethylene glycol ethers.

* EGEE: Ethylene glycol ethyl ether or 2-ethoxyethanol.
** EGME: Ethylene glycol methyl ether or 2-methoxyethanol.

Exposure of pregnant women to some ethylene glycol ethers increases the risk of spontaneous abortions and birth defects

Exposure of pregnant women to ethylene glycol ethers may lead to reproduction toxic effects. Epidemiological studies give indications for an increased risk of spontaneous abortions and malformations after exposure to EGME and EGEE. In addition, experimental animal studies convincingly demonstrated that exposure to ethylene glycol ethers gives causes harmful effects during and after pregnancy (ranging from foetal death to birth defects).

Ethylene glycol ethers are primarily used in the semiconductor industry. Human studies in this industry also provide indications for an increased risk of such effects. Here too, exposure levels have fallen substantially, but the committee cannot exclude the possibility that there are still companies where the airborne concentrations of these substances could exert an effect on the development of the progeny.

Exposure of pregnant women to tetrachloroethylene (PER) and xylene may increase the risk of miscarriage

The committee concludes that there are weak indications that exposure to tetrachloroethylene (PER) and xylene in pregnant women increases the risk of spontaneous abortions. Experimental animal studies also show that exposure to these solvents may harm the unborn foetus. However, the evidence is not strong enough to confirm the epidemiological data.

Exposure to PER is mainly found in dry-cleaning shops (chemical laundries). Studies carried out among employees of dry-cleaning shops and laundries in the 1980s confirm the perception that there might be an increased risk of miscarriage, probably due to exposure to high concentrations of PER. However, PER exposure levels have dropped so markedly in recent years that the committee does not expect current exposure levels to lead to effects on reproduction.

Exposure to xylene mainly occurs in printing companies and in the petrochemical industry. Because the subjects in the available studies were simultaneously exposed to other solvents, the committee is unable to determine whether there is still an elevated risk for those who are currently employed in these industries.

Exposure of men and pregnant women to toluene increases the risk of spontaneous abortion

The committee concludes that there are indications from studies in human subjects that exposure of pregnant women to toluene increases the risk of spontaneous abortion. There are also weak indications for an increased risk of childhood leukaemia. Data from experimental animal studies do not confirm a possible link between exposure to toluene and the occurrence of spontaneous abortions. However, the evidence in these studies is limited and not sufficient for definite conclusions.

Aside from exposure to xylene, those working in printing companies and in the petrochemical industry may also be exposed to toluene. The committee concludes that there are weak indications that pregnant female employees working in printing companies or the petrochemical industry who are exposed to agents such as toluene, amongst others, have an elevated risk of reproductive disorders. As these women are also exposed to other solvents (e.g. xylene and styrene), it is impossible to determine which proportion of that risk can be attributed to toluene.

It is not just the exposure of pregnant women that may have implications for the offspring. There are also weak indications that if men are exposed to toluene (during painting and maintenance work, for example) prior to conception, their partner is at increased risk of a spontaneous abortions.

No details are available concerning the current concentrations of toluene. Accordingly, the committee is unable to determine whether there is still an increased risk of spontaneous abortions in these industries.

The effects of many solvents are still poorly understood

Information concerning the effects of exposure on reproduction is only available for a small fraction of the total group of organic solvents (toluene, xylene, styrene, acetone, ethylene glycol ethers, tetrachloroethylene, benzene and methylene chloride). For a large group of organic solvents (such as N-methylpyrrolidone, butanol, isopropanol, 2-butoxyethanol and many others) no epidemiological or other research has been carried regarding their effects on reproduction. Although people are regularly exposed to these solvents in practice, the committee cannot make any statements concerning the effects of occupational exposure on reproduction.

Even in the case of those solvents for which data are available, the issue is far from cut and dried. Sometimes, the results are very limited or contradictory.

Therefore, it is impossible to draw any conclusions with regard to these solvents, either.

Current limit values cannot be assessed

Do the limit values which currently apply to various organic solvents in the workplace offer sufficient protection against effects on reproduction? Based on the data which has been examined, the committee is unable to answer this question from the State Secretary. By definition, a health-based recommended occupational exposure limit protects employees (and their offspring) against *all* adverse health effects arising from occupational exposure to substances. Therefore, when determining the health-based recommended exposure limits, the effects on reproduction must also be taken into account. But that would require relevant data to be available, and that is often not the case.

Since the introduction of the new Working Conditions Act on January 1, 2007, both public and private limit values have to be pegged at the same level as the health-based recommended occupational exposure limit. Toluene, acetone, xylene, benzene, and some glycol ethers are examples of solvents which are subject to a legally binding limit. In the case of most other solvents, the social partners (employers and trade union confederations) are responsible for deriving of safe limit values.

The committee therefore believes that if an epidemiological study identifies effects on reproduction, it is important to determine the type and concentration of the solvents to which the individuals in question have been exposed. If these details are lacking, it is impossible to determine whether the identified effects on reproduction are due to exposure at a level above the existing limit values, or that the existing limit values simply do not provide sufficient protection.

Better exposure data are needed to identify the potential risks

For a number of solvents (ethylene glycol ethers, toluene, xylene and tetrachloroethylene), there are (weak) indications that exposure may cause reproductive disorders. However, up-to-date date concerning exposure in various branches of industry are not publicly available. Therefore, the committee recommends the following for these substances:

- first, identify those types of industry in which exposure to these solvents still takes place
- next, produce quantitative data on the exposure levels of the individual solvents for those types of industry in which exposure is likely.

Improved data accessibility is also required

For most solvents, little or no exposure data are available. In the Netherlands, most exposure measurements are done at the behest of individual companies, which means that the data are often not publicly available. Therefore, the committee recommends that a national database be created in which all exposure data could be collected. The systems currently in use in Great Britain or Germany could serve as an example.

In addition, the committee established that current registration systems for recording birth defects also have their limitations. Therefore, the committee also recommends the creation of a single national registry which would incorporate and integrate all existing registers.

Furthermore, if it were possible to link these two databases (for exposure and for effects), numerous opportunities would be provided to investigate risks in the workplace more effectively than is currently possible.

Chapter 1

Introduction

1.1 Request for advice

Organic solvents are widely used in the working environment. Occupational exposure to solvents occurs amongst others during painting and in dry-cleaning shops, the printing industry, and in various jobs within the chemical industry. The most frequently used solvents are toluene, xylene, tetrachloroethylene, and glycolethers.

Well-known consequences of exposure to organic solvents are neurological effects. Exposure to volatile organic solvents may cause chronic toxic encephalopathy (CTE), a disease characterized by brain disturbances, concentration loss, fatigue, mental inertia, headache, depression and irritability. These adverse effects have been the subject of many investigations, in particular in the Nordic countries*.

According to the Ministry of Social Affairs and Employment, approximately 2500 employees in the Netherlands were diagnosed with CTE in 1999. In 2003, the Dutch 'Solvent teams' diagnosed 32 new cases of CTE as a result of occupational exposure to solvents.[1]

The possible effects on reproduction after occupational exposure to organic solvents are less obvious. The publication of a case control study by Tielemans *et al*[2]

* Nordic countries comprise Scandinavia, Iceland, and Finland.

in 1999, reporting impaired semen quality following occupational exposure to aromatic solvents, caused a stir in the Netherlands. Several years later, Hooiveld *et al*[3] performed a cross-sectional study among male painters in the Netherlands. The results suggested that ccupational exposure of male painters to solvents is associated with an increased risk of congenital malformations in the progeny.

Both studies were reason for the Ministry of Social Affairs and Employment to turn to the Health Council of the Netherlands for advice concerning the consequences of exposure to organic solvents on human reproduction. The requests for advice can be found in annex A*.

1.2 Questions

In this report, the following questions will be addressed:

1 What is known about the effects of occupational exposure to organic solvents on fertility of men and women?
   - What substantiated effects are found in different types of industry?
   - What substantiated effects are found as a consequence of exposure to a single organic solvent? What is known about the mechanism of action?

2 What is known about the effects of occupational exposure to organic solvents on the development of the progeny of men and women?
   - What substantiated effects are found in different types of industry?
   - What substantiated effects are found as a consequence of exposure to a single organic solvent? What is known about the mechanism of action?

3 What is known about the relation between any of the observed effects on fertility and the development of the progeny on the one hand and peak exposure or exposure during sensitive periods on the other hand?

4 Are workers at risk for fertility problems or problems in the development of their progeny, when the latest scientific knowledge is balanced against the current occupational exposure limits?

5 What measures are recommended to increase the safety of workers and to increase the knowledge of the risks of organic solvents for fertility and the development of the progeny?

To answer these questions, the committee gives an overview of the research on this subject, weighs the strength of the evidence, and, if possible, discusses the

* An initial request was received in 2001, but in mutual agreement with the Ministry of Social Affairs and Employment the Health Council waited for the additional request which was received in 2005.

likelihood of a causal relation between occupational exposure to organic solvents and reproductive effects.

1.3 The committee

On 28 September 2005, the President of the Health Council set up a special committee of experts. The members of this committee, their expertise, and their affiliations are listed in annex B.

In October 2007, a draft of the report was released for public review. The individuals and organizations that commented on this draft are listed in annex C. The committee has taken these comments into account in deciding on the final version of this advisory report.

1.4 Definitions

Reproductive toxicity includes impairment of male and female reproductive functions or capacity and the induction of non-inherited harmful effects in the progeny. The committee distinguishes two main groups among these effects: 1) Effects on male or female fertility, and 2) Developmental toxicity. For the definition of both types of effects the committee adopts the interpretation in Directive (93/21/EEC) of the European Union:

- *Effects on male or female fertility* includes adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or hormonal activity or a physiological response which would interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.
- *Developmental toxicity* is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well as those manifested postnatally. This includes embryotoxic/foetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peri- and postnatal defects, and impaired postnatal mental or physical development up to and including normal pubertal development.

In this report, the committee assesses the effects of occupational exposure to organic solvents on both male and female fertility. Furthermore, for an evaluation of the effects on the development of the progeny, the committee focuses on spontaneous abortion, birth weight, malformations, malignancies and neurodevelop-

ment. The developmental effects after both maternal and paternal exposure are evaluated.

1.5 Structure of the advisory report

To answer the questions formulated in section 1.2, the committee searched for all relevant epidemiological publications and critically judged their quality. Based on the cohort studies gathered, the committee identified seven types of industry in which solvents were used on a regular basis and for which data on the effects on reproduction were available. In addition, for the eight most prevalent individual solvents in these studies, the potential effects on reproduction were studied.

In chapter 2, the committee first describes the search for the relevant publications in the online databases. Secondly, the committee discusses the way in which the quality of these publications is evaluated and how a selection is made.

Chapter 3 gives an overview and an evaluation of the effects on reproduction as they are observed in different types of industry. As industrial cohort studies have the best design to study the effects of occupational exposure to organic solvents, only those industries are selected for which these studies are available. This resulted in seven types of industry. For these industries, the data from the cohort studies are complemented with data from case-control and cross-sectional studies. The evidence is weighed, and conclusions are reached on the effects that have been sufficiently substantiated.

Then a different perspective is chosen. In the consecutive chapters 4 through 11, all data on the effects of exposure to individual solvents used in the seven industries are evaluated and associations (with individual solvents) are described. For each separate solvent, a conclusion is reached on the possible impact that occupational exposure to this compound might have on fertility or the development of the progeny. Furthermore, these chapters briefly discuss the information on individual solvents derived from studies with experimental animals, compare these results with the results from epidemiological studies and describe a mechanism of toxicity, whenever such information is available.

In the final chapter (12) the committee summarizes the effects found after exposure to organic solvents in order to answer the first two questions of paragraph 1.2. Additionally, the committee discusses the matter of peak exposure and sensitive periods for exposure. Finally, the committee establishes whether Dutch occupational exposure limits provide sufficient protection, given the findings on reproductive effects, and gives recommendations for policy and further research.

Chapter 2

Selection of the epidemiological data

2.1 Search for literature

To gather data on the effects of exposure to organic solvents observed in man, the committee searched the databases *Pubmed* and *Toxline* for relevant publications until October 2007. The search terms used are *solvents* and *occupational exposure* in combination with *fertility, infertility, fertilization, sperm, menstrual, ovarian* and *hormone* for the effects on fertility. For the effects on the development of the progeny the terms *solvents* and *occupational exposure* were combined with *reproduction, reproductive, spontaneous (abortion), pregnancy, premature, congenital, teratogen, fetal, abnormalities, birth weight, infant, childhood, and sex ratio*.

In addition, the search terms *reproduction* or *reproductive* or *fertility* were combined with *painters, varnishers, paint manufacturing, paint manufacturers, printers, printing work, rubber manufacturing, rubber manufacturers, rubber workers, metal workers, metal cleaning, metal degreasing, paint spraying, maintenance workers, maintenance technician, garage workers, mechanics, repair, spray painting, body work, textile workers, dry cleaners, textile printers, laundry, laundering, chemical operators, chemical industry, petroleum products, petroleum industry, leather industry, leather workers, shoe makers, semiconductor, embalmer, plastics industry, plastics workers, reinforced plastics, synthetic material industry,* and *synthetic material workers*.

Data on the effects of exposure to the organic solvent 'ethanol' were excluded from the search. In 2005, the Health Council already evaluated the effects of occupational exposure to ethanol.[4]

For data on effects found in experimental animals, the committee used advisory reports of the Health Council's Committee on Compounds Toxic to Reproduction as a starting point, in as far as these were available. Additional data were retrieved from the databases *Pubmed* and *Toxline* until October 2007. The following search terms were used: *name of compound* or *CAS-number* in combination with *reproductive* or *fertility.*

2.2 Assessment of the quality

The search (see above) in the online databases yielded approximately 200 epidemiological studies. For all studies, a critical appraisal of the quality was performed independently by two epidemiologists. The cohort studies were evaluated by two members (epidemiologists) of the committee, and the other studies ((nested) case-control and cross-sectional studies) were judged by two epidemiologists of the Dutch Institute for Risk Assessment Science (IRAS). The goal was to evaluate the most important types of bias in these studies: selection bias in the composition of the study population, information bias in the characterization of the outcome parameters and in the exposure assessment, and confounding bias. For this, the guidelines of the CBO* (Dutch Institute for Health Improvement) were used as a starting point. The committee made some minor refinements to these guidelines (see also annex D).

The results of the critical appraisal are summarized in annex E. The studies of which the quality was judged as insufficient were excluded from further evaluation.

2.3 Results of the selection

The literature search in the online databases resulted in 50 cohort studies and 147 case-control and cross-sectional studies. Of the cohort studies, 10 did not meet the quality criteria of the committee. The quality of approximately 100 case-control and cross-sectional studies was judged as insufficient by the committee and therefore these were not used for the evaluation in this report.

* Guidelines for Cohort studies and for Case Control studies.

2.4 Evaluation of the effects

For each type of industry and for every organic solvent the committee evaluated the strength of the available evidence and drew conclusions about the possible effects on reproduction. The committee distinguished the following categories:

- No data are available; therefore the committee cannot conclude on the possible effects on reproduction
- Insufficient or limited data are available; therefore the committee cannot conclude on the possible effects on reproduction
- Sufficient data are available, but the results are conflicting; therefore the committee cannot conclude on the possible effects on reproduction
- Sufficient data are available, but the committee is of the opinion that there are no indications for an association between exposure and effects on reproduction
- Sufficient data are available, and the committee is of the opinion that there are weak indications for an association between exposure and effects on reproduction
- Sufficient data are available, and the committee is of the opinion that there are indications for an association between exposure and effects on reproduction.

Chapter 3

Exposure to organic solvents in different types of industry

3.1 Introduction

Organic solvents are commonly used in various industries. Most often, workers in those industries are not exposed to just one single solvent, but to a mixture and, in many cases, to other compounds as well. The composition of these mixtures and the magnitude of the exposure are largely dependent on conditions of use and work processes, which can vary from one place of work to another. Nonetheless, occupational exposure patterns within certain jobs are assumed to be more or less comparable.

This enables the committee to discuss the effects of occupational exposure to organic solvents on human fertility and the development of the progeny in two ways. One way is to evaluate the effects that occur after exposure to solvents in different types of industry, the other way is to evaluate the association between effects and specific solvents, regardless of its use in industry. The results of the first approach are presented in this chapter. The latter perspective is chosen in the chapters 4 through 11, where eight different solvents and their effects on fertility and the progeny are discussed.

For the discussion on the effects of organic solvents in different types of industry, the committee weighed the findings of approximately 40 cohort studies. These studies met the quality criteria as described in chapter 2. From these cohort stud-

ies the following types of industry with relevant exposure to solvents were identified:

- Reinforced plastics industry
- Printing industry
- Painting and maintenance trade
- Chemical and petrochemical industry
- Laboratories
- Dry-cleaner's
- Semiconductor industry.

Occupational exposure to organic solvents is, of course, not limited to the above types of industry or jobs; it can also occur in the wood industry[5], textile industry[6], and leather and shoe industry[7,8] as well as among veterinarians[9]. However, findings on exposure in these types of industry were not evaluated, as no cohort studies are available that meet the quality criteria of the committee.

For each type of industry, the committee starts with an evaluation of the industrial cohort and nested case-control studies. In the majority of these epidemiological studies, the committee judges the exposure assessment by the authors as limited. Consequently, 'mixtures of solvents' to which the workers were exposed in these studies are difficult to describe in terms of compounds, frequency and duration of exposure. Furthermore, the definition of 'mixture of solvents' is largely dependent on the design of the study. In industrial cohort studies, this term is used to indicate that the workers in the exposed group are exposed to specific combinations of solvents, whereas in case-control studies, the term concerns a group of combined individual cases and controls exposed to different individual solvents or combinations of solvents. The combination of the cases is mainly done to increase the power of the study.

Nonetheless, in the industry-based case-control studies, the participating individuals were expected to be exposed to mixtures of solvents comparable to the exposure patterns of the workers in cohort studies. In population-based case-control studies, the exposure was more heterogeneous. In addition to the studies carried out in specific types of industry, the committee includes a paragraph on population-based studies, as these also contain information on possible effects of working with organic solvents.

3.2 Reinforced plastics industry

3.2.1 *Occupational exposure*

In the plastics industry, the primary occupational exposure is to styrene (see also chapter 4) with possible concurrent exposure to methylene chloride. The highest styrene exposure occurs in the open and closed mould.

Post *et al*[10] estimated the exposure to styrene and methylene chloride in a small polyester factory in the Netherlands. Personal exposure measurements (8-hour time weighted) were taken. In the preparation department, the geometric means of styrene concentrations ranged from 21 to 59 mg/m^3. In moulding shops, the geometric means ranged between 34 and 139 mg/m^3 for three different jobs. Personal methylene chloride concentrations (geometric means, 8-hour time weighted) in the preparation departments ranged from 315 to 707 mg/m^3.

Van Rooij *et al*[11] studied occupational exposure in the glass fibre reinforced plastics industry in different European countries from 1970 to 2002. Both personal exposure measurements and biological exposure data were searched for in peer reviewed and non-peer reviewed literature. Analyses of the data showed an average decline of styrene concentrations in the breathing zone of European workers of 5.2% per year until 1990, with most recent data not showing a further decline. Exposure data from Central Europe showed a decrease from 500 mg/m^3 in the early seventies to approximately 170 mg/m^3 in the period 1990-2002.

In 2003, Lees *et al*[12] investigated styrene exposure data in four US glass fibre reinforced plastics factories. The individual exposure levels were determined by personal air sampling and through pre- and post shift measurements of urinary metabolites of styrene. The 8-hour time weighted average styrene exposure was 12.6 +/- 10.4 ppm (54 +/- 46 mg/m^3).

3.2.2 *Effects on fertility*

Cohort studies

Three cohort studies[13,14,15] describe the effects on fertility of occupational exposure to organic solvents in the plastics industry. In the study by Lemasters *et al*[13] the effects of exposure of female workers on several parameters of the menstrual cycle were evaluated. The exposure to styrene was estimated by an industrial hygienist and assumed to remain lower than 30 ppm (130 mg/m^3). No statistically significant differences were observed between the exposed and non-

exposed groups concerning severe dysmenorrhea, intermenstrual bleeding, secondary amenorrhea, menstrual blood clots, and hypermenorrhea.

Kolstad *et al*[14,15] (describing the same study) studied the semen quality of 23 male workers in four Danish reinforced plastics factories for six months. Semen samples were collected at the beginning and after six months of working in the plastics industry. A statistically significant decline was observed in sperm density and sperm count in the group exposed to styrene. This time-related effect was not observed in the non-exposed group. Furthermore, in the exposed group the proportion of sperm with normal morphology was reduced, the proportion of non-vital sperm decreased, and the median sperm velocity increased over time. However, none of the sperm parameters, including sperm density and sperm count, were related to the internal styrene concentration, measured as the post-shift urinary mandelic acid concentration. The authors concluded that the outcomes of these two types of analyses, based on internal and external exposure levels, were inconsistent.

Other studies

Kolstad *et al*[16,17] (describing the same study) also performed a cross-sectional study among male workers in the reinforced plastics industry. Fecundity was measured as the reported time to pregnancy. No statistically significantly reduced fecundity was found for men employed in the reinforced plastics industry compared with unexposed men (OR 0.8, 95% CI 0.6-1.1).

Two other studies did not meet the criteria of the committee, since selection bias could not be excluded and the exposure data were not sufficient.[18,19]

3.2.3 *Effects on the development of the progeny*

Cohort studies

Lemasters *et al*[20] analysed the birth weights of children whose mothers worked in the reinforced plastics industry during pregnancy. Exposure information was derived from an historical set of samples obtained from the companies involved. Based on these, mean styrene levels were estimated for 19 job categories. Only limited information was available for exposure to other solvents, i.e. acetone, ethyl-ethyl ketone, methylene chloride, toluene and xylene. Women who worked in jobs with a higher exposure (30 ppm styrene or higher in any month) were found to have had offspring with 4% lower birth weights than the birth weight in offspring of unexposed women (95% CI –7.7% to +0.6%), adjusted for con-

founders. However, no statistically significant exposure-response trend could be established associating higher exposure with decreasing average birth weight of the progeny.

Industry-based case-control studies

In a matched case-control study, Lindbohm *et al*[21] analysed whether maternal occupational exposure in the plastics industry was related to the risk of spontaneous abortion. No increased risks of spontaneous abortion were observed among workers processing polymerized plastics (styrene) (OR 0.4 (95% CI 0.1-1.2)) or heating plastics (OR 0.6 (95% CI 0.2-2.3)), but the statistical power of this study was limited.

3.3 Printing industry

3.3.1 Occupational exposure

In the printing industry, workers are predominantly exposed to inorganic lead and organic solvents. The Health and Safety Executive[22] in the United Kingdom published a report on trends in inhalation exposure in amongst others the printing industry. The geometric mean of toluene exposure among printers was found to be 8 ppm during the period 1985 to 1998. In this period exposure levels declined from 13 to 4 ppm. Furthermore, lead exposure in the printing industry has terminated since the mid 1970s.

3.3.2 Effects on fertility

Cohort studies

No cohort studies are available regarding effects on fertility due to working in the printing industry.

Industry-based case-control studies

The two available case-control studies did not meet the selection criteria of the committee, because there was no correction for confounders, selection bias could not be excluded and there were insufficient exposure data.[23,24]

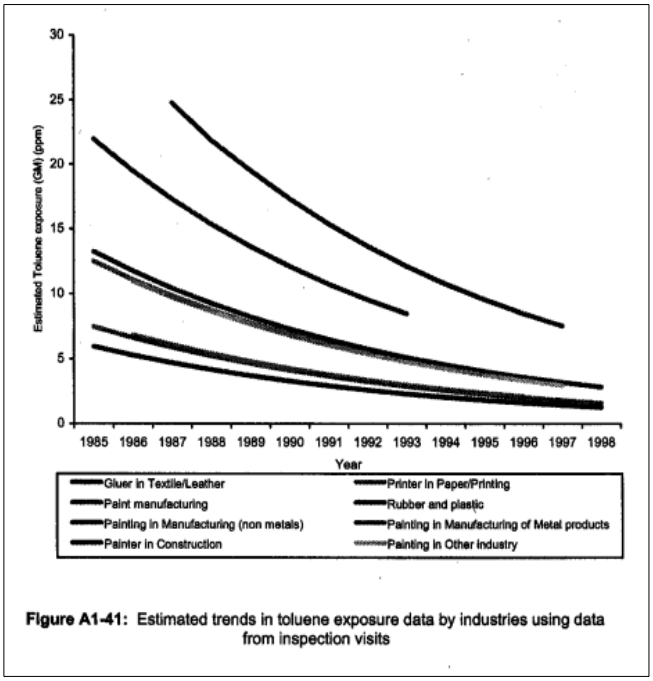

Figure 1 Estimated trends in toluene exposure data by industries using data from inspection visits.[2]

3.3.3 *Effects on the development of the progeny*

Cohort studies

In two cohorts in the printing industry, the effects on the development of the progeny were studied.[25,26] A register linkage study was performed to evaluate the occurrence of cancer in the offspring of male members of the Oslo Unions of Printers.[25] A file of their children, born between 1950 and 1987, was obtained by linking the printers to the Central Population Register. The Union workers were categorized in four groups: lead exposure, solvent exposure, exposure to both lead and solvents and non-exposure. The standard incidence ratio (SIR) for cancer in children born between 1950 and 1987 was not affected (0.7, 95% CI 0.02-3.3) after exposure to solvents only. However, the number of cases was low and

limited information was available on the exposure categories. Furthermore, some exposure misclassification was inevitable.

In 1993, the same cohort was used to study perinatal outcome when wives of men exposed to solvents in the printing industry gave birth. The adjusted odds ratio for early preterm birth (16-27 weeks) was 5.4 (95% CI 1.7-17.4) when there had been paternal exposure to solvents. The odds ratios for 'small for gestational age' (SGA) and 'low birth weight' were not statistically significantly increased for paternal exposure.

Industry-based case-control studies

The two available studies did not meet the selection criteria of the committee, since selection basis could not be excluded.[27,28]

Other studies

An available cross-sectional study of Lindbohm *et al*[29] did not meet the selection criteria of the committee as the exposure data were insufficient.

3.4 Painting and maintenance trade

3.4.1 Occupational exposure

Burstyn and Kromhout[30] analysed trends in inhalation exposure to hydrocarbons among painters in the Netherlands. The solvents most commonly measured among Dutch commercial painters were toluene, xylene, ethylbenzene, n-decane, and n-hexane. Toluene was used as a marker for solvent exposure. The exposure of painters using solvent-based paints was shown to decline 11-fold to less than 1 mg/m^3 in the Netherlands in the period 1980 to 1999. This reduction reflects the decline in solvent content in (solvents-based) paints, and the introduction of water-based paints in the 1990's.

Toluene exposure in a group of construction painters was also described by the Health and Safety Executive of the United Kingdom in 2007. From 1987 to 1997, toluene exposure in this population was estimated to decline from about 25 to 8 ppm (94 to 3 mg/m^3).[22]

3.4.2 *Effects on fertility*

Cohort studies

In 2004, Luderer *et al*[31] assessed the effects of solvent exposure on male fertility and endocrine function (FSH, LH and testosterone levels) in members of the International Brotherhood of Painters and Allied Trades and the United Brotherhood of Carpenters and Joiners. Time-to-pregnancy data and exposure information were gathered by conducting interviews. The authors demonstrated that the exposure to solvents assessed in painters was significantly higher than the exposure assessed in millwrights and carpenters. This difference in exposure was not associated with differences in LH, FSH, and testosterone levels. However, concentrations of FSH increased significantly with increasing exposure indices. No statistically significant difference was observed in time to pregnancy for the painters compared to the carpenters.

Other studies

Lemasters *et al*[32] studied sperm production, structure, and function in 50 male workers (metal workers, painters, jet fuel workers, and flight line crew) exposed to solvents and fuel and in eight unexposed men. Exposure was determined using samples obtained through regular industrial hygiene monitoring and samples of expired breath. Exposure, determined as total solvent, was not statistically significantly related to any change in semen parameters. When job group was a surrogate for exposure, the painters showed a statistically significant decline in sperm motility (19.5%) thirty weeks after exposure started. On the other hand, no statistically significant association was demonstrated with internal dose. Furthermore, the authors indicated that the unexposed group showed declines in a number of sperm parameters as well (sperm length and motility).

Welch *et al*[33] studied fertility in a cross-sectional study among 94 male shipyard painters, who were exposed to a mean concentration of 9.9 mg/m^3 2-ethoxyethanol (EGEE) and 2.6 mg/m^3 2-methoxyethanol (EGME), weighted over eight hours. Among the exposed painters only an increased semen pH was found (7.94 vs 7.88 in controls, $p<0.05$). Exposed non-smokers showed a higher rate of oligozoospermia ($p=0.05$). No differences were found in sperm motility, viability, morphology, or morphometry. The authors stated that the painters were also exposed to other reproductive toxins such as low concentrations of lead, although these remained below the levels for reproductive toxicity.

3.4.3 *Effects on the development of the progeny*

Cohort studies

The available study of Hoglund *et al*[34] did not meet the selection criteria of the committee as selection bias could not be excluded and the exposure data were insufficient.

Industry-based case-control studies

The available studies[35,36] did not meet the selection criteria of the committee as there was no correction for confounders, selection bias could not be excluded, and the exposure data were insufficient.

Other studies

The available study of Hooiveld *et al*[3] did not meet the selection criteria of the committee as selection bias could not be excluded and exposure data were insufficient.

3.5 Chemical and petrochemical industry

3.5.1 *Occupational exposure*

In the chemical and petrochemical industry, the most recent exposure to solvents involves benzene, gasoline and hydrogen sulphide. In addition, exposure to toluene, styrene, and xylene is reported.[37] Exposures in these industries are often relatively low on average and occur intermittently most of the time. A recent study by Van Wendel de Joode[38] in a petrochemical plant in the Netherlands reported geometric mean benzene and toluene concentrations of 0.29 mg/m^3 and 0.38 mg/m^3, respectively.

3.5.2 *Effects on fertility*

Cohort studies

No studies are available concerning effects on fertility due to occupational exposure to solvents in the petrochemical industry.

Industry-based case-control studies

No studies are available concerning effects on fertility due to occupational exposure to solvents in the petrochemical industry.

Other studies

Cho *et al*[37] studied the length of the menstrual cycle in a cross-sectional study carried out among 1,408 female petrochemical workers in China. Exposure (to toluene, styrene, xylene, benzene,, and combinations of these solvents) was assessed by an industrial hygienist. Exposure to 'all aromatic solvents' was associated with oligomenorrhoea. The adjusted odds ratio was 1.8 (95% CI 1.1-2.8).

Two Swedish studies[39,40] did not meet the selection criteria of the committee as the exposure data were insufficient and selection bias could not be excluded.

3.5.3 *Effects on the development of the progeny*

Cohort studies

In 1994, Stücker *et al*[41] published a cohort study on the wives of 823 men working in two chemical plants in France. This study focused on paternal exposure to benzene and the risk of spontaneous abortion. Exposure to benzene was assessed by an industrial hygienist and categorized in three exposure levels. After adjustment for confounders, the odds ratio for paternal exposure in the highest exposure category was close to unity (OR 1.1, 95% CI 0.7-1.8).

Xu *et al*[42] studied the association between spontaneous abortion and occupational exposure of female workers to solvents in a petrochemical plant in Beijing, China. The mean concentration of benzene, toluene, styrene and xylene varied between different workshops, but levels were quite low: 0.86, 0.40, 0.50, and 0.03 ppm respectively. Exposure to petrochemicals was associated with an increased risk of spontaneous abortion (OR 2.7, 95% CI 1.8-3.9).

The association between solvent exposure and birth weight was examined in another Chinese petrochemical plant by Ha *et al*.[43] The exposure to aromatic solvents (toluene, styrene, benzene, or xylene) was assessed by a trained interviewer among 1,222 female employees who had had a live birth. In addition, the exposure of the father was assessed. Maternal exposure to solvents reduced the birth weight of the progeny with 82 grams (95% CI 3.1 to 106 grams). No statistically

significant effects on birth weight were observed when exposure to solvents of the father was considered.

The effects on birth weight after exposure of pregnant women to solvents in a Chinese petrochemical plant was also investigated by Chen *et al*.[44] Exposure was assessed in a three-step procedure, consisting of an assessment by an industrial hygienist, exposure measurements and interviews with employees. The exposure levels of toluene, styrene, xylene, and benzene were all below the occupational exposure limit (OEL) established by OSHA*. Exposure to benzene was not associated with decreased birth weight.

The study from Kallén *et al*[39] did not meet the selection criteria of the committee as the exposure data were insufficient.

Industry-based case-control studies

The available studies[45,46] did not meet the selection criteria of the committee as the exposure data were insufficient and selection bias could not be excluded.

Other studies

The available studies[39,47,48] did not meet the selection criteria of the committee as the exposure data were insufficient and selection bias could not be excluded.

3.6 Laboratories

3.6.1 Occupational exposure

In laboratories, workers are exposed to a variety of solvents. The most common exposures are to acetone, benzene, chloroform, diethylether, and phenol.[49] The exposure in laboratories is miscellaneous, however. Therefore, the exposure to 'mixtures of solvents' in the studies described differs in composition and concentration.

* OSHA: Occupational Safety and Health Association (in USA).

3.6.2 *Effects on fertility*

Cohort studies

Wennborg *et al*[49] studied the time to pregnancy among female personnel in biomedical laboratories in Sweden. Female personnel working in non-laboratory departments served as the reference group. Data on exposure and time to pregnancy were collected retrospectively by postal questionnaire. Working with organic solvents resulted in a decreased adjusted fecundability ratio of 0.79 (95% CI 0.68-0.93). The authors concluded that there were some indications that prolonged time to pregnancy is associated with maternal exposure to solvents.

In 2005, Zhu *et al*[50] published a study on time to pregnancy in a cohort of female laboratory workers from the Danish National Birth Cohort. A group of female teachers from the same cohort was used as reference group. Information about jobs and time to pregnancy was retrieved by telephone interviews. The exposure was assigned using a job-exposure matrix. No difference in time to pregnancy was found between the laboratory workers and the teachers.

Industry-based case-control studies

The available studies[51,52] did not meet the selection criteria of the committee as the exposure data were insufficient and selection bias could not be excluded.

Other studies

No studies are available concerning the effects on fertility due to occupational exposure to solvents in laboratories.

3.6.3 *Effects on the development of the progeny*

Cohort studies

Axelsson *et al*[53] studied pregnancy outcome among female laboratory personnel at the University of Gothenborg from 1968 to 1979. To collect information on the outcome of pregnancy and data on exposure patterns, a questionnaire was distributed among 782 women. Furthermore, information was retrieved from the medical birth register and the register of congenital malformations. No differences were found in the occurrence of prenatal death or the prevalence of malforma-

tions between children of mothers who were exposed to solvents and those of mothers who were not.

In 2000, Wennborg *et al*[54] studied pregnancy outcome among female personnel in a number of biomedical laboratories in Sweden (using the same cohort as Wennborg 2001[49]). Female personnel working in non-laboratory departments were used as reference group. Information on exposure and spontaneous abortion was collected retrospectively by postal questionnaire for the period 1990-1994. Information on birth weight was retrieved from the Swedish Medical Register. With regard to spontaneous abortion, the unadjusted odds ratio for laboratory work in general was 0.9 (95% CI 0.5-1.4). Small-for-gestational age was not statistically significantly associated with exposure to solvents (OR 1.9, 95% CI 0.7-2.5).

In 2002, Wennborg *et al*[55] performed a comparable study among pregnant women working in biomedical research laboratories at a Swedish university from 1970 to 1989. Exposure information was gathered by a questionnaire sent to the heads of the departments involved. Information on the birth weights of firstborns was retrieved by linking the database of the cohort to the Medical Birth Register. Mean birth weight was approximately the same across the groups. However, exposure to solvents was associated with an increased risk of preterm births (< 37 weeks). The estimated odds ratio was 3.4 (95% CI 1.0-11.9).

Finally, Wennborg *et al*[56] investigated major congenital and neural crest malformations in the offspring of a cohort of female laboratory personnel. Exposure information was gathered by means of questionnaires. In addition, information on exposure was provided by the heads of the research groups. Well defined major malformations were identified by linkage with the Medical Birth Register in Sweden. No increased risk of major congenital malformations was found to be related with maternal laboratory work. However, exposure to solvents before the third trimester of pregnancy was associated with major malformations (OR 2.5, 95% CI 1.0-6.0). Paternal exposure to solvents was not considered as a confounder.

Zhu *et al*[57] studied the outcomes of pregnancies in a cohort of 1,025 female laboratory workers and 8037 female teachers in the Danish National Birth Cohort (1997-2003). Information about their jobs and time to pregnancy was retrieved by telephone interview. The exposure was assigned using a job-exposure matrix. Information on pregnancy outcome was obtained by linking the cohort to the Danish national registers. Overall, no statistically significant differences in pregnancy outcome were observed between the laboratory workers and the teachers. However, when an exposure matrix was applied and the analyses were adjusted encompassing all other substances to which the women were

exposed, exposure to solvents showed a statistically significantly higher risk of major malformations (for high exposure: OR 4.5 (95% CI 1.3-16.2)).

The possible effects of paternal exposure to solvents were also studied. Data on *male* employees at Swedish universities from 1970 to 1989 were linked to the Medical Birth Register in Sweden.[58] Men were included when they were exposed to 'laboratory work in general' anytime before the third trimester of the pregnancy of their wives or around the time of conception. Exposure information was retrieved by sending a questionnaire to the heads of the research groups involved. For major malformations, 'laboratory work in general' resulted in an adjusted odds ratio of 1.3 (95% CI 0.8-2.1). For neural crest malformations the odds ratio was 1.1 (95% CI 0.4-2.7).

The other studies[9,59] did not meet the selection criteria of the committee as the exposure data were insufficient and selection bias could not be excluded.

Industry-based case-control studies

The risk of spontaneous abortion was examined in a nested case-control study by Taskinen *et al*[60] among women working in laboratories. An association with spontaneous abortion was found for (self-reported) exposure to aromatic solvents three to five times a week (OR 2.7, 95% CI 1.3-5.6). Exposure to aromatic solvents once or twice a week did not increase the risk of spontaneous abortion. A statistically significant association was also observed for spontaneous abortion and exposure to toluene and xylene three to five times a week. In a second study, Taskinen *et al*[60] studied the effect of exposure to solvent on congenital malformations and birth weight. In this study, no associations were found.

Two other studies[52,61] did not meet the selection criteria of the committee as the exposure data were insufficient and selection bias could not be excluded.

Other studies

The available studies[62,63,64] did not meet the selection criteria of the committee as the exposure data were insufficient, selection bias could not be excluded and correction for confounders was not presented.

3.7 Dry-cleaner's

3.7.1 Occupational exposure

In dry-cleaning shops, occupational exposure to solvents mainly comprises tetrachloroethylene (PER) and machine operators experience the highest exposures. Air concentrations of PER in a dry-cleaning unit in the Netherlands were monitored in three consecutive years (1976-1978) and ranged from 25 to 1,695 mg/m^3.[65] In the 1980s, a breathing zone air level of PER up to 352 mg/m^3 was measured in dry-cleaning units.[66] IARC also listed a large number of exposure concentrations measured in dry-cleaning shops all over the world. In general, the highest exposure levels, ranging from 340 to 680 mg/m^3, occurred in the 1970s and exposure continuously decreased during the 1980s and 1990s to levels ranging from 8 to 150 mg/m^3.[65]

More recent occupational exposure data were reported by Verplanke *et al*[67], Räisänen *et al*[68], and Earnest *et al*[69]. Verplanke *et al*[67] reported a mean concentration of PER of 8.4 mg/m^3 (range 2.2 to 44.6 mg/m^3) in alveolar air samples of Dutch dry-cleaning workers. This corresponded to a mean 8-hour time weighted average of 7.9 mg/m^3 (range 1-221 mg/m^3). Räisänen *et al*[68] measured the occupational exposure of operators of dry-to-dry non-vented dry-cleaning machines in Finland. The mean time weighted average exposures of the operators ranged from 9 to 50 mg/m^3, while exposures of pressers ranged from 3 to 8 mg/m^3. Peak exposures as high as 2,300 mg/m^3 over a 10 minute period occurred when the machine's lint filter was cleaned. A recent paper by Earnest *et al*[69] describes occupational exposure of dry-to-dry non-vented fifth generation dry-cleaning machine operators in the USA. The mean time weighted average exposure was less than 5 ppm (34 mg/m^3) and peak exposure did not exceed 290 ppm (1,998 mg/m^3). This type of dry-cleaning machines was introduced in the 1990s.

3.7.2 Effects on fertility

Cohort studies

In their first paper, Eskenazi *et al*[70] investigated semen quality of 34 exposed dry-cleaners and laundry workers and 48 unexposed laundry workers. Exposure to PER was assessed using job titles and an exposure index assigned by an expert. In addition, PER-levels in exhaled air were monitored. As expected, dry-cleaners exhaled statistically significant higher levels of PER than laundry workers, but

laundry workers reported hotter conditions than dry-cleaners. From a range of sperm parameters, some statistically significant effects on sperm quality were found among the dry-cleaners: higher percentages of round sperm and sperm moving with a higher amplitude of lateral head movement. The other parameters, namely sperm concentrations, sperm counts, volume and percentages of motile sperm did not differ between dry-cleaners and laundry workers.

In the second paper, Eskenazi *et al*[71] examined the reproductive outcomes of the female partners of dry-cleaners and laundry workers. No differences in number of pregnancies and standardized fertility ratios were observed between the wives of dry-cleaners and the wives of laundry workers. According to the authors, dry-cleaners' wives experienced a longer period before getting pregnant or were more likely to consult a doctor for fertility problems (OR=2.5). However, the 95% confidence interval included unity (0.6-10.9). Furthermore, the interpretation of the study is hampered by small group sizes.

Industry-based case-control studies

The available study[72] did not meet the selection criteria of the committee as selection bias could not be excluded and there was no correction for confounders.

Other studies

The available studies of Ferroni *et al*[73] and Bosco *et al*[74] did not meet the selection criteria of the committee as the effects on reproduction were not specifically studied, selection bias could not be excluded and there was no correction for confounders.

3.7.3 Effects on the development of the progeny

Cohort studies

In the only cohort study available of Doyle *et al*[75], 2,711 British women working in dry-cleaning units and 399 women in laundries participated. Each participant reported on her workplace and whether she was a dry-cleaning operator or not. Information about pregnancies and fetal loss (prior to week 20) was reported by the participants and partly verified using their medical records. The authors found no increased risk of spontaneous abortion for dry-cleaning workers as compared to laundry workers. Among the dry-cleaners, however, the odds ratio for spontaneous abortion in pregnancies which occurred between 1980 and 1995

was increased for operators compared to non-operators (1.63; 95% CI 1.01-2.66). The odds ratio was adjusted for maternal age, pregnancy order, and year of birth.

Industry-based case-control studies

Kyyrönen *et al*[76] studied the incidence of spontaneous abortion and the prevalence of congenital malformations among female dry-cleaners and laundry workers in a case-control study in Finland. The study population was selected from the register of the Union of Chemical workers and the Municipal workers' Union of Finland for the period between 1973 and 1983. These data were linked with the National pregnancy database. One hundred and thirty cases of spontaneous abortion were retrieved and compared to 298 controls. 'High' exposure to PER was associated with a statistically significantly increased odds ratio of 3.4 (95% CI 1.0-11.2) for spontaneous abortion. The odds ratio was adjusted for smoking, use of alcohol, working during pregnancy, a temperature in the workplace of more than 24°C, febrile disease, and nulliparity. Dry-cleaning work in general was also associated with spontaneous abortion (unadjusted OR 4.9 (95% CI 1.3-19.5)). For congenital malformations, 24 cases and 93 controls were retrieved. Kyyrönen *et al*[76] found that exposure to 'any level' of PER had no effect on the prevalence of congenital malformations, whereas 'handling of other solvents' (i.e. spot removers, thinner, and acetone) led to a statistically significantly increased odds ratio (5.9; 95% CI 1.0-35.7). However, this part of the study was hampered by the small group size. Associations between congenital malformations and working in dry-cleaning shops in general were not studied.

Ahlborg *et al*[77] also performed nested case-control studies within two cohort of women engaged in laundry or dry-cleaning work. They combined the results of these studies in which the relationship between PER exposure during the first trimester and spontaneous abortion was investigated. The adjusted odds ratio for the combined studies was 1.1 (95% CI 0.6-2.0). The odds ratios for 'low' and 'high' PER exposure were comparable. Ahlborg *et al*[77] also reported cases of perinatal death, congenital malformations and low birth weight. The authors did not perform a statistical analysis of the data since these numbers were too small.

In a large industry-based case-control study on spontaneous abortion, Olsen *et al*[78] combined the Finnish data[76] with cases from Norway, Sweden[77] and Denmark. A partial overlap between the Kyyrönen, and Olsen studies with those by Lindbohm *et al* (1984[79] and 1990[80]), and Taskinen *et al*[81] is likely, since their cases were derived from the same Finnish registers within the same period (1973-1983). The total numbers of cases and controls were 215 and 558 respec-

tively. Combining the data from three countries (Sweden, Denmark, and Finland), the relative risk of spontaneous abortions was demonstrated to be increased with high maternal exposure to PER during the first trimester of pregnancy (adjusted OR 2.88; 95% CI 0.98-8.44). It should be noticed, however, that looking at the data separately, only the Finnish odds ratio ('high exposure' OR 4.5; 95% CI 1.1-18.5) was statistically significantly increased. Furthermore, the total number of cases within this group was small (n=8).

In addition, Olsen *et al*[78] presented odds ratios for the combined data on congenital malformations, still births, and low birth weight (< 1500 g). None of the odds ratios were statistically significantly increased.

Other studies

Lindbohm *et al*[79] performed a cross-sectional study on spontaneous abortions occurring between 1973 and 1976 and the possible association with parental occupations. A total of 68,327 pregnancies from the Finnish hospital discharge register were studied. Information on occupation and exposures (assessed through job titles and type of workplace) were obtained from the national census. Occupations were coded and combined in categories of exposure in cooperation with an industrial hygienist. Laundry workers were classified in the category workers exposed to 'solvents'. The adjusted relative risk for spontaneous abortion, based on 416 pregnancies, turned out to be increased in female laundry workers (OR 1.48; 95% CI 1.09-2.02). The odds ratios for maternal as well as paternal exposure to 'solvents' were smaller than 1.

3.8 Semiconductor industry

3.8.1 Occupational exposure

Occupational exposure in the semiconductor industry predominantly concerns ethylene glycol ethers. However, exposure measurements for glycol ethers in the semiconductor industry have not been found in the peer-reviewed literature.

The Semiconductor Health Study in California involved a retrospective study of a cohort of 904 women employed in 14 semiconductor plants in the USA and a prospective one including 403 women.[82,83,84,85] Among the silicon wafer fabrication room workers, the following three subgroups can be distinguished based on the manufacturing processes:

- Masking: people working in photolithography and etching

- Dopants and thin film: people working with furnaces, thin film, and ion implantation
- Supervisors and engineers.

According to the authors, occupational exposure to ethylene glycol ethers mainly occurred in the subgroup masking and especially among workers occupied with etching. Workers in the dopants and thin film group were predominantly exposed to fluorides and not so much to glycol ethers. Which ethylene glycol ethers were involved in the masking group was not mentioned in the papers.

3.8.2 *Effects on fertility*

Cohort studies

As a part of the Semiconductor Health Study, involving a cohort of female workers from 14 semiconductor plants in the US, Eskenazi *et al*[86] studied *female fecundability* (i.e. probability of conception per menstrual cycle) among 152 women working in silicon-wafer fabrication rooms (also referred to as fab-room workers) over a period of 6 months. Exposure to ethylene glycol ethers did not result in a statistically significantly reduced fecundability (FR 0.4; 95% CI 0.1-1.2). Workers belonging to the 'masking' subgroup showed no statistically significantly effect on fecundability, FR is 0.67 (95% CI 0.25-1.70) for clinically confirmed pregnancies either. However, workers belonging to the 'dopants and thin film' subgroup, who were assumed not to be exposed to ethylene glycol ethers but to fluorides, showed a statistically significantly decreased fecundability of 0.2 (95% CI 0.05-0.96) for clinically confirmed pregnancies.

Gold *et al*[87] investigated the length of the menstrual cycle in the same *female* cohort of workers in fabrication rooms as well as among women working in other areas of semiconductor plants. They found that the mean menstrual cycle length in fab-room workers and non-fab-room workers differed statistically significantly: 28.0 (SD 3.7) and 28.9 (SD 3.7) days, respectively. Fab-room workers exclusively employed in the 'thin film and ion implantation' group experienced a significantly prolonged mean menstrual cycle* of 36.1 days (32.0 days in non-fab-room workers). Fab-room workers and non-fab-room workers did not differ statistically significantly in mean days of bleeding, nor did they show differences in the occurrence of cycles consisting of more than 35 or less than 24 days. The

* Includes women with at least one cycle.

effects on menstrual cycle that were observed could not be related to exposure to ethylene glycol ethers.

Gray *et al*[88] studied a cohort of *male* and *female* workers in clean rooms of two conductor facilities in the Eastern United States. Clean room work with potentially high exposure to ethylene glycol ethers (i.e. EGME, EGMEA and DEGDME) was associated with an increased risk of subfertility (> 1 year waiting time to conception) for female employees (OR 3.9; 95% CI 1.4-11.4). This effect was not observed in the wives of exposed male employees. In the prospective part of this study, no difference was found in the conception rate (CR) per 100 menstrual cycles of workers in a clean room between women with exposure to ethylene glycol ethers and women without such exposure (CR exposed 13.6, non-exposed 11.6).

Correa *et al*[89] investigated spontaneous abortion and subfertility in the same cohort of 561 female and 589 male semiconductor workers in the Eastern USA. The highest exposure to ethylene glycol ethers (i.e. DEGDME and EGEEA) occurred in processes involving photoresist chemical mixtures, such as chemical mixing and photolithography. Female workers who had potentially been exposed to high levels of ethylene glycol ethers, showed an increased risk of subfertility (OR 4.6, 95% CI 1.6-13.3). These effects were not found in the wives of men with a potentially high exposure to ethylene glycol ethers.

Other studies

Male fertility was also investigated in the Semiconductor Health Study. Samuels *et al*[90] performed a cross-sectional study among 241 male fab-room workers en 447 non-fab workers. No decrease in fertility ratio was found, neither in fabrication room workers in general nor in any of the separate working groups. Among the men in the ‘dopants and thin film’ working group, 25% reported that it took their wives more than 1 year to conceive (RR 1.8, 95% CI 1.1-2.9). These workers, however, were mainly exposed to fluorides.

3.8.3 Effects on the development of the progeny

Cohort studies

The Semiconductor Health Study involved a retrospective study of a cohort of 904 women employed in 14 semiconductor plants in the USA and a prospective one including 403 women. In the retrospective study, Schenker *et al*[82], Beaumont

et al[83], and Swan *et al*[84] focused on spontaneous abortion in specific groups. In the prospective study, Eskenazi *et al*[85] also reported on this effect.

Schenker *et al*[82] reported an increased risk of spontaneous abortion in women in the masking group (RR 1.8, 95% CI 1.2-2.6). Although a higher percentage of spontaneous abortions was found in fab-room workers (15%) compared to non-fab-room workers (10.4%), this difference was not statistically significant (RR 1.4, 95% CI 0.95-2.1). Beaumont *et al*[83] differentiated between several groups and concluded that women involved in both photolithography and etching were at a higher risk of spontaneous abortion (RR photo 1.7, 95% CI 1.0-2.6; RR etch 2.1, 95% CI 1.3-3.2).

Swan *et al*[84] reported agent-specific analyses of the group of fab-room workers. Exposure of female workers to ethylene-based glycol ethers or propylene-based glycol ethers was associated with spontaneous abortion rates of 18.4% and 18.8%, respectively. The corresponding relative risks were: 1.6 for ethylene glycol ethers (95% CI 1.0-2.3) and 1.4 for propylene glycol ethers (95% CI 0.8-3.4). Exposure to the highest ethylene glycol ether levels (2-3) resulted in an RR of 2.4 (95% CI 1.2-4.1) for all women and an RR of 3.4 (95% CI 1.6-5.4) for women in the masking subgroup. The authors stated that propylene glycol ethers were used less (<50%) often than ethylene glycol ethers. The risk of spontaneous abortion was found to be dose-related with ethylene glycol ether exposure ($p=0.004$). However, since women in the masking subgroup were also exposed to other chemicals, the effect on spontaneous abortion can not be clearly attributed to ethylene glycol ethers. Finally, Eskenazi *et al*[85] reported on spontaneous abortion in the prospective part of the study, including 152 female fab-room workers and 251 non-fab-room workers. Although a higher percentage of spontaneous abortion was observed in fab-room workers (63.2%) than in non-fab-room workers (45.5%), the relative risk was not statistically significantly increased.

Correa *et al*[89] and Gray *et al*[88] investigated spontaneous abortion and subfertility in a cohort of 561 female and 589 male semiconductor workers in the Eastern USA. The highest exposure to ethylene glycol ethers (i.e. DEGDME and EGEEA) occurred in processes involving photoresist chemical mixtures, such as chemical mixing and photolithography. Female workers who had potentially been exposed to high levels of ethylene glycol ethers, showed an increased risk of spontaneous abortion (RR 2.8, 95% CI 1.4-5.6). These effects were not found in the wives of men with a potentially high exposure to ethylene glycol ethers. Gray *et al*[88] also conducted a prospective study. In this study no statistically significant effect was found on pregnancy loss in female workers potentially exposed to ethylene glycol ethers either.

Elliott *et al*[91] performed a relatively small study among women working in the British semiconductor industry, including 36 cases of spontaneous abortion and 80 controls. No association was found between spontaneous abortion and working in any of the specific fabrication groups.

3.9 Population-based case-control studies

3.9.1 Effects on fertility

Sallmén *et al*[92] performed a retrospective time to pregnancy study among women who were biologically monitored for exposure to organic solvents (styrene, xylene, toluene, trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane) in the past. This study (an extension of the study of Lindbohm *et al*[80]), was performed to investigate whether exposure to mixtures of different organic solvents or working in different types of industry was associated with reduced fertility, measured as prolonged time to pregnancy. One hundred ninety seven women were classified in exposure categories based on their work description, the use of solvents as reported in questionnaires, and biological exposure measurements. The adjusted incidence density ratios (IDR) of clinically recognized pregnancies for exposed workers in different types of industry are presented in table 3.1.

The incidence density ratios (IDR) of clinically recognized pregnancies among women working in the reinforced plastics industry or the graphic industry were not statistically significantly decreased. Any exposure to organic solvents in dry-cleaning shops was statistically significantly associated with prolonged time to pregnancy, bit high exposure was not. The group sizes, however, were rather small and the results should therefore be interpreted with caution. The association between high exposure to organic solvents and reduced fecundability

Table 3.1 Adjusted incidence density ratios (IDR) of clinically recognized pregnancies in different types of industry (modified from Sallmén *et al*[92]).

Industry	Solvent exposure	N	IDR	95% Confidence interval
Reinforced plastics industry	Low or high	15	0.7	0.3-1.3
	High	13	0.6	0.3-1.2
Graphic industry	Low or high	7	0.7	0.3-1.5
	High	4	0.6	0.2-1.8
Dry cleaning shop	Low or high	11	0.4	0.2-0.9
	High	6	0.6	0.2-1.3

was statistically significant among women who were employed in any of these industries when they started trying to conceive (IDR 0.4; 95% CI 0.3-0.7).

Furthermore, Sallmen *et al* 1998[93] performed a retrospective time to pregnancy study among wives of men who were biologically monitored for exposure to organic solvents (styrene, xylene, toluene, trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane) in the past. The study investigated whether exposure to mixtures of different organic solvents or working in different types of industry was associated with reduced fertility, measured as prolonged time to pregnancy. The adjusted fecundability density ratios (IDR) by exposure to organic solvents in the reinforced plastics industry or among painters were not statistically significantly changed.

Veulemans *et al*[94] performed a study among male patients in a clinic for reproductive disorders, including 1,019 cases of diagnosed subfertility or infertility and 475 controls. In 39 cases and 6 controls, urinary ethoxyacetic acid (EAA) was detected (OR 3.1, p=0.004), reflecting exposure to 2-ethoxyethanol (EGEE) or its acetate (EGEEA). These EAA-positive men were clustered among patient subcategories representing complete azoospermia and severe oligozoospermia. After grouping the patients in these categories according to occupation, industry or specific chemicals, EAA-positive men appeared to be associated with paining, motor and car mechanics, wood work, paint products and solvent-containing preparations.

3.9.2 *Effects on the development of the progeny*

In 1989, Taskinen *et al*[81] investigated the occurrence of spontaneous abortion as well as congenital malformations in their offspring among the wives of men occupationally exposed to organic solvents. This nested case-control study was performed in a cohort that was biologically monitored for exposure to six organic solvents to investigate the effects of paternal exposure on pregnancy outcome. The risk of spontaneous abortion was statistically significantly increased in the wives of male painters exposed to solvents (OR 3.3, 95% CI 1.6-6.8). Working in the plastics industry, on the other hand, was not statistically significantly associated with spontaneous abortion (OR 1.9, 95% CI 0.9-4.3). The number of congenital anomalies was too small to draw any conclusions.

Lindbohm *et al*[80] also performed a nested case-control study in a cohort of women who had been monitored for organic solvents. The workers were classified in exposure categories on the basis of their work description, the use of solvents as reported in questionnaires and biological exposure measurements. Data on medically diagnosed spontaneous abortion were retrieved from the Finnish

Registry of Congenital Malformations. The adjusted odds ratio for spontaneous abortion showed a statistically significant association with solvent exposure (OR 2.2 95% CI 1.2-4.1). Where exposure to aliphatic hydrocarbons was concerned, the odds ratio for spontaneous abortion was statistically significantly increased among a subpopulation of graphic workers (OR 5.2, 95% CI 1.3-20.8), but not among a subpopulation of painters (OR 2.4, 95% CI 0.5-13.0). Furthermore, the odds ratio of spontaneous abortion after exposure to tetrachloroethylene was not increased among several subpopulations in dry-cleaning shops. Finally, the odds ratio for spontaneous abortion among laboratory workers who had been exposed to (amongst others) toluene was 0.9 (95% CI 0.2-5.5).

Ha *et al*[95] and Cordier *et al*[96] reported on an European collaborative multicenter case-control study on congenital malformations, including 991 cases of spontaneous abortions and 1,144 controls. Ha *et al*[95] described statistically significant excesses of mothers exposed to glycol ethers in the groups giving birth to children with oral clefts (OR 2.0, 95% CI 1.1-4.1) or central nervous system malformations (OR 1.8, 95% CI 1.1-3.3). Exposure concerned group 2 and 3 glycol ethers, mainly consisting of non-teratogenic compounds. However, this paper was poorly documented. In the much better documented study by Cordier *et al*[96], congenital malformations were divided in 22 subgroups. Glycol ether exposure was shown to result in an increased risk for all congenital malformations included (OR 1.4, 95% CI 1.1-1.9) and for the subgroups of neural tube defects (OR 1.9, 95% CI 1.2-3.2), especially spina bifida (OR 2.4 (95% CI 1.2-4.6), multiple anomalies (OR 2, 95% CI 1.2-3.2) and cleft lip/palate (OR 2.0, 95% CI 1.2-3.3).

In a recent study, Chevrier *et al*[97] examined 164 cases of cleft lip with or without cleft palate, 76 cases of cleft palate, and 236 controls. Maternal exposure to glycol ethers during the first trimester was associated with an increased risk of cleft lip with or without cleft palate (OR 1.9, 95% CI 1.1-3.5). The increased risk appeared to be dose-dependent ($p<0.01$). The authors mentioned that a large number of women exposed to glycol ethers were also exposed to aliphatic alcohols. When these exposures were considered separately, the risk of cleft lip and palate was no longer statistically significantly increased after exposure to glycol ethers.

3.10 Conclusions

3.10.1 Reinforced plastics industry

Effects on fertility

The effect on *female fertility* was studied in a cohort of female workers in the reinforced plastics industry.[98] No effects were observed on several menstrual parameters. In addition, in a study among women who had been monitored for exposure to solvents at some point[92], no association was found between time to pregnancy and employment in the reinforced plastics industry.

The committee concludes that the available data give no indication for an association between working in the reinforced plastics industry and reduced female fertility.

The effect on *male fertility* was studied in a cohort of male workers in four Danish plants where reinforced plastics were produced.[14,15] Several sperm parameters (sperm density and sperm count) were affected. No associations were observed between the sperm parameters and the internal styrene concentration in male workers (measured as post-shift urinary mandelic acid concentration). In an additional cross-sectional study, Kolstad *et al*[16,17] did not observe a statistically significant effect on male fecundity (measured by time to pregnancy). In addition, in a study among men who had at some point been monitored for exposure to solvents, no association was found between time to pregnancy of their wives and employment in the reinforced plastics industry.[93]

The committee considers the available data too limited to draw any conclusions regarding the effect of working in the plastics industry on male fertility.

Effect on the development of the progeny

No effect on birth weight among children in a cohort of mothers working in the plastics industry was found by Lemasters *et al*[20] Lindbohm *et al*[21] performed a nested case-control study in the styrene industry and showed that the risk of spontaneous abortion was not statistically significantly associated with occupational exposure in the plastics industry.

The committee concludes that there are no indications that maternal exposure to solvents in the plastics industry is associated with effects on development (birth weight and spontaneous abortion). With respect to other developmental

effects (congenital malformation, malignancies and neurodevelopment) no data are available.

Furthermore, one population based case control study[81] showed no increased risk for spontaneous abortion among wives of men working in the reinforced plastics industry. However, the committee concludes that the available data are too limited to draw any conclusions regarding the effects of paternal exposure to solvents in the reinforced plastics industry on development.

3.10.2 *Printing industry*

Effects on fertility

No cohort or nested case-control studies were available concerning the effect of working in the printing industry on *male and female fertility.* Only one study[92] was performed among women who had been monitored for exposure to solvents at some point. In this study, no association was found between time to pregnancy and working in the graphic industry. No data are available concerning the effects on male fertility.

The committee concludes that no data are available concerning the effects on male fertility and that limited data are available concerning the effects on female fertility. Therefore, the committee is unable to draw any conclusions with respect to the effects of exposure to solvents in the printing industry on male and female fertility.

Effects on the development of the progeny

No effects on the occurrence of childhood cancer were observed among offspring of a cohort of male printers.[25] In the same cohort, effects on the outcome of pregnancy were also studied.[26] A statistically significant effect on preterm birth was observed (OR 5.4, 95% CI 1.7-17.4). However, the authors were not able to exclude the influence of exposure to lead, which is associated with an increased risk of perinatal death and preterm delivery.[99] In a population-based nested-case control study among women who had at some point been exposed to solvents, Lindbohm *et al*[80] found an increased odds ratio for spontaneous abortion among a subpopulation of graphic workers exposed to aliphatic hydrocarbons (OR 5.2, 95% CI 1.3-20.8).

The committee considers the available data on the effects of paternal exposure on the development of the progeny as insufficient. Therefore the committee

is unable to draw any conclusions regarding the effects of paternal exposure to solvents in the printing industry on development of the progeny.

Regarding the effects on development after maternal exposure, the committee is of the opinion that there are weak indications for an association with spontaneous abortion. No data were available on other developmental effects (birth weight, congenital malformations, malignancies and neurodevelopment).

3.10.3 *Painting and maintenance trade*

Effects on fertility

The effect on *male fertility* (endocrine function and time to pregnancy) was studied in a cohort of male painters, carpenters and joiners by Luderer *et al*.[31] No effect on the time to pregnancy was found in the group of wives of male painters. In addition, in a study among men who had at some point been monitored for exposure to solvents, no association was found between time to pregnancy of their wives and employment as painter.[93] Lemasters *et al*[32] found a decline in sperm motility in a group of male painters 30 weeks after exposure begun. However, no association was observed between internal exposure and sperm motility. Welch *et al*[33] did not find an effect on semen parameters motility, viability, morphology and morphometry among male shipyard painters, who were predominantly exposed to 2-ethoxyethanol (EGEE) and 2-methoxyethanol (EGME). On the other hand, semen pH was increased, and the exposed non-smokers showed a higher rate of oligozoospermia. In a population-based case-control study, Veulemans *et al*[94] showed that in patients diagnosed as infertile or subfertile, the urinary EEA (ethoxy aceticacid) concentration was statistically significantly increased. The presence of urinary EEA was associated with exposure to paint products.

Based on the studies by Lemaster *et al*[32], Welch *et al*[33] and Veulemans *et al*[94], the committee concludes that there are weak indications for an association between working in the painting industry and effects on male fertility.

No data are available concerning female fertility. Therefore, the committee is unable to draw any conclusions regarding the effects of exposure to solvents in the painting industry on fertility in women.

Effects on the development of the progeny

No cohort studies among painters were available concerning effects on the development of the progeny. Two population-based case-control studies[80,81] were avail-

able. Taskinen *et al*[81] demonstrated an association between exposure of male painters and spontaneous abortion in the wives of these men. Lindbohm *et al*[80] observed no increased risk of spontaneous abortion for female painters exposed to aliphatic hydrocarbons.

The committee is of the opinion that the available data on effects on the development of the progeny due to maternal exposure are insufficient. Therefore, the committee is unable to draw any conclusions regarding the effects of maternal exposure to solvents in the painting industry on development.

For the developmental effects after paternal exposure to solvents, the committee concludes that there are weak indications for an association with spontaneous abortion. No data are available concerning other developmental effects.

3.10.4 *Chemical and petrochemical industry*

Effects on fertility

Effects on *fertility* have not yet been investigated in cohorts from the petrochemical industry. In one cross-sectional study, the effect of exposure to solvents in the petrochemical industry on female fertility was studied.[37] Exposure to solvents was statistically significantly associated with oligomenorrhea. As only one study is available and the consequences of oligomenorrhea for female fertility are not clear, the committee concludes that the available data are too limited to draw any conclusions regarding the effects on female fertility of exposure to solvents in the petrochemical industry.

No data concerning the effects of exposure to solvents in the petrochemical industry on male fertility were available. Therefore the committee is unable to draw any conclusions about the effects on male fertility of exposure to solvents in the petrochemical industry.

Effects on the development of the progeny

The risk of spontaneous abortion was studied in a female cohort working in the petrochemical industry in China.[42] Maternal exposure to petrochemicals was demonstrated to be statistically significantly associated with an increased risk of spontaneous abortion.[42] In another Chinese study, reduced birth weight was found after maternal exposure to solvents in the petrochemical industry.[43] How-

ever, this effect on birth weight was not observed in a cohort of Chinese women by Chen *et al*[44]

In conclusion, based on the effects found in the studies by Xu *et al*[42] and Ha *et al*[43], the committee is of the opinion that the available data give weak indications for an association between maternal exposure to solvents in the petrochemical industry and effects on spontaneous abortion. No data are available concerning other effects on development.

No increased risk of spontaneous abortion was observed in the wives of men occupationally exposed to solvents in the petrochemical industry in France.[41] In addition, no effect on birth weight was observed after paternal exposure to solvents in the Chinese petrochemical industry.[43]

Therefore, the committee is of the opinion that the available data on paternal exposure do not indicate an association with developmental effects on birth weight and spontaneous abortion. No data are available concerning other effects on development.

3.10.5 Laboratories

Effects on fertility

Both Wennborg *et al*[49] and Zhu *et al*[50] studied time to pregnancy in a cohort of female laboratory workers. In the study of Wennborg *et al*, time to pregnancy was statistically significantly increased after female exposure to 'organic solvents' while this effect was not observed by Zhu *et al*[50] after exposure to 'solvents'.

The committee is of the opinion that the available data are conflicting and is therefore unable to draw any conclusion regarding the effects on *female fertility* after exposure to solvents in laboratories.

With respect to *male fertility*, the committee concludes that no data are available. Therefore the committee is unable to draw any conclusion regarding the effects on *male fertility* after exposure to solvents in laboratories.

Effects on the development of the progeny

Three cohort studies carried out among female laboratory personnel showed no effect of exposure to solvents on the risk of spontaneous abortion and 'small for gestational age' [54], prenatal death and malformations[53] or pregnancy outcome[57]. However, Wennborg *et al*[55] did show an association between female exposure to

solvents and preterm births, and Taskinen *et al*[60] found an increased risk of spontaneous abortion after maternal exposure to aromatic solvents. Furthermore, Wennborg *et al*[56] and Zhu *et al*[57] found that female exposure to solvents was associated with major malformations.

Among male workers studied by Magnusson *et al*[58], no association was observed between exposure to solvents and major malformations in offspring.

In conclusion, the committee is of the opinion that the available data on female exposure to solvents in laboratories and the effects on the development of the progeny are conflicting. Therefore, the committee is unable to draw any conclusion regarding the effects on development after maternal exposure to solvents in laboratories.

Where male exposure is concerned, insufficient data are available. Therefore, the committee is also unable to draw any conclusion regarding the effects on development of paternal exposure to solvents in laboratories.

3.10.6 Dry-cleaner's

Effects on fertility

The effects on *male fertility* were studied in two cohort studies by Eskenazi *et al*.[70,71] Within a range of semen parameters, subtle effects were found on the two parameters of sperm quality among male dry-cleaners. The relevance of these effects, however, remains unclear as all other parameters were unaffected. In the second study, time to pregnancy of wives of male dry-cleaners was not affected. In a study, Sallmén *et al*[92], found a prolonged time to pregnancy among a small group of female drycleaners who were biologically monitored for exposure to solvents.

The committee concludes that the available data are limited and therefore the committee is unable to draw any conclusions regarding the effects of exposure to solvents in dry-cleaning shops on male fertility.

The data on *female fertility* are also insufficient and therefore the committee is unable to draw any conclusions regarding the effects on female fertility either.

Effects on the development of the progeny

In one cohort of women working in dry-cleaning shops, no increased incidence of spontaneous abortion was observed compared to a control group of laundry workers.[75] However, within the group of dry-cleaners, the odds ratio for spontaneous abortion was statistically significantly increased for operators versus non-

operators. Several industry based case-control studies were performed.[76,77,78] Kyyrönen *et al*[76] found that high maternal exposure to tetrachloroethylene (PER) in dry-cleaning shops and laundries in Finland was statistically significantly associated with an increased risk of spontaneous abortion, but not with congenital malformations. However, an association between spontaneous abortion and maternal exposure to PER in dry-cleaning shops was not observed by Ahlborg *et al*[77] and Olsen *et al*[*] [78]. In a cross-sectional study, Lindbohm *et al*[79] found an increased risk of spontaneous abortion in laundry and dry-cleaning workers after maternal exposure. On the other hand, a population-based case-control study by Lindbohm *et al*[80] showed no increased risk of spontaneous abortion in a subgroup of female dry-cleaning workers exposed to tetrachloroethylene. Furthermore, Olsen *et al*[78] showed no effects on congenital malformations, still birth and low birth weight (< 1500 g). For congenital malformation, however, all outcomes were combined, which means the results cannot be used according to the committee.

In conclusion, the committee is of the opinion that the studies of Doyle *et al*,[75] Kyyrönen *et al*,[76] and Lindbohm *et al*[79] give weak indications for an association between an increased risk of spontaneous abortion and female exposure to PER in dry-cleaning shops. No sufficient data are available concerning other effects on development.

For the effects on development after paternal exposure no data are available. Therefore, the committee is not able to draw any conclusion regarding the effects on development after paternal exposure to solvents in dry-cleaning shops.

3.10.7 *Semiconductor industry*

Effects on fertility

In four cohort studies in the semiconductor industry, the effect on *female fertility* was evaluated.[86,87,88,89] Eskenazi *et al*[86] observed a decreased fecundability ratio (probability of conception per menstrual cycle) in a subgroup of 'dopants and thin film' fabrication workers (predominantly exposed to fluorides and not to solvents). This decrease was not observed in the 'masking' subgroup (primarily exposure to solvents). Gold *et al*[87] observed a prolonged menstrual cycle length in the subgroup 'thin film and ion implantation' *female fab-room workers* as opposed to non-fab-room workers, but not in other subgroups of fab-room wor-

* An association between spontaneous abortion and exposure to PER in dry-cleaning shops was observed for the subgroup of Finnish dry cleaners. These data, however, were derived from the study of Kyyrönen.[76]

kers. Gray *et al*[88] observed an increased risk of subfertility (delayed conception) in *female* semi-conductor workers. This effect was not observed when *male workers* were exposed. Correa *et al*[89] confirmed an increased risk of subfertility among the same female workers. Again, this effect was not observed when male workers were exposed. Furthermore, no effects on male fertility were observed in a cross-sectional study by Samuels *et al*.[90]

The committee is of the opinion that the available data concerning female fertility seems to be conflicting. This might be explained by different composition of the exposure in the subgroups. Given that, the committee is not able to draw any conclusions regarding the effects of exposure to solvents in semiconductors on *female fertility.*

Based on the available data with respect to effects on *male fertility*, the committee concludes that there are no indications for an association.

Effects on the development of the progeny

Several cohort studies carried out among female workers in semiconductor industries are available concerning the effects on spontaneous abortion.[82,83,84,86,88,89] Schenker *et al*[82] reported a statistically significantly increased incidence of spontaneous abortion in the masking subgroup of female fab-room workers. Beamont *et al*[83] found an increased incidence in both subgroups of the female 'masking' group. Swan *et al*[84] reported increased incidences of spontaneous abortion in different subgroups (among which the subgroup classified under masking) of female fab-room workers. Correa *et al*[89] and Gray *et al*[88] found an increased incidence of spontaneous abortion in female fab-room workers. This effect was not observed when male workers were exposed. Finally, Eskenazi *et al*[86] did not observed a statistically significantly increased incidence in spontaneous abortion in female fab-room workers versus non-fab-room workers.

In conclusion, the committee is of the opinion that there are indications for an association between maternal exposure to solvents in the semiconductor industry and spontaneous abortions. No data are available concerning other developmental effects.

The studies of Correa *et al*[89] and Gray *et al*[88] evaluated the effects of male exposure in the semiconductor industry. Both studies found no association between exposure to solvents and spontaneous abortion. Therefore, the committee concludes that the available data give no indications for an association between paternal exposure and the risk of spontaneous abortion. No data are available concerning other developmental effects on the progeny of exposed men.

Chapter 4

Associations with individual chemicals: Styrene

4.1 Introduction

Styrene (CAS nr 100-42-5) is an aromatic hydrocarbon (C_8H_8) which is, amongst others, used as an organic solvent and as a cross linking agent. Occupational exposure to styrene can occur during manufacturing of the monomer, producing of the polystyrene or ether styrene-based polymers, processing of styrene-based polymers and the manufacturing of glass-reinforced plastics.

In the Netherlands, the Dutch Expert Committee on Occupational Standards (DECOS)[234] recommended an health based occupational exposure limit for styrene of 107 mg/m^3 (25 ppm) as an eight hour time weighted average. This exposure limit was based on preventing neurological effects (i.e. critical effect). No short term exposure limit (STEL), ie an occupational exposure limit as a fifteen-minute time weighted average, has been recommended.

In 2001, the Committee on Compounds toxic to reproduction of the Health Council of the Netherlands recommended for styrene no classification for effects on fertility due to a lack of appropriate data.[100] No classification for the effects on development was recommended as well, due to a lack of appropriate data.

4.2 Occupational exposure

The highest styrene exposure is found in the open and closed mould production in the reinforced plastics industry. Besides occupational exposure to other sol-

vents, the styrene monomer is one of the predominant components of the exposure. Occupational exposure to styrene also occurs in the petrochemical industry, however this always in co-exposure with several other solvents (eg toluene, xylene, benzene).

Post *et al*[10] estimated the exposure to styrene in a small polyester factory in the Netherlands. Van Rooij *et al*[11] reported styrene exposure concentrations in the European glass-reinforced plastics industry and Lees *et al*[12] in the US glass reinforced industries (see chapter 3).

Jensen *et al*[101] assessed the occupational exposure to styrene and associated chemicals in Denmark, where the main use of styrene was in the reinforced plastics industry production. A total of 2,528 air samples from 256 workplaces were collected and analyzed during the years 1955-1988. The mean concentration decreased from 714 mg/m^3 in the early years to 172 mg/m^3 in the eighties.

4.3 Fertility

4.3.1 Effects on human fertility

Seven studies, 2 cohort studies (Kolstad *et al*[17] (describing another study of Kolstad *et al*[16] in more detail) and Lemasters *et al*[13]), one nested case control study[92], three cross-sectional studies[14,15,19,37] (describing the same study) and one other study[18] describe the effects of inhalatory exposure to styrene on male or female fertility. Five of these studies met the selection criteria of the committee.[13,14-16,17,37,92] Two studies were rejected for various reasons including: no correction for confounders[18], selection bias not excluded[18,19] , no sufficient exposure data[18,19] and no definition of a hypothesis[19].

Female fertility

Lemasters *et al*[13] evaluated several menstrual cycle parameters of 174 women exposed to (predominantly) styrene in an US reinforced plastics industry. No statistically significant increase in menstrual effects was found after exposure to high levels of styrene (> 52 ppm) (and other solvents).

Cho *et al*[37] studied the menstrual cycle length in a cross-sectional study among 1408 petrochemical workers in China. Exposure was assessed by an industrial hygienist. Besides the effects of styrene, also the consequences of exposure to benzene, toluene, xylene and combinations were evaluated. Exposure to ‘all aromatic solvent’ was associated with an (adjusted) OR for oligome-

norrhea of 1.8 (95% CI 1.1 to 2.8) and exposure to styrene was associated with an OR of 1.7 (95% CI 1.1-2.6).

Sallmén *et al*[92] investigated time-to-pregnancy in a nested case control study among 197 women who had been monitored for exposure to specific organic solvents (styrene, xylene, toluene, trichloroethylene, tetrachloroethylene and 1,1,1-trichloroethane) in the past. Exposure assessment was based on classification of the reported jobs and on biological measurements. The adjusted Incidence density ratio (IDR) of clinically recognized pregnancies was not statistically significantly associated with women exposed to styrene or working in the reinforced plastics industry.

Male fertility

Kolstad *et al*[14,15] followed the semen quality of 23 men who were exposed to predominantly styrene in four Danish reinforced plastics industries for six months. Semen samples were collected at the beginning and after six months of working in the plastics industry. In addition, semen samples were collected from 21 non-exposed farmers. A statistically significant decline was observed in sperm density and sperm count in the styrene exposed group. This time-related effect was not observed in the non-exposed group. Furthermore, the proportion of sperm with normal morphology was reduced, the proportion of nonviable sperm decreased and the median sperm velocity increased in time in the styrene exposed workers. However, none of the sperm parameters, including sperm density and sperm count, were related to the internal styrene concentration, measured as the post shift urinary mandelic acid concentration. The authors concluded that the findings for the analyses of the internal and external exposure were inconsistent.

Finally, Kolstad *et al*[16,17] examined the effect of styrene exposure on male fecundity, measured as the time to pregnancy of their wives. No reduced fecundity was found for men employed in the reinforced plastics industry. The committee concluded that no association was observed between time to pregnancy and male exposure to styrene.

4.3.2 *Effects on fertility in experimental animals*

Summary of the data from the report of the Committee Compounds toxic to reproduction (2001)

In a three-generation reproduction study, Beliles *et al*[102] evaluated the reproductive performance in Sprague Dawley rats receiving (low concentrations) styrene in their drinking water. No effects on male and female fertility were observed. Srivastava *et al*[103] studied the effect of 60 days styrene exposure (two levels administered by gavage) in male Wistar rats. No effects on body weight, testes and epididymis weight were observed. Alteration in testicular enzyme activities and epididymal sperm concentration occurred in males exposed to styrene 400 mg/kg bw/day. Rats in this exposure group also showed abnormal testicular histopathology (shrunken seminiferous tubules with some Sertoli only tubules sections). Waalkens-Berendsen found no significant effects on sperm parameters, organ weights and histopathology of reproductive organs after 28 days of oral exposure to styrene (400 mg/kg bw/day).[104]

Data since 2001

Cruzan *et al*[105] (part b) exposed male and female rats in a two-generation reproduction toxicity study to 0, 50, 150, and 500 ppm 6 hour/day, 7 days/week for at least 70 consecutive days. There was no indication that the reproductive capability of the F0 and F1 parental animals was affected. Male and female mating and fertility indices, pre-coital intervals, spermatogenic endpoints, reproductive organ weights, length of oestrous cycle and gestation, live litter size and postnatal survival were similar in all exposure groups. Systemic toxicity (reduced body weight gain) in the F0 and F1 males and females was observed in the 500 ppm exposure group during the premating period.

4.3.3 *Effects on fertility: Conclusions*

Two studies describe the effects of female exposure to solvents (amongst others styrene) on menstrual parameters.[13,37] In the cohort study of Lemasters, no effects on menstrual parameters were observed after high exposure (>51 ppm, ~218 mg/m^3) to predominantly styrene in the reinforced plastics industry. On the other hand, in a cross-sectional study in a Chinese petrochemical industry, with exposure to lower levels of styrene in combination with numerous other solvents, a

statistically significant association was observed between exposure to styrene and oligomenorrhoea. A statistically significant association was observed for oligomenorrhoea and exposure to 'aromatic solvents' as well. No other menstrual disorders were observed in the Chinese petrochemical industry. In a population based case-control study[92], no statistically significant association was observed between time to pregnancy and exposure to styrene or working in the reinforced plastics industry. From experimental data in animals, the committee did not find indications for effects on female fertility. The human data of Cho *et al*[37] suggest that exposure to low levels of styrene might cause oligomenorrhoea; this effect was however not confirmed in the study of Lemaster *et al*[13] after exposure to higher levels of primary styrene, nor in experimental animal data. Therefore, the committee concludes that there are no indications for an association between exposure to styrene and effects on female fertility.

The effect of working in the reinforced plastics industry on *male fertility* was studied in a cohort of male workers in four Danish reinforced plastics industries.[14,15] Several sperm parameters (sperm density and sperm count) were affected in men exposed to styrene in the plastics industry. However, no associations were observed between the sperm parameters and the internal styrene concentration in male workers (measured as post shift urinary mandelic acid concentration). In an additional cross-sectional study, Kolstad *et al*[16,17] did not observe an effect on male fecundity (time to pregnancy of their wives). In conclusion, the committee considered the available data too limited and therefore the committee is unable to draw any conclusions about the possible effects of exposure to styrene on male fertility.

4.4 Development of the progeny

4.4.1 Effects on human development

Ten studies, two cohort studies[20,44], seven (nested) case control studies[21,28,35,80,81,106,107] and one cross-sectional study[108] describe the effects of inhalatory exposure to styrene on development. Of these, five studies[20,21,44,80,81] met the criteria for selection.

Five studies were rejected for various reasons amongst others: No adequate description of the exposure[35], no correction for confounders[106,108], selection bias not excluded[28,108], no hypothesis[35], and only preliminary results[107].

Lemasters *et al*[20] analysed the birth weights of children whose mothers worked during their pregnancy in the reinforced plastics industry, where styrene was the primary exposure. Besides styrene there was exposure to acetone, ethyl ethyl

ketone, methylene chloride, toluene and xylene as well. There was not a statistically significant dose-response trend in decreasing average birth weight. Women who worked in the higher exposed jobs (30 ppm or higher in any month) had offspring with adjusted birth weight of 4% less than the offspring of unexposed women (95% CI -7.7% to +0.6%).

Chen *et al*[44] studied the association between birth weight and prenatal exposure to benzene and other solvents (amongst others to styrene) in the Yanshan petrochemical cooperation in Beijing China (BYPC). The exposure was assessed by an industrial hygienist and the exposure levels of toluene, styrene, xylene and benzene were all below the OEL established by OSHA*. Exposure to styrene containing solvents (except for benzene) was not significantly associated with changed birth weight. Associations with individual solvents were not studied (except for benzene). Birth weight was negatively associated with exposure to benzene; this effect was more pronounced in the presence of work stress.

Lindbohm *et al*[21] analyzed in a matched case-control study whether maternal occupational exposures in the plastics industry were related to the risk of spontaneous abortion. No increased risk of spontaneous abortion was observed among workers processing polymerized plastics (styrene) (OR 0.4 (95%CI 0.1-1.2)) or heating plastics (OR 0.6 (95% CI 0.2-2.3)). However the statistical power of this study was limited.

In a population based case-control study, Lindbohm *et al*[80] studied the effects of (self reported) occupational exposure to styrene and other solvents on spontaneous abortion among women ever monitored for solvents exposure. The (adjusted) odds ratio of spontaneous abortion for solvent exposure was significantly increased (2.2 95% CI 1.2-4.1). The adjusted odds ratio for aromatic hydrocarbons was 1.6 (95% CI 0.8-3.3) and for styrene 0.3 (95% CI 0.1-1.0).

Finally, Taskinen *et al*[81] evaluated pregnancy outcome of the wives of men occupationally exposed to styrene and other industrial chemicals in a population based case control study. The authors concluded that no association between spontaneous abortion and paternal styrene exposure was found and that the number of congenital anomaly cases was too small for drawing conclusions. Paternal exposure to organic solvents in general was on the other hand statistically significantly associated with spontaneous abortion (OR 2.3 95% CI 1.1-5.0).

* OSHA: Occupational Safety and Health Association (USA).

4.4.2 *Effect on development in experimental animals*

Summary of the data from the report of the Committee Compounds toxic to reproduction (2001)

Murray *et al*[109] treated pregnant Sprague-Dawley rats (by inhalation and gavage) and New Zealand white rabbits (by inhalation) with styrene. Although maternal toxicity was observed, no embryotoxic, foetotoxic or teratogenic effects were observed. In a study by Kankaanpää *et al*[110] developmental effects (number of dead and resorbed foetuses was statistically significantly increased) were observed in mice at high maternally toxic dose levels (styrene exposure by inhalation). In a two-generation study, Beliles *et al*[102] showed a significant adverse effect on pup survival. However, these effects could be attributed to two litters only. In a study by Srivastava, styrene exposure resulted in a decreased number of implantations at concentrations that cause maternal toxicity as well.[111] At higher concentrations, the number of dead and resorbed foetuses increased and the foetal weight was decreased. Waalkens-Berendsen *et al* did not observe any effects on development in rats exposed to styrene from GD 6 to PN day 14.[112]

Data since 2001

In 2005, a developmental neurotoxicity study as part of a two-generation reproductive study was performed by Cruzan *et al*[105] Male and female rats were exposed to styrene by inhalation to 0, 50, 150 and 500 ppm for 6 hours/day, 5 days/week for at least 70 days. A slightly decreased body weight was observed in the F0 and F1 animals of the 500 ppm exposure group (and to a lesser extent in the 150 ppm group). There was a decrease of F2 offspring body weights from birth through PND 70 in the 500 ppm group. In the 150 ppm group pup body weight was deceased from PND 7 to adulthood. Functional observational evaluations for all F1 dams during gestation and lactation and for the F2 offspring were not affected by styrene exposure. There was a decrease in fore and hindlimb grip strength on PND 45 and 60 in the F2 offspring of the 500 ppm exposure group. PND 24 swimming ability was slightly decreased in the F2 offspring of both sexes of the 500 ppm exposure group.

Effects on development: Conclusions

Two human studies evaluated the effect of maternal exposure to styrene (and other solvents) on the birth weight of the progeny[20,44]. Lemasters *et al*[20] found a statistically non-significant decline (less than 5%) in birth weight in the high exposed female groups in a cohort in the reinforced plastics industry. No statistically significant effect on birth weight was observed after maternal exposure to styrene containing solvents[44] in a cohort of female petrochemical workers. In conclusion, the committee is of the opinion that there are no indications for an association between maternal exposure to styrene and effects on the birth weight of the progeny.

Three studies evaluated the effects of maternal or paternal exposure to styrene in relation to the risk of spontaneous abortion. Lindbohm *et al*[21,80] did not find an increased risk for spontaneous abortion among female workers exposed to styrene in a nested case control study in the plastics industry. When on the other hand, the maternal exposure to 'total solvents' was linked to spontaneous abortion, a positive association was determined (OR 2.2 (95% CI 1.2-4.1)). Taskinen *et al*[81] found no association between paternal exposure to styrene and spontaneous abortion in a population based case control study. However, paternal exposure to 'organic solvents in general' was statistically significantly associated with spontaneous abortion.

Several studies in experimental animals did not find any effects on development in the presence of maternal toxicity. Two studies showed small effects on development, however these effects were found either in the presence of severe maternal toxicity or at high exposure levels.

Therefore, the committee is of the opinion that there are no indications for an association between maternal exposure to styrene and developmental effects (spontaneous abortion and birth weight). No data were available concerning the relationship between maternal exposure and the other developmental effects (malformations, neurobehavioural effects, and malignancies). Therefore, the committee is unable to draw any conclusions about these effects after maternal exposure.

Furthermore, for paternal exposure to styrene, the committee concludes that the available data are too limited to draw any conclusions regarding the effects of paternal exposure on development.

Chapter 5

Data on individual chemicals: Toluene

5.1 Introduction

Toluene (CAS nr 108-88-3), an aromatic hydrocarbon ($C_6H_6CH_3$), is used in the manufacturing of several organic compounds. Furthermore toluene is applied as a solvent and as a thinner for paints, glues, varnish and rubber. Besides, it is used as a cleaning agent. Finally, toluene is a component of (jet) fuel for cars and airplanes.

In the Netherlands, the Dutch Expert Committee on Occupational Standards (DECOS) recommended an health based occupational exposure limit for toluene of 150 mg/m^3 (40 ppm) as an eight hour time weighted average. This exposure limit was based on preventing effects on the central nervous system (i.e. critical effect). No short term exposure limit (STEL), ie an occupational exposure limit as a fifteen-minute time weighted average, has been recommended.[235]

In 2001, the Committee on Compounds toxic to reproduction recommended for toluene no classification for effects on fertility due to a lack of appropriate data. For the effects on development the committee recommended classifying toluene in category 3 (substances which cause concern for humans owing to possible developmental effects).[236]

5.2 Occupational exposure

Occupational exposure to toluene may occur in several industries and jobs. Highest toluene concentrations in air have been found in the printing industry where toluene is used as a solvent for inks and dyes. Occupational exposure may also occur during paint stripping operations. Concentrations of 5-50 ppm (19-190 mg/m^3) are common at the workplace with some values as high as 250 ppm (1000 mg/m^3) (NCI 1995 in ATSDR 2000). The exposure level of aircraft maintenance personnel to toluene in jet fuel vapour was amounted to be 6.3 ppm (44 mg/m^3) (Smith, 1997 in ATSDR 2000).[113] A group of Dutch carpet-layers using water-based adhesives were exposed to an 8-hour average concentration of toluene in the range of 0.27-77 ppm (1.9-290 mg/m^3), while carpet-layers using contact adhesives were exposed to 4-161 ppm (15-604 mg/m^3) (Muijser 1996 in ATSDR 2000).[114]

Burstyn and Kromhout[30] analyzed trends in inhalation exposure to hydrocarbons among painters in The Netherlands (1980-1999). The solvents most commonly measured among Dutch commercial painters were toluene, xylene, ethylbenzene, n-decane and n-hexane. As toluene correlated well with the aromatic solvent exposure it was selected as a marker for solvent exposure. Exposure to toluene measured during the application of solvent based paints declined by 12% per year over the period 1980-1999.

In 2006, the Health and Safety Executive (HSE) published trends in inhalation exposure from the mid 1980 till 2006.[22] For toluene a total of 3,323 results from toluene measurement were available in the NEDB (National Exposure DataBase). The highest exposure levels by industry for toluene were observed in the '*manufacture of pulp, paper and paper products, publishing and printing*' industry and in the '*Manufactory of rubber and plastic products*' industry. The highest level in terms of occupation occurred amongst workers in the '*rubber and plastic'* industry, '*painters and decorator industry'* and '*packing/assembling /warehouse and finishing'* workers (see table 5.1). Data on exposure concentration in time were also available; however, the trend in time was dependent on the source of the data. A potential problem with the toluene exposure data in the NEDB is that it contains measurements of solvent mixtures where toluene is only a minor constituent.

Table 5.1 Summary of toluene exposure (ppm) by industry (1985-2002).

Industry	N[a]	AM[b]	GM[c]	GSD[d]	Min[e]	Max[f]
Manufacture of textiles and leather products	285	12.1	2.8	8.8	<0.01	158.0
Manufacture of pulp, paper and paper products, publishing and printing	385	77.2	12.5	10.9	0.1	2684.0
Manufacture of chemicals, chemical products and man-made fibers	395	72.9	2.0	17.6	<0.01	8698.0
Manufacture of rubber and plastic products	639	53.0	10.6	8.5	<0.01	1600.0
Manufacture of other non-metallic mineral products, electrical, optical, transport, not elsewhere classified.	698	23.5	7.4	5.5	0.1	1050.0
Manufacture of basic metals and fabricated metal products	251	9.5	1.7	5.7	<0.01	250.3
Construction, real estate, renting and business activities	189	168.2	3.8	13.9	0.1	4880.0
Other	168	12.8	1.2	12.4	<0.01	260.0

[a] N: Number of measurements
[b] AM: Arithmetic mean
[c] GM: Geometric mean
[d] GSD: Geometric Standard Deviation
[e] Min: Lowest observed exposure
[f] Max: Highest observed exposure

5.3 Fertility

5.3.1 *Effects on human fertility*

Fifteen studies, 1 cohort studies[115] (describing the same study as Xiao *et al*[116] in more detail)), 1 experimental study[117], 2 (nested) case-control studies[2,92] and 11 cross-sectional studies[3,23,24,32,37,118,119,120,121,122,123] describe the effects of inhalatory exposure to toluene on male or female fertility. Nine of these studies met the selection criteria of the committee[2,32,37,92,115,117,118,119,122]. The other studies were rejected for various reasons amongst others: no correction for confounders[23,24,120,121,123], selection bias not excluded[3,23,24,121,123], and insufficient exposure data[24,121,123].

Effects on female fertility

Luderer *et al*[117] studied reproductive endocrine effects of acute exposure to toluene in men and women in an experimental study. Women in the follicular phase (n=9) or luteal phase (n=9) were exposed to 50 ppm toluene (190 mg/m^3) for 3 hours. No statistically significant effects were observed on the individual LH

(luteinising hormone) and FSH (follicle stimulating hormone) levels of women exposed to toluene. Only subtle effects on LH secretion in women during the luteal phase were found. The clinical relevance of this finding is unclear.

Reutman *et al*[122] assessed the potential reproductive endocrine effects of exposure to low levels of aromatic and aliphatic hydrocarbons among female personnel of the US air force industry in a cross sectional study. The exposure was quantified by measuring the (exhaled) breath levels of several compounds (benzene, toluene, ethylbenzene, xylene etc). No effects of (self-reported) exposure to toluene were found on endocrine levels. Preovulatory LH levels were significantly lower among women with high breath aliphatic hydrocarbon levels and aromatic hydrocarbon levels.

Sallmén *et al*[92] investigated time-to-pregnancy in a nested case control study among 197 women who had been monitored for exposure to specific organic solvents (styrene, xylene, toluene, trichloroethylene, tetrachloroethylene and 1,1,1-trichloroethane) in the past. Exposure assessment was based on classification of the reported jobs and on biological measurements. The adjusted Incidence density ratio (IDR) of clinically recognized pregnancies was significantly decreased for women working in the shoe factory (low and high exposure IDR 0.3; 95% CI 0.1-0.7). The authors suggested that the most common organic solvents in the shoe factory are toluene, hexane and acetone. However, no statistically significant effect was found for high exposure to toluene (IDR 0.7; 95% CI 0.4-1.3). The group sizes, however, were rather small.

Cho *et al*[37] studied the menstrual cycle length in a cross-sectional study among 1408 petrochemical workers in China. Exposure was assessed by an industrial hygienist. Besides the effects of toluene, also the consequences of exposure to benzene, styrene, xylene and combinations were evaluated. Exposure to 'all aromatic solvent' was associated with oligomenorrhoea, with an adjusted OR of 1.8 (95% CI 1.1 to 2.8). Exposure to toluene did not statistically significantly increase oligomenorrhoea (OR of 1.4 (95% CI 0.9-2.2).

Effects on male fertility

Luderer *et al*[117] studied reproductive endocrine effects of acute exposure to toluene in men and women in an experimental study. Women in the follicular phase (n=9) or luteal phase (n=9) were exposed to 50 ppm toluene (190 mg/m^3) for 3 hours. No statistically significant effects were observed on the individual LH (luteinising hormone) and FSH (follicle stimulating hormone) levels of women exposed to toluene. Only subtle effects on LH secretion in women during the luteal phase were found. The clinical relevance of this finding is unclear.

Xiao *et al*[115,116] examined the effect of short- and long-term exposure to benzene, toluene and xylene on the semen quality and the function of accessory glands of occupationally exposed men. The mean concentration of benzene, toluene and xylene in the air was determined (103, 43 and 8.2, mg/m^3, respectively). 24 exposed workers and 37 controls were included in the study and donated blood and semen samples. The mean sperm activity (p=0.01) and acrosin activity (p=0.001) in the exposed was statistically significantly reduced. Xylene, toluene, and benzene concentrations were detected only in the blood and semen of workers, but were not detected in the controls. The associations with individual solvents were not studied.

Lemasters *et al*[32] studied the sperm production, structure and function in 50 male workers (metal workers, painters, jet fuel workers and flight line crew) exposed to solvents and fuel and 8 unexposed men. Exposure was determined by industrial hygiene sampling and expired breath sampling. Exposure measures as total solvent was not significantly related to any change in semen parameters. When jobgroup was a surrogate for exposure, several outcome parameters were significantly changed. The painters group showed a significant decline in sperm motility (19.5%) at 30 weeks of exposure and the flight line workers demonstrated a significant increase in sperm concentration (33%). No associations between sperm parameters and individual solvents were studied.

De Celis *et al*[118] examined the effects of exposure to hydrocarbons on semen quality in 48 men at a rubber factory exposed at the time of investigation to concentrations xylene, ethylbenzene, toluene and benzene of respectively ~50 mg/m^3, ~230 mg/m^3, ~200 mg/m^3 and ~40 mg/m^3. The number of exposed persons with ejaculates with normal characteristics was 17% compared with 76% in the control group consisting of 42 male office workers from the same company. In addition, more abnormalities were found in the semen of the exposed group. These included alterations in viscosity (odds ratio 4.0; 95% CI 1.5-10.6), liquefaction capacity (odds ratio 4.0; 95% CI 1.5-11.4), sperm count (odds ratio 14.3; 95% CI 3.6-78.7), sperm motility (odds ratio 9.7; 95% CI 3.1-32.9), and the proportion of sperm with normal morphology (odds ratio 27.8).

Hanaoka *et al*[119] determined the urinary bisphenol A and plasma hormone concentration in 42 male workers exposed to bisphenol A diglycidyl ether (BADGE) and mixed organic solvents (amongst others toluene). The urinary metabolites of organic solvents were found more frequently in the epoxy resin workers compared with the controls. Different concentrations of follicle stimulating hormone (FSH) were found in the epoxy sprayers (5.3 mIU/ml) and the controls (7.6 mIU/ml). FSH showed a mild correlation with urinary bisphenol A,

but not with metabolites of organic solvents. No correlations were found between other hormones and urinary metabolites.

Tielemans *et al*[2] studied the association between abnormal semen parameters and occupational exposure to amongst others solvents. In a case control study, semen parameters were studied of male partners of couples with fertility problems. Exposure was assessed using job-specific questionnaires, a job exposure matrix and measurements of metabolites of solvents in urine. A statistically significant association was found for aromatic solvents and reduced semen quality. Urine metabolites of solvents (including hippuric acid a metabolite of toluene) were more often detected in the cases than in the controls. The authors reported a high odds ration of 6.8 (95% CI 0.8-58.4) for urine metabolites of toluene and/or xylene and abnormal semen parameters. However, this study was underpowered as a result of the small number of cases.

5.3.2 *Effects on fertility in experimental animals*

Summary of the data from the report of the Committee Compounds toxic to reproduction (2001)

Ono *et al*[124] found no effects on mating and fertility of male and female Sprague Dawley rats at dose levels which induce general toxicity. In Ono *et al*[125] effects on male fertility (decreased number of epidydimal sperm cells, sperm motility, sperm quality and in vitro sperm penetration) were only observed at dose levels at which general toxicity was observed. In the study of Yamada *et al*[126], no effects of toluene exposure (the concentration of toluene was not measured but the animals were kept in an inhalation chamber until anaesthesia was achieved after about 4-6 min twice a day for 7 days) on testis weight, epididymis and accessory reproductive organs were observed. Also the number of spermatozoa was not affected after exposure to toluene. Nylen *et al*[127] observed no effects on testes, accessory glands and hormone levels. However, the data presented do not indicate whether toluene, at the dose level used, induced general toxic effects.

Data since 2001

Dalgaard *et al*[128] performed two studies. In the first, pregnant Wistar rats were exposed to 1200 ppm toluene by inhalation 6 hours per day from gestational day 7 to postnatal day 18. Sperm analysis was performed in the adult male offspring at postnatal day 110. Toluene exposure did not affect the sperm quality. In the second study, pregnant Wistar rats were exposed to 1800 ppm from GD 7 to GD

20, and the male offspring were killed at PND 11, 21, 90. Absolute and relative testes weight was reduced in all three age groups, although not statistically significant. Histopathological examinations did not reveal any differences. In no studies, clinical signs of toxicity were observed in the dams during the exposure period.

Nakai *et al*[129] treated male Sprague Dawley rats subcutaneously with 50 mg/kg bw and 100 mg/kg bw toluene (in olive oil) once a day for 10 days. Controls were injected with the same amount of olive oil. Low dose of toluene exposure had little effects on body and tissue weight, but high dose toluene treatment decreased the body, heart and prostate weights. Furthermore, the number of sperm was significantly lower in the high dose toluene group than in the controls group. The testes of the high dose toluene group showed moderately degenerating spermatogonia.

In a two generation test, Roberts *et al*[130] evaluated the reproduction toxicity of toluene exposure in male and female Sprague Dawley rats, exposed by inhalation to 0, 100, 500, and 2000 ppm toluene. Exposure (6 h/day, 5 days) of the parental and first generation started 80 days premating to 15 days of mating and pregnant females were exposed from gestation day1 to 20 and lactation day 5 to 21. Toluene did not induce adverse effects on fertility (male and female fertility index), and reproductive performance in male and females of the parental and first generation.

5.3.3 *Effects on fertility: Conclusions*

Four studies evaluated the effects of female exposure to toluene on fertility.[37,92,117,122] No association was found between female exposure to toluene and effects on endocrine hormones (LH, FSH)[117,122]. In a population based case-control study, Sallmén *et al* found no association between exposure to toluene and prolonged time to pregnancy.[92] Cho *et al*[37] studied the association between exposure to toluene and oligomenorrhoea. No statistically significant association was found for exposure to toluene and oligomenorrhoea. Data from experimental animals did not reveal effects on female fertility as well. In conclusion, the committee is of the opinion that there are no indications for an association between exposure to toluene and effects on female fertility.

Exposure to aromatic solvents (including toluene) affected several sperm parameters. [2,32,115,118] In a cohort of occupationally exposed men, Xiao *et al*[115] observed a statistically significant association between aromatic solvent exposure and sperm vitality, sperm activity and acrosin activity. Moreover, in a cross sectional study

by Lemasters *et al*[32], a statistically significant association was observed between job exposure (painters) and sperm parameters. Limited data were available concerning the association between toluene (or hippuric acid, a metabolite) and sperm parameters.[2] Data from experimental animals did not show any effects of exposure to toluene on sperm parameters, or only in the presence of general toxicity.

In conclusion, the committee is of the opinion that the available data indicate an association between organic solvents (including toluene) in general and sperm parameters. However, the effects on sperm parameters could not be attributed to toluene, due to the limited power of the study of Tielemans *et al*.[2] Therefore, the committee is of the opinion that the data concerning associations with toluene are insufficient. Consequently, the committee is unable to draw any conclusions about the effects of paternal exposure to toluene on male fertility.

5.4 Development of the progeny

5.4.1 Effects on human development

Sixteen studies, 3 cohort studies[42,43,44] nine (nested) case-control studies[60,80,81,107,131,132,133,134,135] and four cross-sectional or descriptive studies[3,136,137,138] describe the effects of inhalation exposure to toluene on the development of the progeny. Eight of these studies met the selection criteria of the committee.[43,44,60,80,81,131,135,137] The other studies were rejected for various reasons amongst others: no correction for confounders[132,139] selection bias not excluded[3,132,133,136,139], insufficient exposure data[132,136,139], no definition of a hypothesis[139] and preliminary results.[107]

The effect of exposure to solvents on spontaneous abortion was studied by several authors.[42,60,80,81,135,137]

Lindbohm *et al*[80] performed a population based nested case control among women monitored for organic solvents. Data on medically diagnosed spontaneous abortion has been retrieved from the Finish Registry of Congenital Malformations. The (adjusted) odds ratio (OR) of spontaneous abortion for solvent exposure was 2.2 (95% CI 1.2-4.1). No statistically significant association has been found by linking spontaneous abortion to individual solvents (for toluene the OR was 1.6 (95% CI 0.7-3.8)). Furthermore, the odds ratio for spontaneous abortion was increased in toluene exposed shoe workers (OR 9.3, 95% CI 1.0-84.7). The shoe workers were exposed to concentration of toluene ranging from 1-33 ppm, but they were also exposed to acetone and hexane.

Ng *et al*[137] determined the rates for (self reported) spontaneous abortion in a cross-sectional study among 309 women in an audio speaker industry. The mean exposure to toluene in the final bond process was 88 (50-150) ppm. Other production workers were exposed to levels of 0-25 ppm. The OR for the association between spontaneous abortion and high exposure to toluene was 4.8 (95% CI 1.0-22.9, versus low exposure) or 2.8 (95% CI 1.3-5.9, versus no exposure).

Xu *et al*[42] studied the association between spontaneous abortion and occupational exposure to solvents among female workers in a petrochemical industry in Beijing, China. The mean concentration of benzene, toluene, styrene and xylene varied between the different workshops but were quite low, 0.86, 0.40, 0.50, 0.03 ppm respectively. Exposure to petrochemicals was statistically significantly associated with an increased risk for spontaneous abortion, OR 2.7 (95% CI 1.8-3.9). In analyses for exposure to specific chemicals, an increased risk for spontaneous abortion was found with exposure to most chemicals, but did not reach significance.

Taskinen *et al*[135] conducted a register based study on spontaneous abortion among female workers in eight pharmaceutical factories. The odds ratio for spontaneous abortion was neither statistically significantly associated with the cases exposed to toluene (OR 1.6, 95% CI 0.6-4.5) nor with cases related to other individual compounds (except for methylene chloride). On the other hand, the odds ratio was significantly increased among those exposed to four or more solvents, ie 3.5 (95% CI 1.0-12.4).

In 1989, Taskinen *et al* investigated the spontaneous abortion and congenital malformations among the wives of men occupational exposed to toluene and other organic solvents.[81] The risk for spontaneous abortion after high and frequent paternal exposure to toluene was significantly increased (OR 2.3, 95% CI 1.1-4.7). Exposure to organic solvents in general or to miscellaneous solvents significantly increased to risk for spontaneous abortion as well. The number of congenital anomalies was too small to draw any conclusions.

Finally, the risk for spontaneous abortion was examined in a nested case-control study among women working in laboratories by Taskinen *et al*.[60] Significant associations with spontaneous abortion were found for (self-reported) exposure to toluene 3 to 5 times a week (OR 4.7, 95% CI 1.4-15.9). Exposure to toluene 1 or 2 times per week did not increase the risk for spontaneous abortion. In a second study, Taskinen[60] studied the effect of exposure to solvent on congenital malformation and birth weight. No associations were found in this study.

The effects on birth weight after exposure to (toluene containing) solvents among pregnant women in a Chinese petrochemical industry was investigated by

Chen *et al*.[44] Exposure was assessed in a three-step procedure (by industrial hygienist, and by exposure measurements, by interview). The exposure levels of toluene, styrene, xylene and benzene were all below the OEL established by OSHA*. Exposure to toluene containing solvents (except for benzene) was not significantly associated with changed birth weight. Associations with individual solvents were not studied (except for benzene). Birth weight was negatively associated with exposure to benzene; this effect was more pronounced in the presence of work stress.

The effects on birth weight after exposure to (toluene containing) solvents in a Chinese petrochemical industry was investigated by Ha *et al*[43] as well. The exposure to aromatic solvents (toluene, styrene, benzene or xylene) of 1222 female employees who had a live birth, was assessed in three steps (industrial hygienist, exposure measurements and by interview). In addition, the exposure of the father was assessed as well. No data were available concerning associations between exposure to individual compounds and the birth weight. Maternal exposure to solvents reduced the birth weight of the progeny with 82 gram (95% CI –106 to –3.1). No effects on birth weight were observed after exposure of the father.

Shu *et al*[134] evaluated the effect of occupational exposure to hydrocarbons and the risk of acute lymphocytic leukaemia (ALL) in offspring. Parental exposure data were collected by interview. No significant association was found between exposure anytime to toluene and ALL (OR 1.0 (95% CI 0.8-1.4)). Maternal exposure to 'solvents' and 'paints or thinners' during the preconception period (OR 1.8 (95% CI 1.3-2.5) and 1.6 (95% CI 1.2-2.2) respectively) and during pregnancy (OR 1.6 (95% CI 1.1-2.3) and 1.7 (95% CI 1.2-2.3) respectively) were related to an increased risk for ALL.

Infante-Rivard *et al*[131], evaluated the effect of maternal exposure to solvents on childhood leukemia in a population based case-control study. An increased risk for childhood leukaemia after exposure to toluene (starting two years before pregnancy up to birth) was found, OR 1.9 (95% CI 1.01-3.5). In addition, an increased risk for childhood leukaemia was also found after exposure to mononuclear aromatic hydrocarbons, OR 1.6 (95% CI 1.1-2.4).

* OSHA: Occupational Safety and Health Association (USA).

5.4.2 *Effect on development in experimental animals*

Summary of the data from the report of the Committee Compounds toxic to reproduction (2001)

Studies with experimental animals (rats, mice, rabbits and Hamsters) exposure to toluene caused skeletal retardation, extra ribs, decreased foetal/pup weight and/ or foetal mortality at or near dose levels which caused maternal toxicity as well.[140,141,142,143,144,145,146,147-149,150,151,152,153]

Data since 2001

Dalgaard *et al*[128] performed two studies. In the first, pregnant Wistar rats were exposed to 1200 ppm toluene by inhalation 6 hours per day from gestational day 7 to postnatal day 18. In the second study, pregnant Wistar rats were exposed to 1800 ppm from GD 7 to GD 20, and the male offspring were killed at PND 11, 21, 90. In non of these studies clinical signs of toxicity were observed in the dams during the exposure period. No statistically significant differences were found between the toluene-exposed en control groups concerning the number of neonatal deaths, sex distribution, external malformations, postimplantations and litter size. However, prenatal exposure to 1800 ppm toluene did increase neuronal apoptosis in the cerebellum of weaned male rats.

In a two generation test, Roberts *et al*[130] evaluated the reproduction toxicity of toluene exposure in male and female Sprague Dawley rats, exposed by inhalation to 0, 100, 500, and 2,000 ppm toluene (0, 375, 1,875 and 7,500 mg/m^3). Exposure (6 h/day, 5 days) of the parental and first generation started 80 days premating to 15 days of mating and pregnant females were exposed from gestation day 1 to 20 and lactation day 5 to 21. Toluene did inhibit growth in the F1 and F2 offspring in the 2,000 ppm groups (both sexes treated or female treated). At lactation day 21, groups mean body weight of the 2,000 ppm treated group was decreased from 41.1 gram (control males) and 40.4 gram (control females) to 33.0 and 31.6 gram respectively.

5.4.3 *Effects on development: Conclusions*

An association between maternal exposure to toluene and spontaneous abortion was found in several studies.[60,137] A cross sectional study of Ng *et al*[137], showed a statistically significant association between maternal exposure to toluene and

spontaneous abortion. In a nested case-control among women in laboratories of Taskinen *et al*[60], a statistically significant association between exposure to toluene and spontaneous abortion was observed as well. In both studies the effect seemed to be dose related. No significant effect on spontaneous abortion after exposure to toluene was observed in the study of Lindbohm *et al*[80], Xu *et al*[42] and Taskinen[135]. However, in the study of Lindbohm *et al*[80], a positive association was found for toluene exposed shoe workers and spontaneous abortion. An association for exposure to solvents and spontaneous abortion was found by Lindbohm *et al*[80], Xu *et al*[42] and Taskinen *et al*[135]. In conclusion, the committee is of the opinion that the available data indicate an association between maternal exposure to toluene and spontaneous abortion. This effect was not confirmed in experimental animals at dose levels which cause no maternal toxicity.

In a population based case-control study, paternal exposure to toluene was statistically significantly associated with increased spontaneous abortion as well.[81] Therefore, the committee is of the opinion that the available data give weak indications for an association between paternal exposure to toluene and spontaneous abortion.

Furthermore, no association was observed between maternal exposure to toluene and malformations, as the number of malformations was too small for drawing any conclusions.[60] In experimental animals, exposure to toluene caused skeletal malformations and extra ribs at or near dose levels which cause maternal toxicity. Therefore, the committee concludes that the available data are insufficient and preclude an assessment for malformations.

With respect to an association between exposure to toluene and birth weight, two studies are available. Chen *et al*[44] found no association for maternal exposure to (toluene containing) solvents and birth weight. Association with individual compounds were not determined. Ha *et al*[43] found a statistically significantly reduced the birth weight of the progeny after maternal exposure to solvents. Associations for individual compounds and birth weight were not determined. No effects on birth weight were observed after exposure of the father to solvents.

In conclusion, the committee is of the opinion that the available human data are insufficient. In addition, animal data show a reduced birth weight at or near dose levels which caused maternal toxicity. Therefore, the committee concludes that insufficient data are available and therefore preclude an assessment of the effects of exposure to toluene on birth weight.

Finally, maternal exposure to solvents is associated with childhood leukaemia.[131,134] This association was also found for maternal exposure to toluene

and leukaemia.[131] No association (with acute leukaemia) was found after paternal exposure to toluene.[134] Therefore, the committee is of the opinion that the available data give weak indications for an association between maternal exposure to toluene and childhood leukaemia. Furthermore, the committee concludes for paternal exposure that the available data on the effects of exposure to toluene are insufficient. Therefore, the committee is unable to draw any conclusions about these effects.

Summarizing, with respect to effects on development, the committee is of the opinion that the available data give indications for an association between maternal exposure to toluene and spontaneous abortion and give weak indications for an association between maternal exposure to toluene and childhood leukemia.

In addition, the committee is of the opinion that the available data give weak indications for an association between paternal exposure to toluene and spontaneous abortion.

Finally, the committee is of the opinion that there are insufficient data concerning the effects of exposure to toluene on other developmental effects (decreased birth weight (maternal and paternal exposure), malformations (maternal and paternal exposure) and childhood leukaemia (paternal exposure)). Therefore, the committee is unable to draw any conclusions concerning these effects.

Chapter 6

Data on individual chemicals: Xylene

6.1 Introduction

Xylene (CAS No. 1330-20-7) is an aromatic hydrocarbon (C_8H10) occurring in three isomers: *ortho*- (CAS No. 95-47-6), *meta*- (CAS No. 108-38-3), and *para*-xylene (CAS No. 106-42-3). Industrial grade xylene consists of 40-60% *m*-xylene, with ethylbenzene, *o*-xylene, and *p*-xylene each accounting for a further 10-20%.[154]

Mixed xylene is primarily (ca.89%) used in extraction and chemical processing (viz. breakdown into its components) and further as a solvent (ca. 11%) and a gasoline component (ca. 1%). The most important isomer is *p*-xylene (ca. 69%) followed by *o*-xylene (ca. 25%); ethylbenzene accounting, among others, for the remaining 6% (1995 data for Western Europe). The xylene isomers are oxidised into the corresponding phthalic acids, which are used as precursors for polyesters.[154]

In the Netherlands, the Dutch Expert Committee on Occupational Standards (DECOS) recommended an health based occupational exposure limit for xylene of 210 mg/m^3 (50 ppm) as an eight hour time weighted average. This exposure limit was based on preventing effects on the central nervous system (i.e. critical effect). In addition, a short term exposure limit (STEL), ie an occupational exposure limit as a fifteen-minutes time weighted average, of 442 mg/m^3 (100 ppm) has been recommended.[237]

In 2001, the Committee for Compounds Toxic to Reproduction could not classify xylene with respect to effects on fertility due to a lack of appropriate data. For developmental effects, the committee recommended to classify xylene in Category 3 (*substances with cause concern for humans owing to possible developmental effects*) and to label xylene with R 63 (*possible risk of harm to the unborn child*).[155]

6.2 Occupational exposure

In occupational settings, exposure to xylenes alone is rare. There is usually simultaneous exposure to other compounds, often organic solvents.[154,156]

High exposure levels were found in histological laboratories. Levels (8-hour time-weighted averages) of about 11 to over 300 mg/m^3 (2.5 to over 70 ppm) were reported and in a hospital laboratory up to 1740 mg/m^3 (400 ppm). Exposure levels for one work shift in various kinds of painting shops are generally below 5 ppm (22 mg/m^3). In printing shops and in chemical plants, brief exposures of up to 100-200 ppm (400-900 mg/m^3) may occur, while levels up to 7,000 ppm (30,000 mg/m^3) have been reported around installation of flooring.[154,156]

6.3 Fertility

6.3.1 Effects on human fertility

Nine studies, one cohort study[115,116] (describing the same study), one case-control study[92], five cross-sectional studies[32,37,80,119,122], and one experimental intervention study[118] describe the effects of inhalation exposure to xylene on male or female fertility. All the studies met the selection criteria of the committee.

Female fertility

Cho *et al*[37] studied the effects of exposure to organic solvents on the menstrual cycle length in a cross-sectional study among 1408 petrochemical workers in China. Exposure was assessed by an industrial hygienist. Besides the effects of xylene, also the consequences of exposure to benzene, toluene, styrene and combinations were evaluated. Exposure to ‘all aromatic solvent’ was associated with oligomenorrhoea (adjusted OR 1.8 (95% CI 1.1 to 2.8) and exposure to xylene was associated with oligomenorrhoea as well (OR 1.6 (95% CI 1.0-2.5)).

Reutman *et al*[122] assessed the potential reproductive endocrine effects of exposure to low levels of aromatic and aliphatic hydrocarbons among female personnel of the US air force industry in a cross sectional study. The exposure was quantified by measuring the (exhaled) breath levels of several compounds (xylene, toluene, ethylbenzene, benzene, etc). No effects of (self-reported) exposure to xylene were found on endocrine levels. Pre-ovulatory LH levels were significantly lower among women with high breath aliphatic hydrocarbon levels and aromatic hydrocarbon levels.

Sallmén *et al*[92] investigated time-to-pregnancy in a nested case control study among 197 women who had been monitored for exposure to specific organic solvents (styrene, xylene, toluene, trichloroethylene, tetrachloroethylene and 1,1,1-trichloroethane) in the past. Exposure assessment was based on classification of the reported jobs and on biological measurements. 105 women (53%) were exposed during pregnancy, 46 of them were classified as high exposed. No statistically significant association was found for high exposure to xylene and time to pregnancy (10 cases; IDR 0.9 95% CI 0.5-1.8).

Male fertility

Xiao *et al*[115,116] examined the effect of short and long-term exposure to benzene, toluene and xylene on the semen quality and the function of accessory glands of occupationally exposed men. The mean concentration of benzene, toluene and xylene in the air was determined (103, 43 and 8.2 mg/m^3 respectively). 24 Exposed workers and 37 controls were included in the study and donated blood and semen samples. The mean sperm activity and acrosin activity in the exposed men was reduced. Benzene, toluene and xylene concentrations were detected only in the blood and semen of exposed workers, but were not detected in the controls.

Lemasters *et al*[32] studied the sperm production, structure and function in 50 male workers (metal workers, painters, jet fuel workers and flight line crew) exposed to solvents and fuel and 8 unexposed men. Exposure was determined by industrial hygiene sampling and expired breath sampling. Exposure measures as total solvent was not significantly related to any change in semen parameters. When job group was a surrogate for exposure, several outcome parameters were significantly changed. The painters group (exposed to solvents containing benzene) showed a significant decline in sperm motility (19.5%) 30 weeks after the start of exposure and the flight line workers (exposed to solvents containing benzene) demonstrated a significant increase in sperm concentration (33%). Internal dose measures, however, did not show a statistically significant association.

Hanaoka *et al*[119] determined the urinary bisphenol A and plasma hormone concentration in 42 male workers exposed to bisphenol A diglycidyl ether (BADGE) and mixed organic solvents (amongst others xylene). The urinary metabolites of organic solvents were found more frequently in the epoxy resin workers compared with the controls. Different concentrations of follicle stimulating hormone (FSH) was found in the epoxy sprayers (5.3 mIU/ml) and the controls (7.6 mIU/ml). FSH showed a mild correlation with urinary bisphenol A, but not with metabolites of organic solvents. No correlations were found between other hormones and urinary metabolites.

De Celis *et al*[118] examined the effects of exposure to hydrocarbons on semen quality in 48 men at a rubber factory exposed at the time of investigation to concentrations of ca. 50 mg/m^3 of xylene, of ca. 230 mg/m^3 of ethylbenzene, of ca. 200 mg/m^3 of toluene, and of ca. 40 mg/m^3 benzene. The number of exposed persons with ejaculates with normal characteristics was 17% compared with 76% in the control group consisting of 42 male office workers from the same company. In addition, more abnormalities were found in the semen of the exposed group. These included alterations in viscosity (odds ratio 4.0; 95% CI 1.5-10.6), liquefaction capacity (odds ratio 4.0; 95% CI 1.5-11.4), sperm count (odds ratio 14.3; 95% CI 3.6-78.7), sperm motility (odds ratio 9.7; 95% CI 3.1-32.9), and the proportion of sperm with normal morphology (odds ratio 27.8).

6.3.2 *Effects on fertility in experimental animals*

Summary of the data from the report of the Committee for Compounds Toxic to Reproduction (2001)

Yamada *et al*[126] reported decreases in weights of testes and accessory reproductive organs and in plasma testosterone levels, prostate acid phosphatase activity, and epididymal spermatozoa number in male Wistar rats. Animals were exposed to “xylene” (composition and concentration not specified) by inhalation twice a day for 7 days until anaesthesia occurred (after about 10 minutes). On day 7 when they were sacrificed body weights were decreased.

Biodynamics (1983) exposed male and female Sprague-Dawley rats to concentrations of mixed xylenes of 0-500 ppm (0-2,210 mg/m^3), 6 hours/day, during a 131-day pre-mating and a 20-day mating period.[157] Additionally, exposed males from the high-concentration group were mated with unexposed females and vice versa. There were no treatment related effects on fertility end points. Apart from increases in body weights of the female animals of the low- and high-concentration groups, no effects indicative of parental toxicity were reported.

Nylen *et al*[127] did not find effects on testes, accessory glands, and circulating male hormone levels or fertility in Sprague-Dawley rats exposed to concentrations of mixed xylenes of 1,000 ppm (4,420 mg/m^3), 18 hours/day, 7 days/week, for 61 days. Treatment did not cause mortality or body weight changes.

Data since 2001

No additional data on effects on fertility in experimental animals were found.

6.3.3 *Effects on fertility: Conclusions*

Three studies described the effects of exposure to solvents, among which xylene, on female fertility.[37,92,122] In a cross-sectional study among female petrochemical workers in China, who were exposed to low levels of xylene in combination with numerous other solvents, a statistically significant association was found between exposure to aromatic solvents and oligomenorrhoea and between exposure to xylene and oligomenorrhoea.[37] However, the relevance of this effect for female fertility is not clear. Another cross-sectional study among female personnel in the US air force industry exposed to several solvents, showed no association between xylene exposure and endocrine hormone levels (LH, FSH).[122] In a population based case-control study of Sallmén *et al*[92], no association between prolonged time to pregnancy and exposure to xylene were found.

Experimental studies in rats did not reveal effects on female fertility end points.

In conclusion, the committee is of the opinion that there are insufficient data and therefore the committee is unable to draw conclusions concerning the effects of exposure to xylene on female fertility.

With respect to the effect of exposure on male fertility, four studies are available.[32,115,118,119] Exposure to aromatic solvents (including xylene) affected several sperm parameters.[32,115,118] In a cohort of occupationally exposed men, Xiao[115] observed a statistically significant association between aromatic solvent exposure and sperm activity and acrosin activity. Moreover, in a cross sectional study by Lemasters[32], an statistically significant association was observed between job exposure (painters) and sperm parameters. No data were available concerning the association between xylene and sperm parameters. Experimental studies in rats did not reveal effects on male fertility end points.

In conclusion, the committee is of the opinion that the available data indicate an association between organic solvents (including xylene) in general and sperm

parameters. However, the effects on sperm parameters could not be attributed to xylene. In conclusion, the committee is of the opinion that there are insufficient data concerning associations with individual solvents and therefore the committee is unable to draw conclusions concerning the effects of exposure to xylene on male fertility.

6.4 Development of the progeny

6.4.1 Effects on human development

Twelve studies, four cohort studies[43,44,84,89] and eight (nested) case-control studies [60,81,107,132,133,135,158] describe the effects of inhalation exposure to xylene on the development of the progeny. Nine of these studies met the selection criteria of the committee.[43,44,60,80,81,84,89,135,158] The study of Kurppa *et al*[107] was rejected for various reasons amongst others: no correction for confounders, selection bias not excluded, insufficient exposure data, or preliminary results.

The effect of exposure to solvents on spontaneous abortion was studied by several authors.[42,60,80,81,84,89,135,158]

Lindbohm *et al*[80] performed a population based nested case-control study among women ever monitored for organic solvents. Data on medically diagnosed spontaneous abortion has been retrieved from the Finish Registry of Congenital Malformations. Spontaneous abortion was significantly associated with solvent exposure (adjusted OR 2.2 (95% CI 1.2-4.1)). No significant association has been found by linking spontaneous abortion to individual solvents (for xylene the OR was 1.3; 95% CI 0.4-4.5; based on 5 cases vs. 7 controls).

Taskinen *et al*[135] conducted a register-based study on spontaneous abortion among female workers in eight pharmaceutical factories. Spontaneous abortion was neither statistically significantly associated with the cases exposed to xylene (OR 2.0; 95% CI 0.4-10.6) nor with cases related to other individual compounds (except for methylene chloride). On the other hand, the odds ratio was significantly increased among those exposed to four or more solvents, i.e. 3.5 (95% CI 1.0-12.4).

The risk for spontaneous abortion was examined among women working in laboratories by Taskinen *et al*[60] Significant associations with spontaneous abortion were found for exposure to xylene 3 to 5 times a week (OR 3.1; 95% CI 1.3-7.5; $p<0.05$). Exposure to xylene 1 or 2 times per week did not increase the risk for spontaneous abortion.

Windham *et al*[158] conducted a large study of 626 cases of spontaneous abortion (by week 20) and 1,300 controls at the US west coast. The cases were retrieved from hospital records. Based on the exposure information reported in the interviews occupation codes were assigned by experienced coders. Maternal exposure to xylene during pregnancy resulted in a crude odds ratio for spontaneous abortion of 1.6 (95% CI 0.7-3.8; based on 9 cases vs. 12 controls). When aromatic solvents were considered together (n = 37), the adjusted odds ratio was 1.2 (95% CI 0.6-2.4)

In a retrospective study, Swan *et al*[84] examined the risk of spontaneous abortion among 891 women working in semiconductor manufacturing in the western USA. Women were usually exposed to multiple chemicals. Chemicals were divided into four functionally similar subgroups. One of these, the photoresist and developer solvents (PDS), consisted of ethylene-based and propylene-based glycol ethers, xylene and n-butyl acetate. When each compound was considered singly, xylene, ethylene-based glycol ethers and n-butyl acetate were among the compounds that appeared to be associated with spontaneous abortion. Unadjusted relative risks for xylene were 1.0 (95% CI 0.6-1.8), 2.0 (95% CI 1.0-3.7), and 2.7 (95% CI 1.5-4.8) for women with low, intermediate and high exposure, respectively. The adjusted relative risks were 1.4 (0.9-2.3) for all women exposed to xylene and 2.3 (95% CI 1.4-3.6) for women with intermediate and high exposure. Exposure to these three individual agents was difficult to be separated because of the high degree of overlap: all women exposed to n-butyl acetate were also exposed to xylene; all but 12 women exposed to xylene were exposed to n-butyl acetate; most women exposed to xylene and n-butyl acetate were exposed to ethylene-based glycol ethers. According to Swan *et al*. the associations between spontaneous abortion and xylene (or n-butyl acetate) may reflect concurrent exposure to ethylene-based glycol ethers.[84] Among the few women exposed to xylene and/or n-butyl acetate and not to ethylene-based glycol ethers, the spontaneous abortion rate was only 4% (1/23); it was also low (8%) for the few women (n=13) exposed to ethylene-based glycol ethers and not to xylene or n-butyl acetate.

In a similar study in two semiconductors manufacturing plants in eastern USA, Correa *et al*[89] found that potential exposure to mixtures containing ethylene glycol ethers was associated with increased risks of spontaneous abortion. In addressing the question whether the associations noted were specific to ethylene glycol ethers, Correa *et al*[89] stated (without showing data) that in the absence of ethylene glycol ethers-based mixtures the risks of spontaneous abortion were not significantly associated with exposure to, amongst others, xylene or n-butyl acetate.

In 1989, Taskinen *et al* investigated the spontaneous abortion and congenital malformations among the wives of men occupationally exposed to xylene and other organic solvents.[81] There was no increased risk for spontaneous abortion after high and frequent paternal exposure to xylene (OR 1.6; 95% CI 0.8-3.2). On the other hand, exposure to organic solvents (high and frequent exposure) in general or to miscellaneous solvents (high and frequent exposure) statistically significantly increased the risk for spontaneous abortion (OR 2.6; 95% CI 1.2-5.9 and OR 2.1; 95% CI 1.1-3.9 respectively). The number of congenital anomalies was too small to draw any conclusions.

Chen *et al*[44] studied the association between birth weight and prenatal exposure to benzene and other solvents (among which xylene) in the Yanshan petrochemical cooperation in Beijing China (BYPC). The exposure was assessed by an industrial hygienist and the exposure levels of xylene, toluene, styrene, and benzene were all below the OEL established by OSHA. Exposure to xylene containing solvents (except for benzene) was not significantly associated with changed birth weight. Associations with individual solvents were not studied (except for benzene). Birth weight was negatively associated with exposure to benzene; this effect was more pronounced in the presence of work stress.

The effects on birth weight after exposure to (xylene containing) solvents in a Chinese petrochemical industry was investigated by Ha *et al*[43] as well. The exposure to aromatic solvents (toluene, styrene, benzene or xylene) of 1222 female employees who had a live birth, was assessed in three steps (industrial hygienist, exposure measurements and by interview). In addition, the exposure of the father was assessed as well. No data were available concerning associations between exposure to individual compounds and the birth weight. Maternal exposure to solvents reduced the birth weight of the progeny with 82 gram (95% CI –106 to –3.1). No effects on birth weight were observed after exposure of the father.

6.4.2 *Effects on development in experimental animals*

Summary of the data from the report of the Committee for Compounds Toxic to Reproduction (2001)

Hudak *et al*[140] observed foetal skeletal effects but no maternal toxicity in CFY rats exposed to concentrations of mixed xylenes of 1000 mg/m^3 (230 ppm), 24 hours/day, from gestational day 9-14. However, these effects were thought to be caused by the higher number of foetuses per litter in the exposed group (14.30 vs. 11.25 in the control group).

Ungváry *et al*[159] exposed CFY rats to concentrations of 0, 150, 1,500, or 3,000 mg/m^3 (0, 35, 345, 690 ppm) of either *o*-, *m*-, or *p*-xylene, 24 hours/day on gestational days 7-14. They reported developmental effects (foetal loss, decreased foetal weight, skeletal retardation, increased incidence of extra ribs) at maternally toxic concentrations.

In CFY rats, foetal weights were decreased after exposure to 3,000 mg/m^3 (690 ppm) *p*-xylene, 24 hours/day on gestational day 9 and 10, but not after exposure on gestational day 9. No data on maternal toxicity were presented.[159]

Biodynamics (1983) exposed male and female Sprague-Dawley rats to concentrations of mixed xylenes of 0-500 ppm (0-2,210 mg/m^3), 6 hours/day, during a 131-day pre-mating and a 20-day mating period, during gestation and during lactation days 5 to 20.[157] Apart from a decrease in body weight in pups from parents both exposed to 500 ppm, no effects on developmental end points were seen in any of the groups. No data on maternal toxicity were presented.

Mirkova *et al*[160] exposed Wistar rats to concentrations of mixed xylenes of 0-500 mg/m^3 (0-115 ppm), 6 hours/day, 5 days/week, on gestational days 1-21. Several developmental end points were affected. However, the relevance of these results were questioned since all groups, including the controls, showed very high incidences of haemorrhages in the thoracic and abdominal cavities, indicating poor health condition of the rats.

Ungváry and Tátrai[143] exposed CFY rats to concentrations of mixed xylenes of 0-3400 mg/m^3 (0-780 ppm), 24 hours/day, on gestational days 7-15, New Zealand White rabbits to concentrations of 0, 500, or 1000 mg/m^3 (0, 115, 230 ppm) of *p*-xylene or of mixed xylenes and of 0 and 500 mg/m^3 (0, 115 ppm) of *o*- or *m*-xylene, 24 hours/day on gestational days 7-20, and CFLP mice to concentrations of 0 and 500 mg/m^3 of *o*-, *m*-, or *p*-xylene and of 0, 500, and 1,000 mg/m^3 of mixed xylenes, 3x4 hours/day on gestational days 6-15. Treatment induced developmental effects but generally at maternally toxic concentrations.

Rosen *et al*[161] did not observe effects on developmental end points, including neurobehaviour, in Sprague-Dawley rats exposed to concentrations of 0, 3,500, and 7,000 mg/m^3 (0, 805, 1610 ppm), 6 hours/day on gestational days 7-16. The high concentration caused reduced maternal weight gain.

Hass *et al*[162-164] observed neurobehavioural effects in the offspring of female Wistar rats exposed to concentrations of mixed xylenes as low as 200 and 500 ppm (884, 2,210 mg/m^3), 6 hours/day on gestational days 4 or 7 to 20.

Saillenfait *et al*[165] examined the developmental effects of *o*-, *m*-, and *p*-xylene (purity of each isomer: ≥99.5% and technical xylene (composition: 21.3% *o*-xylene, 43.9% *m*-xylene, 19.4% *p*-xylene, 15.3% ethylbenzene) in pregnant Sprague-Dawley rats. Animals (n=20-26/group) were exposed to target concentrations of either of these agents of 0, 100, 500, 1,000, or 2,000 ppm (0, 442, 2,210, 4,420, 8,840 mg/m^3), 6 hours/day, on gestational days 6-20. Food consumption was recorded for the gestational days 6-13 and 13-21. Dams were weighed on gestational days 0, 6, 13, and 21, and body weight changes were calculated for the periods gestational days 0-6, 6-13, and 13-21. On gestational day 21, females were killed. The uteri were removed and weighed, and the number of corpora lutea, implantation sites, resorptions, and dead and live foetuses were recorded. Live foetuses were weighed, sexed, and examined for external anomalies including those of the oral cavity. For statistical analysis of foetal variables, the litter was used as a basis.

For all agents tested, decreased maternal weight gain was seen at 1,000 and 2,000 ppm. There also were decreases in corrected weight gain (i.e., body weight gain during gestational days 6-21 minus gravid uterine weight) and food consumption in the groups exposed to 1,000 and 2,000 ppm of the isomers and in the group exposed to 2,000 ppm of technical xylene. No statistically significant changes were found in any of the treated groups with respect to the number of corpora lutea, implantation sites, resorptions, and dead and live foetuses, to the sex ratio, and to the incidence of malformations and external and visceral variations. All agents caused dose-related, statistically significant decreases in foetal body weights at 1,000 and 2,000 ppm, while *o*- and *p*-xylene also induced statistically significant increases in the mean percentage of foetuses per litter with skeletal variations at 2,000 ppm. At 500 ppm, in the absence of maternal toxicity, slightly decreased foetal body weights per litter were seen for *o*-xylene (5.49±0.33 g vs. 5.76±0.31 in controls; $p<0.05$) and technical xylene (5.60±0.27 vs. 5.83±0.29).

6.4.3 Effect on development: Conclusions

Seven studies described the effects of exposure to solvents (including xylene) on spontaneous abortion.[60,80,81,84,89,135,158] No statistically significant association was determined in (population based or industry based) case-control studies for maternal exposure to xylene and spontaneous abortion.[80,89,135,158] In a case-control study among female laboratory workers, there was an increased risk for sponta-

neous abortion and female workers who (self)reported to handle xylene 3 to 5 times a week (but not 1 or 2 times).[60] Furthermore, the studies of Lindbohm *et al*[80] and Taskinen *et al*[135], showed a statistically significant association for spontaneous abortion and exposure to solvents.

In two cohort studies that concerned women in semiconductor industry[84,89], an increased risk for spontaneous abortion was found in one study for women with intermediate and high exposure to xylene. However, the aetiological role of other compounds to which these women were concomitantly exposed could not be ruled out. In the other study, there was no association between spontaneous abortion and xylene exposure.

Finally, a statistically significant association between spontaneous abortion and male exposure to solvents has been found by Taskinen *et al*.[81] This statistically significant association was not observed for exposure to xylene.

In conclusion, the committee is of the opinion that the available data indicate an association between maternal exposure to organic solvents (including xylene) and spontaneous abortion. However, in these studies the increased incidence of spontaneous abortion could not be attributed to xylene. The nested case-control study of Taskinen *et al*[60] among women exposed to solvents in laboratories on the other hand, showed an association with xylene. Therefore, the committee is of the opinion that the available data give weak indications for an association between maternal exposure to xylene and spontaneous abortion. In rats no effects on foetal loss was observed at dose levels that caused no maternal toxicity.

In one population based case-control study, paternal exposure to xylene was not associated with increased spontaneous abortion.[81] No other data were available. The committee is of the opinion that the available data on paternal exposure to xylene and spontaneous abortion is too limited and therefore the committee is unable to draw any conclusions about these effects.

With respect to an association between exposure to xylene and birth weight, two studies are available.[43,44] Chen *et al*[44] found no association for maternal exposure to (xylene containing) solvents and birth weight. Association with individual compounds were not determined. Ha *et al*[43] found a statistically significantly reduced the birth weight of the progeny after maternal exposure to solvents. Associations for individual compounds and birth weight were not determined. No effects on birth weight were observed after exposure to solvents of the father.

In conclusion, the committee is of the opinion that the available human data on the effects of maternal and paternal exposure to xylene on birth weight are insufficient. In addition, animal data show a reduced birth weight at or near dose levels that caused maternal toxicity. The committee concludes that insufficient

data are available and therefore the committee is unable to draw any conclusions concerning the effects of (maternal and paternal) exposure to xylene on birth weight.

No human data are available with respect to the effects of exposure to xylene on congenital malformations and (childhood) malignancies. Therefore, the committee is unable to draw any conclusions regarding these effects.

Summarizing, with respect to effects on development, the committee is of the opinion that the available data indicate a weak association between maternal exposure to xylene and spontaneous abortion. Furthermore, the committee is of the opinion that the available data after paternal exposure to xylene on spontaneous abortion are insufficient and therefore the committee is not able to draw any conclusions regarding the effects of paternal exposure to xylene.

Data on other developmental effects (decreased birth weight, malformations and malignancies) are insufficient as well and therefore the committee is unable to draw any conclusions concerning these effects of (maternal and paternal) exposure to xylene.

Chapter 7

Data on individual chemicals: Benzene

7.1 Introduction

Benzene (CAS nr 71-43-2), an aromatic hydrocarbon (C_6H_6), is an important industrial chemical, a petroleum by-product, a component of unleaded gasoline.

In the Netherlands, the occupational exposure limit (MAC-Value) for benzene was 3.25 mg/m^3 (1 ppm) as an eight hour time weighted average. No short term exposure limit (STEL), ie an occupational exposure limit as a fifteen-minute time weighted average, has been established.

In the Netherlands, the Dutch Expert Committee on Occupational Standards (DECOS) concluded that occupational exposure to benzene causes leukaemia.[238] The Health Council concluded in 1997 that benzene is a genotoxic carcinogen with an unusual genotoxic profile. Therefore, the committee calculated that 40 year exposure to a concentration of 3.25 mg/m^3 (1 ppm) for benzene as an eight hour time weighted average corresponds to four additional death cases (as a result of leukaemia) per 1000 deaths.[239]

In 2003, the Commission Working Group on the Classification and Labelling of Dangerous Substances of the European Union has recommended in a draft proposal not to classify benzene for its effects on fertility and development.

Furthermore, benzene is a carcinogenic solvent classified as a group 1 substance* according to IARC**.

* Group 1: The agent is *carcinogenic to humans*.

7.2 Occupational exposure

Certain jobs, such as gasoline station workers, fire-fighters, and dry cleaners, are believed to put people at a higher risk of benzene exposure. It was estimated that workers in the area of crude petroleum and natural gas are exposed to 0.13 mg/m^3 (0.04 ppm) benzene, while workers in petroleum refining, gas stations, and crude petroleum pipelines are exposed to 0.22, 0.12 and 0.25 ppm (0.7, 0.4, 0.8 mg/m^3) benzene, respectively. Fire-fighters are exposed to an average of 0.38 ppm (1.2 mg/m^3) benzene.[166]

Workers from four different dry cleaning facilities in Korea had mean benzene air concentrations ranging from 2.7 to 3.2 ppb (8.8-10.4 µg/m^3). Their exposure to benzene was dependent upon the type of solvent used for cleaning.[167]

Concawe* measured benzene concentrations (8 hour twa) in the air of refineries in the period 1993-1998 and 1999-2000. Mean concentrations varied between 0.3 mg/m^3 and 5.8 mg/m^3. Furthermore, in the petroleum industry, mean benzene concentrations (8 hour twa) varied between 0.1 mg/m^3 and 1.3 mg/m^3. In the automobile industry concentrations were comparable.[168,169]

7.3 Fertility

7.3.1 Effects on human fertility

Five studies, 2 cohort studies[49,115,116] and 3 cross-sectional studies[32,37,122] describe the effects of inhalatory exposure to benzene on male or female fertility. All studies met the selection criteria of the committee.

Female fertility

Cho *et al*[37] studied the menstrual cycle length in a cross-sectional study among 1408 petrochemical workers in China. Exposure was assessed by an industrial hygienist. Besides the effects of benzene, also the consequences of exposure to toluene, styrene, xylene and combinations were evaluated. Exposure to 'all aromatic solvent' was associated with an (adjusted) OR for oligomenorrhoea of 1.8

** IARC: International Research on Cancer.
* CONCAWE: CONservation of Clean Air and Water in Europe, The oil companies' European association for environment, health and safety in refining and distribution.

(95% CI 1.1 to 2.8). Exposure to benzene did not increase oligomenorrhoea statistically significantly (OR of 1.4 (95% CI 0.9-2.0).

Wennborg *et al*[49] studied the effects of exposure to solvents on time to pregnancy (TTP) in a cohort study among female personnel in biomedical laboratories. Exposure information was gathered using a self administered questionnaire. Acetone, benzene, chloroform, diethylether and phenol were the most commonly used solvents. Exposure to benzene was not statistically significantly associated with the fecundability ratio (FR ie time to pregnancy in exposed versus control workers), 0.8 (CI 0.4 to 1.3). Exposure to 'solvents in general' or acetone on the other hand, was significantly associated with a lower fecundability ratio, 0.8 (95% CI 0.7-0.9) and 0.7 (95% CI 0.5-0.97), respectively.

Reutman *et al*[122] assessed the potential reproductive endocrine effects of exposure to low levels of aromatic and aliphatic hydrocarbons among female personnel of the US air force industry. The exposure was quantified by measuring the (exhaled) breath levels of several compounds (benzene, toluene, ethylbenzene, xylene etc). No effects of (self-reported) exposure to benzene were found on endocrine levels. Preovulatory LH levels were significantly lower among women with high breath aliphatic hydrocarbon levels and aromatic hydrocarbon levels.

Male fertility

Xiao *et al*[115,116] examined the effect of short and long-term exposure to benzene, toluene and xylene on the semen quality and the function of accessory gonad of occupationally exposed men. The mean concentration of benzene, toluene and xylene in the air was determined (103, 43 and 8.2 mg/m^3 respectively). Twenty four exposed workers and 37 controls were included in the study and donated blood and semen samples. The mean sperm activity in the exposed men was statistically significantly reduced from 3.17(+/- 0.75) to 2.52 (+/- 0.96). Acrosin activity was statistically significantly reduced in the exposed men from 30.74 U/L (+/-10.05) to 18.02 U/L (+/- 7.24). Benzene, toluene and xylene concentrations were detected only in the blood and semen of exposed workers, but were not detected in the controls.

Lemasters *et al*[32] studied the sperm production, structure and function in 50 male workers (metal workers, painters, jet fuel workers and flight line crew) exposed to solvents and fuel and 8 unexposed men. Exposure was determined by industrial hygiene sampling and expired breath sampling. Exposure measures as total solvent was not significantly related to any change in semen parameters. When job group was a surrogate for exposure, several outcome parameters were

significantly changed. The painters group (exposed to solvents containing benzene) showed a significant decline in sperm motility (19.5%), 30 weeks after the start of exposure and the flight line workers (exposed to solvents containing benzene) demonstrated a significant increase in sperm concentration (33%). Internal dose measures, however, did not show a statistically significant association.

7.3.2 *Effects on fertility in experimental animals*

Summary of the data from the report of the Committee Compounds toxic to reproduction

The Health Council of the Netherlands has not evaluated the reproduction toxic effects after exposure to benzene.

Animal data

In CFY rats exposed to benzene (125 ppm, 406 mg/m^3) for 24 hours per day from gestation day 7 through 14, no effect on implantation number was observed.[170]

The American Petroleum Industry and the Chemical Manufacturers Association performed an inhalation study in which female Wistar rats were exposed to 1, 10, 30, 300 ppm benzene (6 h/day, 5 days/week) (3, 30, 96, 960 mg/m^3) during premating and mating (10 weeks) gestation and lactation up to postnatal day 21.[171] No effects on fertility (percentage pregnant animals, mean gestational length) were observed. General toxicity (maternal body weight(gain) was absent as well.

No other data of sufficient quality are available.

7.3.3 *Effects on fertility: Conclusions*

Three studies evaluated the effects of female exposure of solvents (including benzene) on fertility.[37,49,122] Cho *et al*[37] found a statistically significant association between exposure to solvents and oligomenorrhoea. No association was observed for benzene and oligomenorrhoea. In a cross-sectional study among female US airforce workers exposed to solvents, Reutman *et al*[122] did not observe a statistically significant association between exposure to benzene and endocrine hormones. Preovulatory LH levels were lowered due to exposure to aliphatic and aromatic hydrocarbons. Wennborg[49], did not observe an association between benzene and time to pregnancy in a cohort of female laboratory workers exposed to solvents. On the other hand time to pregnancy was statistically significantly pro-

longed after exposure to solvents. In conclusion, the committee is of the opinion that the available data indicate an association between organic solvents (including benzene) in general and female fertility. However, the effects could not be attributed to benzene. The committee is of the opinion that the data concerning associations with benzene are insufficient. Therefore, the committee is unable to draw any conclusions concerning the effects exposure to benzene on female fertility.

Exposure to aromatic solvents (including benzene) affected several sperm parameters.[32,115] In a cohort of occupationally exposed men, a significant association was observed between aromatic solvent exposure and sperm activity and acrosin activity.[115] Moreover, in a cross sectional study, an association was observed between job exposure (painters) and sperm parameters.[32] No data were available concerning the effects of exposure to benzene alone on sperm parameters. No data on the effect of exposure to benzene on male fertility were available.

In conclusion, the committee is of the opinion that the available data indicate an association between organic solvents (including benzene) in general and sperm parameters. However, the effects on sperm parameters could not be attributed to benzene. The committee is of the opinion that the data concerning associations with benzene are insufficient. Therefore, the committee is unable to draw any conclusions concerning the effect of exposure to benzene on male fertility.

7.4 Development of the progeny

7.4.1 Effects on human development

Twelve studies, 5 cohort studies[41,42,43,44,56],7 (nested) case-control studies [28,60,107,134,135,172,173] describe the effects of inhalatory exposure to benzene on the development of the progeny. Eight of these studies met the selection criteria of the committee.[41,42,43,44,56,60,134,135] The other studies were rejected for various reasons amongst others: Insufficient exposure data[172], preliminary results[107], selection bias was not sufficiently excluded[28], and no definition of a hypothesis[173].

Stucker *et al*[41] performed a cohort study on the risk of spontaneous abortion among wives of 823 male workers occupationally exposed to predominantly benzene. Information on their pregnancy was obtained using a questionnaire. Occupational physicians estimated the exposure to benzene in every function (none (0 ppm), low (1-5 ppm) and moderate (>5 ppm)). The frequency of sponta-

neous abortion was not significantly higher in the exposed groups (at any time) than in the non-exposed groups. The authors concluded that paternal exposure to benzene did not increase the risk of spontaneous abortion.

Xu *et al*[42] studied the association between spontaneous abortion and occupational exposure to solvents in a cohort of female workers in the petrochemical industry in Beijing, China. The mean concentration of benzene, toluene, styrene and xylene varied between the different workshops but were quite low, 0.86, 0.40, 0.50, 0.03 ppm respectively. Exposure to petrochemicals was statistically significantly associated with an increased risk for spontaneous abortion, OR 2.7 (95% CI 1.8-3.9). In analyses for exposure to specific chemicals, an increased risk for spontaneous abortion was found with exposure to most chemicals, and reached significance for benzene (OR 2.5 (95% CI 1.7-3.7)), gasoline and hydrogen sulphide.

Taskinen *et al*[135] conducted a register based study on spontaneous abortion among female workers in eight pharmaceutical factories. No statistically significant association was found for spontaneous abortion with the cases exposed to benzene (OR 2.4 (95% CI 0.5-12.0) nor with cases related to other individual compounds (except for methylene chloride). On the other hand, the odds ratio was significantly increased among those exposed to four or more solvents, ie 3.5 (95% CI 1.0-12.4).

Finally, the risk for spontaneous abortion was examined among women working in laboratories by Taskinen *et al*.[60] No significant associations with spontaneous abortion were found for exposure to benzene (OR 0.8, 95% CI 0.4-1.7). In a second study, Taskinen *et al*[60] studied the effect of exposure to solvent on congenital malformation and birth weight. No associations were found in this study.

In addition, Chen *et al*[44] studied the association between birth weight and prenatal exposure to benzene (in combination with occupational stress) in the Yanshan petrochemical cooperation in Beijing China (BYPC). 792 pregnant workers in a petrochemical industry (Beijing Yanshan Petrochemical Corporation (BYPC)) were followed up through delivery. Exposure to benzene and other solvents were assessed by an industrial hygienist based on job title and workplace information. Birth weight was not statistically significantly associated with exposure to benzene, (-15 gram (95% CI –82 to +52). Adjusted mean birth weight was 3,445 g (95% CI 3,401-3,489) for the non exposed workers, 3,430 g for those with exposure to benzene only, 3,426 g for those with work stress only and 3,262 g (95% CI 3,156 to 3,369) for those with both exposures. The authors concluded that low level of exposure to benzene and work stress interacts to reduce birth weight. A

statistically significant association was observed between birth weight and exposure to benzene in combination to stress (-149 gram, 95% CI -296 to -1).

The association between exposure to solvents (including benzene) and birth weight was examined in a Chinese petrochemical corporation by Ha *et al*.[43] The exposure to aromatic solvents (toluene, styrene, benzene or xylene) of 1,222 female employees, who had a live birth, was assessed by a trained interviewer. In addition, the exposure of the father was assessed as well. No data were available concerning the effect of exposure to individual compounds on the birth weight. Maternal exposure to solvents reduced the birth weight of the progeny with 82 gram (95% CI –106 to –3.1). No effects on birth weight were observed after exposure of the father.

Finally, Wennborg *et al*[56] investigated the major congenital and neural crest malformations in the offspring of a cohort of laboratory personnel. Exposure information was gathered with questionnaires. No increased risk for major congenital malformations in relation with laboratory work was found. Exposure to solvents before the third trimester of pregnancy was associated with major malformations (OR 2.5 (95% CI 1.0-6.0). Current exposure to benzene was significantly associated with neural crest malformations (OR 5.3 (95% CI 1.4-21.1)). However, the results are potentially biased by dependent outcomes and should be interpreted cautiously.

Shu *et al*[134] evaluated the effect of parental occupational exposure to hydrocarbons and the risk of acute lymphocytic leukaemia (ALL) in offspring. Parental exposure data were collected by interview. No significant association was found between exposure anytime to benzene and ALL (OR 1.2 (95% CI 0.8-1.6)). Maternal exposure to ‘solvents’ and ‘paints or thinners’ during the preconception period (OR 1.8 (95% CI 1.3-2.5) and 1.6 (95% CI 1.2-2.2) respectively) and during pregnancy (OR 1.6 (95% CI 1.1-2.3) and 1.7 (95% CI 1.2-2.3) respectively) were related to an increased risk for ALL.

7.4.2 *Effect on development in experimental animals*

Summary of the data from the report of the Committee Compounds toxic to reproduction (2001)

The Health Council of the Netherlands has not evaluated the reproduction toxic effects after exposure to benzene.

Tatrai *et al*[170] did not find significant skeletal malformations in pups of CFY rats continuously exposed (24 h/day) to benzene air concentrations of 150, 450, 1,500, or 3,000 mg/m³ (equivalent to about 50, 150, 500, or 1,000 ppm) during day 7 to day 14 of gestation. Decreased mean foetal body weights, signs of skeletal retardation (i.e. delayed ossification) were observed at all dose levels. At the three higher concentration levels significant post-implantation foetal loss was revealed and an increasing trend in the incidence of skeletal variants. However, at all dose levels maternal toxicity (decreased body weight gain, decreased mean placental weight) was observed as well.

In an experiment conducted by Green *et al*[174], pregnant Sprague-Dawley rats were exposed to 0, 100, 300, and 2200 ppm benzene (6 h/day) during day 6 to day 15 of gestation. Maternal weight gain was equivalent between controls and the 100 ppm and 300 ppm experimental groups. A significant depression in maternal mean body weight gain was observed in the 2200 ppm exposure group. Implantation sites/litter, live foetuses/litter, percentage absorptions/implantation sites, percentage of litters with absorptions, and number of litters totally absorbed were comparable between controls and exposed groups even at the high concentration level of 2200 ppm. Mean foetal body weight, mean foetal crown-rump length were statistically significantly reduced at the highest concentration in comparison to the control.

In a study by Murray *et al*[175], CF-1 mice were exposed (whole chamber; 7 h/day, g.d. 6-15) to either 0 or 500 ppm benzene (technical grade). Furthermore, New Zealand white rabbits were similarly exposed from days 6 through 18 of gestation to 0 or 500 ppm benzene. Exposure to 500 ppm benzene was reported to have no significant effects on the appearance of dams or does, their body weight or their body weight gain (data not given). Food and water intake (data not given) was increased for the benzene exposed rabbits but was unaffected in mice. No significant effect on the average number of live foetuses or resorptions per litter was discerned in either species. Mean foetal body weight was decreased significantly among litters of mice exposed to benzene. In rabbits, foetal body weights were not altered significantly by exposure to benzene. No malformations were observed in the offspring of mice and rabbits. However, increases in the occurrence of several minor skeletal variants including delayed ossification of stern brae, skull bones and of non-fused occipital bones of the skull were observed in litters of the benzene-exposed mice.

The American Petroleum Industry and the Chemical Manufacturers Association performed an inhalation study in which female Wistar rats were exposed to

1, 10, 30, 300 ppm benzene (6 h/day, 5 days/week) during premating and mating (10 weeks) gestation and lactation up to postnatal day 21.[171] No maternal toxicity (maternal body weight(gain) was observed, including the highest dose level. A trend towards reduced mean offspring body weights during the lactation period and reduced mean (absolute) organ weights (testes, liver and kidney) on postnatal day 21 was observed at the 30 and 300 ppm exposure level. However, these effects were not statistically significant.

7.4.3 *Effects on development: Conclusions*

The association between exposure to benzene and spontaneous abortion was studied in four studies.[41,42,60,81] In the cohort study of Stücker *et al*[41], male workers were predominantly exposed to high levels of benzene. No association was observed for benzene and spontaneous abortion in the wives of these men. Taskinen *et al*[81] found no association between paternal exposure to benzene and spontaneous abortion in a population based case control study.

In a cohort of female workers exposed to relatively low levels of a mixture of solvents in the petrochemical industry, Xu *et al*[42] found a statistically significant association between exposure to benzene and spontaneous abortion. Taskinen *et al*[60] found no association between benzene and spontaneous abortion in a nested case control study among female workers exposed to solvents.

Therefore, based on the study of Stücker *et al*[41], the committee concludes that the available data give no indications for an association between paternal exposure to benzene and spontaneous abortion. For maternal exposure to benzene, the committee concludes that the available data are conflicting. No effect was found in experimental animals at dose levels which cause no maternal toxicity. Therefore, the committee is of the opinion that there are no indications for an association between the effects of maternal exposure to benzene and spontaneous abortion.

Furthermore, two cohort studies in a petrochemical industry in Beijing in China concerning maternal exposure to solvents (including benzene) and decreased birth weight were available.[43,44] Chen *et al*[44] found no association between maternal exposure to benzene and birth weight. A statistically significant association was observed between birth weight and the combined exposure of benzene and working stress. Ha *et al*[43] found a statistically significantly reduced the birth weight of the progeny after maternal exposure to solvents. Associations for individual compounds and birth weight were not determined. No effects on birth weight were observed after exposure of the father to solvents. Animal data show

an effect of exposure to high levels of benzene on foetal birth weight, predominantly in the presence of reduced maternal weight. In conclusion, the committee is of the opinion that the available human data are insufficient. Therefore, the committee is unable to draw any conclusions concerning the effects of maternal and paternal exposure to benzene on birth weight.

Two human studies were available concerning the effect of exposure to benzene on major congenital and neural crest malformations and the risk on acute lymphocytic leukaemia.[56,176] In the cohort study of Wennborg among female laboratory personnel exposed to solvents, exposure to benzene was statistically significantly associated with neural crest malformations. The nested case control study of Shu *et al*[134] showed no association between benzene and acute lymphocytic leukaemia. The committee is of the opinion that the available data are insufficient. Therefore, the committee is unable to draw any conclusions concerning the effects of maternal and paternal exposure to benzene on (childhood) malignancies.

Summarizing, with respect to effects on development, the committee is of the opinion that the available human data (a cohort study) concerning the effects of maternal exposure to benzene and spontaneous abortion are conflicting. Futhermore, there are insufficient human data on other developmental effects (birth weight, malformations, childhood malignancies and neurobehavioral effects) and maternal exposure to benzene. On the other hand, the available data in experimental animals show no indications for effects on development after maternal exposure. Therefore, the committee concludes that there are no indications for an association between maternal exposure to benzene and the developmental effects.

In addition, the committee is of the opinion that the data on the effects after paternal exposure to benzene give no indications for an association with spontaneous abortion. Furthermore, no data are available concerning the other developmental effects after paternal exposure to toluene. Therefore, the committee cannot draw any conclusions concerning these other developmental effects.

Chapter 8

Data on individual chemicals: Acetone

8.1 Introduction

Acetone (CAS nr 67-64-1) is an aliphatic ketone (C_3H_6O) which is, amongst others, used as a solvent. It is used as raw material in the chemical synthesis of many commercial products. Acetone is also endogenously produced in the human body.

In the Netherlands, the occupational exposure limit (MAC-Value) for acetone was 1,210 mg/m^3 (502 ppm) as an eight hour time weighted average. The short term exposure limit (STEL), ie an occupational exposure limit as a fifteen-minute time weighted average, has been established to be 2420 mg/m^3 (1004 ppm).

The Committee on Compounds toxic to reproduction of the Health Council of the Netherlands has not evaluated the effects on fertility and development after exposure to acetone.

8.2 Occupational exposure

Occupational exposure occurs in industries that manufacture or used acetone. Furthermore, professional painters and commercial and household cleaners and laboratory workers are also likely to be exposed to acetone. For example, the concentration of acetone in the breathing zone air of a paint factory, a plastics factory and a synthetic fibre factory in Italy were > 3.5 mg/m^3.[177] The inhalation

exposure for workers to acetone in a shoe factory in Finland ranged from 25.4 to 393.4 mg/m^3.[178]

8.3 Fertility

8.3.1 Effects on human fertility

Two studies, one cohort study[49], and one cross-sectional study[19] describe the effects of inhalatory exposure to acetone on fertility. Of these, one study[49] met the criteria for selection. The study of Jelnes[19] was rejected because selection bias could not be excluded and the exposure data was insufficient.

Female fertility

Wennborg *et al*[49] studied the effects of exposure to solvents on time to pregnancy (TTP) in a cohort study among female personnel in biomedical laboratories. Exposure information was gathered using a self administered questionnaire. Acetone, benzene, chloroform, diethylether and phenol were the most commonly used solvents. Exposure to acetone was statistically significantly associated with the fecundability ratio (FR ie time to pregnancy in exposed versus control workers), 0.7 (95% CI 0.5 to 0.97). Exposure to 'solvents in general' was statistically significantly associated with a lower fecundability ratio, 0.8 (95% CI 0.7-0.9) as well.

8.3.2 Effects on fertility in experimental animals

No effects were observed on the fertility of male Wistar rats treated for 6 weeks with drinking water containing acetone at 1.1 mg/kg/day.[179] Number of successful matings with untreated females, number of pregnancies, number of foetuses, testicular weight, seminiferous tubule diameter, and testicular lesions were investigated.

Male Sprague-Dawley rats treated with 3.4 mg/kg bw/day acetone in drinking water for 13 weeks had significantly increased ($p<0.01$) relative testis weight, probably because body weight was reduced, and significantly decreased sperm motility, caudal weight and epididymal weight, and increased incidences of abnormal sperm.[180] No testicular lesions were observed upon histological examination. Vaginal cytology examinations of the female rats revealed no effects. No effects on sperm morphology and vaginal cytology were observed in

mice similarly treated with drinking water containing acetone at doses <4,858 mg/kg/day in males and <11,298 mg/kg/day in females.

8.3.3 *Effects on fertility: Conclusions*

In one cohort study in biomedical laboratories with exposure to several solvents, female exposure to acetone was statistically significantly associated with a prolonged time to pregnancy among female personnel.[49] However, the association between 'solvents in general' and prolonged time to pregnancy was comparable in the same study. Therefore the committee questions whether the association with acetone is specific. Effects on male fertility in Sprague Dawley rats were only observed in the presence of general toxicity.

In conclusion, the committee is of the opinion that the available data are insufficient, Therefore, the committee is unable to draw any conclusions regarding the effects of exposure to acetone on male and female fertility.

8.4 Development of the progeny

8.4.1 *Effects on human development*

Three studies, one cohort study[53], and two (nested) case control studies[60,172] describe the effects of inhalation of solvents containing acetone on development. Of these, two studies[53,60] met the criteria for selection. One study[172] was rejected due to the absence of correction for confounders.

The risk for spontaneous abortion was examined among women working in laboratories by Taskinen *et al*.[60] Exposure information was self-reported and to several organic solvents, amongst others toluene, xylene, benzene and acetone etc. No statistically significant association was found for exposure to acetone and spontaneous abortion (OR 1.4, 95% CI 0.4-4.7). In a second study, Taskinen *et al*[60] studied the effect of exposure to solvent on congenital malformation and birth weight. No associations were found in this study.

Pregnancy outcome was studied in a cohort among personnel employed in laboratories at the University of Gothenburg.[53] 782 women were send a questionnaire (92% response) and information on the women was also gathered from the Medical Birth Register in Sweden. No statistically significant effect on spontaneous abortion was observed in women exposed to 'solvents' (RR 1.3 (95% CI 0.9-1.9)). Furthermore, exposure to acetone did not induce the rate of spontaneous abortion.

8.4.2 *Effect on development in experimental animals*

In 1988, the National Toxicology Program (NTP) performed an inhalation developmental study in Sprague Dawley rats and Swiss (CD-1) mice. Presumed pregnant rats were exposed to 0, 440, 2,200 or 11,000 ppm (0, 1,056, 5,280, 26,400 mg/m^3) and mice to 0, 440, 2,200 and 6,600 ppm (0, 1,056, 5,280 and 15,840 mg/m^3), 6 hours per day, 7 days per week. Positively mated animals were exposed on day 6-17 of gestation (mice) or day 6-19 of gestation (rats). No reproductive effects (effects on number of implants per litter, percent of live pups per litter or mean percent resorption per litter) were observed in rats or mice in an inhalation developmental study.[181]

8.4.3 *Effects on development: Conclusions*

Two studies are available concerning the effects of maternal exposure to solvents (containing acetone) and development (spontaneous abortion, congenital malformation and birth weight).[53,60] No association was found for maternal exposure to acetone and spontaneous abortion in both studies. In addition, an NTP-study in rats and mice gave no indications for effects on the development of the progeny. Therefore, the committee is of the opinion that the available data give no indications for an association between maternal exposure to acetone and effects on development after exposure to acetone.

Furthermore, no data are available on the effects on development after paternal exposure to acetone. Therefore the committee is unable to draw any conclusions regarding the effects of paternal exposure to acetone on development.

Chapter 9

Data on individual chemicals: Tetrachloroethylene (PER)

9.1 Introduction

Tetrachloroethylene or PER (C_2Cl_4; CAS nr 127-18-4) is a chlorinated hydrocarbon solvent. The major uses of PER are in dry cleaning of textiles, in metal cleaning or as a chemical intermediate.[182] Minor uses of PER include processing and finishing in the textile industry, as an extraction solvent, in paint removers, printing inks, adhesive formulations, paper coatings, leather treatments, as a carrier solvent for silicones, and as a pesticide.[65,183] The IPCS document mentions a decreasing PER consumption within Europe, from 230 kilotonnes in 1990 to about 80 kilo tonnes in 2004. In 1990, 49% of the production volume in the EU was used for dry cleaning, 26% as a chemical intermediate and 21% in metal cleaning.[184]

In 2003, the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council, recommended a health-based occupational exposure limit (HBROEL) for tetrachloroethylene of 138 mg/m^3 (20 ppm) as an 8 hour time weighted average (TWA), and 250 mg/m^3 (36 ppm) as a 15 min TWA (STEL). DECOS considered neurotoxicity to be the critical effect of PER.[66]

In 2003, the Committee for compounds toxic to reproduction of the Health Council recommended not to classify PER for effects on fertility due to lack of sufficient human data and sufficient animal data showing that no classification is needed. For developmental effects, the committee recommended to classify PER in category 3 based on the animal data (*substances which cause concern for*

humans owing to possible developmental effects) and to label PER with R63 (*possible risk of harm to the unborn child*).[190]

9.2 Occupational exposure

The majority of the data on occupational exposure to PER, concerns dry-cleaning shops (see chapter 3). Machine operators experience the highest exposures.

Occupational exposure data on other uses of PER were scarce. Von Grote *et al*[185] reviewed the changes in occupational exposure to PER (and trichloroethylene) in metal degreasing in Germany. The authors calculated emission concentrations of different types of degreasing machines. Especially with the introduction of closed-loop non-vented machines in the 1990s calculated-near-field concentrations decreased to about 20 mg/m^3. In the 1980s when open-top machines were in use in Germany, near-field concentrations could have been as high as 1,000 mg/m^3. The calculations were in good agreement with measured data. The authors also estimated a large decrease in the number of workers exposed, from more than 25,000 workers in 1985 to less than 3,000 in 1996. In the DECOS document[186] the following breathing zone air levels of PER were measured: up to 269 mg/m^3 in metal industries and 110 mg/m^3 in offset printing facilities. In metal cleaning, chemical industry or offset printing there is always co-exposure to a variety of other compounds next to PER.

Kauppinen *et al*[187] described a large international database of exposure data in the pulp, paper and paper product industries (> 30,000 measurements from 13 countries). This database included a small number of 60 measurements of PER. The actual concentrations were not given but the authors mentioned that 30% of these measurements exceeded the TLV (170 mg/m^3, 8 h TWA).

In summary, in two major applications of PER exposure concentrations have decreased since the 1970s and 1980s. Recent data from dry cleaning shops[67,68,69] indicate that average PER concentrations hardly ever exceed 50 mg/m^3. In metal degreasing not only concentrations decreased to about 20 mg/m^3, but also the number of workers exposed has diminished considerably.[185]

9.3 Fertility

9.3.1 Effects on human fertility

The six studies describing effects on fertility included two cohort[70,71], two (nested) case-control[92,72] and two cross-sectional studies[73,188]. The two papers by

Eskenazi *et al*[70,71], describing the same population of dry cleaners and laundry workers, and the study by Sallmén *et al*[92] met the selection criteria of the committee. The other studies were rejected for different reasons: poor documentation, not specifically aimed at effects on reproduction, not specifically aimed at organic solvents, and self-reported exposure to chemicals.

In their first paper Eskenazi *et al*[70] investigated semen quality of 34 exposed dry-cleaners and laundry workers and 48 unexposed laundry workers. Exposure to PER was assessed using job titles and having an exposure index assigned by an expert. Also PER-levels in exhaled air were monitored. From a range of 17 sperm parameters, statistically significant effects on sperm quality of the dry cleaners were found: a higher percentage of round sperm and a lower percentage of narrow sperm, compared to laundry workers. Sperm concentrations, sperm counts, volume or percentage motility did not differ between dry-cleaners and laundry workers.

In the second paper Eskenazi *et al*[71] examined the reproductive outcomes of the female partners of the dry-cleaners (n=17) and laundry workers (n=32). No differences in number of pregnancies and standardized fertility ratios were observed between wives of dry cleaners and laundry workers. According to the authors, dry-cleaners' wives did experience a longer period before getting pregnant or consulting a doctor for a fertility problem (OR=2.5). However, the 95% confidence interval included unity (0.6-10.9) and therefore was not statistically significant.

Sallmén *et al*[92] investigated time-to-pregnancy in a population based case-control study among 197 women from a cohort that was monitored for exposure to organic solvents (a.o. tetrachloroethylene, trichloroethylene, styrene, xylene, toluene, 1,1,1-trichloroethane). Exposure assessment was based on classification of the reported jobs and on biological measurements. 105 women (53%) were exposed during pregnancy, 46 of them were classified as high exposed. The adjusted incidence density ratio (IDR) of clinically recognized pregnancies was significantly decreased for women working in dry cleaning shops (low and high exposure IDR 0.44; 95% CI 0.22-0.86). The authors remarked that the decreased fecundability may have been related to PER, but no association was found either with low or high exposure to PER (IDR 0.63; 95% CI 0.34-1.17; IDR 0.69; 95% CI 0.31-1.52).

9.3.2 *Effects on fertility in experimental animals*

The committee also used several criteria documents on PER.[65,189,190] More detailed information about the animal studies can be found in these criteria documents.

An online literature search in Toxline and Pubmed (period: January 2000 until December 2006; key words: CAS nr and (fertility or reproductive)) did not result in additional animal studies, only one *in vitr*o study was found.

Summary of the data from the report of the Committee Compounds toxic to reproduction (2003)

In mice the proportion of sperm with aberrant morphology was increased four weeks after a 5 day exposure to 500 ppm (3,445 mg/m^3). This effect was not present at the lower concentration (100 ppm; 689 mg/m^3) tested, or at 10 weeks post exposure. No effect was observed in a similar study in rats.[192]

In a two-generation study in rats, exposed from 11 weeks prior to mating up to lactation to either 100, 300 or 1,000 ppm (689, 2,067, or 6,890 mg/m^3) of PER, no effect was observed on fertility in any of the exposed groups from either generation. In the animals of the highest dose group (1,000 ppm; 6,890 mg/m^3) some clinical observations and systemic toxic effects were observed.[193]

Data since 2003

In a series of studies the effect on in vitro rat oocyte fertilizability was investigated. Prior to retrieval of the oocytes the female rats were exposed to PER either by inhalation or via drinking water during a period of two weeks. Inhalation of 1,700 ppm PER (11,713 mg/m^3, the only concentration tested) resulted in a slightly, but significantly, reduced in vitro fertilizability of the oocytes and a reduced number of penetrated sperm per oocyte. Exposure via drinking water had no effect on these parameters.[191]

9.3.3 Effects on fertility: conclusions

The human data on effects of PER on male and female fertility are sparse. Eskenazi *et al*[70] found significant differences on two out of 17 sperm parameters in dry cleaners. The relevance of more round sperm and less narrow sperm, however, is unclear. Eskenazi *et al*[71] found that the time to pregnancy of wives of male dry-cleaners was not affected. In animal studies, there was one single finding of abnormal sperm in mice that could not be reproduced in rats. In a two-generation study in rats no treatment-related effects on fertility were observed. The committee concludes that the available data are limited and is therefore unable to draw any conclusions regarding the effects of exposure to PER on male fertility.

In the epidemiological paper on female fertility by Sallmén *et al*[92], a subgroup of women working in dry-cleaning shops showed a significantly reduced fertility. However, no association was found with the level of PER exposure. Animal data on female fertility concerned a negative two-generation study in rats and a positive in vitro test, the relevance of the latter being unclear. The committee concludes that the available data is limited and is therefore unable to draw any conclusions regarding the effects of exposure to PER on female fertility.

9.4 Development of the progeny

9.4.1 Effects on human development

17 papers concerned developmental effects of exposure to PER, including one cohort[75], nine case-control[76,77,78,80,81,131,134,158,194] and seven cross-sectional studies[59,74,79,195,196,197,198]. In two of these studies exposure occurred via drinking water contamination.[195,198]

Thirteen studies[75,76,77,78,79,80,81,131,134,158,194,195,198] met the selection criteria. The large nested-case-control study by Olsen *et al*[78] combined populations from Denmark, Norway, Sweden en Finland, including those studied by Kyyrönen *et al*[76] and Ahlborg[77]. Also a partial overlap between the Kyyrönen- and Olsen-studies with those by Lindbohm *et al*[79,80] and Taskinen *et al*[81] is likely, since their cases were derived from the same Finnish registers within the same period 1973-1983. Four studies were rejected for different reasons such as: insufficient documentation of the study, methods or exposure assessment; or unclear relevance of the effect in relation to reproductive toxicity.

Spontaneous abortion is the most studied developmental effect.[75,76,77,78,79,80,81,158] An increased risk for spontaneous abortion associated with maternal exposure to PER was observed by Doyle *et al*[75], Lindbohm *et al*[199], Kyyrönen *et al*[76], Olsen *et al*[78] and Windham *et al*[158]. Except for the study by Windham *et al*[158], where it was not specified, occupational exposure had occurred in dry cleaning shops.

In the only cohort study available[75] 2,711 women working in dry cleaning units and 399 from laundries in the UK participated. Each participant reported her workplace and whether she was a dry-cleaning operator or not. Information about pregnancies and foetal loss (prior to week 20) was reported by the participants and partly verified from their medical records. The authors found no increased risk in spontaneous abortion for dry-cleaning workers compared to laundry workers. Within the dry cleaners, however, the odds ratio for ‘pregnancies completed between 1980-95’ was significantly increased for operators ver-

sus non-operators (1.6; 95% CI 1.01-2.7). The odds ratio was adjusted for maternal age, pregnancy order and year of birth.

Kyyrönen *et al*[76] studied the prevalence of spontaneous abortion and congenital malformations among female dry-cleaner and laundry workers, in a case-control study in Finland. 130 cases were selected from the Finnish hospital discharge register between 1973 and 1983 and a register of congenital malformations, and compared to 298 controls. 'High' exposure to PER was associated with a significantly increased odds ratio of 3.4 (95% CI 1.0-11.2) for spontaneous abortion. The odds ratio was adjusted for smoking, use of alcohol, working during pregnancy, temperature of the workplace > 24°C, febrile disease and nulliparity.

In a large case-control study, Olsen *et al*[78] combined the Finnish data[76] with cases from Norway, Sweden[77] and Denmark. The total numbers of cases and controls were 215 and 558, respectively. The relative risk for spontaneous abortions (n=8) in three countries (Sweden, Denmark and Finland) was increased with high maternal exposure to PER during the first trimester of pregnancy (OR adj 2.9; 95% CI 0.98-8.4). Of the separate countries only the Finnish odds ratio ('high exposure' OR 4.5; 95% CI 1.1-18.5) was significantly increased.

Lindbohm *et al*[79], performed a cross-sectional study on spontaneous abortions (between 1973-1976) and the possible association with parental occupations. 68,327 pregnancies from the Finnish hospital discharge register were studied. Information on occupation and exposures (job titles and type of workplace) were obtained from the national census records. Occupations were coded and combined in categories of exposure in cooperation with an industrial hygienist. Laundry workers* were part of the category 'solvents'. The adjusted odds ratio for spontaneous abortion, based on 416 pregnancies, was statistically significantly increased in female laundry workers (OR 1.5; 95% CI 1.1-2.0). The odds ratio for the association between maternal exposure to solvents and spontaneous abortion was 0.8 (95% CI 0.6-1.1). The (adjusted odds ratio for the association between paternal exposure to solvents and spontaneous abortion was 0.9 (95% CI 0.7-1.1).

Windham *et al*[158] conducted a large study of 626 cases of spontaneous abortion (by week 20) and 1,300 controls at the US west coast. The cases were retrieved from hospital records. Based on the exposure information reported in the interviews occupation codes were assigned by experienced coders. Maternal exposure to PER during pregnancy resulted in a crude odds ratio for spontaneous

* Though not described in the paper, the committee assumes that laundry work includes dry cleaning as well, since the authors mention a potential exposure of laundry workers to PER.

abortion of 4.7 (95% CI 1.1-21.1). This result was based on seven cases and four women were exposed to trichloroethylene as well. When halogenated solvents were considered together (n = 103) no increase in the prevalence of spontaneous abortion was observed. Exposure to PER most likely occurred in other occupations than dry cleaning, since the authors only mention one laundry and dry cleaning worker.

In contrast to the studies abovementioned, the ones by Ahlborg[77], Lindbohm *et al*[80] and Taskinen *et al*[81] did not reveal an association between spontaneous abortion and parental PER exposure.

Ahlborg[77] combined the results of two case-control studies in which the relationship between maternal PER exposure during the first trimester and spontaneous abortion was investigated. The adjusted odds ratio for the combined studies was 1.1 (95% CI 0.6-2.0). The odds ratios for 'low' and 'high' PER exposure were comparable.

Within their nested case-control study Lindbohm *et al*[80] found no association between maternal exposure to PER and spontaneous abortion (OR adj 1.4; 95% CI 0.5-4.2). Also exposure to halogenated hydrocarbons did not result in an increased risk (OR adj 1.4; 95% CI 0.6-3.2).

Taskinen *et al*[81] performed a case-control study within the same cohort as Lindbohm *et al*[80] with a focus on paternal exposure. No increases in spontaneous abortions were observed among the wives of men exposed to PER or halogenated hydrocarbons, crude odds ratios being 0.5 (95% CI 0.2-1.5) and 1.1 (95% CI 0.6-1.8) respectively.

Other developmental effects studied after parental occupational exposure to PER were: still birth or perinatal death, low birth weight, congenital malformations and childhood cancer.[76,77,78,131,134,194] None of these effects could be related to either maternal or paternal occupational exposure to PER.

Ahlborg[77] reported cases of perinatal death, congenital malformations and low birth weight. The authors did not perform an analysis of the data since the numbers were too small.

Kyyrönen *et al*[76] found that maternal exposure to 'any level' of PER had no effect on the prevalence of congenital malformations, 'handling of other solvents' (i.e. spot removers, thinner, acetone), however, led to a significant increase in the odds ratio (5.9; 95% CI 1.0-35.7).

Olsen *et al*[78] presented relative risks for the combined data on congenital malformation, still birth and low birth weight (< 1,500 g). None of the odds ratios

was significantly increased. Because the outcomes were combined, the committee considers the data not useful.

Three case-control studies investigated the relationship between parental occupational exposure and childhood cancer.[131,134,194] These studies involve large numbers of cases, however, the numbers with parental exposure to PER are small.

In their study[194] estimated the effects of parental exposure on the prevalence of neuroblastoma in their offspring. Parental exposure to halogenated hydrocarbons did not increase this risk. A separate analysis of paternal exposure to PER was negative as well.

Shu *et al*[134] studied the effect of parental exposure to PER during preconception, pregnancy, postnatally or 'anytime'. Maternal nor paternal exposure resulted in an increased risk of acute lymphocytic leukaemia in their children.

These findings were confirmed by Infante-Rivard *et al*[131] No effect of maternal exposure to PER two years before pregnancy up to pregnancy and during pregnancy on the prevalence of acute lymphoblastic leukaemia in their offspring was found.

In contrast to, the above described studies involving occupationally exposed persons, developmental effects of oral exposure to PER were observed in two studies in the general population.[195,198]

Bove *et al*[195] investigated the effects of chemical contaminants in drinking water on birth outcomes in a cross sectional study in New Jersey including 80,938 live births and 594 foetal deaths. Tap water sample data from 49 water companies were used to estimate monthly levels of a number of contaminants. The monthly exposure level of PER was estimated to be 26 ppb. Of the 13 different parameters for birth outcome only one showed an association with high level PER exposure (>10 ppb) of the mothers: oral cleft defects (OR 3.5, 90% CI 1.3-8.8). The number of cases was small (n = 4). The highest concentrations in the drinking water concerned trihalomethanes, the second highest trichloroethylene.

In a study by Sonnenfeld *et al*[198], conducted at a marine corps base in North Carolina, PER was the major drinking water contaminant (concentration range 10-1,580 ppb). The population consisted of 11,798 live-born infants. Parental exposure to PER caused a decrease in the offspring's mean birth weight (-26 g; 90% CI -43 to -9) and an increase in the prevalence of infants small-for-gestational-age (SGA) (OR 1.2; 90% CI 1.0-1.3). Older mothers (>35 y) had an odds ratio for SGA infant of 2.1 (90% CI 0.9-4.9) and maternal exposure caused a lower birth weight of the infants (mean difference -130 g, 90% CI -236, -23). Mothers with a history of more than two previous foetal losses had an odds ratio

for SGA of 2.5 (90% CI 1.5-4.3) and their infants had a lower birth weight (mean difference -104 g, 90% CI -174, -34 g). No association was found between parental PER exposure and preterm birth. The limited exposure data are a shortcoming of this study.

9.4.2 *Effects on development in experimental animals*

Summary of the data from the report of the Committee Compounds toxic to reproduction (2003)

Four animal studies on developmental effects of PER were available.[192,193,200,201]

In the two-generation study in rats, that was negative on fertility effects, exposure to the highest concentration tested (1,000 ppm; 6,890 mg/m^3) caused a reduction in the number of pups born alive, pup survival and pup weight in the first and second generation. The dams, however, showed signs of toxic effects. Reduced body weights were also observed in first generation pups that were exposed to the next lower concentration of 300 ppm (2,067 mg/m^3) of PER. Maternal toxicity at this concentration was not reported.[193]

Exposure of rats and mice during pregnancy to 300 ppm (2,067 mg/m^3) of PER (the only concentration tested) resulted in maternal toxicity. In the exposed rats the foetal resorption rate was increased. In the mice study foetal bodyweight was decreased and ossification of the skull bones and stern brae delayed.[200]

Exposure of pregnant rats to 500 ppm (3,445 mg/m^3) of PER at gestational days 0-18 or 6-18 did not result in foetotoxicity or teratogenicity. A comparable study in rabbits, exposed at gestational days 0-21 or 7-21, was negative as well.[192]

Exposure of pregnant rats to 6,201 mg/m^3 during gestation resulted in a decreased neuromuscular function in 2-wk old pups, in reduced acetylcholine and dopamine in the brains of 3-wk old pups and a higher activity in a behavioural test of pups at 4 weeks. Maternal body weight gain and food intake were decreased. These effects were not found with the lower concentration tested.[201]

Data from the IPCS document

When rats were exposed to PER by inhalation during pregnancy no effects were found on the foetuses at a concentration of 1,500 mg/m^3. At the next higher and highest concentrations, 4,500 and 8,500 mg/m^3 respectively, foetal weight was reduced, and skeletal retardation and supernumerary ribs increased. Of the exposed offspring female animals showed minimal and temporary changes in neurobehavioral tests. At both concentrations signs of maternal toxicity were

found.[202] Exposure of pregnant mice to 1,500 mg/m^3 of PER throughout the period of organogenesis reduced the number of live foetuses and increased visceral malformations. Maternal toxicity was observed as well. Exposure of pregnant rabbits to a concentration of 4,500 mg/m^3 caused maternal toxicity and an increased post implantation loss and increased number of completely reabsorbed litters.[202]

9.4.3 *Effects on development: conclusions*

There are several epidemiologic studies on developmental effects of PER, especially concerning spontaneous abortion among workers in dry cleaning shops. In dry cleaning shops in the Nordic countries PER replaced trichloroethylene in the 1960s and thus became the principle compound of occupational exposure in these workplaces. Around dry-cleaning machines concentrations exceeding 30 ppm (= 207 mg/m^3) of PER had been measured.[78] In five out of eight studies the risk of spontaneous abortion was significantly associated with maternal exposure to PER.[75,76,78,158,199] However, three of these studies[76,78,199] derived their cases from the same population which means they are not independent. Also the 'negative' study by Lindbohm *et al*[80] on spontaneous abortion was based on cases from the same cohort of solvents-exposed workers. In the studies by Kyyrönen *et al*[76] and Olsen *et al*[78] only an association with high PER exposure was found, defined as dry cleaning or spot removing for at least 1 hour a day on average, or reporting of handling PER at least once a week. In the cohort study by Doyle *et al*[75] only an increased risk for female operators in dry-cleaning shops was found. The committee considers the study by Windham *et al*[158] less informative than the dry-cleaning studies, since co-exposure to trichloroethylene occurred. Despite the shortcomings in the database, the committee concludes from the epidemiological data that there are weak indications for an association between maternal exposure to PER and spontaneous abortion.

Other developmental effects studied included stillbirth or perinatal death, low birth weight, small-for-gestational age, congenital malformations and childhood cancer.[76,77,78,131,134,194,195,198] Only in the two studies concerning the general population an association was found between high exposure to PER and oral cleft defects[195], low birth weight and small for gestational age infants[198]. These effects were not observed after occupational exposure to PER. The relevance of the findings by Bove *et al*[195] and Sonnenfeld *et al*[198] is unclear, since exposure occurred via drinking water, an oral route, and other contaminants were present as well. In conclusion, the committee considers the human data on other developmental effects as limited and therefore the committee is unable to draw any conclusions concern-

ing the association between maternal exposure to tetrachloroethylene and the other developmental effects.

In studies in rats, mice and rabbits several developmental effects have been observed, however, these were always accompanied by signs of maternal toxicity. There might be one exception, a decreased bodyweight in pups of dams exposed to 2,067 mg/m^3 in the two generation rat study.[193] In 2003, the Committee on compounds toxic to reproduction recommended to classify PER in category 3 ('substances which cause concern for humans owing to possible developmental toxic effects') based on the available animal data. No animal data were found that could provide information on a possible mechanism of action for the spontaneous abortions observed in humans.[190]

With respect to the effects on development after paternal exposure, two studies (a population based case control study of Taskinen *et al*[81] and a cross-sectional studies by Lindbohm *et al*)[80] were available. Both studies showed no effects of paternal exposure to PER on spontaneous abortion. The committee therefore concludes that the available data are too limited to draw any conclusions concerning the association between paternal exposure to tetrachloroethylene and developmental effects.

Chapter 10

Data on individual chemicals: Methylene chloride

10.1 Introduction

Methylene chloride or dichloromethane (CH_2Cl_2; CAS nr 75-09-2) is a chlorinated hydrocarbon solvent (CH_2Cl_2). Methylene chloride has a broad range of applications, like in aerosol formulations, paint strippers, and degreasing agents; in plastics processing, metal and textile treatment; in food, pharmaceutical, film and electronics industries.[203] Production of this chemical was estimated to be about 570,000 tonnes worldwide (in 1980 and 1992), and ranging from 331,500 tonnes in 1986 to 254,200 tonnes in 1991 in Western Europe. Usage of methylene chloride in Western Europe has decreased from 200,000 tonnes per year in the 1970s and 1980s to about 150,000 tonnes in 1992. In 1984 the major use (about 50%) of methylene chloride in Western Europe was in paint strippers.[203]

In 1992, the Dutch Expert Committee on Occupational Standards (DECOS) recommended a health-based occupational exposure limit (HBROEL) for methylene chloride of 350 mg/m^3 (100 ppm) as an 8 hour time weighted average (TWA), and 1,750 mg/m^3 (500 ppm) as a 15 min TWA. The 8 hour limit was based on systemic effects in the liver, whereas the 15 minute limit was based on an upper limit of 4% CO-Hb in blood of non-smokers.[240]

Methylene chloride has not been submitted to the Committee for compounds toxic to reproduction of the Health Council for classification.

10.2 Occupational exposure

The WHO-document (1996)[203] provides a list of exposure levels in various occupational situations. The data were reported during the 1980s and early 1990s from the US and several European countries (eg UK):

- production: 219-374 mg/m^3, 8 h TWA
- aerosol filling : 95-628 mg/m^3, 8 h TWA
- paint stripping: 18-1,765 mg/m^3, 8 h TWA; (UK, 1992) 25-3,810 mg/m^3, 8 h TWA
- cleaning, degreasing: 0-460 mg/m^3, 8 h TWA
- printing: 3.5-558 mg/m^3, 8 h TWA
- fibre glass manufacture: 0-6,693 mg/m^3, 8 h TWA
- film manufacturing: 180-3,442 mg/m^3, 8 h TWA
- rubber products manufacturing: 208-304 mg/m^3, 8 h TWA
- foam industry: 7-1,090 mg/m^3, 8 h TWA
- laboratory work: 71-1,370 mg/m^3, 8 h TWA
- pharmaceuticals manufacture: 0-3,749 mg/m^3, 8 h TWA.

Post *et al*[10] measured methylene chloride concentrations in different departments of a polyester factory. At several work places in the preparation department the geometric means ranged from 315 to 707 mg/m^3; in the laboratory the mean was 151 mg/m^3, as an 8 h TWA.

Vincent *et al*[204] measured occupational exposure to methylene chloride during paint stripping in the aeronautical industry. For an 8 h working day concentrations ranged from 86 to 1239.5 mg/m^3.

In a recent paper, Enander *et al*[205] reported methylene chloride concentrations ranging from 26 to 120 ppm (92-424 mg/m^3, 8 h TWA) among automotive repair technicians, measured by personal air sampling.

10.3 Fertility

10.3.1 Effects on human fertility

Only two studies evaluated the effects of exposure to methylene chloride or to solvents containing methylene chloride on human fertility, including one cohort[206] and one cross-sectional study[32].

Kersemaekers *et al*[206] compared reproductive disorders between a cohort of 9,000 female hairdressers and 9,000 clothing salesclerks. The authors distin-

guished two conception periods: 1986-1988 and 1991-1993. In the latter period the authors expected less exposure to formulations that were supposed to contain methylene chloride. Time to pregnancy was not statistically significantly prolonged in any of the periods (1986-1988: OR 1.5, 95% CI 0.8-2.8; 1991-1993: OR 1.2, 95% CI 0.8-1.6).

Lemasters *et al*[32] studied the sperm production, structure and function in 50 male aircraft maintenance workers (metal workers, painters, jet fuel workers and flight line crew) exposed to solvents and jet fuel, and 8 unexposed men. Exposure was determined by personal monitoring and expired breath sampling. Internal dose measures were not significantly related to any change in semen parameters. When job group was taken as a surrogate for exposure, several outcome parameters were significantly changed. The painters group showed a significant decline in sperm motility (19.5%) at 30 weeks of exposure and the flight line workers demonstrated a significant increase in sperm concentration (33%). According to the authors the sheet metal workers experienced the highest exposure of total solvents and fuel. The only statistically significant effect observed in this group was a decline in sperm length at 15 and 30 weeks (-2.1% and –2.9% resp). It should be noticed, however, that also the unexposed workers had a decline in sperm length at wk 15 (-2.5%). Sperm motility was decreased by 3.2% in the sheet metal workers, which was not statistically significant. Separate data on methylene chloride were not presented in this study.

10.3.2 Effects on fertility in experimental animals

Raje *et al*[207] performed a dominant lethal test in male mice, exposed to 100, 150 or 200 ppm methylene chloride (353, 529, 706 mg/m^3) for 2 hours a day, 5 days a week for 6 weeks. After each exposed male mice was mated with an adult virgin female during two weeks, no statistically significant differences were found on the fertility index.

In a two-generation study by Nitschke *et al*[208], female and male rats were exposed to 0, 100, 500, or 1,500 ppm (0, 353, 1,765, 5,295 mg/m^3) methylene chloride for 6 hours per day, 5 days per week for 14 weeks. F_0 animals were allowed to mate to produce the F_1 generation which was exposed for 17 weeks. Next the F_1 generation was allowed to mate to produce the F_2 generation. No effect was found on the fertility indices of either generation or sex.

10.3.3 *Effects on fertility: conclusions*

Epidemiologic data evaluating effects of methylene chloride on human fertility are limited. In the only study concerning female fertility, Kersemaekers *et al*[206] did not observe a prolonged time to pregnancy (>12 months) among hairdressers supposedly exposed to methylene chloride-containing products. Animal data on female fertility were restricted to the two generation study in rats by Nitschke *et al*.[208] In this study no effect of methylene chloride exposure up to a concentration of 5,295 mg/m^3 on female fertility was found. The committee concludes that the available human data are limited. The committee concludes that there are no indications for an association between exposure to methylene chloride and effects on female fertility in experimental animal studies. Therefore, the committee concludes that there are no indication for an association between female exposure to methylene chloride and female fertility.

On male fertility, an epidemiological study by Lemasters *et al*[32] is available. Though some statistically significant effects were found on sperm parameters of painters, sheet metal workers and flight line workers, these could not be attributed to methylene chloride exposure or any of the other single solvent used by these workers. Animal studies performed by Raje *et al*[207] and Nitschke *et al*[208] showed that the fertility indices of methylene chloride-exposed male rats were not affected. The committee concludes that that the available human data are limited, which preclude an assessment of the effects of occupational exposure to methylene chloride on male fertility. Based on the animal data, the committee concludes that there are no indications for an association between exposure to methylene chloride and male fertility.

10.4 Development of the progeny

10.4.1 *Effects on human development*

Seven studies evaluated the effects of exposure to methylene chloride or to solvents containing methylene chloride on human development, including two cohort[20,206], four (nested) case-control studies[60,107,135,158] and one cross-sectional study[209]. Six papers met the criteria of the committee[20,60,135,158,206,209] The paper by Kurppa *et al*[107] provided a global description of preliminary results. Only in the studies by Taskinen *et al*[135] and by Kersemaekers *et al*[206] developmental effects were found that were associated with methylene chloride[135] or with supposedly

methylene chloride-containing formulations[206]. The other studies either gave negative results on methylene chloride[60,158,209] or no separate analyses for this solvent were presented[20].

Taskinen *et al*[135] conducted a register-based study on spontaneous abortion among female workers in eight pharmaceutical factories, including 44 cases and 130 controls. Information on occupational exposure to a series of solvents was obtained by questionnaire from the factory physicians. Only for methylene chloride a significant association with spontaneous abortion was found (OR 2.3, 95% CI 1.0-5.7). Also the odds ratio among women exposed to four or more solvents was significantly increased, ie 3.5 (95% CI 1.0-12.4).

Kersemaekers *et al*[206] compared reproductive disorders between a cohort of 9,000 female hairdressers and 9,000 clothing salesclerks. The authors distinguished two conception periods: 1986-1988 and 1991-1993. In the latter period the authors expected less exposure to formulations that were supposed to contain methylene chloride. In the 1986-1988 period hairdressers experienced an increased risk of spontaneous abortion (OR 1.6, 95% CI 1.0-2.4). No effects were found on the prevalence of low birth weight infants, prematurity or major structural malformations. The odds ratio for spontaneous abortion in the 1991-1993 period was 0.9 (95% CI 0.7-1.1).

In another paper by Taskinen *et al*[60], two studies were presented concerning the risk for spontaneous abortion, low birth weight and congenital malformations among women working in laboratories. There was no association between low or high exposure to methylene chloride and spontaneous abortion (OR low 1.2; 95% CI 0.5-3.0; OR high 1.7; 95% CI 0.6-5.0). Working in a laboratory resulted in a lower birth weight (-133 g; CI -246 to -20 g) of infants. The authors did not mention whether this effect was statistically significant. In their second study, Taskinen *et al*[60] found no association between congenital malformations and solvent exposure.

Windham *et al*[158] conducted a large study of 626 cases of spontaneous abortion (by week 20) and 1,300 controls at the US west coast. The cases were retrieved from hospital records. Based on the exposure information reported by the women, occupation codes were assigned by experienced coders. No association was found between maternal occupational exposure to methylene chloride and spontaneous abortion (OR 0.9; 95% CI 0.5-1.7).

In contrast to, the above described studies involving occupationally exposed persons, Bell *et al*[209] studied birth weight in offspring of the general population of Monroe County in the US. Their exposure to methylene chloride emissions from a photographic industry was assessed by a computer generated air dispersion

model. Among a total of 91,302 births, including 3,850 low birth weight, 6,044 were classified as exposed to low, 1,795 as exposed to moderate and 1,085 as exposed to high levels of methylene chloride. The authors did not find an association between any level of methylene chloride exposure and birth weight.

10.4.2 Effects on development in experimental animals

In the DECOS document (1992) three animal studies were mentioned.[200,210,211] DECOS concluded that methylene chloride did not cause maternal, embryonal or foetal toxicity, and teratogenicity in pregnant rats and mice exposed during gestation to 1250 ppm (4,413 mg/m^3; on d 6-15 of pregnancy for 7 h/d) and 4,500 ppm (15,885 mg/m^3; before and/or during 17 d of pregnancy for 6 h/d). The effect of methylene chloride exposure (4,500 ppm, 15,885 mg/m^3) of female rats on behavioral habituation of their offspring was considered to be inconclusive by DECOS. In 1996, this conclusion was confirmed by WHO.

From the online literature search additional studies were retrieved.

In a two-generation study by Nitschke *et al*[208], female and male rats were exposed to either 0, 100, 500, or 1,500 ppm (0, 353, 1,765, 5,295 mg/m^3) methylene chloride for 6 h/d, 5 d/w for 14 weeks. F_0 animals were allowed to mate to produce the F_1 generation which was exposed for 17 weeks. Next the F_1 generation was allowed to mate to produce the F_2 generation. No effect was found on the following reproductive parameters: gestation index, gestation survival index, sex ratio on day 1, litter size, pup body weight. No exposure-related gross-pathological or histopathological changes were observed in either of the generations.

Raje *et al*[207] performed a dominant lethal test in male mice, exposed to 100, 150 or 200 ppm (353, 529, 706 mg/m^3) methylene chloride for 2 hours a day, 5 days a week for 6 weeks. After each exposed male mice was mated with an adult virgin female during two weeks, no statistically significant differences were found on number of litters; implants, live fetuses, percent dead or percent resorbed per litter; or litters with two or more resorbed fetuses.

In vitro embryotoxic/teratogenic potential of methylene chloride on developing rat embryo showed a dose-related response (solvent concentration in medium related to decreased crown-rump length, somite number, protein (µg/embryo)). According to the authors, fatal or near fatal solvent levels would be required in the industrial situation for the embryotoxic level to be reached.[212]

10.4.3 *Effects on development: conclusions*

The risk for spontaneous abortion was significantly increased in female pharmaceutical workers[135], and in female hairdressers[206]. It is unclear to what extent these results can be attributed to methylene chloride exposure, since pharmaceutical workers and hairdressers are exposed to a number of other compounds as well. An effect of methylene chloride exposure was found on spontaneous abortion among female laboratory workers.[60] No effect of methylene chloride exposure was found on spontaneous abortion among women participating in a hospital-based case-control study at the US west coast.[158], and among the general population of Monroe county, New York[209]. In animal studies no statistically significant developmental effects of maternal exposure to methylene chloride were observed. The committee is of the opinion that the available human data are conflicting and the animal data gave no indication for an association between maternal exposure to methylene chloride and developmental effects. Therefore the committee concludes that there are no indications for an association between maternal exposure to methylene chloride and developmental effects.

There are no epidemiologic data concerning developmental effects in offspring of exposed male workers. In two animal studies with rats[208] and mice[207] exposure of the males did not result in developmental effects. The committee concludes that there are no indications for an association between paternal exposure to methylene chloride and developmental effects.

Chapter 11

Data on individual chemicals: Ethylene glycol ethers

11.1 Introduction

Ethylene glycol ethers are used as solvents in oil-water compositions like in resins, lacquers, paints, varnishes, dyes, inks, printing pastes, cleaning agents, deicing additives, liquid soap, cosmetics, and as chemical intermediates (DECOS 1996).[241] According to the French National Institute of Health and Medical Research (INSERM), the use of glycol ethers increased dramatically in the 1960s with the introduction of water-based paints. They reported a world production of 900,000 tonnes in 1997, of which 40% were used in Europe.[213] Since 1994 short chain ethylene glycol ethers have been gradually replaced by less toxic long-chain ethylene glycol ethers (eg EGBE) or propylene glycol ethers due to EU regulations.[214]

In a Technical Report, ECETOC (1995) reviewed the toxicology of 35 glycol ethers, 24 based on ethylene glycol and 11 on propylene glycol. ECETOC based their selection on either commercial interest or toxicological significance.[215] From this selection the short chain ethylene glycol methyl and ethyl ethers and their acetates, as well as glycol ethers that can be metabolised into ethylene glycol methyl and ethyl ethers, are recognized reproductive toxic compounds in several animal species, causing testicular atrophy, teratogenicity and fetotoxicity. Of the propylene glycol ethers only two are known to be teratogenic, however, these are not commercially available.

Crucial in the reproduction toxicity of the ethylene glycol ethers is the metabolic oxidation by alcohol dehydrogenase resulting in the formation of alkoxy acetic acid metabolites, with methoxyacetic acid (MAA) and ethoxyacetic acid (EAA) being the most potent ones. MAA is thought to disrupt cell proliferation and differentiation by affecting energy metabolism (entering the TCA-cycle as methoxy acetyl coenzyme A) and/or the availability of small carbon units necessary for purine and pyrimidine base synthesis.[215]

In 1996, the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council, recommended the following health-based occupational exposure limits (HBROEL):[241]

- Ethylene glycol monomethyl ether or 2-methoxy ethanol (CAS 109-86-4; EGME): 1 mg/m^3 (0.3 ppm), 8 h time weighed average (TWA); skin notation
- Ethylene glycol monomethyl ether acetate or 2-methoxy ethanol acetate (CAS 110-49-6; EGMEA): 1.5 mg/m^3 (0.3 ppm), 8 h TWA; skin notation
- Diethylene glycol monomethyl ether (CAS 111-77-3; DEGME): 45 mg/m^3 (9 ppm), 8 h TWA; skin notation
- Diethylene glycol monoethyl ether (CAS 111-90-0; DEGEE): 180 mg/m^3 (32 ppm), 8 h TWA; skin notation.

For EGME, EGMEA and DEGME testicular and developmental effects, observed in animal studies, were considered to be the critical effects. The limit value for DEGEE was derived from a NOAEL for maternal and reproductive toxicity in a three generation rat study.[241] Skin notations were assigned to these glycol ethers because of the substantial contribution of dermal uptake to systemic effects, ie >10% of the total body burden after inhalation.

On January 1, 2007, a new OEL system came into force in the Netherlands, that includes a legal OEL for diethylene glycol monomethyl ether (DEGME), similar to the health-based OEL recommended by DECOS in 1996. Also for 2-butoxy ethanol (EGBE) and its acetate (EGBEA) legal OELs were introduced, corresponding with the health-based OELs recommended by the European Scientific Committee on Occupational Exposure Limitis (SCOEL) in 1996. The SCOEL considered haematologic effects to be the critical effect of EGBE and EGBEA:

- Ethylene glycol n-butyl ether or 2-butoxy ethanol (CAS 111-76-2; EGBE): 100 mg/m^3 (20 ppm), 8 h TWA; 246 mg/m^3 (50 ppm), 15 min TWA; skin notation

- Ethylene glycol n-butyl ether acetate or 2-butoxy ethyl acetate (CAS 112-07-2; EGBEA): 135 mg/m^3 (20 ppm), 8 h TWA; 333 mg/m^3 (50 ppm), 15 min TWA; skin notation.

The European SCOEL recently (2007) released a document on 2-ethoxyethanol (EGEE) and its acetate (EGEEA) and recommended a health-based OEL of 2 ppm (8 mg/m^3 and 11 mg/m^3, resp), 8 h TWA, and a skin notation, for both substances. The SCOEL considered effects on haematopoiesis and developmental toxicity as critical effects. In time, these OELs will result in legal OELs in the Netherlands.

Several glycol ethers have been classified in category 2, for substances that should be regarded as if they impair fertility in humans or as if they cause developmental toxicity in humans, either by the Committee on compounds toxic to reproduction of the Health Council and/or by the European Committee:
- R60, may impair fertility: EGEE, EGEEA, EGME, EGMEA
- R61, may cause harm to the unborn child: EGEE, EGEEA, EGME, EGMEA, 1PG2ME , 1PG2MEA, DEGME.

DEGME, DEGEE: no classification for fertility based on sufficient animal data.
DEGEE: no classification for development based on sufficient animal data.

11.2 Occupational exposure

Occupational exposure to glycol ethers mainly occurs by using industrial products containing these chemicals. Multigner *et al*[213] summed up a long list of professions in which occupational exposure may occur, eg painters; printers; automobile, aeronautical, naval, furniture, building, textiles and dying, packaging and transformation, hair dressers and perfume, metal, and agricultural industry workers; printed circuit manufacturers; producers of metallic packaging; road builders; mechanics; car cleaners; graffiti removers; photographers.

The following concentrations of ethylene glycol ethers in workplaces in the US and Europe in the 1980s were summarized by ECETOC (1995)[215] and DECOS (1996):[241]
- EGME: painting: geometric mean (GM)=31.3 mg/m^3 (range 5.6-136.9); car repair: GM=7.9 mg/m^3 (range 3.4-15.9)
- EGMEA: printing: GM=4.3 mg/m^3 (3.9-4.7); various: GM=11.6 mg/m^3 (0.4-143.3)

- EGEE: printing: GM=9.8 mg/m^3 (0.7-182.0); painting: GM=9.5 mg/m^3 (1.4-210.3); various: GM=17.1 mg/m^3 (3.1-1,224)
- EGEEA: printing: GM=4.1 mg/m^3 (1.5-17.7); painting: GM=18.8 mg/m^3 (3.4-93.6); car repair: GM=5.9 mg/m^3; various: GM=10.6 mg/m^3 (8.9-11.7).

Söhnlein *et al* (1993)[216] monitored occupational exposure to glycol ethers of 19 workers of a varnish production plant. The mean concentrations in workroom air, obtained by personal air monitoring, were:

- 2-ethoxy ethanol (EGEE): Monday: 2.9 ppm (10.7 mg/m^3, range 2.2-55.9 mg/m^3); Tuesday: 2.1 ppm (7.7 mg/m^3, <0.4-22.8 mg/m^3)
- 2-ethoxy ethyl acetate (EGEEA): Monday: 0.5 ppm (2.8 mg/m^3, <0.6 –20.3 mg/m^3); Tuesday: 0.1 ppm (0.6 mg/m^3, <0.6-2.2 mg/m^3)
- 2-butoxy ethanol (EGBE): Monday: 0.5 ppm (2.5 mg/m^3, range <0.5-6.9 mg/m^3); Tuesday: 0.6 ppm (2.9 mg/m^3, range <0.5-4.9 mg/m^3).

The mean concentrations of metabolites in the varnish worker's urine samples were: 2-ethoxyacetic acid: Monday: 53.2 mg/L urine (2.3-180.0); Tuesday: 53.8 mg/L (11.1-143.7); 2-butoxyacetic acid: Monday 0.2 mg/L urine (<0.02-1.3); Tuesday: 16.4 mg/L urine (0.8-60.6).

Vincent *et al*[204] measured exposure to a range of organic solvents, including EGEEA, in an aeronautical workshop in France. Personal air samples on three different days resulted in mean concentrations varying between 63.2-110.2 mg/m^3. The sampling period ranged from 95 to 250 min. Internal exposure was determined by measuring the metabolite ethyoxyacetic acid in urine samples of the painters. Pre-shift mean concentrations were 108.4 ± 58.3 mg/g creatinine, and post-shift 139.4 ± 57.1 mg/g creatinine.

Koontz *et al*[217] estimated occupational exposure to EGBE from commercial floor stripper, cleaner and coating by computer modelling. Depending on the floor product used, the peak air concentrations varied between <1 to 2.5 mg/m^3, and would result in a modelled 8 h TWA of 1.2 mg/m^3.

Since the 1970s the use of ethylene glycol ethers EGME and EGEE has decreased dramatically and nowadays contributes less than 5% to the European usage of glycol ethers. The toxic EGME and EGEE were replaced by ethylene glycol ethers of the butyl family (EGBE(A) and DEGBE(A)) in water-based paints, and by propylene glycol ethers in inks, detergents and consumer products.[218]

11.3 Fertility

11.3.1 Effects on human fertility

Female fertility

Seven studies evaluated the effects of occupational exposure to ethylene glycol ethers on female fertility, including six cohort[82,86,87,88,89,219] and one cross-sectional study[220]. The studies by Eskenazi *et al*[86], Gold *et al*[87], Gray *et al*[88] and Correa *et al*[89] met the selection criteria by the committee. The other studies were rejected because of poor documentation of exposure.

As a part of the Semiconductor Health Study, consisting of a cohort of workers from 14 semiconductor plants in the US, Eskenazi *et al*[86] studied the fecundability (i.e. probability of conception per menstrual cycle) of 152 women working in silicon-wafer fabrication rooms during 6 months. Compared to a non-fabrication group of 251 women, working in fabrication or exposure to ethylene glycol ethers did not result in a significantly reduced fecundibility ratio for clinically confirmed pregnancies (FR 0.5, 95% CI 0.2-1.1, and FR 0.4; 95% CI 0.1-1.2 resp). Fab workers belonging to the 'dopants and thin film' group with a predominant exposure to fluorides, showed a statistically significantly decreased FR of 0.2 (95% CI 0.05-0.96).

Gold *et al*[87] investigated menstrual cycle length of the same cohort of fab and non-fab workers. They found that self-reported mean menstrual cycle length was significantly shorter in fab workers compared to non-fab workers, 28.0 and 28.9 days respectively (p=0.02). However, self-reported median menstrual cycle length did not differ between fab and non-fab workers. According to the results of the daily diaries and urine sampling, fab workers of the 'thin film and ion implantation' subgroup experienced a significantly prolonged mean menstrual cycle of 36.1 days (32.0 days in non-fab workers). Fab and non-fab workers did not differ significantly in mean days of bleeding or risk of having cycles longer than 35, or shorter than 24 days. The effects could not be related to exposure to ethylene glycol ethers.

Gray *et al*[88] studied a cohort of male and female workers in clean rooms in two semiconductor manufacturing plants. In the retrospective part of the study the authors noticed that clean room work with potentially high exposure to ethylene glycol ethers (ie EGME, EGMEA and DEGDME) was associated with an increased risk for subfertility (>1 year time to conception) of female employees (OR 3.9; 95% CI 1.4-11.4). This effect was not observed in wives of exposed

male employees. In the prospective part of the study, no difference was found between the conception rate (CR) per 100 menstrual cycles for working in a clean room either with or without exposure to ethylene glycol ethers (CR EGE exposed: 13.6, CR non-EGE exposed: 11.6).

Correa *et al*[89] investigated spontaneous abortion and subfertility (ie. taking more than one year of unprotected intercourse to conceive) in a cohort of 561 female and 589 male semiconductor workers in Eastern USA. The highest exposure to ethylene glycol ethers (ie DEGDME, EGEEA) occurred in processes involving photoresistent chemical mixtures, like chemical mixing and photolithography. Female workers, potentially exposed to high levels of ethylene glycol ethers, showed an increased subfertility (OR 4.6, 95% CI 1.6-13.3). This effects were not found in wives of men with potentially high exposure to ethylene glycol ethers.

Male fertility

Effects of occupational exposure to ethylene glycol ethers on male fertility were investigated in six studies, including one case-control[94] and five cross-sectional studies [33,90,119, 214,221]. All studies met the selection criteria by the committee.

Also male fertility was investigated in the Semiconductor Health Study. Samuels *et al*[90] performed a cross-sectional study among 241 male fab workers en 447 non- fab workers. No decrease in fertility ratio of either fabrication room workers in general or any of the distinguished subgroups was found. 25% of the men in the 'dopants and thin film' group reported a period ≥1 year in trying of their wives to conceive (RR 1.8, 95% CI 1.1-2.9). These workers, however, were mainly exposed to fluorides.

Welch *et al*[33] studied fertility in a cross-sectional study among 94 male shipyard painters, exposed to a mean concentration of 9.9 mg/m^3 2-ethoxyethanol (EGEE) and 2.6 mg/m^3 2-methoxyethanol (EGME), as 8 h time weighted average. Among the exposed painters only an increased semen pH was found (7.94 vs 7.88 in controls, $p<0.05$). Exposed non-smokers showed a higher rate of oligozoospermia ($p=0.05$). No differences were found in sperm motility, viability, morphology or morphometry. The painters were also exposed to other reproductive toxins like e.g. low concentrations of lead, though the authors stated that these were below reproductive toxicity levels.

Also Shih *et al*[221] observed an effect on semen pH in a small group of workers from two factories producing copper clad laminate. However, instead of an increase, semen pH of exposed workers was decreased (7.08 vs 7.51 in controls, $p=0.005$). No other sperm parameter was affected by occupational exposure to

ethylene glycol monomethyl ether (EGME; mean air concentration of 12.6-13.5 mg/m^3).

In their cross-sectional study, Hanaoka *et al*[119] found reduced FSH concentrations in 42 male epoxy resin spray painters. This effect was significantly associated with bisphenol A metabolites in their urine and not with a glycol ether metabolite (ie 2-butoxyacetic acid).

Veulemans *et al*[94] performed a study among male patients of a clinic for reproductive disorders, including 1,019 cases of diagnosed subfertility or infertility, and 475 controls. In 39 cases and 6 controls urinary ethoxyacetic acid (EAA) was detected, reflecting exposure to 2-ethoxyethanol (EGEE) or its acetate (EGEEA). This resulted in a highly significant OR of 3.11 (p=0.004). These EAA- positive men were significantly clustered among sperm subcategories representing complete azoospermia and severe oligozoospermia (χ_2 probability 0.0087). After grouping the patients according to occupation, industry or chemicals, EAA-positive men appeared to be clustered around paint products and solvents-containing preparations, and some occupations, like paint work, motor and car mechanics, and wood workers.

In a cross-sectional study, Multigner *et al*[214] determined semen quality and hormones in men working for the Paris Municipality during the period 2000-2001. The authors determined the percentage of glycol ethers-containing chemical preparations in several product categories: 100% of water-proofing products; 50% of paints, anti-graffiti, brake fluids and floor coatings; 25% of cleaning agents, hardeners, inks, diluents, oil removers, antifreezes and varnishes; 10% of photographic developers, pesticides, paint strippers, scale removers and disinfectants. 48 men were exposed to glycol ethers, 50 were non-exposed. Exposure to glycol ethers was also biologically monitored by measuring six alkoxycarboxylic acid metabolites in the urine. The metabolites BAA (derived from EGBE) and 2-MPA (derived from propylene glycol ethers) were present in the majority of urine samples, but only BAA-levels differed between the exposed and non-exposed workers. Metabolites from toxic short chain ethylene glycol ethers (EGEE and EGME) were measured in 8-34% of the urine samples. Sperm concentration (p<0.0001), total sperm count (p=0.0002), percentage of rapid progressive sperm (p=0.0008), and percentage of morphologically normal sperm (p=0.005) were statistically significantly decreased in the exposed men. Compared to WHO semen reference values, associations with glycol ether exposure were found for low percentages of: rapid progressive sperm motility OR 4.5 (95% CI 1.3-15.0) and morphologically normal sperm OR 3.6 (95% CI 1.3-9.7). A dose-response for these associations was found when the authors distinguished between moderate and high exposure. Of the hormones, only a statistically sig-

nificant increase in FSH levels (p=0.05) was observed in the exposed men. No correlation was found between the sperm or hormone effects and BAA or 2-MPA urinary levels. According to the authors, the effects on sperm reflect past exposure (before mid 1990s) to the toxic short chain ethylene glycol ethers EGME and EGEE and an incomplete restoration of testicular function.

11.3.2 *Effects on fertility in experimental animals*

There are numerous studies on ethylene glycol monoalkyl ethers and their acetates causing dose-dependent testicular atrophy in male animals of all species tested, and independent of the route of administration.

For testicular toxicity (degeneration of the germinal epithelium in the seminiferous tubules and impairment of sperm maturation) the rank order of potency of ethylene glycol ethers appears to be: ethylene glycol monomethyl ether, EGME > ethylene glycol monomethyl ether acetate, EGMEA > ethylene glycol ethyl ether, EGEE > ethylene glycol ethyl ether acetate, EGEEA. The testicular toxicity of EGME in rats is well characterized, the primary spermatocyte undergoing pachytene development is the most severely damaged cell type, but all stages of spermatocyte development and some stages of spermatid development are affected by EGME.[215]

EGME is the best studied substance. In oral studies with mice, rats, hamsters and guinea pigs the NOAELs for testicular effects were ~50 mg/kg bw/d (cited in DECOS: Foster 1984, Nagano 1984, Chapin 1985, Oudiz 1993). In rabbits a NOAEL of 12.5 mg/kg bw/d was found for disruption of spermatogenesis.[242] Inhalatory exposure to 30 ppm (93.3 mg/m^3) EGME was a minimum effect level in male rabbits (cited in ECETOC: Miller 1983), and 100 ppm (=311 mg/m^3) and 300 ppm (933 mg/m^3) were found to be no effect levels in male rats (cited in DECOS: Doe 1984, Hanley 1984) and mice (cited in ECETOC: Miller 1981), respectively. There are indications that several diethylene and triethylene glycol ethers cause testicular toxicity, with diethylene glycol dimethyl ether (DEGDME) being the most potent one, followed by diethylene glycol methyl ether (DEGME), diethylene glycol ethyl ether (DEGEE) and triethylene glycol dimethyl ether (TEGDME). 110 ppm (=612.7 mg/m^3) of DEGDME was a LOAEL in male rats.[215,243]

When male rats were dosed orally with EGEE (200 mg/kg bw/d, for 6 d/w, during 4 w) together with toluene (250 mg/kg bw/d) and xylene (500 mg/kg bw/d), testicular atrophy of EGEE was diminished by 25%. The total amount of EAA, the metabolite of EGEE, in plasma of these rats was significantly decreased by 29% ($p<0.01$), and the highest concentration of EAA by 45%

($p<0.01$).[222] The authors hypothesize that toluene and xylene compete with the metabolism of EGEE (ie alcohol and aldehyde dehydrogenases, NAD^+). In a second study, also testicular tubular degeneration by EGEE in male rats was reduced by co-administration of toluene and xylene.[223]

Only one paper was available on the effects of EGME on female fertility. Davis *et al*[224] found a LOAEL of 300 mg/kg bw/d (administered for 7 or 14 days) for effects on rat cyclicity and serum hormone levels (ie decreases in estradiol, FSH, LH, and prolactin; increase in progesterone). These effects were not observed at 100 mg/kg bw/d, the NOAEL. From an *in vitro* study with MAA, the metabolite of EGME, the authors concluded that the luteal cell is a target.

11.3.3 Effects on fertility: conclusions

The only clear effect on female fertility from epidemiological studies was the observation by Gray *et al*[88] and by Correa *et al*[89] that exposure to ethylene glycol ethers increased the risk for subfertility. The Semiconductor Health Study[86,87] showed effects on length of menstrual cycle and female subfertility in a subgroup (dopants and thin film), were the primary exposure appeared to be to fluorides instead of ethylene glycol ethers.

In the only study on female fertility performed in experimental animals, EGME affected rat cyclicity, and serum hormone levels.[224] From an *in vitro* study the authors concluded that luteal cells might be a target.

The committee is of the opinion that the data on effects of exposure to ethylene glycol ethers on female fertility seem to be conflicting, although the results might be explained by differences in exposure compositions. Therefore, the committee is of the opinion that based on the available data the committee is unable to draw any conclusions regarding the effects of exposure to ethylene glycol ethers on female fertility.

The studies by Welch *et al*[33] and Veulemans *et al*[94] indicate that occupational exposure to ethylene glycol ethers reduced male fertility, by increasing the risk of oligozoospermia and azoospermia. These findings are supported by the significant decrease in sperm concentration and total sperm count in glycol ethers-exposed men, observed by Multigner *et al*.[214] Data on other sperm parameters are conflicting. Welch *et al*[33] and Shih *et al*[221] found no effect on sperm parameters like motility, viability, morphology and morphometry after occupational exposure to 2.5-13.5 mg/m^3 EGME or 10 mg/m^3 EGEE. On the other hand, Multigner

et al observed significant decreases in sperm motility and normal sperm morphology.[214]

In all species of experimental animals tested ethylene glycol monoalkyl ethers caused dose-dependent testicular atrophy that was independent of the route of exposure.

The committee concludes that the epidemiological and animal data indicate an association between exposure to ethylene glycol monoalkyl ethers and male fertility.

11.4 Development of the progeny

11.4.1 Effects on human development

14 epidemiological studies evaluated the effects of occupational exposure to ethylene glycol ethers on development, including six cohort[82,83,84,85,88,89], six case-control [91,95,96,97,158,225] and two cross-sectional studies[226,227]. Of these studies, eleven met the selection criteria of the committee.[82,83,84,85,88,89,91,95,96,97,158] The other studies were rejected for different reasons: poor documentation of exposure, or of the study. The majority of the available studies focused on spontaneous abortion.

The studies by Beaumont *et al*[83], Eskenazi *et al*[85], Schenker *et al*[82] and Swan *et al*[84] involved the same cohort of semiconductor workers (Semiconductor Health Study). Cordier *et al*[96] and Ha *et al*[95] investigated a population in an European collaborative multicenter study.

The Semiconductor Health Study involved a retrospective study of a cohort of 904 women employed in 14 semiconductor plants in the USA, and a prospective one including 403 women. Schenker *et al*[82], Beaumont *et al*[83], and Swan *et al*[84] focused on spontaneous abortion in specific subgroups in the retrospective cohort study, whereas Eskenazi *et al*[85] reported about the prospective part. Within the silicon wafer fabrication room workers, the following three groups, based on processes, were distinguished:

- Masking group: including the subgroups photolithography, and etching
- Dopants and thin film group: including the subgroups furnace, and thin film and ion implantation
- Supervisors and engineers group.

According to the authors, occupational exposure to ethylene glycol ethers mainly occurred in the masking group and especially the etching subgroup. Data on exposure levels were not presented.

Schenker *et al*[82] reported an increased risk of spontaneous abortion in women of the masking group (RR 1.8, 95% CI 1.2-2.6). Though a higher percentage of spontaneous abortions was found in fab workers (15%) compared to non-fab workers (10.4%), this difference was not statistically different (RR 1.4, 95% CI 0.95-2.1). Beaumont *et al*[83] differentiated between the subgroups and concluded that women of both subgroups, photolithography and etching, within the masking group, were at higher risk of having a spontaneous abortion (RR photo 1.7, 95% CI 1.0-2.6; RR etch 2.1, 95% CI 1.3-3.2). Swan *et al*[84] reported agent-specific analyses of the group of fab workers. Exposure of female workers to ethylene-based glycol ethers or propylene-based glycol ethers resulted in rates of spontaneous abortion of 18.4% and 18.8% respectively The corresponding relative risks were: RR ethylene 1.6 (95% CI 1.0-2.3) and RR propylene 1.4 (95% CI 0.8-3.4). Combining the two highest ethylene glycol ethers-exposed groups resulted in an RR of 2.4 (95% CI 1.2-4.1) for all women, and an RR of 3.4 (95% CI 1.6-5.4) for women of the masking group. The risk of spontaneous abortion was found to be dose-related with ethylene glycol ether exposure (p=0.004). The authors stated that propylene glycol ethers were used less (<50%) than ethylene glycol ethers. Which ethylene glycol ethers were involved was not mentioned in the paper. Also for other solvents, like xylene, n-butyl acetate, acetone and isopropyl alcohol, and physical factors like work stress, the risk of spontaneous abortions was significantly increased. Since women of the masking group were exposed to other chemicals as well, the effect on spontaneous abortion can not be clearly attributed to ethylene glycol ethers. Finally, Eskenazi *et al*[85] reported on spontaneous abortion in the prospective part of the study, including 152 female fab and 251 non-fab workers. Though they observed a higher percentage of spontaneous abortion in fab workers (63.2%) compared to non-fab workers (45.5%), the relative risk was not significantly increased.

Correa *et al*[89] investigated spontaneous abortion and subfertility in a cohort of 561 female and 589 male semiconductor workers in Eastern USA. The highest exposure to ethylene glycol ethers (ie DEGDME, EGEEA) occurred in processes involving photoresistent chemical mixtures, like chemical mixing and photolithography. Female workers, potentially exposed to high levels of ethylene glycol ethers, showed an increased number of spontaneous abortion (RR 2.8, 95% CI 1.4-5.6) and subfertility (OR 4.6, 95% CI 1.6-13.3). These effects were not found in wives of men with potentially high exposure to ethylene glycol ethers.

In a cohort of female and male employees working at two semiconductor manufacturing plants, Gray *et al*[88] found an increased risk of spontaneous abortion in women with potentially high ethylene glycol ether exposure (ie EGME, EGMEA, DEGDME) (RR 2.8, 95% CI 1.4-5.6). The prevalence of spontaneous

abortion was not increased in wives of men with potential exposure to ethylene glycol ethers. No significant effect was found on pregnancy loss of female workers.

Elliott *et al*[91] performed a relatively small study in the British semiconductor industry, including 36 cases of spontaneous abortion and 80 controls. No association was found between spontaneous abortion and working of the women in any of the specific fabrication workgroups.

Windham *et al*[158] studied 626 cases of spontaneous abortion from hospital records and 1,300 controls. No association was found with maternal occupational exposure to glycol ethers (7 cases and 9 controls), or to 'all solvents'.

Ha *et al*[95] and Cordier *et al*[96] both reported on the same European collaborative multicenter case-control study on congenital malformations, including 991 cases of abortions and 1,144 controls. Ha *et al* (1996)[95] described a significant excess of mothers exposed to glycol ethers in the group of oral clefts (OR 2.0, 95% CI 1.1-4.1) and of central nervous system malformations (OR 1.8, 95% CI 1.1-3.3). Exposure involved class 2 and 3 glycol ethers, mainly consisting of non-teratogenic compounds. Cordier *et al* (1997)[96] reported more details of the same multicenter study. For in-dept analyses malformations were divided in 22 subgroups. Glycol ether exposure resulted in an increased risk of all congenital malformations (OR 1.4, 95% CI 1.1-1.9) and for the subgroups of neural tube defects (OR 1.9, 95% CI 1.2-3.2), especially spina bifida (OR 2.4 (95% CI 1.2-4.6); multiple anomalies (OR 2, 95% CI 1.2-3.2) and cleft lip/palate (OR 2.0, 95% CI 1.2-3.3).

In a recent study, Chevrier *et al*[97] examined 164 cases of cleft lip with or without cleft palate, 76 cases of cleft palate and 236 controls. They found that maternal exposure to glycol ethers during the first trimester resulted in a increased risk of cleft lip with or without cleft palate (OR 1.9, 95% CI 1.1-3.5). The increased risk appeared to be dose-dependent ($p<0.01$). The authors mentioned that a large number of women exposed to glycol ethers were also exposed to aliphatic alcohols. When these exposures were considered separately, the risk for cleft lip (and palate) was no longer significantly increased for exposure to glycol ethers.

11.4.2 *Effects on development in experimental animals*

Numerous studies on developmental effects of ethylene glycol ethers in experimental animals have been performed. The most potent substances are EGME and EGMEA. Other glycol ethers that were shown to be developmental toxicants are: EGEE, EGEEA, EGDME, EGDEE, DEGME, DEGDME, TEGME, TEGDME, 1PG2ME and 1PG2MEA.[215] These glycol ethers caused developmental effects in

the absence of maternal toxicity. Metabolism of the parent compounds into their alkoxy acetic acid metabolites is assumed to mediate the developmental effects. MAA, derived from EGME, EGMEA, EGDME, DEGDME, TEGDME, and EAA, derived from EGEE, are similarly potent embryotoxicants and teratogens.[215]

Inhalatory exposure of female rabbits to 10 ppm (31.3 mg/m^3) EGME resulted in an increased resorption rate and delayed ossification in the foetuses (cited in DECOS: Hanley 1984). The no-effect level of inhaled EGME was 3 ppm (9.5 mg/m^3) in rabbits and 10 ppm (31.1 mg/m^3) in rats and mice (ECETOC 1995, cited in DECOS: Hanley 1984). Using the benchmark dose approach, the lower bound confidence limits for developmental effects of EGME were: 5 ppm (16 mg/m^3) for resorptions in rabbits, 15 ppm (47 mg/m^3) for congenital malformations in rabbits, and 53 ppm (165 mg/m^3) for malformations in rats.[228]

Oral dosing of female rats resulted in a NOAEL of EGME of 12.5 mg/kg bw/d (cited in DECOS: Morrissey 1989). In pregnant female mice repeated oral administration of 31 mg/kg/day of EGME, the lowest dose tested, caused skeletal defects. At 250 mg/kg/day these defects consisted of exencephaly, paw anomalies, abnormal digits and other anomalies. The types of malformation depended on the stage of development during the exposure period (ECETOC 1995, cited in DECOS: Nagano 1984).

For EGEE and EGEEA no-effect levels of 50 ppm (186.5 and 273.5 mg/m^3, resp) were found in inhalatory studies in female rats and rabbits (cited in ECETOC: Tinston 1983, Doe 1984, Tyl 1988). The potency of EGEE(A) is estimated to be five fold lower compared to EGME and its acetate.[215]

The NOAEL for developmental effects of DEGME was 50 mg/kg/day in female rabbits dermally exposed, and 200 mg/kg/day in female rats after oral administration (cited in DECOS: Scorthichini 1986, Yamano 1993).

Based on several PBPK models using data from animal studies, human NAELs for developmental toxicity of EGEE and EGEEA of 25 ppm (92 mg/m^3 and 137 mg/m^3, 8 h TWA), and of 12 ppm (37 mg/m^3, 8h TWA) for EGME were estimated. The human LAEL for inhaled EGEEA was determined to be 55 ppm (301 mg/m^3).[229,230] Also occupational exposure limits were proposed based on these models: EGEE(A): 2 ppm (8 and 11 mg/m^3), 8 h TWA; and EGME: 0.9 ppm (3 mg/m^3), 8 h TWA.[230] When these models were used to predict human blood levels after simulated inhalation of 5 ppm (16 mg/m^3) EGME, its Treshold Limit Value, human blood levels were well below blood levels causing adverse reproductive effects in rats and mice.[231]

11.4.3 Effects on development: conclusions

Spontaneous abortion is the best studied developmental effect in the epidemiological studies available. The majority of studies were carried out in the semiconductor manufacturing industry. There is a consistent finding of a significantly increased risk of spontaneous abortion in women working in processes with a high potential for exposure to ethylene glycol ethers.[82,83,88,89] Agent-specific analyses performed by Swan[84] among women in the semiconductor industry, revealed an association between spontaneous abortion and maternal occupational exposure to ethylene-based glycol ethers. In the study by Elliott *et al*[91], no effect on spontaneous abortion was found; however, the number of cases might have been too small.

In the semiconductor industry, the women of the subgroups associated with an increased risk of spontaneous abortion were not exclusively exposed to ethylene glycol ethers but to a variety of other solvents, fluorides, and metals as well.[84] Data on the levels of exposure to ethylene glycol ethers were not available in these studies. Overall, the committee concludes that the available epidemiological studies indicate an association between female exposure to ethylene glycol ethers and the risk of spontaneous abortion.

Two studies[88,89] evaluated paternal exposure in the semiconductor industry, but no association was found with the prevalence of spontaneous abortion in their wives. Therefore, the committee concludes that the available data do not indicate an association between paternal exposure to ethylene glycol ethers and the risk of spontaneous abortion.

From the epidemiological data on congenital malformations, Ha *et al*[95] and Cordier *et al*[96] found associations between maternal glycol ether exposure and neural tube defects (i.e. spina bifida), multiple anomalies and cleft lip/palate in the European collaborative multicenter study. However, exposure involved glycol ethers that are not teratogenic in experimental animals. The study by Chevrier *et al*[97] supports the positive finding on cleft lip/palate, though it is not clear to what extent the women exposed to glycol ethers were exposed to aliphatic solvents as well. The committee concludes that there are indications for an association between maternal exposure to glycol ethers and an increased risk of neural tube defects and cleft lip or palate.

In experimental animals, the evidence for developmental toxicity, ranging from fetal death and resorptions to malformations, of a number of ethylene glycol

ethers is overwhelming. Female rabbits were found to be the most sensitive animal species, and EGME and its acetate the most potent ethylene glycol ether with a no effect level of 9.5 mg/m^3.

Overall, the committee concludes that the epidemiological and animal data indicate an association between female exposure to ethylene monoalkyl glycol ethers and developmental effects. Since the epidemiological studies lack data on occupational exposure levels, and co-exposure to other chemicals is involved, no-adverse-effect- levels (NAELs) of ethylene glycol ethers cannot be derived from these studies. However, health-based OELs recommended by the Health Council or the European SCOEL for the most toxic short-chain ethylene glycol ethers (ie EGME(A), EGEE(A), DEGME) are based on reproduction toxicity in animal studies. The OELs are in agreement with the results of PBPK-modeling using the animal data.

Chapter 12

Conclusions and recommendations

12.1 Complexity of epidemiological studies concerning reproductive outcomes

To study the relationship between occupational exposure to organic solvents and effects on reproduction is a difficult task. On the one hand, exposure characterization is complex due to the combined presence of a vast number of solvents (at varying exposure levels), and the interactions between these solvents and other chemicals and risk factors related to e.g. life style. On the other hand, reproductive toxicity may result in a wide range of different reproductive outcomes.

Furthermore, only controlled experimental studies are able to definitively answer the questions regarding possible causal relations. Performing such studies in humans, however, is not possible for obvious ethical reasons but observational epidemiological studies all have their methodological limitations.

Multiple compounds and varying exposure levels

The working environment is difficult to describe as numerous chemical, physical, and biological risk factors for reproductive outcomes may be present. In addition, within most workplaces a variety of organic solvents are used and, thus, workers are usually exposed to a combination of organic solvents which may vary with place and time. The available epidemiological studies have to deal with all of these limitations. Consequently, epidemiological data can not be used to

draw definitive conclusions about possible causal relations between specific organic solvents and particular reproductive outcomes.

Furthermore, organic solvent exposure scenarios (a set of facts and assumptions about how exposure takes place) at the workplace may range from single high peak exposures to chronic low exposures, with variable intermediate scenarios being most likely in actual occupational situations. The possible differences in biological and toxicological effectiveness of different scenarios are not well known. In addition, studies on occupational exposures are usually unable to establish which scenarios actually occurred in a given situation.

Wide range of endpoints of reproductive toxicity

The reproductive cycle includes a wide variety of regulatory mechanisms and endpoints, which complicates the assessment of reproductive toxicity. Endpoints of relevance include, among others, sexual behavior, sperm and oocyte parameters, ovarian function, menstrual cycle pattern, time to pregnancy, spontaneous abortion, birth defects, pre-term birth, birth weight, psychomotor development, childhood malignancies, and sexual maturation.

These endpoints all have their specific windows of sensitivity within the reproductive cycle. In sensitive windows of exposure the tissue specific potency of a reproductive toxicant is higher than outside this time period. The sensitivity for reproductive effects is also dependent on the genetic background and can be gender-specific. Furthermore, most effects are dose-dependent and are assumed to have a specific threshold below which compounds are ineffective. In view of this complexity, studies in which different reproductive effects are lumped together in effect assessment or where the reproductive effects are not well defined, are considered of limited value by the committee.

12.2 Availability and quality of data

Organic solvents are among the most prevalent sources of chemical exposure among male and female workers. A wealth of observational epidemiological research has been performed on the effects of occupational exposure to solvents on reproduction. However, the committee is of the opinion that only a part of these studies is of sufficient quality to evaluate exposure-response relations. Furthermore, studies on the consequences of occupational exposure to single solvents are rare.

Quantitative and qualitative exposure data on exposure are often lacking

A major problem in the interpretation of the available epidemiological studies is the lack of quantitative data on exposure to individual solvents and mixtures. In most studies, exposure information is limited to a description of the jobs performed. In some cases, an occupational hygienist assessed the solvents involved and, if possible, attempted to estimate the intensity, frequency, and duration of exposure to individual solvents encountered by the study population. In only a few studies, actual exposure measurements were conducted. However, in workplaces with strongly varying exposure patterns, quantification of the exposure remains difficult.

Reproductive endpoints are insufficiently defined

In several epidemiological studies, the characterization of reproductive endpoints was insufficient or described inadequately. This may have been due to absent or incomplete definitions of effect categories, or to the combination of very different types of effects into one category, probably to increase the power. The underlying assumption that diverse effects may have the same etiology is at least an oversimplification, which complicates the interpretation of the findings.

12.3 Effects of occupational exposure to organic solvents

What is known about the effects of occupational exposure to organic solvents on male and female fertility and on the development of the progeny?

This question is answered using two different perspectives. First, the findings on the associations between effects and exposure to individual solvents are discussed. Second, for each type of industry in which these solvents are used, the committee discusses whether the effects have been observed in that industry as well. If possible, the committee establishes whether current exposure levels in those industries in the Netherlands give reason for concern in view of these results. However, given the lack of exposure data in most studies, the latter is only occasionally possible. Finally, this section provides summary tables in which a complete overview of the findings is given.

12.3.1 *Effects on fertility*

Exposure to ethylene glycol ethers and effects on male fertility

From the available human studies, there are indications that exposure to ethylene glycol ethers (especially EGEE* and EGME**) is associated with effects on sperm parameters. The epidemiological data, however, cannot give an answer to the question whether the solvents are actually the cause of these effects. Animal studies show that exposure to EGME causes testicular atrophy. Inhalatory exposure for 13 weeks to 93 mg/m^3 EGME was the minimum effect level in male rabbits. Similar effects were observed after exposure to EGEE. Therefore, the committee is of the opinion that a causal relation between exposure to ethylene glycol ethers and testicular atrophy is apparent based on the animal studies.

Based on the findings in the literature, the Health Council of the Netherlands recommended in 1996, an Health Based Occupational Exposure Limit (HBROEL***) for 2-methoxyethanol (EGME) of 1 mg/m^3. In addition, the European SCOEL**** recommended an occupational exposure limit for 2-ethoxyethanol (EGEE) of 8 mg/m^3 in 2007. Both exposure limits are based on the prevention of reproduction toxic effects.

The *painting and maintenance trades* are types of industry where occupational exposure to ethylene glycol ethers (especially EGEE and EGME) occured and where the association between effects on fertility and exposure to solvents has been studied. There are weak indications for an association between exposure to solvents and effects on male fertility. A reduction in male fertility and effects on sperm in male shipyard painters are observed after exposure to concentrations of ~10 mg/m^3 EGEE and ~3 mg/m^3 EGME.

In 1980, the occupational exposure levels to ethylene glycol ethers in the painting industry in the US and Europe varied from 1 to 210 mg/m^3 for EGEE (GM is 10 mg/m^3) and from 6 to 140 mg/m^3 for EGME (GM 31 mg/m^3). Since the ban of short chain ethylene glycol ethers in consumer products in the 1990s, occupational exposure has drastically declined. Recent data concerning exposure to short chain ethylene glycol ethers in the Netherlands are, however, not available. Therefore, the committee cannot exclude that the current exposure levels in

* EGEE: Ethylene glycol ethyl ether or 2-ethoxy ethanol.
** EGME: Ethylene glycol monomethyl ether or 2-methoxy ethanol.
*** HBROEL: Health Based Recommended Occupational Exposure Limit.
**** SCOEL: Scientific Committee on Occupational Exposure Limit.

Table 12.1 (Weak) Indications for effects on fertility after exposure to solvents or employment in different types of industry.

Exposure	Exposure (male/female)[a]	Effect[b]	Exposure level showing effects in human studies	Recent exposure levels in Netherlands
Ethylene glycol ethers	Male	*Indications for* subfertility and sperm effects		Not known
Painting and maintenance trade	Male	*Weak indications for* subfertility and sperm effects	9.9 mg/m^3 EGEE 2.6 mg/m^3 EGME	Not known

[a] Exposure of female or male workers.
[b] Effects on male and female fertility are evaluated.

the painting and maintenance trades in the Netherlands might cause effects on fertility.

Although ethylene glycol ethers are also frequently used in the semiconductor industry and exposure to these solvents is expected (despite the lack of exposure data), no indications were found for effects on male fertility in this occupational setting.

No indications for effects on fertility

Studies in female workers, mainly exposed to styrene in the reinforced plastics industry, showed no indications for effects on time to pregnancy or menstrual parameters (see table 12.2). Animal studies likewise failed to show effects on female fertility.

For female workers predominantly exposed to toluene, no associations were found between toluene and time to pregnancy or menstrual parameters. Animal data did not reveal effects on female fertility either.

Finally, there are no indications for an association between exposure to methylene chloride and effects on male or female fertility.

No conclusions possible for other solvents and effects on fertility

Concerning the possible effects on fertility of exposure to other solvents and exposure to solvents in other types of industry, no conclusions can be drawn. This is due to a lack of data, insufficient data or conflicting outcomes in different studies (see Table 12.2). Furthermore, no conclusion can be drawn concerning

Table 12.2 'No indications for effects on fertility' or 'no conclusions possible' about effects of exposure to solvents or employment in different types of industry.

Exposure	No conclusions	No indications for association		
	No data	Insufficient or limited data	Sufficient but conflicting data	Sufficient data
Styrene		Male[a]		Female (TTP[b] and menstrual parameters)
Toluene		Male		Female (TTP and menstrual parameters)
Xylene		Male/female		
Benzene		Male/female		
Acetone		Male/female		
Tetrachloroethylene		Male/female		
Methylene chloride				Male/female
Ethylene glycol ethers			Female (subfertility)	
Reinforced plastics industry		Male		Female (TTP and menstrual parameters)
Printing industry	Male	Female		
Painting and maintenance trade	Female			
Petrochemical and chemical industry	Male	Female		
Laboratories	Male		Female (TTP)	
Dry-cleaning		Male/female		
Semiconductor industry			Female (delayed conception)	Male (delayed conception)

[a] Exposure of female or male workers; Effects on male and female fertility are evaluated.
[b] TTP: Time To Pregnancy.

the effects of exposure to ethylene glycol ethers, styrene, toluene, and methylene chloride in relation to fertilityparameters other than those discussed above.

12.3.2 *Effects on the development of the progeny*

What is known about the effects of occupational exposure to organic solvents on the development of the progeny of men and women?

Maternal exposure to ethylene glycol ethers and developmental effects on the progeny

In view of the literature, the committee concludes that women occupationally exposed to mixtures of ethylene glycol ethers may have an increased risk of spontaneous abortion and malformations in offspring (see Table 12.3). However,

the epidemiological data can not give an answer to the question whether these solvents are actually the cause of the observed effects and at what level of exposure these effects occur. On the other hand, in experimental animal studies the evidence for effects on the development of the progeny (from resorptions to malformation) is overwhelming. Therefore, based on animal studies, NOAELs* for developmental toxicity have been established for several ethylene glycol ethers (NOAEL for rats/mice is 30 mg/m^3 and for rabbits 9.5 mg/m^3). In 1996, the Health Council of the Netherlands recommended a Health Based Occupational Exposure Limits (HBROELs) for several ethylene glycol ethers based on these NOAELs. In addition, the European SCOEL recommended an occupational exposure limit for EGEE in 2006.

Exposure to ethylene glycol ethers and developmental toxicity occurs among others in the semiconductor industry. There are indications that female workers exposed to solvents in the semiconductor industry have an increased risk of spontaneous abortion and congenital malformations (neural tube defects and cleft lip or palate) in their progeny. Ethylene glycol ethers are the principal component of solvent exposure in this industry. Unfortunately, data on exposure levels are not available in these studies.

After the ban of short chain ethylene glycol ethers in consumer products in the 1990s, the exposure drastically declined in the Netherlands in ten years' time. Nevertheless, the committee concludes that the available data do not exclude that effects might still occur in the progeny of women exposed to current levels of ethylene glycol ethers in the Netherlands.

Maternal exposure to tetrachloroethylene and developmental effects on the progeny

Epidemiological studies in the Nordic countries in the period 1973-1983 showed weak indications for an increased risk of spontaneous abortion after exposure to 'high levels' of tetrachloroethylene (PER), probably higher than 30 ppm (207 mg/m^3) (see Table 12.3). However, exposure data are limited. Studies in rats, mice, and rabbits have shown several developmental effects (eg. reduced pup survival), but these animal data cannot provide conclusive information, because the effects were observed in the presence of marked maternal toxicity.

* NOAEL: No Observed Adverse Effect Level

Tetrachloroethylene is the principal solvent to which workers in dry-cleaning shops are exposed. Indications for an association between effects on the development of the progeny and this type of industry are weak. Since the 1980s, the occupational exposure to PER has shown a significant decline. Current occupational exposure levels in the Netherlands range from 2 to 45 mg/m^3 (mean 8 mg/m^3).

Therefore, the committee concludes that under current PER exposure levels in the Netherlands, adverse effects on the development of the progeny due to occupational exposure of women are not likely.

Paternal and maternal exposure to toluene and developmental effects on the progeny

A range of epidemiological studies are available concerning the exposure to mixtures containing toluene. Several of these give indications for an association between maternal exposure to toluene and spontaneous abortion (see Table 12.3). Furthermore, there are weak indications that maternal exposure to toluene is associated with childhood leukemia. The epidemiological data do not answer the question at which toluene concentration the developmental effects were observed. Studies in rats and mice also show developmental effects after exposure to toluene, but the quality of these studies is limited and therefore the studies do not give evidence for a causal link between toluene exposure and the observed effects in man.

Paternal exposure to toluene has also been linked to an increased risk of spontaneous abortion occurring in the pregnancies of their wives. The indications for this association are weak. The studies which support this observation are once again deficient in exposure data.

Exposure to toluene can amongst others be found in the printing industry, the painting and maintenance trade, and the petrochemical industry. For the printing and the petrochemical industry, weak indications were observed for an association between maternal exposure to solvents and spontaneous abortion. For the painting and maintenance trade, weak indications were found for an association between paternal exposure to solvents and spontaneous abortion.

The solvent exposure in the printing industry varies in composition and exposure level. Benzene, styrene, toluene, and xylene are the most common solvents to which women in this industry are exposed. More detailed information on exposure levels is not available. Therefore, the committee cannot conclude whether exposure to toluene is responsible for the observed effects. As no infor-

mation is available on current exposure levels, the committee cannot establish whether the present occupational situation in the Dutch printing industry is such that female workers are at increased risk of spontaneous abortion.

In the petrochemical industry, women are primarily exposed to benzene, styrene, toluene, and xylene – just as is the case in the printing industry. More detailed information on the composition and levels of exposure is once again lacking. The committee cannot conclude whether exposure to toluene is responsible for the observed effects. Therefore, the committee cannot establish whether the present occupational exposure in the Netherlands poses an increased risk of spontaneous abortion among women working with toluene in the petrochemical industry.

For male workers in the *painting and maintenance trade,* comparable conclusions can be drawn as an increased risk of spontaneous abortion has been found among their wives. Again, the assessment of the exposure of the male painters and maintenance workers in the available studies is incomplete. Therefore, the committee is not able to establish whether the present occupational situation in this type of industry constitutes a risk for the development of the progeny of male workers.

Maternal exposure to xylene and developmental effects on the progeny

From the epidemiological data, there are weak indications that maternal exposure to xylene is associated with an increased risk of spontaneous abortion (see table 12.3). The available data give no indications about the exposure levels involved. In experimental animals, the effect was not found.

Exposure to xylene occurs in the petrochemical and printing industry. In both types of industry, there are weak indications that maternal exposure to solvents is associated with an increased risk of spontaneous abortion. However, detailed information on exposure is lacking. Therefore, the committee cannot determine whether xylene is responsible for the observed effects in these industries. In addition, the committee is not able to establish whether the present occupational situation in these types of industry constitutes a risk for the development of the progeny of female workers.

Table 12.3 (Weak) indications for effects on the development of the progeny after exposure to solvents or employment in different industries.

Exposure	Exposure (male/female)	Effect	Exposure level showing effects in human studies	Recent exposure levels in the Netherlands
Toluene	Paternal[a]	*Weak indications* for spont. abortion	'High exposure'[b]	Toluene as marker. Decline to less than 1 mg/m^3
	Maternal	*Indications* for spont. abortion and *weak indications* for childhood leukemia	Not determined High exposure	
Xylene	Maternal	*Weak indications* for spont. abortion	Exposure 3 to 5 times/week[c]	
Ethylene glycol ethers	Maternal	*Indications* for spont. abortion, malformations		
Tetrachloroethylene	Maternal	*Weak indications* for spont. abortion		8 mg/m^3 PER (mean)
Printing industry	Maternal	*Weak indications* for spont. abortion	Not determined	
Painting and maintenance	Paternal	*Weak indications* for spont. abortion	Not determined, exposure to combination of compounds	
Petrochemical industry	Maternal	*Weak indications* for spont. abortion	Benzene: 0.86 ppm[d] Toluene:0.40 ppm Styrene:0.50 ppm Xylene: 0.03 ppm	
Dry-cleaners	Maternal	*Weak indications* for spont. abortion	Exposure to high PER concentrations > 207 mg/m^3	8 mg/m^3 PER (mean)
Semiconductor industry	Maternal	*Indications* for spont. abortion	Not specified	

[a] Exposure of female (maternal) or male (paternal) workers; Effects on development are evaluated.
[b] Not further specified by Taskinen *et al*.[81]
[c] Not further specified by Taskinen *et al*.[60]
[d] Data from Xu *et al*.[42]

No indications for developmental effects on the progeny after maternal exposure

For female workers in the reinforced plastics industry with primarily exposure to styrene, the committee found no indications for associations with reduced birth weight and spontaneous abortion (see Table 12.4). No indications for increased risks of spontaneous abortion were found for female workers exposed to methylene chloride, benzene, or acetone either.

No indications for developmental effects on the progeny after paternal exposure

No indications for an association with spontaneous abortion in their wives were found for male workers exposed to methylene chloride or benzene (see Table 12.4). For male workers in the semiconductor industry, mainly exposed to ethylene glycol ethers, no indications for an association with reduced birth weight in offspring or spontaneous abortion in their wives were found after exposure to solvents.

Finally, in the petrochemical industry, no indications were found for an association between paternal exposure to solvents and spontaneous abortion in their wives or reduced birth weight of their offspring either. In this type of industry, exposure to solvents mainly involves toluene, xylene, benzene, and styrene. Although for toluene weak indications for an effect on development were found, this effect was not observed in the petrochemical industry.

Table 12.4 'No indications for associations' between developmental effects and solvent exposure or employment in different types of industry or 'no conclusions possible'.

Exposure	No conclusions			No indications for association
	No data	Insufficient or limited data	Sufficient but conflicting data	Sufficient data
Styrene		Paternal		Maternal[a]
Benzene				Paternal (SA[b])/ Maternal
Xylene		Paternal		
Acetone	Paternal			Maternal
Tetrachlororethylene		Paternal		
Methylene chloride				Paternal/maternal
Ethylene glycol ethers				Paternal (SA)
Reinforced plastics industry		Paternal		Maternal (RBW[c] and SA)
Printing industry		Paternal		
Painting and maintenance		Maternal		
(petro)Chemical industry				Paternal (RBW and SA)
Laboratories		Paternal	Maternal (SA, preterm birth)	
Dry-cleaners	Paternal			
Semiconductor industry				Paternal (SA)

[a] Exposure of female (maternal) or male (paternal) workers; Effects on the development are evaluated
[b] SA: Spontaneous abortion
[c] RBW: Reduced body weight

No conclusions possible for other solvents or developmental effects

Concerning the possible effects of exposure to other solvents in the above mentioned or other types of industry, no conclusions can be drawn. This is due to a lack of data, insufficient data or conflicting outcomes in different studies. Furthermore, no conclusions can be drawn concerning the effects of maternal and paternal exposure to toluene, and maternal exposure to xylene, ethylene glycol ethers and tetrachloroethylene in relation to reproductive outcomes other than those discussed above.

12.4 Effects of peak exposure, exposure during sensitive periods, and exposure to mixtures

The influence of peak exposure is not known

What is known about the relation between any of the observed effects on reproduction on the one hand and peak exposure on the other hand?

Occupational hygiene data indicate that exposure to organic solvents at the workplace is highly variable and generally occurs as peaks*. In addition, exposure can occur in concentrations over ten times the existing occupational exposure limits (eight hours time weighted average).

In 1999, the Health Council of the Netherlands studied whether peak exposures to organic solvents might be an important factor in the development of chronic neurotoxicological effects.[244] At that time, the Council concluded that no sufficient data were available to explain the relationship between peak exposures to organic solvent vapours and the occurrence of chronic neurotoxicological effects, including CTE**. In other words, if CTE occurs after peak exposures it is not clear whether the total dose or the maximum concentration determines the development of CTE.

The present committee is of the opinion that the same conclusion holds true for toxic effects on reproduction. However, the potential influence of peak exposure versus time weighted average exposures might differ from outcome to outcome. No data are currently available to conclude whether peak exposures are responsible for effects on reproduction. Much more research is needed to understand the relationship between external peak exposures and internal dose. Physi-

* In 1999, the Health Council defined the term ‘peak exposure’ or ‘peak concentration’ as the mean exposure over a period of 15 minutes (reference).

** CTE: Chronic toxic encephalopathy.

ologically based pharmacokinetic models might be helpful in this regard. On the other hand, the committee realizes that it is difficult to investigate whether observed risks are attributable to an average exposure or to several peaks, since peak frequency and intensity at the workplace will largely determine the average exposure over the workday (e.g. 8 hour time weighted average) as was recently shown in a study on spraying tasks.[232]

No data are available on the sensitive periods for exposure

What is known about the relation between any of the observed effects on fertility and the development of the progeny on the one hand and exposure during sensitive periods on the other hand?

The committee is not able to answer this question due to lack of data. One must bear in mind that workers are most likely exposed before conception and during all periods in pregnancy (except for the final weeks). Hence, sensitive periods are difficult to identify. However, it is well known that the three months period before conception is the sensitive period for the spermatogenesis, whereas implantation and embryonal period (until 14 weeks after conception) are crucial in the etiology of spontaneous abortion and birth defects. In the beginning of pregnancy, many women are not yet aware of being pregnant. The last five months of pregnancy and the breast feeding period may be sensitive periods concerning intra-uterine growth and the development of the central nervous system.

In an advice concerning preconceptional care in the Netherlands that appeared in September 2007, the Health Council recommended to inform parents-to-be about possible health risks due to occupational exposure prior to and just after conception. The Health Council advised to set up a national coordinated program of preconceptional care. Information on possible occupational health risks could be included in this program, including a role for the occupational health physician.

The present committee agrees with this advice and recommends informing parents-to-be through preconceptional counseling about the possible reproductive effects of occupational exposure to some specific solvents (see paragraph 12.6).

Much is unclear concerning the effects of exposure to combinations of solvents

It remains unclear whether exposure to mixtures of solvents causes different effects than might be expected on the basis of the reproduction toxic properties of the individual solvents. Interactions between chemicals are possible. Effects might be additive, but inhibition or potentiation cannot be excluded *a priori*.

12.5 Occupational exposure limits

In the study of Hooiveld *et al*[3], effects on reproduction were suggested after exposure to concentrations below the occupational exposure limit for one of the solvents, i.e. toluene. Therefore, the Minister of Social Affairs and Employment asked the Health Council whether the present occupational exposure limits for the individual solvents protects against the effects on reproduction.

Since the new Health and Safety Act came into effect in 2007, both the public and private occupational exposure limits should be health based. For only a few solvents a public occupational exposure limit is available (i.e. toluene, xylene, actone, benzene and some ethylene glycolethers). By definition, a health based recommended occupational exposure limit should protect workers against *all* health effects caused by exposure to substances, including the effects on reproduction. Therefore, if available, human and animal data regarding the effects on reproduction are considered in the derivation and recommendation of an occupational exposure limit. In case of the recommendation of occupational exposure limits for solvents, only for a few of them data (mostly experimental data in animals) were available on the effects on reproduction and this was included in the discussion.

Therefore, if effects on reproduction are found in epidemiological studies after exposure to solvents, it should be decided whether the observed effect is a result of exposure above the health based occupational exposure limit or whether the effect is caused by the fact that the occupational exposure limit does not protect against the reproduction toxic effects. This decision is only possible if quantitative exposure data on the individual solvents is available. However, detailed information on the exposure levels is not available in the majority of the studies. Therefore, the committee cannot determine whether the present health based occupational exposure limits also protect against the effect on reproduction.

12.6 Conclusions

There are indications for an association between:

- Occupational exposure to ethylene glycol ethers and reduced male fertility. Effects on male fertility were found in the painting and maintenance trade, but not in the semiconductor industry
- Maternal exposure to ethylene glycol ethers and increased risk of spontaneous abortion and malformations. These effects were found in the semiconductor industry.

In general, occupational exposure to ethylene glycol ethers declined during the past ten years as a result of the replacement of the short chain ethylene glycol ethers by the less toxic long chain ethylene glycol ethers or propylene glycol ethers. Nevertheless, no data are available concerning current exposure levels in the Netherlands. Therefore, the committee cannot exclude that the current occupational exposure levels in the Netherlands still cause effects on male fertility or show an increased risk of spontaneous abortion after maternal exposure.

There are weak indications for an association between:

- Maternal exposure to tetrachloroethylene and increased risk of spontaneous abortion. This effect was observed in dry-cleaning shops
- Maternal or paternal exposure to toluene and increased risk of spontaneous abortion. Increased risks of spontaneous abortion were found in the printing industry, the petrochemical industry, and the painting and maintenance sector. However, the data give no definite proof whether toluene exposure was the cause of the observed effects in these industries
- Maternal exposure to xylene and increased risk of spontaneous abortion. An increased risk of spontaneous abortion was found in the petrochemical industry. However, the data give no indications whether xylene exposure was the cause of the observed effects in these industries.

The committee concludes that the current exposure levels to tetrachloroethylene in the Netherlands are not likely to result in an increased risk of spontaneous abortion. Data on the current exposure levels of toluene and xylene in the Netherlands are not available. Therefore, the committee cannot establish whether the current occupational levels of xylene and toluene would lead to an increased risk of spontaneous abortions.

In addition, the committee concludes that there is a lack of (sufficient) data concerning exposure levels and effects on reproduction for many organic solvents used in occupational settings.

12.7 Recommendations

What measures are recommended to increase the safety of workers or to increase the knowledge of the risks of organic solvents for fertility and for the development of the progeny?

Monitoring of present exposure levels of ethylene glycol ethers, tetrachloroethylene, toluene, and xylene in the Netherlands is needed

The committee concluded that there are (weak) indications that exposure to ethylene glycol ethers, tetrachloroethylene, toluene and xylene is associated with effects on fertility or on the development of the progeny. If a causal relationship does indeed exist, the occurrence of effects depends predominantly on the level and pattern of exposure. Therefore, the committee recommends a two step approach. First, a survey in the Netherlands should be carried out to determine in which type of industry occupational exposure to these solvents occurs. Second, for those situations where exposure to solvents is likely, the committee recommends carrying out a monitoring study to quantify encountered exposure levels of the individual solvents, for instance by performing inhalation and dermal exposure measurements.

Depending on the outcome of this exposure assessment study, it should be decided whether a risk of effects on reproduction exists in the Netherlands at present. As indicated before, the committee has no information to doubt that the present health based occupational exposure limits in the Netherlands protect against all health effects (including reproduction). Therefore, as long as occupational exposure to solvents remains below the health based occupational exposure limit, no effects are expected to occur. If the prevailing exposure levels to these solvents would appear to be close to or above the existing exposure limits, more research into the possible health effects is recommended.

Finally, the committee recommends providing parents-to-be with more information about possible reproductive hazards of occupational exposure to solvents. This information should be given before conception. Therefore, the committee supports the concept of a coordinated program of preconception care.[233]

Occupational exposure data in national database

For most solvents, data concerning possible effects on fertility or on the development of the progeny are lacking. The committee is concerned about the absence of the quantitative exposure data. Consequently, the committee cannot assess whether exposure to solvents in today's working place conditions in the Netherlands pose a risk on reproduction.

Therefore, the committee is of the opinion that routine exposure monitoring in general would be of great value to detect possible occupational risks in the future. The exposure measurements presently performed in the Netherlands, generate company-owned data which are not generally accessible. For this reason, the committee recommends setting up a national exposure database in which occupational exposure measurements should be an integral part. The national databases of the Health and Safety Executive of the United Kingdom (NEDB*) and the BGIA** in Germany (MEGA***) can serve as examples with the Dutch STEAM-base**** as a possible starting point.

More information on reproductive health effects

The committee recommends exploring the possibilities to link the exposure database to detailed information on developmental effects in the progeny. Present registers (the Netherlands Perinatal Register and/or the Eurocat birth defects registry) all have their specific limitations, eg registers may focus on a specific region or may have a limited follow-up period. Therefore, the committee is of the opinion that a national register comprising more extensive and longer term birth and developmental effects would be of great value to facilitate the detection of possible occupational exposure risks on fertility and development of the progeny.

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* NEDB: National Exposure Data Base.
** Berufsgenossenschaftliches Institut für Arbeitsschutz.
*** MEGA: Messdaten zur Exposition gegenüber Gefahrstoffen am Arbeitsplatz (in German).
**** STEAM-base: Stoffenmanager Exposure and Modelling database.

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A Request for advice

B The committee

C Comments on the public draft

D Guidelines of the Dutch Institute for Healthcare Improvement (CBO)

E Results of the quality appraisal

F Human studies on fertility

G Human studies on developmental toxicity

Annexes

Annex **A**

Request for advice

Date of request: March 13, 2001. Reference: ARBO/AMIL/01 14479.

Dear sir,

Various studies indicate that there is a link between working with (aromatic) solvents and reduced fertility. A report to that effect emerged in 1999, resulting in some media consternation as well as questions in parliament. In my answers to these questions (TK 1326, 1998-1999), I indicated that I would ask the Health Council for an advisory report on this problem.

Background

In 1999, the publication of a Dutch study generated concern about the possible harmful effects on fertility and on employees' children of occupational exposure to organic (aromatic) solvents. This study* showed that there was a correlation, in men attending fertility clinics with fertility problems, between the quality of sperm and working with certain organic (aromatic) solvents.

In addition to the study in question, the last decade has also seen international research into the link between working with solvents and reduced fertility, particularly in men. For the time being, the available data does not seem to result in a clear picture. Positive findings in one study are contradicted by negative results in another. Virtually all the studies concern exposure to complex mixtures of substances.

* Occupationally Related Exposures and Male Reproductive Function, E. Tielemans, University of Utrecht, 1999.

There is a shortage of concrete data showing that individual solvents can affect fertility. Recently, your Council's Committee for Compounds Toxic to Reproduction initiated, pursuant to the reports referred to above and at my request, individual assessments of a number of solvents in widespread use. The purpose of these assessments was to determine the toxic effects of the solvents on reproduction (the substances in question were toluene, xylene, styrene, trichloroethylene and tetrachloroethylene) on the basis of the European Union criteria. However, it proved awkward to classify the solvents according to compound on the basis of human data since exposure mainly involves mixtures of these solvents.

The epidemiological reports of possible detrimental effects on fertility as a result of exposure to organic solvents should, as such, be taken seriously. However, combining these reports with toxicological data about individual substances does not, for the time being, point in any definite direction. I would like the Health Council to provide an assessment of the consistencies and inconsistencies in the scientific data and to provide an overview of the gaps and uncertainties it finds in the field in question. If the Health Council finds that there is cause for concern about the link between occupational exposure to solvents and damage to fertility, this could be a reason for extending the current strategy for the reduction of occupational exposure to organic solvents – which is based on the neurotoxic effects of these compounds – to include the risk relating to damage to fertility. As you are aware, the Ministry of Social Affairs and Employment has been engaged in an active approach to the prevention of organic psychosyndrome (OPS) for a number of years now. This occupational disease is caused by occupational exposure to organic solvents. In the context of this prevention policy, sectors in which solvents are used and in which there is a high risk of OPS are now subject to statutory requirements in order to reduce exposure to solvents. The aim of limiting peak exposures in response to your recent advisory report on this issue* fits in with this strategy. A request for an advisory report on this subject has now been submitted to the Social and Economic Council.

The policy of the Ministry of Social Affairs and Employment therefore focuses on the avoiding as far as possible the presence of harmful concentrations of solvents in the workplace. Your advisory report will make it clear whether this strategy is adequate to deal with any existing or suspected threat to fertility from organic solvents or whether additional policy measures are required. An example of the latter is the reevaluation, where necessary, of existing standards for organic solvents which do not include data about the possible toxic effects on reproduction.

Request

I therefore request your Council to formulate answers to the following questions:

1 What is the view of the Health Council with respect to the ‘power’ of the epidemiological link between damage to fertility and occupational exposure to solvents on the basis of the existing scientific literature?

---

* Peak exposures to organic solvents, Health Council, 1999/12.

2 If the Health Council considers an epidemiological link of this kind to be plausible, can this link be explained on the basis of the mechanism of action of the compounds in question? In other words, does the Council find a *causal* link plausible?

3 If an epidemiological or causal link is plausible, is it possible to state with some degree of certainty when a link of this kind is present or absent in the case of specific organic solvents (or groups of solvents)?

4 What are the uncertainties/gaps in scientific knowledge in the answers to questions 1 to 3?

5 In the opinion of the Health Council, what kinds and levels of precautions should these uncertainties result in, given the observed effects? I am thinking in particular of your opinion with respect to the level of protection provided by existing limit values from the point of view of effects on fertility as well as possible gaps in the prevailing system for the classification of individual compounds as toxic for reproduction. This latter issue should be considered in the light of the fact that, in practice, exposure almost exclusively involves mixtures of solvents.

Yours faithfully,
The State Secretary of Social Affairs and Employment,
(signed)
J.F.Hoogervorst

Date of request: March 21, 2005. Reference: ARBO/P&G/2005/17432.

Dear Prof. Knottnerus,

In March 2001, I sent you a letter (ARBO/AMIL01 14479) containing a request for advice with respect to the issue of solvents and fertility.

Recent epidemiological studies* have produced data which strongly suggests that exposure to solvents has additional effects, namely physical and mental abnormalities in painters' offspring. The classification (with regard to reproduction toxicity) of some solvents also pointed in this direction, a finding that was supported by epidemiological studies. I would appreciate it if you would incorporate these effects in the above-mentioned advisory report that is to be dealt with by your council, employing the same five questions that were put forward in connection with the relationship with fertility.

Additional questions:

- researchers have estimated that the average level of exposure is below current MAC values. Do current MAC values offer sufficient protection against effects on offspring? Is there any new information that sheds a different light on the established MAC value? Could continuous low-level exposure account for the observed effects?
- are the identified effects related to peak exposure, as indicated on several occasions by the same researchers with regard to exposure to inhaled anaesthetics?

As previously stated, I would appreciate it if this request for advice could be dealt with as a matter of great urgency. Please send me details of the projected delivery date of this advisory report.

Yours sincerely,
the State Secretary of Social Affairs and Employment
(signed)
(H.A.L. van Hoof)

* Press conference on February 23, 2005, Study by Hooiveld and Roeleveld, Radboud University Nijmegen Medical Centre. Data presented on that occasion. Study report not available, submitted for publication

Annex **B**

The committee

- B.J. Blaauboer, *chairman*
  Toxicologist, Institute for Risk Assessment Sciences, Utrecht
- A.M. Bongers, *advisor*
  Ministry of Social Affairs and Employment, Den Haag
- S. Buitendijk
  Epidemiologist, TNO Quality of life, Leiden
- A. Burdorf
  Epidemiologist, Erasmus MC, Rotterdam
- J.H.J. Copius Peereboom-Stegeman
  Toxicologist, Radboud University Nijmegen Medical Centre, Nijmegen
- H.F.P. Joosten
  Toxicologist, NV Organon, Department of Toxicology and Drug Disposition, Oss
- H. Kromhout
  Professor of exposure assessment and occupational hygiene, Institute for Risk Assessment Sciences, Utrecht
- D. Lindhout
  Professor of Medical Genetics, paediatrician, University Medical Centre, Utrecht
- A.H. Piersma
  Professor of Reproductive and developmental toxicology, National Institute for Public Health and the Environment, Bilthoven

- N. Roeleveld
  Epidemiologist, Radboud University Nijmegen Medical Centre, Nijmegen
- D.H. Waalkens-Berendsen
  Reproductive toxicologist, TNO Quality of Life, Zeist
- P.J.J.M. Weterings
  Toxicologist, Weterings Consultancy BV, Rosmalen
- C.A. Bouwman, *scientific secretary*
  Health Council of the Netherlands, Den Haag
- A.S.A.M. van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag

J.T.J. Stouten, scientific secretary at the Health Council of the Netherlands, prepared a first draft of chapter 6.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public draft

A draft of the present report was released in 2007 for public review. The following organizations and persons have commented on the draft report:

- R. Zumwalde, National Institute for Occupational Safety and Health, USA
- M. Terpstra, Vereniging van verf en drukinktfabrikanten (VVVF), Leidschendam
- M. Korteweg Maris, European Chlorinated Solvent Association (ESCA), Belgium
- M. Sallmén, Finish Institute of Occupational Health, Finland
- J. Waage, FNV Bouw, Woerden
- E. Gonzalez-Fernández, Minsterio de Trabajo y Asuntos Sociales, Spain.

Annex **D**

Guidelines of the Dutch Institute for Healthcare Improvement (CBO)

Assessment of the quality of a patient and control study

Name assessor: ..... Date: .....
Title: .....
Authors: .....
Source: .....

General characteristics

Study design: [ ] Prospective cohort [ ] Retrospective cohort
[ ] Case control [ ] Nested case control
[ ] Cross-sectional [ ] Descriptive
Population: [ ] General population
[ ] Professional, which profession (or professions) …….
Exposure: [ ] Maternal [ ] Paternal [ ] Both
Effect on: [ ] Fertility [ ] Development [ ] Both

Assessment of validity

Brief description of the exposure or prognostic factor (or factors): ……
.....

1 Is the hypothesis clearly focused on a relationship between the solvent (profession) and the reproductive toxic effects (fertility and development)?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

2 Is the patient group clearly and adequately defined?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

3 Is the control group clearly and adequately defined?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

4 Can selection bias be effectively excluded?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

5 Has the exposure been clearly defined and is the exposure assessment method adequate?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

6 Has the exposure blind to disease status been established?
[ ] Yes
[ ] No → will this affect the assessment of exposure?
[ ] Yes
[ ] No

[ ] Too little information in the article to provide an answer

7 Have the main confounders been identified and has this been adequately taken into account in the design of the study or in the analysis?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

Verdict

8 Is the outcome of the study valid and practically applicable?
[ ] Sufficiently valid and applicable
[ ] Questionable
[ ] Insufficiently valid and applicable

Explanatory notes accompanying patient-control study form

Studies involving patients and controls are based on the group of patients that have already developed the outcome in question (such as an illness or an adverse effect of therapy) and a control group from the same population, which has not developed the outcome in question. The exposure to a particular factor (aetiological, prognostic or intervention) is then compared between these two groups, using case history studies or interviews, for example. Patient-control studies are particularly suited to outcomes which are rare and which require a long time to develop. This type of study usually involves less time and effort than a cohort study.

Question 1. Clear research hypothesis

Prior to the study, the researchers should have formulated an hypothesis setting out the relationship between exposure to solvents or a particular profession and possible effects on fertility or development.

Questions 2 and 3. Descriptions of patient group and control group

The definitions of the patient and control groups should incorporate descriptions of their major characteristics. Take particular note of the details of the setting, time period, definition of outcome and the factor to be investigated.

Question 4. Selection bias

Selection bias occurs when exposed patients and non-exposed patients have different probabilities of being included in the experimental group. This could result in significant differences between the experimental groups to be compared, differences that are associated with the ultimate outcomes of the participants. The criteria used to select the groups and the original source population should be clearly described. The experimental groups should adequately reflect the source population. In addition, the control group should be drawn from the same type of population.

It is also important that the response percentage should exceed 50%.

Question 5. Exposure

Exposure should be clearly defined. Details should also be provided of the instrument used to identify the degree of exposure, and of when and under what circumstances this was done. In addition, this exposure should be assessed using valid means.

Question 6. Blinded measurement of exposure

Exposure must be determined in the same way in both groups, independent of disease status. Exposure must be determined independently of any knowledge about the outcome of the participant in question. If different methods are used to measure exposure in each group, this will result in inequality of information (information bias). "Recall bias" is a special form of information bias. It occurs when participants in the patient group (aided by the method of determination) are better able to recall details of their exposure than those in the control group.

Question 7. Confounders

The term ‘confounding’ refers to a situation in which the relationship between exposure and outcome is distorted by another factor, one which is associated (in statistical terms) with both outcome and exposure. Accordingly, observational research will almost always involve an unequal distribution of confounders (prognostic factors or co-interventions) between the groups. Clinical insight should help to assess whether all of the major confounders have been taken into consideration. An indication should be given of which confounders were considered, and of how this was taken into account in the analysis (stratified analysis or multivariate analysis).

Question 8. General opinion

Here, an assessment is required of the study’s validity (Is this a good study?) and applicability (Is this study about my PICO: Patient Intervention Comparison Outcome?). Take careful note of any flaws in the study that undermine its validity (*red flags*, *fatal flaw*s). It is not possible to frame any rules about which items should be scored positively or about the minimum number of items that must achieve a positive score. This is partly dependent on the state-of-the-art" with respect to the topic in question. It comes down to the final judgement concerning what the reviewer wishes to report to the working group regarding the usefulness of the article for decision-making purposes.

Assessment of the quality of a cohort study

Name assessor: ..... Date: .....
Title: .....
Authors: .....
Source: .....

General characteristics

Study design: [ ] Prospective cohort [ ] Retrospective cohort
[ ] Case control [ ] Nested case control
[ ] Cross-sectional [ ] Descriptive

Population: [ ] General population
[ ] Professional, which profession (or professions) …….

Exposure: [ ] Maternal [ ] Paternal [ ] Both

Effect on: [ ] Fertility [ ] Development [ ] Both

Assessment of validity

Brief description of the exposure or prognostic factor (or factors): ……
.....

1 Is the hypothesis clearly focused on a relationship between the solvent (profession) and the reproductive toxic effects (fertility and development)?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

2 Have the exposed groups to be compared been clearly defined?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

3 Can selection bias be effectively excluded?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

4 Has the exposure been clearly defined and is the exposure assessment method adequate?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

5 Has the outcome been clearly defined and is the outcome assessment method adequate?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

6 Has the outcome blind to exposure status been established?
[ ] Yes
[ ] No → will this affect the assessment of outcome?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

7 Can selective loss-to-follow-up be effectively excluded?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer / loss-to-follow-up not described

8 Have the main confounders been identified and has this been adequately taken into account in the design of the study or in the analysis?
[ ] Yes
[ ] No
[ ] Too little information in the article to provide an answer

Verdict

9 Is the outcome of the study valid and practically applicable?
[ ] Sufficiently valid and applicable
[ ] Questionable
[ ] Insufficiently valid and applicable

Explanatory notes accompanying cohort study form

In a cohort study a defined group of patients is followed for a given time and observed to determine whether a given outcome occurs. In addition to questions pertaining to the field of aetiology or that of adverse effects ('Harm'), cohort studies can also involve questions of a prognostic nature. Cohort studies investigate the relationship between a determinant (an aetiological factor, a specific intervention or a prognostic factor) and the outcome. A comparison is made between one group of participants with a given determinant and another group in which it is absent. This assessment list will deal with participants who have been exposed to a particular determinant (an aetiological or a prognostic factor of an intervention) and those who have not.

Cohort studies may be either prospective (the exposure was defined and the participants selected before the outcome occurred) or retrospective (historical cohort study; exposure was examined, mostly in case histories, after the outcome was known). Retrospective cohort studies are more susceptible to bias.

Cohort studies can involve two distinct situations:

- The relationship between a single central determinant and the outcome is examined. This situation occurs in aetiological research or in studies into adverse reactions. Prognostic studies sometimes focus on just a single determinant. In situations of this kind an estimate is made of the strength of the relationship between the determinant and the outcome, which incorporates corrections for a wide range of other possible factors (confounders).
- The relationship between multiple determinants and outcome is investigated. This method is used specifically in prognostic research. On the basis of an estimated prognostic model, the effect of the various determinants on the outcome is investigated.

This distinction is particularly important in assessing question 8.

Question 1. Clear research hypothesis

Prior to the study, the researchers should have formulated an hypothesis setting out the relationship between exposure to solvents and a particular profession and possible effects on fertility or development.

Question 2. Definition of the study groups

The main features of the exposed and non-exposed groups should be clearly described.

Prognostic studies must involve a cohort composed of patients at the same point in the course of their disease (inception cohort).

Question 3. Selection bias

Any attempt to detect selection bias requires that the experimental groups' inclusion and exclusion criteria be clearly defined. One way in which selection bias can arise is if a cohort is assembled retrospectively, when details of the outcome are already known. The decision to include a specific participant might then be influenced by the outcome. As a result, the probability of an "ill" exposed individual being selected for the cohort will differ from the probability of an "ill" non-exposed individual being selected. Selection bias may also arise when only part of the original cohort is selected, on the basis of subconscious prior knowledge about a relationship between certain factors and the outcome, for example. This could result in significant differences between the experimental groups to be compared (in addition to exposure), differences that are associated with ultimate outcomes for which no corrections can be made.

It is also important that the response percentage should exceed 50%.

Question 4. Exposure

Exposure should be clearly defined. Details should also be provided of the instrument used to identify the determinant, and of when and under what circumstances this was done. This should be performed in the same way in each experimental group.

Question 5. Outcome

The outcome itself and the criteria used to determine the outcome should be sufficiently clearly defined to enable the work to be reproduced by other researchers. The outcome should be determined using a valid measurement method.

Question 6. Blinded measurement of outcome

Blinding is achieved by measuring the outcome without any knowledge of the exposure status. Blinding is used to ensure that the outcome measurement is not influenced by knowledge about the exposure in question. If no blinding was used, are there any indications that this knowledge of the exposure status influenced the assessment of the outcome?

Question 7. Selective loss-to-follow-up

There should be no major differences between the experimental groups in terms of drop-out rate. The numbers of subjects dropping out must be reported, along with their reasons for doing so. Even if there were no drop-outs at all, this information must be included. It is not possible to say in advance exactly what drop-out percentage is acceptable, per area of indication. A relatively high drop-out rate renders a cohort study susceptible to selective drop-out. This may be the case if the absolute numbers of individuals dropping out, and their reasons for doing so, differ from one experimental group to another.

Question 8. Confounders

Study into the effect of a single, central determinant:

The term "confounding" refers to the distortion of a relationship between exposure and outcome by another factor, one which is associated with both outcome and exposure. Cohort studies will almost always involve an unequal distribution of confounders between the groups. Clinical insight should provide some assistance in determining whether all of the major confounders have been taken into consideration. An indication should be given of which confounders have been considered, and of how this has been taken into account in the study design and in the analysis. In the design phase, this can be achieved by restriction or matching, and in the analysis phase by stratified analysis or multivariate analysis. If gender, for example, is a potential confounder, then one course of action would be for the study to focus solely on men (restriction), alternatively equal numbers of men could be included in the exposed and non-exposed groups (matching). The analysis can also be retrospectively corrected for "gender" by performing a stratified analysis or a multivariate analysis which incorporates gender.

One example of confounding was when a relationship was found between use of the contraceptive pill and the occurrence of a myocardial infarction. However, it was subsequently found that this relationship might be attributable to the fact that the group using contraceptive pills also included a higher percentage of smokers. When the analysis was corrected for "smoking", there was no longer a detectable relationship between myocardial infarction and use of the contraceptive pill. In this example, smoking (a determinant of myocardial infarction) is a confounder of the relationship between myocardial infarction and use of the contraceptive pill.

Question 9. General opinion

Here, an assessment is required of the study's validity (Is this a good study?) and applicability (Is this study about my PICO: Patient Intervention Comparison Outcome?). Take careful note of any flaws in the study that could undermine its

validity (red flags, fatal flaws). It is not possible to frame any rules about which items should be scored positively or about the minimum number of items that must achieve a positive score. This is partly dependent on the state-of-the-art" with respect to the topic in question. It comes down to the final judgement of what the reviewer wishes to report to the working group concerning the usefulness of the article for decision-making purposes.

Annex **E**

Results of the quality appraisal

Reference	Design	Effect	Validity and applicability
Abadi-Korek 2006	case-control	development	dubious
Agnesi 1997	case-control	development	dubious
Alborg 1990	nested case control	development	valid and applicable
Ali 2004	case-control	development	insufficient validity and applicability
Axelsson 1984	retrospective cohort	fert/develop	dubious
Beaumont 1995	cohort	fertility	dubious
Bell 1991	cross-sectional	development	dubious
Bianchi 1997	case-control	development	insufficient validity and applicability
Blatter 1993	cross-sectional	fertility	insufficient validity and applicability
Bosco, 1987	cross-sectional	fert/develop	insufficient validity and applicability
Bouyer 1998	case-control	fert/develop	dubious
Bouyer 2002	case-control	fertility	insufficient validity and applicability
Bradley 1995	case-control	development	insufficient validity and applicability
Bove 1995	cross-sectional	development	dubious
Brender, 1990	case-control	development	insufficient validity and applicability
Brender 2002	case-control	fert/develop	insufficient validity and applicability
Buckley 1989a	case-control	development	insufficient validity and applicability
Buckley 1989b	case-control	development	insufficient validity and applicability
Buckley 1994	case-control	development	insufficient validity and applicability
Bull 1999	cross-sectional	fertility	insufficient validity and applicability
Chen 2000	prospective cohort	development	valid and applicable
Chen 2002	cross-sectional	fertility	insufficient validity and applicability
Cherry 2001	case-control	fertility	dubious
Chevrier 2006	case-control	development	dubious

Chia 1994	case-control	fertility	insufficient validity and applicability
Chia 1996	cross-sectional	fertility	insufficient validity and applicability
Chia 1997	cross-sectional	fertility	insufficient validity and applicability
Cho 2001	cross-sectional	fertility	dubious
Cordier 1992	case-control	development	insufficient validity and applicability
Cordier 1997	case-control	development	valid and applicable
Correa 1996	retrspective cohort	fert/develop	valid and applicable
Czeizel 2003	cross-sectional	development	insufficient validity and applicability
Czeizel 2004	cross-sectional	development	insufficient validity and applicability
Doyle 1997	retrospective cohort	development	insufficient validity and applicability
El Zein 2002	cross-sectional	development	insufficient validity and applicability
Elliot 1999	nested case control	development	dubious
Eskenazi 1991a	cohort	fertility	dubious
Eskenazi 1991b	cohort	fertility	dubious
Eskenazi 1993	cross-sectional	development	insufficient validity and applicability
Eskenazi 1995a	cohort	development	dubious
Eskenazi 1995b	cohort	fertility	dubious
Fedrick 1976	cohort	development	Insufficient validity and applicability
Ferroni 1992	cross-sectional	fertility	insufficient validity and applicability
Figa-Talamanca 2000	cross-sectional	fertility	dubious
Fixler 1998	cross-sectional	development	insufficient validity and applicability
Forkert 2003	case-control	fertility	insufficient validity and applicability
Funes-Cravioto 1977	cross-sectional	development	insufficient validity and applicability
Garcia 1998	case-control	development	insufficient validity and applicability
Goh 1998	cross-sectional	fertility	insufficient validity and applicability
Gold 1995	cohort	fertility	valid and applicable
Goulet 1991	nested case control	development	insufficient validity and applicability
Gray 1996	retrospective cohort	fert/develop	dubious
Green 2005	cross-sectional	fertility	dubious
Ha 1996	case-control	development	dubious
Ha 2002	prospective cohort	development	dubious
Hanaoka 2002	cross-sectional	fertility	valid and applicable
Heidam 1983	cross-sectional	fert/develop	insufficient validity and applicability
Heidam 1984	cross-sectional	fert/develop	insufficient validity and applicability
Hoglund 1992	retrospective cohort	development	insufficient validity and applicability
Holmberg 1979	case-control	development	insufficient validity and applicability
Holmberg 1980	case-control	development	insufficient validity and applicability
Holmberg 1982	case-control	development	insufficient validity and applicability
Hooiveld 2006	cross-sectional	development	insufficient validity and applicability
Hourani 2000	cross-sectional	development	insufficient validity and applicability
Hruba 1999	cohort	fert/develop	Insufficient validity and applicability
Hsieh 2005	cross-sectional	fertility	insufficient validity and applicability
Huel 1990	case-control	development	Insufficient validity and appicabillity
Ichihara 1999	cross-sectional	fertility	insufficient validity and applicability
Infante-Rivard 2005	case-control	development	valid and applicable

Irgens 2000	cohort	development	Insufficient validity and applicability
Jeiness 1988	cross-sectional	fertility	insufficient validity and applicability
Kallen 1994	retrospective cohort	fert/develop	insufficient validity and applicability
Kardaun 1991	case-control	development	insufficient validity and applicability
KcKean-Cowdin 1998	case-control	development	insufficient validity and applicability
Kenkel 2001	case-control	fertility	insufficient validity and applicability
Kerr 2000	case-control	development	insufficient validity and applicability
Kersemaekers 1997	cohort	development	dubious
Khattak 1999	prospective cohort	development	insufficient validity and applicability
Kim 1996	cross-secional	fertility	insufficient validity and applicability
Koh 1998	prospective cohort	fertility	insufficient validity and applicability
Kolmodin-Hedman 1981	cross-sectional	fertility	insufficient validity and applicability
Kolstad 1999a	case control	fertility	dubious
Kolstad 1999b	case control	fertility	dubious
Kolstad 1999c	cohort	fertility	dubious
Kolstad 2000	cohort	fertility	dubious
Kristensen 1992	cohort	development	dubious
Kristensen 1993	cohort	development	valid and applicable
Kurinczuk 2001	case-control	fertility	insufficient validity and applicability
Kurppa 1983	case-control	development	insufficient validity and applicability
Kyyrönen 1989	case-control	development	dubious
Laslo 2004	prospective cohort	development	dubious
Laumon 1996	case-control	development	insufficient validity and applicability
Lehmann 2002	cross-sectional	development	dubious
Lemasters 1985	cross-sectional	fertility	dubious
Lemasters 1989	retrospective cohort	developent	valid and applicable
Lemasters 1991	cross-sectional	fertility	insufficient validity and applicability
Lemasters 1999	cross-sectional	fertility	valid and applicable
Lindbohm 1983	cross-sectional	development	insufficient validity and applicability
Lindbohm 1984	case-control	development	valid and applicable
Lindbohm 1985	case-control	development	dubious
Lindbohm 1988	cross-sectional	development	insufficient validity and applicability
Lindbohm 1990	case-control	development	dubious
Lindbohm 1991	cross-sectional	development	insufficient validity and applicability
Lipscom 1991	cross sectioneel	development	valid and applicable
Lowengart 1987	case-control	development	valid and applicable
Luderer 1999	prospective cohort	fertility	dubious
Luderer 2004	cross-sectional	development	valid and applicable
Magnussen 2004	cohort	development	valid and applicable
Makowiec-Dabrowska 1998	case-control	development	insufficient validity and applicability
McAbee 1993	cross-sectional	development	dubious
McDonald 1987	retrospective cohort	development	insufficient validity and applicability
McDonald 1987	cross-sectional	development	insufficient validity and applicability
McDonald 1988	cross-sectional	fert/develop	insufficient validity and applicability
Mckinney 2003	case-control	development	insufficient validity and applicability

Multigner 2007	cross-sectional	fertility	valid and applicable
Naccarati 2003	cross-sectional	fertility	insufficient validity and applicability
Ng 1992	cross-sectional	fert/develop	dubious
Ng 1992	cross-sectional	fertility	insufficient validity and applicability
Oliva 2001	cross-sectional	fertility	insufficient validity and applicability
Oliva 2002	cross-sectional	fertility	insufficient validity and applicability
Oliveira 2002	case-control	development	insufficient validity and applicability
Olsen 1991	case-control	development	insufficient validity and applicability
Olsen 1990	nested case control	fert/develop	valid and applicable
Olshan 1999	case-control	development	insufficient validity and applicability
Olshan 1991	case-control	development	insufficient validity and applicability
Park 1997	cross-sectional	fertility	insufficient validity and applicability
Pastides 1988	cross-sectional	development	insufficient validity and applicability
Patel 2004	retrospective cohort	fertility	insufficient validity and applicability
Peters 1981	case-control	development	valid and applicable
Plenge-bonig 1999	cross-sectional	fertility	insufficient validity and applicability
Rachootin 1983	case-control	fertility	insufficient validity and applicability
Rasmussen 1988	cross-sectional	development	insufficient validity and applicability
Rendon 1994	cross-sectional	fertility	insufficient validity and applicability
Reutman 2002	cross-sectional	fertility	valid and applicable
Richardson 1992	case-control	development	insufficient validity and applicability
De Roos 2001	case-control	development	valid and applicable
Samuels 1995	cross-sectional	fertility	dubious
Sallmén 1995	case control	fertility	valid and applicable
Sallmén 1998	case-control	fertility	dubious
Sanjose 1991	prospective cohort	development	insufficient validity and applicability
Savitz 1989	case-control	development	insufficient validity and applicability
Savitz 1996	case-control	development	valid and applicable
Savitz 1997	prospective cohort	development	insufficient validity and applicability
Schaumburg 1992	cross-sectional	fertility	insufficient validity and applicability
Schenker 1995	prospective cohort	development	valid and applicable
Schenker 1996	retrospective cohort	fertility	insufficient validity and applicability
Seidler 1999	prospective cohort	development	dubious
Shaw 1999	case-control	development	dubious
Shaw 2002	case-control	development	insufficient validity and applicability
Shih 2000	cross-sectional	fertility	dubious
Shu 1995	case-control	development	insufficient validity and applicability
Shu 1999	case-control	development	valid and applicable
Shu 2004	case-control	development	valid and applicable
Shusterman 1993	case-control	development	insufficient validity and applicability
Smith 1997	case-control	fertility	insufficient validity and applicability
Smulevich 1999	case-control	development	insufficient validity and applicability
Sonnenfeld 2001	cross-sectional	development	dubious
Strandberg, 1978	case-control	development	insufficient validity and applicability
Stücker 1994	cross sectional	ontwiikkeling	valid and applicable

Svensson 1992	cross-sectional	fertility	insufficient validity and applicability
Swan 1995	cohort	development	valid and applicable
Taskinen 1986	case control	development	dubious
Taskinen 1989	cross sectional	development	valid and applicable
Taskinen 1994	cross sectional	development	valid and applicable
Taskinen 1999	cross sectional	fertility	valid and applicable
Testud 2001	prospective cohort	development	insufficient validity and applicability
Tielemans 1999	case-control	fertility	dubious
Tielemans 2000	cross-sectional	fertility	valid and applicable
Tikkanen 1988	case-control	development	insufficient validity and applicability
Tikkanen 1991	case-control	development	insufficient validity and applicability
Tikkanen 1992	case-control	development	insufficient validity and applicability
Till 2001	prospective cohort	development	dubious
Tilll 2002	prospective cohort	development	dubious
Till 2003	cross-sectional	development	insufficient validity and applicability
Till 2005	prospective cohort	development	dubious
Torchia 1994	cross-sectional	development	insufficient validity and applicability
Torfs 1996	case-control	development	valid and applicable
Tuntiseranee 1998	cross-sectional	fertility	insufficient validity and applicability
Valery 2002	case-control	development	valid and applicable
Vasama-Neuvonen 1999	cross-sectional	fertility	insufficient validity and applicability
Veulemans 1993	case-control	fertility	dubious
Welch 1988	cross-sectional	fertility	dubious
Welch 1991	cross-sectional	fertility	insufficient validity and applicability
Wennborg 2000	cohort	development	valid and applicable
Wennborg 2001	cohort	development	valid and applicable
Wennborg 2002	cohort	development	valid and applicable
Wennborg 2005	cohort	development	valid and applicable
Wilkins 1991	cross-sectioal	development	insufficient validity and applicability
Wilkins 1998	retrospective cohort	development	dubious
Wilson 1998	case-control	development	dubious
Windham 1991	case-control	development	valid and applicable
Xiao 1999	prospective cohort	fertility	dubious
Xiao 2001	prospective cohort	fertility	dubious
Xu 1998	cohort	fertility	insufficient validity and applicability
Xu 2003	cross-sectional	fertility	insufficient validity and applicability
Zhu 2005	prospective cohort	fertility	insufficient validity and applicability
Zhu 2006	prospective cohort	fertility	valid and applicable

Annex **F**

Human studies on fertility

Reference	Study design, population, number	Exposure	(adjusted) Confounders	Effects and risk estimates OR or RR (95% CI)
Cho 2001	Cross-sectional study in petrochemical industry. 1408 female workers seeking childbirth permission or receiving marriage health examination (20-40 years). All working in selected plants (refinery, chemical #1 to #3, synthetic rubber, polyester, fibre and carpet, water treatment and power plants)	Female exposure to benzene, toluene, styrene and/or xylene. Years of exposure based on time spent in a workshop. Three-step procedure : - walk-through by IH - subset of 132 had exposure measured and compared with self-reported exposure and IH assessment (see paper Hu (AJIM 2002). - Interview data in algorithm resulting in benzene, not benzene but other solvents and not-exposed (blind for outcome)	Age, body weight and height, date of marriage, current and past contraceptive use, parity, history of active and passive smoking, presence of indoor coal combustion and cooking oil fumes, alcohol consumption, diet use of herbal medicines, heavy lifting, body position during work, rotating shift work, perceived work stress and physical activities outside work and exposure to other chemicals	Self-reported p*rolonged menstrual cycle length* in year previous to time of enrollment (by interview). OR for 'any exposure to all aromatic solvents' 1.8 (CI 1.1-2.8)). OR for styrene 1.7 (CI 1.1-2.6) OR for xylene 1.6 (CI 1.0-2.5) OR for other individual aromatic solvents (benzene en toluene) were lower and not statistically significant. OR for benzene 1.4 (0.9-2.0) 7% increase in oligomenorrhea per year of exposure to any aromatic solvent (OR 1.1 (CI 1.0-1.1))

De Celis 2000	Experimental study with 48 males at a rubber plant with exposure to hydrocarbons; 42 office workers at the same plant	Exposure to Xylene: ~50 mg/m³; Ethylbenzene ~230 mg/m³; Toluene ~200 mg/m³; Benzene ~40 mg/m³	no data presented	decreased number of exposed persons with "normal" ejaculate (17% vs. 76% in controls); Increase in sperm abnormalities: *abnormal viscosity*: OR 4.0 (95% CI 1,5-10.6) abnormal liquefaction: OR 4.0 (95% CI 1.5-11.4) incomplete sperm count: OR 14.1 (95% CI 3.7-78.7) diminished sperm motility: OR 9.7 (95% CI 3.1-32.9) *diminished normal sperm form*: OR: 27.8
Eskenazi 1991a	Cross-sectional 34 male dry cleaners and laundry workers (exposed) and 48 male laundry workers (unexposed)	Male exposure Based on self-reported job title and tasks (personal interview), exposure index (from 1 to 11) was assigned by industrial hygienist; also measurements of PER in exhaled air: Exposed: 7,893 µg/m³ (1.5-54,949) Unexposed: 77 µg/m³ (0.6-1,562)	Age; quetelet index; ethnicity; religion; years of education; marital status; number of years smoking; for past 3 months: number of cigarettes per day, use of marijuana, amount of alcohol, amount of caffeine, frequency of hot baths, history of fever; history of mumps, alcoholism, prostate or kidney infection, sexually transmitted diseases; abnormalities in physical examination; number of days of abstinence; number of days working in high temperatures (> 80 F)	17 semen parameters determined: *% round sperm*: exposed: 4.1 ± 3.1 (p=0.002) unexposed: 2.5 ±2.1 *% narrow sperm*: exp.: 8.5 ± 8.1 (p=0.02) unexposed.: 12.6 ± 9.5 sperm swimming with greater amplitude of head: exp.: 2.1 ± 0.7 (µm) (p=0.09) unexposed.: 1.8 ± 0.5 (µm) no differences in sperm concentrations, total count, volume or motility between exposed and unexposed workers
Eskenazi 1991b	Cross-sectional Wives of dry cleaners and laundry workers (see Eskenazi 1991a); 17 'exposed' and 32 'unexposed'	Male exposure Mean PER concentrations in exhaled air of husbands: Exposed: 10,245 µg/m³ (range not given; unexposed not given)	Maternal age; ethnicity; alcohol, caffeine, cigarette consumption; quetelet index; frequency of intercourse; years of education; number of days husband worked in high temperatures (> 80 F)	Pregnancy outcomes (reported by the women): Time to pregnancy > 12 months and/or consulted doctor for fertility problem: exposed women 41% (prevalence); unexposed 22% (prevalence); OR = 2.5 (95% CI 0.6-10.9) No differences in number of pregnancies, live births, spontaneous abortions and standardized fertility rations

Eskenazi 1995c	Prospective cohort study: 6 months. Semiconductor Health Study (SHS) 152 female fabrication room workers, 251 female non fab workers at five different US semiconductor companies	Self reported occupation, jobtitle and observation by expert Silicon wafer fabrication room vs.non fabrication room; within fabrication room workers supergroups were distinguished: masking (including photolithography, etching), dopants and thin film (including furnace, thin-film and ion implantation); exposure to ethylene based glycol ethers, fluorides In 'dopants and thin film' supergroup mainly exposure to non-etching fluorides	Age, ethnicity, education, smoking, history of reproductive disease, recent pregnancy, recent oral contraceptive use	Self reported (daily diary of menstrual cycles) plus medical records; urinary analysis FR (fecundability ratio, ie probability of conception per menstrual cycle) fab vs non-fab ranged from 0.59-0.72 (4 different log regression models) for all pregnancies, and from 0.43-0.50 for clinical pregnancies (p = 0.04) Masking, all pregnancies (early fetal loss, abortions, ectopic pregn, live and still births) FR 1.02 (95% CI 0.52-2.02) Masking, clinical pregnancies (all, except early fetal loss) FR 0.67 (95% CI 0.25-1.70) Dopants, all pregnancies FR 0.61 (95% CI 0.27-1.40) Dopants, clinical pregnancies FR 0.84 (95% CI 0.05-0.96) EGE, all pregnancies FR 0.37 (95% CI 0.11-1.19)
Figa Talamanca 2000	Cross-sectional study among workers of a Italian coin-producing Mint. Of the 235 eligible workers, 167 participated (67% of the administrative staff and 72% of the technical manual workers)) Limited statistical power (25%)	Paternal exposure to metal fumes and solvents by face-to-face interviews. Additional information from environmental monitoring. Workers were classified in job categories. Paternal exposure to metal fumes (ie. nickel, chromium, lead) in foundry and solvents (aldehydes, acetone and glycol ethers) in stamping. Exposure assessment rather limited and procedures not clear	Age of partner, smoking and alcohol consumption, of partners at time of the pregnancy, educational level of male partner, whether pregnancy was planned or not Possibly not completely corrected for metal exposure	Self-reported time to pregnancy: Highest pregnancy delay: stampers (21%) and exposed to solvents (21.5) tov administrative (11.8%) and unexposed (12%) Hazard ratio for a delay of more than 6 months 1.7 (0.6-4.6)

Gold 1995	Prospective cohort: one menstrual cycle up to 6 cycles. SHS 152 female fab, 250 female non-fab workers from five semiconductor companies	Self reported, site visits and assessment by industrial hygienist Fab vs non fab 5 working groups : supervisors/engineers, furnace, thin film and ion implantation, photolithography, etching; 4 exposure categories (0-3); exposure to ethylene glycol ethers, fluorides Thin film: also exposure to arsenic compounds; photolithography: also exposure to organic solvents	Age, ethnicity, education, income, body mass index, (passive) smoking, alcohol, marijuana, caffeine, physical activity, age at menarche, gravidity, parity, history of infertility	Menstrual cycle length: self reported by daily diaries plus urine samples Mean usual cycle length (days): fab 28.0 (SD 3.7), non fab 28.9 (SD 3.7), p = 0.02 Crude mean cycle length (MCL), thin film group 34.1 d vs 30.6 d in non fab After adjustment: thin film MCL 36.1 +/- 2.04 d; non fab MCL 32.0 +/- 1.38 d, p=0.017 Mean of log SD cycle length : thin film 6.7 +/- 1.3 d (p=0.013); photolito graphy 5.7 +/- 1.2 d (p=0.019); non fab 4.1 +/- 1.2 d Crude RR for short cycle: Photolithography crude RR 1.63 (95% CI 1.05-2.32), after adjustment no longer significantly elevated No significant differences in mean cycle length, SD of cycle length, % any long cycles, % any short cycles, mean days of bleeding between fab vs non fab No trends with EGE or fluorides exposure
Gray 1996	Retrospective cohort: '80-'90 561 pregnancies of female employees, 589 pregnancies of wives of male employees working in clean rooms of semiconductor manufacturing industry Prospective cohort study 148 women working at IBM for at least 6 months	Female and male exposure; daily self-reported work-related exposures and assessment of ethylene glycol exposure (by the authors?) based on chemicals-process-matrix: high EGE (working with photoresist and in chemical mixing), intermediate EGE (working with combination of photoresist and non-photoresist processes) and unexposed (clean room work without EGE use)	Age, smoking, alcohol use, previous pregnancy and infertility, intercourse during the cycle, conception year, plant	Reproductive outcomes from medical records, daily urinary sampling of hCG and ovarian steroid hormones Subfertility (> 1 year delay) female employees High EGE OR 3.9 (95%CI 1.4-11.4) Intermed EGE OR 1.6 (95% CI 0.7-3.9) No effect was found on subfertility of wives of male employees Rate of conception per 100 cycles EGE exposure in clean room Conc R 13.6, OR 0.9 (95% CI 0.2-3.2) No EGE exposure in clean room Conc R 11.6, OR 0.5 (95% CI 0.2-1.0)
Green 2005	480 Chinese, 494 white women in western USA plant	Job title judged by expert. Organic solvents (xylene, toluene, tetrachloroethylene, trichloroethylene)	Occupational status, age, smoking, education, ethnicity, physical activity, BMI, parity, history of arthritis, marital status	Selfreported menopausal symptoms: Low exposure RR 0.5 (CI 0.2-1.2) High exposure RR 2.5 (CI1.2- 5.6)

Hanaoka 2002	Cross-sectional study among 42 male workers spraying epoxy resin hardening agents including BADGE with mixed organic solvents, and 42 controls who did not use BADGE. Subjects were randomly selected from 1202 assembly workers from the same plant	Bisphenol A diglycidylether, mixed organic solvents (toluene, xylene, 2-ethoxyethanol, 2-butoxyethanol, methyl isobutyl ketone Bisphenol-A (measured in urine)	Matched by age, number of cigarettes per day. For regression metabolites and hormones: age, alcohol, drinking habits	Significant difference of urinary bisphenol A in exposed and controls (average difference 2.5 µmol/mol creatinine, 95% CI 1.4-4.7). Urinary metabolites of solvents were detected more frequently in cases than controls: 2-butoxyacetic acid (62% vs 0%); median concentration 0.6 mmol/mol creat. (range ND-70). Significant difference in FSH concentrations between sprayers and controls (-1.3 ; 95%CI -1.5- -1.0). Significant relationship between FSH and bisphenol A (p=0.045), but not with 2-butoxyacetic acid (p=0.12). No differences in free testosterone or LH concentrations
Kolstad 1999 a/b	Prospective cohort in reinforced plastics industry 21 unexposed man 23 exposed man	Paternal exposure to styrene. Biomonitoring (urine sample; mandelic acid MA)	Abstinence time, spillage during semen sampling, fever	Relative change in: Semen concentration: -28% (sign) Total sperm count: -41% (sign) % normal sperm: -13%)(sign) % non-vital sperm: -55% (sign)* sperm velocity: +18% (sign)* SCSA mean: -1% *opposite trend! No dose-response MA and outcome variables
Kolstad 1999c and 2000	Cross-sectional study in reinforced plastics industry. 382 unexposed man 96 low exposure 69 medium exposure 53 high exposure	Paternal exposure to styrene: Linkage exposure measurements with questionnaire	Maternal age, parity, oral contraceptives, study center and Smoking (maternal and paternal)	Fecundability ratio (time to pregnancy) (Cox regression) FR low exposure: 0.7 (0.5-0.97) FR medium exposure: 0.7 (0.5-1.0) FR high exposure: 1.09 (0.7-1.7)
Lemasters 1985	Retrospective cohort in reinforced plastic industry. 174 exposed and 449 non-exposed	Female exposure. Linkage of expert opinion and detailed exposure measurements Styrene: High exposure: 52 ppm Low exposure: 13 ppm No exposure: 0 ppm (in analyses yes/no)	Age, marital status, parity, smoking, chronic disease	Self-reported effects, 6 months follow up. Severe dysmenorrhea (prevalence 14%): RR 0.9 (0.5-1.5). Intermenstrual bleeding (prevalence 16%): RR 1.1 (0.7-1.8) Secondary amenorrhea (prevalence 7%): RR 0.5 (0.2-1.2) Menstrual blood clots (prevalence 40%): RR 1.1 (0.8-1.6) Hypermenorrhea (prevalence 31%) : RR 1.1 (0.8-1.7)

Lemasters 1999	Cross-sectional study in “newly employed/ exposed” men working in aircraft maintenance at an Air Force Installation (n=50) and 8 unexposed men. 79.5% response!	Personal IH monitoring (including expired breath samples) at 15 and 30 weeks in concordance with semen sample Job categories were used as well. Jet fuel, benzene, 1,1,1,-TCE, MEK, xylenes, toluene and methylenechloride Mean industrial hygiene total solvent exposure, including methylene chloride, MEK, xylenes, toluene and TCA: Sheet metal: 5.9 ppb (0.0-106.9) Paint shop: 2.4 ppb (0.0-16.6) Jet fuel: 1.2 ppb (0.0-25.3) Flight line: 0.46 ppb (0.0-11.8)	Age, race, smoking, having a sexually transmitted disease, alcohol consumption, hot baths and season	Sperm production, structure and function based on three semen samples (baseline, 15 and 30 weeks. Only associations with job group, not with measure external or internal exposure Flight line group: Sign increase (33%) in sperm concentration at week 30. Sheet metal group: Significant decline in sperm length: 2.1% at week 15 and 2.9% at week 30. However, a significant decline was also observed in the unexposed group at week 30. Painter shop: Decline in sperm motility (19.5%) at week 30
Luderer 1999	Experimental study: 9 women in the follicular phase, 9 women in the luteal phase, 9 men	Exposed experimentally to 50 ppm toluene for 3 hours (pure toluene with air)	Women and men were of fertile age. Captive group (regular menstruation)	Subtle effects on LH secretion in men and women in the luteal phase. Clinical relevance is unclear
Luderer 2004	Retrospective cohort in members (male and female) of International Brotherhood of Painters and Allied Trades and the United Brotherhood of Carpenters and Joiners (n=180 were enrolled out of an estimated 624, Age 21-75	Structured occupational history elicited info on jobs, number of years in each job, and time spent in painting-related tasks in each job. Mixed solvents, chlorinated solvents, aromatic solvents, total solvent use (yes/no) Lead concentrations in whole blood	Age, BMI, education	Time-to-pregnancy (by interview): - Hormone levels (LH, FSH) in plasma: Significant effect of log total solvent and log chlorinated solvent on log FSH. Painters and millwrights had a non statistically significant lower probability of pregnancy than the carpenters No relation with exposure Small number of first pregnancies prohibited a more useful and potentially less-biased analysis of only first pregnancies. Very low participation rate

Multigner 2007	Cross-sectional among 48 exposed and 50 non-exposed men employed at the Paris Municipality; period 2000-2001 Response rate: 24%. Non-responder analysis not possible (data from 34% of non-participants)	Exposure to glycol ethers determined by interview; classifying glycol ether containing products into categories; review by industrial hygienist; calculating continuous exposure index; and biological monitoring of six glycol metabolites in urine % of glycol ether containing products per category: 100% of water-proofing; 50% of paints, anti-graffiti, brake fluids, floor coatings; 25% of cleaning agents, hardeners, inks, diluents, oil removers, antifreezes, varnishes; 10% of photographic developers, pesticides, paint strippers, scale removers, disinfectants Urinary metabolites in mg/g creatinine: BAA (from EGBE): non-exposed <dect.lim-0.40; exposed <dl-0.61 2-MPA (from propylene glycol ethers): non-exp <dl-2.5; exp <dl-5.14. No differences in MAA, EAA, PAA and PhAA levels, detected in <40% of the samples	Age, body mass index, alcohol and tobacco consumption, duration of ejaculation abstinence, season of sperm analysis, past history of genital infections	Sperm parameters: Mean concentration (10^{-6}/mL): non-exp 119.1 ; exp 74.0 ($p<0.0001$) ; OR 3.1 (95% CI 0.8-12.5), when compared to WHO semen reference value; no dose response (moderate, high exposure) Count (10^{-6}) : non-exp 416.3 ; exp 277.4 ($p=0.0002$) Rapide progressive motility (%) : non-exp 18.4 ; exp 12.8 ($p=0.0008$) ; OR 4.5 (95% CI 1.3-15.0) ; dose response (mod, high exp) Normal morphology (%) : non-exp 54.2 ; exp 47.1 ($p=0.005$); OR 3.6 (95% CI 1.3-9.7); dose response (mod, high exp) No differences in seminal volume, pH, total progressive motility, multiple anomaly index, viability. Serum hormones: FSH (IU/L): non-exp 3.9; exp 5.5 ($p=0.05$) No differences in testosterone, LH and inhibin B levels. No association between sperm parameters or FSH, and BAA or 2-MPA urine levels
Reutman 2002	Cross-sectional study in 335 eligible women (air force personnel), 170 participated (51%)	Female exposure, self reported and exhaled air measurements (n=63) for hydrocarbons (HC) with a ninefold difference in exhaled breath levels. Aromatic and aliphatic HC among which toluene, xylene, benzene, ethyl benzene. Benzene levels up to 97.5 ppb. Toluene levels up to 52 ppb.	A large series of potential confounders was included in regression models (BMI, smoking, age, race, coffee, caffeine consumption, job srain, illness, fever, etc). Significant covariates ($p<0.05$) remained in the model	Urinary LH, E13G, Pd3G, creatinine levels. Aliphatic and aromatic hydrocarbons: Preovulatory LH levels were significantly lower in high exposed women in a regression analysis (app 7 mLU/mg creatinine difference; $p<0.007$). No effects of toluene on endocrine levels

Sallmén 1995	Case-control study within cohort of women biologically monitored for exposure to organic solvents (styrene, xylene, toluene, trichloroethylene, tetrachloroethylene, 1,1,1,-trichloroethane): 197 women (similar population as in Lindbohm 1990) of whom 105 were exposed during their pregnancy	Female exposure Classification by industrial hygienist based on self-reported work description, solvent usage, and biological measurements: none, low exposure (handling solvents 1-4 days a week without measurements, or handling solvents less than once a week), high exposure (handling solvents 1-4 days a week and measurements indicating exposure)	Spontaneous abortion, infertility in the context of other time-to-pregnancy periods, recent contraceptive use, unplanned pregnancy, caffeine intake	Time to pregnancy (self reported number of menstrual cycles required to become pregnant). Adjusted incidence density ratio (IDR) of clinically recognized pregnancies: Toluene: Low exp: 1.0 (95% CI 0.6-1.5) High exp: 0.7 (95% CI 0.4-1.3) Styrene: Low exp: 0.6 (95% CI 0.3-1.4) High exp: 1.0 (95% CI 0.5-1.9) Tetrachloroethylene Low exposure (n=13): 0.63 (95% CI 0.34-1.17) High exp. (n=7): 0.69 (0.31-1.52) Halogenated hydrocarbons Low exp. (n=33): 1.00 (0.68-1.48) High exp. (n=15): 0.53 (0.29-0.97) ($p<0.05$) Organic solvents Low exp. (n=59): 0.69 (0.48-0.99) ($p<0.05$) High exp. (n=46): 0.41 (0.27-0.62) ($p<0.001$) Reinforced plastic industry Low/high exp: 0.7 (95% CI 0.3-1.3) High exp: 0.6 (95% CI 0.3-1.2) Dry cleaning shop Low or high exp. (n=11): 0.44 (0.22-0.86) ($p<0.05$) High exp. (n=6): 0.57 (0.24-1.34) Shoe factory Low/high exp: 0.3 (95% CI 0.1-0.7) High exp: 0.2 (95% CI 0.05-0.8)
Samuels 1995	Cross-sectional study. SHS 241 wafer fabrication room workers (male), 447 non fabrication (eight companies) Fertility of male semiconductor workers	Self report of job area; fabrication room super groups and working groups: supervisors/ engineers, maintenance, masking (etching and photolithography), dopants and thin film (furnace and thin-film/ion implantation)	Wife's parity, smoking status, age and age squared, employment status; worker's ethnic group, years of education, company site, months of prior fab work, calendar year, number of yrs from most recent birth	Self reported (number of) births Fab/non fab: Fertility rate 0.98 (95% CI 0.80-1.19), $p=0.79$ Dopefilm working group/non fab : period trying to conceive ≥ 1 year 25%, RR 1.79 (95% CI 1.09-2.94), $p=0.04$, and FR 0.73 (95% CI 0.50-1.09), $p=0.12$ Power of study adequate Maintenance workers had a higher (un)adjusted FR than other groups. EGE exposure mainly in masking group

Shih 2000	Cross-sectional study among male and female workers from two factories producing copper clad laminate: 53 exposed, 121 controls Semen study: 14 exposed, 13 controls	Male exposure; self-reported (questionnaire); personal air sampling of ethylene glycol monomethyl ether and biological sampling of urinary methoxyacetic acid Mean air concentrations EGME: 3.98 ppm (SD 2.88, n=55; range 0.65-30.07 ppm) 4.27 ppm (SD 2.19, n=11; range 1.70-20.00 ppm) control group (n=9): range ND-0.28 ppm (1 ppm EGME = 3.16 mg/m^3) Urinary MAA from EGME exposed: 19.95 mg/g creatinine (SD 2.19, n=30) 20.89 mg/g creat (SD 2.19, n=15) control group: 1.26 mg/g creat (SD 1.62, n=32)		Semen analysis: pH exposed: 7.08 (SD 0.29), p=0.005 pH controls: 7.51 (SD 0.43) No effect on volume, sperm count or morphology. (Study was mainly focused on haematological effects)
Tielemans 1999	Male partners of couples with fertility problems n=899. cases and controls based on semen analysis. Different case definitions (lenient, strict, rigid) based on outcomes	Paternal exposure assessed using questionnaires (two phase), plus application of a job exposure matrix, plus urine metabolite assessment in a restricted subset Generic definition of solvent exposure	Age partner, education, hospital, with a stratified analysis (restruction to) primary infertile men	Odds Ratios ranging between 1.14 (0.7-1.7) and 7.3 (1.4-38) dependent on the exposure criterion used in the analysis (any exposure lowest, questionnaires and JEM concordant highest) and the patient group (lenient versus strict and primary infertile men). Strong associations were also observed in the subgroup in which urine metabolites of toluene and xylene were measured, but number were low especially for controls leading to instable estimates. Measurement in urine: All metabolites: OR 7.1 (0.8-60.0) HA/MHA: OR 6.8 (0.8-58.4)
Veulemans 1993	Case-control study among patients of university outpatients clinic for reproductive disorders 1019 cases of clinically diagnosed subfertility or infertility, 475 controls	Male exposure; self reported occupation and chemicals (questionnaire), and classification (by the authors?). Biological monitoring of metabolites ethoxyacetic acid and methoxyacetic acid in urine	Alcohol, smoking ?	In urine of 39 cases and 6 controls EAA was detected: OR 3.11, p=0.004 Highly significant clustering of EAA positive subjects among subcategories of complete azoospermia and severe oligospermia (χ^2 prob 0.0087) Clustering of EAA positive subjects in: paint workers (6 out of 18, p<0.0001); motor mechanics and car repairers (5/29, p=0.0015); wood workers (3/25, p=0.039), or with occupational exposure to paint products (20/32, p<0.0001) and all preparations containing solvents (29/38, p<0.001)

Welch 1988	Cross-sectional study among shipyard painters Fertility study: 94 painters, 55 controls Semen study: 73 painters, 40 controls Response rate 80%	Male exposure; personal monitoring (8 h TWA) for 2-ethoxyethanol and 2-methoxyethanol and biological monitoring of the metabolites in urine. Mean 2-EE: 9.9 mg/m^3 (0-80.5), Mean 2-ME: 2.6 mg/m^3 (0-17.7) Also exposure to other reproductive toxins like lead (45 out of 94 painters), although authors stated that exposure levels were below effect levels	Smoking	Exposed men: Increased semen pH 7.94 ± 0.15 vs 7.88 ± 0.16 in controls, p<0.05; Decreased sperm count per ejaculate: OR 1.85 (95% CI 0.6-5.6) Exposed non-smokers had higher rate of oligospermia (p=0.05) No significant differences in sperm motility, percent motile or velocitiy, viability, morphology, morphometry. No differences in FSH, LH and testosterone serum levels
Wennborg 2001	Retrospective cohort in female biomedical research laboratory workers + non laboratory workers in universities. N=697, 71% respons	(1) Postal questionnaire job + exposure, classified as organic solvents or (2) by industrial hygienist.	Cycle order, age mother, age father, laboratory work father, fertility problems	Time to pregnancy: Laboratory work: FR 1.1 (0.9-1.2) Solvents in general: FR 0.8 (0.7-0.9) Acetone: FR 0.7 (0.5-0.97) Benzene: FR 0.8 (0.4-1.3) Chloroform: FR 1.0 (0.8-1.2) Diethylether: FR 1.1 (0.8-1.5) Phenol: FR 0.9 (0.7-1.2)
Xiao 1999 and 2001	Prospective cohort. Paternal exposure to benzene, toluene and xylene in shoemaking, spray painting and paint manufacturing industry 24 cases (43%) 37 controls (93%)	Measuring mean concentrations in the air. Benzene : 103.34 mg/m^3 (0-7070.3); Toluene: 42.73 mg/m^3 (0-435.8) Xylene: 8.21 mg/m^3 (0-133.1) Biological monitoring from benzene toluene and semen in blood and semen (µmol/L) : Blood: Benzene: 4.40±0.63 Toluene: 1.42±0.30 Xylene: 1.32±0.19 Semen Benzene: 1.85±0.38 Toluene: 0.22±0.19 Xylene: 5.67±0.28	Matched to age and occupation	Sperm activity and acrosin activity were statistically significantly reduced in exposed group. Benzene, toluene and xylene was only detected in the blood and semen of the exposed. Multiple regression showed relationship between liquefaction time and toluene in semen, sperm vitality and work history, sperm motility and work history, sperm concentration and benzene in blood. Sign lower levels of LDH-C4 in exposed. Multiple regression analyses showed relationships with additional variables and semen and blood values
Zhu 2005	Prospective cohort, Danish National Birth Cohort, 1,025 female laboratory workers and 8,037 female teachers	Female exposure, interview during pregnancy, job-exposure-matrix, exposure to organic solvents	Maternal age, gravidity, history of spontaneous abortion, prepregnancy CMI, smoking, alcohol, paternal lab work, sex of child, other substances at work	Medical Birth Register No differences in time to pregnancy

Annex **G**

Human studies on developmental toxicity

Reference	Study design, population, number	Exposure	(adjusted) Confounders	Effects and risk estimates OR or RR (95% CI)
Ahlborg 1990	Case-control study Primary study: female laundry and dry-cleaning workers, with n= 48 or without n=110 adverse pregnancy outcome. Complementary study: 68 cases and 131 referents Response rate: 75-88%	Female exposure, tetrachloroethylene in dry cleaning, self-reported job title or tasks (operating dry cleaning machine, spot removing) and classification (by the authors?) in high (operating machine or spot removing with tetrachloroethylene at least 2 h/w, or ironing/pressing >20 h/w, or cleaning/filling machine >3 times), and low (other work in dry cleaning with tetrachloroethylene) exposure	Smoking, alcohol consumption, medical complications, history of adverse pregnancy outcome	Spontaneous abortion, perinatal death, congenital malformation from medical birth registry Combined studies: Approximately 20% of the cases was high exposed, approx 30% was low exposed, depending on the endpoint studied OR for 'any' tetrachoroethylene exposure during the first trimester of pregnancy: 1.1 (95% CI 0.6-2.0). OR for high or low exposure were comparable
Axelsson 1984	Retrospective cohort in laboratory workers. N=745 (95% response)	Information by postal questionnaire. Exposure to toluene, acetone, xylene, benzene, methylene chloride	Pregnancy number, age, year of pregnancy, previous spontaneous abortion, and shift work	No association between solvents and spontaneous abortion with frequency of type of solvent exposure (RR 1.3 (95% CI 0.9-1.9). However, high potential for misclassification bias

Beaumont 1995	Retrospective cohort: '86-'89. SHS 904 women from 14 semiconductor plants	Ethylene based glycol ethers (primary), etching fluorides (secondary) Relationship between different fab groups and risk of spontaneous abortion Self report/ occupation, job title plus observation by expert; fabrication super groups and working groups: -supervisors/ engineers, maintenance, - masking (etching and photolitho-graphy), -dopants and thin film (furnace and thin-film/ion implantation); 4 exposure categories (level 0-3)	Age, smoking, ethnicity, pregnancy history, stress, income, education	Spontaneous abortion: self report plus medical records Fab vs. non fab 15% vs. 10.4% SAB 17.5% SAB in masking, 22,2% in etching, Fab vs non fab : RR 1.43 (95% CI 0.95-2.09) Masking : RR 1.78 (95% CI 1.17-2.62) Etching : RR 2.08 (95% CI 1.27-3.19) Photo: RR 1.67 (95% CI 1.04-2.55) Dopefilm workers no significantly increased risk Also increased RR for workplace stress: 2.18 (95% CI 1.39-3.26)
Bell 1991	Cross-sectional study ; general population based; 91,302 white singleton births (1976-1987), including 3,850 low birth weights	Female exposure; computer generated air dispersion model for methylene chloride exposure (low, moderate, high) from manufacturing processes of the photographic industry (Monroe county, NY)	Maternal age	Birth weight from birth certificate. High exposure: mean 3396.1 g (SE 16.2); OR 1.00 (95% CI 0.8-1.2) Moderate: 3400.8 g (SE 20.5); OR 1.06 (95% CI 0.9-1.2) Low: 3403.9 g (SE 17.6); OR 1.05 (95% CI 0.9-1.2) None: 3407.7 g (SE 16.3) No statistically significant relation with low birth weight (p=0.79)
Bove 1995	Cross-sectional study, population-based including 80,938 live births and 594 fetal deaths	Drinking water contaminants from 49 water companies (in New Jersey, USA) were monitored from 1985-1988 Tetrachloroethylene (monthly estimate): 26 ppb; 4 exposure categories	Maternal age, race, education; primipara, previous stillbirth or miscarriage, sex of the birth, adequacy of prenatal care, month when prenatal care began, number of prenatal visits	Birth outcome (13 parameters) from birth and fetal death certificates Tetrachloroethylene >10 ppb: Oral cleft defects (4 cases) OR 3.54 (90% CI 1.28-8.78); no dose response relationship Very low birth weight OR 1.49 (50% CI 1.13-1.97) 'all surveillance birth defects' OR 1.14 Tetrachlooethylene >5-10 ppb: Neural tube defect OR 1.16 (50% CI 0.69-1.83) Major cardiac defects OR 1.13

Chen 2000	Prospective cohort in petrochemical industry, 792 female workers (20-40 years) exposed or not exposed to benzene (women exposed to other solvents excluded!)	Three-step procedure : (1) walk-through by IH, (2) subset of 132 had exposure measured and compared with self-reported exposure and IH assessment (see paper Hu (AJIM 2002). (3) (trained) Interview data in algorithm resulting in benzene, not benzene but other solvents and not-exposed (blind for outcome) TWA benzene ranging from 0.17 ppm rubber plant to 0.191 ppm in chemical plant ; overall mean 0.033 ppm, styrene, toluene and xylene all below 1 ppm	Age, BMI, education, parity, sex child, noise, physical exertion and exposure to other hazards	Birth weight as measured in delivery room. Adjusted mean birth weight non exposed: 3445 gram (CI 3401-3489). Overall mean birth weight for the low risk population is 3427 (sd 441) gram. Benzene group 82 grams lighter and gestational age 0.2 week shorter, after adjustment this went down to 15 grams lower (-85 to 52). Only an interaction with stress resulted in a significant effect of –149 gram (-296 to -1). However, this group was relatively small (n=57). Exposure to other hazards were not significantly associated with birth weight
Chevrier 2006	Case-control study 164 cases of cleft lip (and palate) and 76 cases of cleft palate from surgery departments, 236 hospital controls	Female exposure; self-reported (questionnaire) occupation and tasks, exposure classification (first trimester of pregnancy) by expert chemist	Location hospital, child's sex, mother's geographic origin	Cleft lip (and palate) total glycol ethers OR 1.88 (95% CI 1.1-3.5) very low glyc eth OR 1.30 (95% CI 0.5-3.1) low OR 1.88 (95% CI 0.9-3.8) medium-high (7 cases) OR 4.77 (95% CI 1.2-19.5) p trend<0.01 Cleft palate total glycol ethers OR 1.5 (95% CI 0.7-3.2) very low glyc eth OR 0.40 (95% CI 0.1-1.9) low OR 1.83 (95% CI 0.8-4.4) medium-high OR 4.06 (95% CI 0.8-20.3) p trend =0.06 Occupations of mothers with medium-high glycol ether exposure: sales worker, cleaning worker, hairdresser/beautician Majority of women exposed to glycol ethers was also exposed to aliphatic alcohols or other types of oxygenated solvents Exposure to glycol ethers separately: Cleft lip (and palate) OR 1.33 (95% 0.7-2.5) Cleft palate OR 1.38 (95% CI 0.6-3.0)

Cordier 1997	Case-control study 984 cases (50%), 1134 controls; European collaborative multicenter study (see Ha 1996)	Female exposure: selfreported job history. Evaluation of job description by industrial hygienist and classification of 314 exposures. Exposure to glycol ethers during first trimester. Not exposed vs. exposed, but also categories of exposure levels, frequency, and reliability	Center, maternal age, social economic status, residential area, country of origin	6 EUROCAT registries for major congenital malformations, 22 subgroups identified First trimester exposure to glycol ethers: Cleft lip/palate: 23 cases and 137 controls, OR 1.97 (1.20-3.25) Overall exposure first trimester for mothers of children with cleft lip/palate: Very low OR 1.35 (95% CI 0.51-3.56), low 3.06 (95% CI 1.36-6.92), medium to high 1.80 (95% CI 0.68-4.75). Small number of cases per exposure group. Central nervous system: 40 cases exposed and 140 controls, OR 1.91 (1.23-2.96); subgroup spina bifida OR 2.37 (1.22-4.62) Small number of exposed cases
Correa 1996	Retrospective cohort Workers from 2 semi conductor plants in Eastern USA: 1150 pregnancies; 561 maternal and 589 paternal (wives of male employees) Follow up: 10 yrs	Ethylene glycol ethers and acetates (i.e. diethylene glycol dimethyl ether, ethylene glycol monoethyl ether acetate) Selfreported job area, shifts, processes and tools (chemicals or instruments). Verified with personnel record and with assistance of plant industrial hygienists. Matrices of plant processes and chemicals in combination with exposure during critical periods; resulted in low, medium, high group	Yr of conception, age at conception, order of index pregnancy, previous spontaneous abortions, yrs of education, race, smoking, alcohol use, study plant	Spontaneous abortion: self report and medical records Maternal: 33.3% spont abortion in high exposed vs. 14.8 in non-exposed; 27.3, 13.3 and 13.3% subfertile in high, medium, low exposed vs. 9.2 in non-exposed Paternal: 14.3, 14.5 and 11.8% subfertile in high, medium, low exposed vs 9.8 in non-exposed Maternal: Spont abort (high exp): RR 2.8 (CI 1.4-5.6) Subfert (high): OR 4.6 (CI 1.6-13.3) Paternal exposure: no effect on SA, possibly increased risk on subfertility (not sign). No association with exposure to n-butyl acetate, xylene, n-methyl-2-pyrrolidine. Recall of process-level information was with acceptable levels of reliability
Doyle 1997	Retrospective cohort study (past and current workers): 2,711 dry cleaning; 399 laundry workers Follow up: max 15 years	Female exposure; dry cleaning; laundry workers in the companies surveyed were not exposed to tetrachloroethylene Self-reported workplace and job-title (operator / non-operator)	Maternal age, pregnancy order, year of birth	Fetal death (<20 wk): self-reported and partly verified from medical records Dry cleaning vs laundry: OR all pregnancies 0.97 (95% CI 0.55-1.69) OR first pregn 1.03 (95% CI 0.48-2.21) OR last pregn 1.28 (95% CI 0.43-3.81) Dry cleaning operator vs non-operator: OR all pregn 1.63 (95% CI 1.01-2.66), p=0.04 OR first pregn 1.51 (95% CI 0.81-2.84) OR last pregn 1.72 (95% CI 0.74-4.01)
Elliott 1999	Case-control study 36 cases, 80 controls	Maternal, self reported job title, qualitative exposure assessment by case-by-case expert Ranking low, medium, high based on likely exposure assessment based on site visits	Maternal age at conception, smoking, alcohol, lifting, bending, stress	Spontaneous abortion, self reported and ascertained by GP. 2 cases and 10 controls exposed to EGE in 1st trimester. OR [95%CI] = 0.45 [0.05-3.89] Small study in UK

Eskenazi 1995	Prospective cohort: 6 months. SHS 152 fab, 251 non fab female employees	Self report/occupation, jobtitle plus observation by expert Fab vs.non fab. Fab: mask, etch, dopant, thin film; Ethylene based glycol ethers Fab vs. non-fab, risk of reduced fecundability (SAB and early fetal losses)	Age, ethnicity, education, smoking, gravidity, household income, prior SAB	Spontaneous abortion: self report plus medical records, urinary analysis SAB in 63% of FAB, 46% of non-FAB RR 1.25 (CI 0.63-1.76) Similar RR for dopant or thin-film
Gray 1996	Retrospective cohort: '80-'90 561 pregnancies of female employees, 589 pregnancies of wives of male employees working in clean rooms of semiconductor manufacturing industry Prospective cohort: 148 women working at IBM for at least 6 months	Female and male exposure; self-reported (questionnaire) area of the plant and work processes, and exposure assessment (by the authors?) based on chemicals-process-matrix. Exposure to EGE (ethylene glycol monoethyl ether and its acetate, diethylene glycol dimethyl ether): high EGE (working with photoresist and in chemical mixing), intermediate EGE (working with combination of photoresist and non-photoresist processes) and unexposed (clean room work without EGE use)	Conception year, age, plant	Self-reported reproductive health, partly verified by medical records. Spontaneous abortion in female employees: High EGE: 33.3%, control: 14.8% RR 2.8 (95% CI 1.4-5.6) Intermediate EGE: 18.9% RR 1.4 (95% CI 0.8-2.6) Wives of male employees : RRs ≤ 1 Pregnancy loss of female workers: EGE exposure in clean room: rate 66.6, no EGE exp in clean room: rate 55.3, non-clean room work: rate 43.8 EGE exp: OR 2.5 (95% CI 0.8-8.5) No EGE, clean room: OR 1.7 (95% CI 0.7-4.3)
Ha 1996	Case-control study 991 cases (abortions because of malformations), 1144 controls, European collaborative multicenter study (see Cordier 1997)	Female exposure; selfreported job history, exposure assessment by industrial hygienist. Exposure to glycol ethers, month before conception and trimesters of pregnancy. Four classes of glycol ethers: 1) 2-methoxy- and 2-ethoxyethanol and acetates, polyethylenic compounds; 2) 2-butoxy- and propoxyethanol and acetates; 3) methoxypropanol and acetates, polyethylenic and propylenic compounds; 4) other glycol ethers. Exposure occurred most often to 2-butoxyethanol and propoxyethanol and acetates and methoxypropanol and acetates and polyethylenic and propylenic compounds	SES of mother and study center	6 EUROCAT registries on major congenital malformations: cardiac; musculoskeletal; central nervous system; oral clefts, digestive anomalies, genital and urinary. Exposure had occurred in 26% of the job titles (averaged over cases and controls) Significant associations for oral cleft OR 2 (95% CI 1.1-4.1) ; central nervous system malformations OR 1.8 (95% CI 1.1-3.3) ; Muskuloskeletal malformations OR 1.6 (95% CI 0.9-2.8) Only (borderline) statistically significant associations are given, detailed presentation of numbers in categories was not present. Women were exposed to class 2 and 3 glycol ethers that are not teratogenic in experimental animals

Ha 2002	Prospective cohort in 1222 female employees of BYPC who had a live birth at BYPC staff hospital between May 1996 and December 1998 (same population as Chen!, but now including women exposed to other solvents than benzene)	Exposed or unexposed to benzene, toluene, styrene and or xylene based on self-report (see also Chen 2000) Approximately only 10% exposed	Parental age, BMI, weight, height, education, infant gender, alcohol consumption, passive smoking	Birth weight as measured in delivery room. Women in organic solvents groups 81.6 gram lower (p=0.042) exposed fathers showed no effect. No association with styrene alone
Infante-Rivard 2005	Case-control study, population-based; 790 cases and 790 controls	Female exposure; self reported industry, job-title, task, chemicals; exposure coding by chemists and industrial hygienists. Also home exposure to solvents self reported. Solvents : 13 specific chemicals, 4 mixtures, 6 chemical families	Maternal age, level of schooling	Childhood acute lymphoblastic leukemia from hospital records Maternal exposure to tetrachloroethylene: '2 years before pregnancy up to birth' Ratio Discordant Pairs = 11:11 OR 0.96 (95% CI 0.41-2.25) 'during pregnancy' RDP = 7:8 OR 0.84 (95% CI 0.30-2.34) Maternal exposure to solvents: '2 years before pregnancy up to birth' Ratio Discordant Pairs = 154:141 OR 1.09 (95% CI 0.87-1.38) 'during pregnancy ' RDP = 125:125 OR 1.00 (95% CI 0.78-1.28) Maternal exposure to toluene (both before and during pregnancy), mineral spirits, alkanes and MAH resulted in increased OR's (CI >1)
Kersemaekers 1997	Retrospective cohort study, 9,000 hairdressers, 9,000 age-matched clothing salesclerks; two conception periods: 1986-1988, 1991-1993	Female exposure; self reported; occupation identified through database of trade association for service jobs; methylene chloride and dye formulations, use was limited or banned in 1990	Educational level, maternal age at conception and gravidity. Crude effect measures presented, because difference with adjusted was less than 10%	Self reported spontaneous abortion, live births, congenital malformations Hairdressers, conception between 1986-1988: time to pregnancy >12 mo: OR 1.5 (95% CI 0.8-1.6); spontaneous abortion (<20 w): OR 1.6 (95% CI 1.0-2.4); low birth weight (≤2500 g) OR 1.5 (95% CI 0.7-3.1); prematurity (<37 w): OR 0.5 (95% CI 0.1-2.2); major structural malformations: OR 1.6 (95% CI 0.3-8.4) Hairdressers, conception between 1991-1993: time to pregnancy >12 mo: OR 1.2 (95% CI 0.8-1.6); spontaneous abortion: OR 0.9 (95% CI 0.7-1.1); low birth weight: OR 1.2 (95% CI 0.8-1.9); prematurity: OR 1.20 (95% CI 0.8-1.4); major structural malformations: OR 1.9 (95% CI 0.5-6.9)

Kristensen 1992	Retrospective cohort, record linkage, 12,440 children born between 1950-1987 from 10,992 members of Oslo unions of printers (1930- 1974, and alive at 1960 census)	Paternal exposure, lead, solvents, based on employment description in union record, each worker was a priori categorized into one of four groups; lead, solvents, both, other exposures	Maternal age, year of birth, sex and birth order	Childhood cancer, 33 cases from registry Solvents: SIR 0.7 (95% CI 0.02-3.3); Other categories: SIR not significant, range from 0.25 to 1.36
Kristensen 1993	Retrospective cohort, see Kristensen 1992	Paternal exposure, see Kristensen 1992	See Kristensen 1992	Early preterm births (n=39): solvents OR 5.4 (95% CI 1.7-17.4), Lead and solvents OR 8.6 (95% CI 2.7-27.3); Late abortion (n=17): solvents OR 5.5 (95 CI 1.8-17.2); Small for gestational age: solvents SMR 1.4 (1.1-1.8); All birth defects (n=246): lead categories SMR 4.1 (1.8-8.1)
Kyyrönen 1989	Case-control study Spontaneous abortion study : 243 cases and 680 controls. Malformation study : 32 cases, 153 controls Response rate: 77-81%	Female exposure, tetrachloroethylene in dry cleaning, self reported and classification of work tasks (by the authors?) in high (work tasks included dry cleaning for at least one hour daily on average, or reporting handling tetrachoroethylene al least once a week) and low exposure (work tasks including pressing at a dry cleaners' or spot removing. Or reporting handling tetrachoroethylene less than once a week.) 7 study objects with blood measurements of tetrachloroethylene as a 'validation'	Smoking, alcohol, working during pregnancy, temperature of the workplace ≥ 24°C, febrile disease, nulliparity, exposure to other solvents	Spontaneous abortion, congential malformation (between 1973-1983) from Finnish hospital discharge register and medical registers Spontaneous abortion: 23% of cases (n=9) had high exposure to tetrachloroethylene vs 9% of the controls (n=6): OR 3.4 (95% 1.0-11.2), p<0.05 Congenital malfomations: 50% of cases (n=2) had low exposure to tetrachloroethylene vs 36 % of controls (n=8): OR 0.8 (95% CI 0.2-3.5); there were no cases with high exposure. Handling of other solvents (acetone, thinner, spot remover) with congenital malformations (n=4): OR 5.9 (95% 1.0-35.7), p<0.05
Lemasters 1989	Retrospective cohort in reinforced plastics industry 229 exposed live, single births 819 not exposed live, single births	Maternal exposure: Linkage expert opinion and detailed exposure measurements. Major occupational exposure styrene : (1) High 30+ ppm Low exp < 30 ppm No exp = 0 (2) cumulative exp (3) 2 with time-windows Also exposure to methylene chloride, acetone, methyl ethyl ketone, toluene and xylene. 'Highest exposure to styrene generally had the highest exposure to other substances'	Age, marital status, parity, smoking, chronic disease, drinking, time-to-pregnancy, prior abortion, family income, prior birth weight, sex of offspring, race and weeks of gestation.	Birth weight in grams (by self-reported interview): High exposure: -4% (-7.7 to +0.6) Low exposure : -1% (-3.6 to +1.8)* *(calculated from regression model)

Lindbohm 1984	Cross-sectional study among 68,327 pregnancies including 4,896 cases of spontaneous abortion	Female and male exposure; self reported (questionnaire), and grouping in 7 categories by industrial hygienist	Age, place of residence, parity, marital status, other exposures	Spontaneous abortion (between 1973-1976) from Finnish hospital discharge register Maternal exposure: Solvents OR 0.79 (95% CI 0.58-1.07) Laundry workers OR 1.48 (95% CI 1.09-2.02), p<0.05 Paternal exposure: Solvents OR 0.86 (95% CI 0.69-1.08)
Lindbohm 1985	Nested case control in plastics industry 44 cases 123 controls	Maternal exposure to Styrene plastics, Polyvinyl-chloride, and Poly-urethane. Expert opinion (occupational physician & researchers)	Compounds for each other. Matching on age at start pregnancy	Spontaneous abortion (from hospital discharge register) *Processing*: Styrene 0.4 (0.1-1.2) Pvc 1.1 (0.3-4.2) Poly-urethane 3.0 (1.2-7.8) *Heating*: Styrene 0.6 (0.2-2.3) Pvc 1.2 (0.3-5.4)
Lindbohm 1990	Nested case-control study in women ever monitored for organic solvents 73 cases, 167 controls Response rate 85.5%	Female exposure; self reported (questionnaire) work task and solvents, and classification of exposure (by the authors) as unexposed, potentially exposed or exposed, and three exposure levels (high, low, none) Maternal exposure (low/ medium/ high/frequent) to: Aromatic hydrocarb Styrene, Toluene, Xylene Halogenated hydroc Tetrachloroethylene Trichloroethylene 1,1,1Trichloroethane, aliphatic hydrocarb. Total solvents	Matching on age at start pregnancy, previous spontaneous abortion, parity, smoking, alcohol, exposure to other solvents	Spontaneous abortion (between 1973-1983) from Finnish register Aromatic hydrocarbons OR 1.6 (95% CI 0.8-3.3) Styrene 0.3 (0.1-1.0) Toluene 1.6 (0.7-3.8) Xylene 1.3 (0.4-4.5) Halogenated hydrocarb 1.4 (0.6-3.2) Tetrachlorethylene 1.4 (0.5-4.2) Trichloroethylene 0.6 (0.2-2.3) Trichloroethane 3.4 (0.7-16.9) Aliphatic hydrocarb 2.1 (0.9-5.1) Solvents-total 2.2 (1.2-4.1) (p=0.01) Aliphatic-high 3.9 (1.1-14.2) Graphic work 5.2 (1.3-20.8) Shoe work 9.3 (1.0-84.7)
Magnusson 2004	Retrospective cohort (1970-1989), biomedical research lab and non-lab workers in universities (n=4,170), see Wennborg 2002	Male exposure, exposure information from employee records and postal questionnaires to the head of department and exposure in 5 year period to several compounds including solvents	Age mother, previous spontaneous abortion, pregnancy number, smoking	Swedish Medical Birth Register and Register of Congenital Malformations Major malformations OR 1.3 (95% CI 0.8-2.1), neural crest malformations OR 1.1 (95% CI 0.4-2.7)

Ng 1992	309 women (90%) of whom 55 were married and worked in the relevant departments of audiospeaker factory with toluene exposure	Exposure information is limited and not described in detail. Low exposure 0-25 ppm. High exposure mean 88 (range 50-150 ppm). Toluene, other agents might have been present, but this is not clear from the paper (toluene was a glue compound)	Age and gravidity	Spontaneous abortions/pregancies : *High exposure*: Before employment 2/32 After employment 11/73 (p not sign) *Low exposure*: Before employment: 0/38 After employment: 2/30 (p not sign) *All exposure* : Before employment: 2/70 After employment: 13/103 (p = 0.020) High exposure OR (versus low exp): 4.8 (95% CI 1.0-22.9) OR (versus no exp): 2.8 (95% CI 1.3-5.9)
Olson 1990	Case-control study combining several studies: total of 215 cases, 558 controls Sweden: 47 cases, 79 controls; Denmark: 12/131; Finland: 142/320; Norway: 14/28. Response rate 77%-81.1%	Female exposure; dry cleaning; self reported or reported by employers and classification by industrial hygienist in high (dry cleaning work and spot removing at least 1 h/d) or low (work in dry cleaning but not in high exposure group)	Parity, smoking and drinking habits	Birth weight less than 1500 g, congenital malformation (specified), spontaneous abortion, still birth from hospital and medical registers All reproductive failure: OR low exposure: 1.23 (95% CI 0.81-1.87) OR high exp (9 cases): 2.95 (95% CI 1.14-7.65) Spontaneous abortion: OR low exposure: 1.17 (95% CI 0.74-1.85) OR high exp (8 cases): 2.88 (95% CI 0.98-8.44) OR high exp Finland (6 cases): 4.53 (95% CI 1.11-18.5) *Congenital malformations, still birth and low birth weight:* OR low exposure: 1.72 (95% CI 0.40-7.12) OR high exp: 0.87 (95% CI 0.20-3.69)
De Roos 2001	Case-control study Female exposure: 538 cases and 504 controls; Male exposure: 405 cases and 302 controls	Female or male exposure Halogenated hydrocarbons and volatile hydrocarbons Self reported occupation, job title, chemicals or review by industrial hygienist	Child age, demographic covariates, paternal co-occurrence in exposure	Neuroblastoma from hospital records Self reported, maternal exposure: 5%-10% exposed cases Halogenated hydrocarbons OR 0.7 (95% CI 0.4-1.5) Self reported, paternal exposure: 15%-40% exposed cases Halogenated hydrocarbons OR 1.2 (95% CI 0.-1.7) Tetrachloroethylene OR 0.5 (95% CI 0.2-1.4) Industrial hygienist, maternal exposure: 1%-5% exposed cases Halogenated hydrocarbons OR 0.7 (95% CI 0.2- 2.1) Industrial hygienist, paternal exposure: 10%-30% exposed cases Halogenated hydrocarbons OR 0.9 (95% CI 0.5-1.5) Tetrachloroethylene OR 0.5 (95% CI 0.1-1.7)

Schenker 1995	Retrospective cohort: ’86-‘89 Prospective cohort: ’89-‘91 Semiconductor Health Study 904 women (historical), 403 (prospective) from 14 semiconductor plants	Ethylene based glycol ethers (primary), etching fluorides (secondary) Fabrication room work associated with increased risk of spontaneous abortion Self report/occupation, jobtitle plus assessment by industrial hygienist: 1) silicon-wafer fabrication room (fab), non fab; 2) super or work groups based on process and equipment; 3) exposure to chemical, physical and ergonomic agents	Ethnicity, education, smoking, income, previous pregnancies	Spontaneous abortion: self report plus medical records Fab vs. non fab RR of SAB 1.43 (95% CI 0.95-2.09) retrosp 1.25 (95% CI 0.63-1.76) prosp Masking RR: 1.78 (95% CI 1.17-2.62) retrosp 1.30 (95% CI 0.59-1.84) prosp Dopefilm workers no significantly increased risk
Shu 1999	Case-control study 1842 cases of acute lymphocytic leukemia (ALL) and 1986 controls	Female or male exposure Self-reported job title, industry, duties, chemicals: ‘solvents, degreasers or cleaning agents’	Maternal education, race, family income	ALL from hospital records Maternal: 7.5 % cases exposed to one of the solvents Paternal: 33 % cases exposed to one of the solvents Exposed ‘anytime’ to tetrachloroethylene: Maternal (4 cases): OR 0.4 (95% CI 0.1-1.4) Paternal (25 cases): OR 0.9 (95% CI 0.5-1.6) Exposed ‘anytime’ to chlorinated solvents: Maternal: OR 3.5 (95% CI 0.6-18.9) Paternal: OR 0.8 (95% CI 0.4-1.6) Exposed ‘anytime’ to ‘possible organic solvents’: Maternal: OR 1.3 (95% CI 0.9 – 1.9) Paternal: OR 1.0 (95% CI 0.8 – 1.3) Exposed ‘anytime’ to ‘all solvents’: Maternal: OR 1.3 (95% CI 1.0 – 1.7) Paternal: OR 1.0 (95% CI 0.9 – 1.2) Small non significant effects for specific solvents (CCl_4, TriCE) *Maternal exposure during pregnancy to turpentine, paint remover or paint thinner* OR’s sign increased (3.5, 5.2, 3.3 resp). *Paternal exposure during and after pregnancy to turpentine* OR (1.7, 1.5 resp) increased

Sonnenfeld 2001	Cross-sectional study, population-based including 11,798 birth certificates.	Tetrachloroethylene concentrations in drinking water in US Marine Corps Base (North Carolina): 10-1580 ppb	Infant's sex, year of birth; mother's race, age, educational level, parity, adequacy of prenatal care, marital status, history of fetal death; father's age, educational level, military pay grade	Mean birth weight, small-for-gestational-age, preterm birth (<37 w) from birth records Tetrachloroethylene exposed: Mean difference in birth weight, all births – 26 g (90% CI -43, -9); mother's age ≥35 yrs -130 g (90%CI, -236, -23); mother's ≥2 previous fetal losses -104 g (90% CI 174,-34) SGA, all births OR 1.2 (90%CI 1.0-1.3); mother's age ≥35 yrs OR 2.1 (90% CI 0.9-4.9); mother's ≥2 previous fetal losses OR 2.5 (90% CI 1.5-4.3) Preterm birth OR 1.0 (90% CI 0.9-1.1) No obvious pattern with duration of exposure for these outcomes
Stücker 1994	Retrospective cohort in two organic chemocil factories in the Rhones Alpes region of France. All married men under 45 years (n= 823 out of 1077)	Job histories covering all jobs were provided by the personnel departments Benzene exposure was based on jobs which were assigned to three levels of benzene exposure (0 ppm), low (1-5 ppm) and moderate (>5 ppm) by occupational physicians	Age, pregnancy order, tobacco consumption	Self reported spontaneous abortion: 1) Group exposed at any time before conception RR = 1.1 (0.7-1.8). 2) Group exposed during the three months immediately before conception RR=0.9 (0.4-1.7). No data are available on the reliability of the exposure assessment method
Swan 1995	Retrospective cohort: '86-'89. SHS 891 women from 14 semiconductor plants	Relationship between occupational exposures in semiconductor manufacturing and SAB risk; agent specific Selfreport job title plus assessment by industrial hygienist; for super groups and working groups, see Beaumont et al.; 4 subgroups of chemicals: -PDS (EGE, PGE, xylene, n-butyl acetate), exposure occurring in masking (photolithography, etching); -fluorides (etch and non-etch), exposure occurring in masking (etching) and dopants (furnace); -cleaning solvents (methanol, acetone, isopropyl alcohol), exposure occurring in all working groups; -dopants (arsenic, antimony, boron, phosphorus), exposure occurring in dopants (furnace); 4 exposure categories	Age, smoking, ethnicity, pregnancy history, stress, income, education	Spontaneous abortion: self report plus medical records EGE-exposed all levels, all women: 18.4%, RR 1.56 (95% CI 1.02-2.31); masking women: RR 2.54 (95% CI 1.41-4.12) EGE-exposed level 2-3, all women: RR 2.40 (95% CI 1.24-4.11); masking women: RR 3.38 (95% CI 1.61-5.37) PGE-exposed all levels, all women: 18.8%, RR 1.39 (95% CI 0.76-2.37); masking women: RR 1.27 (95% CI 0.65-2.35). PGE were used less often (<50%) than EGE. Mask, high level xylene: Adj RR 2.72 (CI 1.51-4.37) Mask, high level nBA : Adj RR 2.05 (CI 1.42-5.35) Dose response EGE, xylene and nBA; Xylene and nBA results may reflect EGE exposure; no elevated risk from cleaning solvents only High stressed women had higher SAB rates in almost all working groups: etching RR 5.3 (95% CI 2.7-7.7); photo RR 3.7 (95% CI 1.9-5.9); furnace RR 3.7 (95% CI 1.5-6.6). Low stressed women: etching RR 1.75 (95% CI 1.0-2.8)

Taskinen 1986	Nested case-control in female pharmaceutical industry personnel: 44 cases, 130 controls	Maternal exposure: Expert opinion (occupational physician & researchers) and exposure index based on frequency of solvents. Total solvents, alicyclic hydrocarbons, aliphatic hydrocarbons, benzene, toluene, xylene, chloroform, methylene chloride and other solvents.	Matching on age at start pregnancy	Spontaneous abortion from hospital discharge register (1973-1981): total solvents: OR 1.5 (95% CI 0.7-3.5) ≥ 4 solvents: OR 3.5 (95% CI 1.0-12.4) Alicyclic hyd: OR 2.5 (0.6-9.6) Aliphatic hyd: OR 3.2 (0.7-13.8) Benzene: OR 2.4 (0.5-12.0) Toluene: OR 1.6 (0.6-4.5) Xylene: OR 2.0 (0.4-10.6) Chloroform: OR 1.6 (0.6-4.2) other: OR 1.6 (0.6-4.2) Methylene chloride: OR 2.3 (95% CI 1.0-5.7) < once a week, 5 cases: OR 2.0 (95% CI 0.6-6.6) ≥ once a week, 6 cases: OR 2.8 (95% CI 0.8-9.5)
Taskinen 1989	Nested case-control study in men and women ever monitored for organic solvents Paternal exposure: 103 cases 182 controls Maternal exposure: 11 cases, 18 controls Response rate 73-79%	Female or male exposure; self reported (questionnaire) occupation and solvents, and classification of exposure (by the authors?) as unexposed, potentially, likely exposed, and high/frequent, intermediate or low/rare	Previous abortion, heavy lifting	Spontaneous abortion (between 1973-1983) from Finnish hospital discharge register; malformation from Finnish register of congenital malformations Paternal exposure: *Spontaneous abortion*: Not adjusted OR for: * Tetrachloroethylene (4 cases) OR 0.5 (95% CI 0.2-1.5) * Halogenated hydrocarbons OR 1.1 (95% CI 0.6-1.8); no dose response with exp. Class Adjusted OR for: * Organic solvents (high frequent exposure): OR 2.6 (95% CI 1.2-5.9), p<0.01; no dose response with exp. Class Organic solvents (total) OR 2.3 (95% CI 1.1-5.0) * Styrene (high frequent exposure): OR 0.7 (0.4-1.5) * Toluene (high, frequent exposure): OR 2.3 (1.1-4.7) * Xylene (high, frequent exposure): OR 1.6 (0.8-3.2) * Miscell. (high, frequent exposure): OR 2.1 (1.1-3.9) *For SA and paternal exposure*: Painters: OR=3.3(1.6-8.8) Woodwork: OR=3.8 (1.2-11.9) Congenital malformation: Org. solvents OR 0.6 (95% CI 0.2-2.0) Toluene 1.5 (0.4-5.4) Miscell. 2.0 (0.6-6.1) Xylene 1.6 (0.4-5.7) Maternal exposure: Org solvents OR 1.4 (95% CI 0.6-3.0)

Taskinen 1994	Nested case-control in female laboratory personnel 1. 206 cases of spontaneous abortion, 329 controls 2. 36 cases of malformations, 105 controls	Maternal exposure; self-reported (questionnaire), and exposure index calculated by occupational hygienist. Exposure to: acetone, acetonitrile, benzene, carbon tetrachloride, chloroform, ethanol, ether, ethyl, acetate, formalin, heptane, isoproponal, methanol, methylene chloride, petroleum, toluene, trichloroethane, white spirit, xylene	Employment, smoking, alcohol, parity, miscarriages, failed birth control, febrile disease	Spontaneous abortion from hospital discharge register (1973-1986); malformations from Finnish Register of Congenital malformations *Spontaneous abortion* : Working in laboratory: OR 1.4 (95% CI 0.9-2.2) methylene chloride: Low, 11 cases, 14 referents: OR 1.2 (95% CI 0.5-3.0) High, 7 cases, 8 referents: OR 1.7 (95% CI 0.6-5.0) Toluene 4.7 (1.4-15.9) Xylene 3.1 (1.3-7.5) Formalin 3.5 (1.1-11.2) Aceton: 1.4 (0.4-4.7) Other compounds: no association found Low birth weight : Working in laboratory: -133 g (CI –246 to –20 g) *Malformation* : No associations
Wennborg 2000	Retrospective cohort (1990-1994) in female biomedical research laboratory workers and non laboratory workers in universities (n=697), 71% response, see Wennborg 2001	Female exposure to several compounds, incl organic solvents, (1) Postal questionnaire job and exposure, classified as organic solvents or (2) by industrial hygienist	High blood pressure chronic, gynaecological ans sexually transmited diseases, smoking, lab work father, small children, previous spontaneous abortion, pregnancy number	Swedish Medical Birth Register, Spontaneous abortion: OR 0.9 (95% CI 0.5-1.4) Small for gestational age: OR 1.9 (95% CI 0.7-2.5)
Wennborg 2002	Retrospective cohort (1970-1989) in female biomedical research laboratory workers and non- laboratory workers in universities (n=1629)	Female exposure, exposure information from employee records and postal questionnaires to the head of department and exposure in 5 year period to several compounds including solvents	Age mother, previous pregnancy not resulting in life birth, smoking	Swedish Medical Birth Register, Birth weight < 2500 g: lab work in general: OR 1.5 (95% CI 0.7-3.1); solvents in general: OR 1.3 (95% CI 0.3-6.0) Birth weight > 4000 g: solvents OR 0.5 (95% CI 0.2-1.4) Preterm birth < 37 w: solvents OR 3.4 (95% CI 1.0-11.9) Postterm birth 42 w: solvents OR 1.7 (95% CI 0.7-3.8)

Wennborg 2005	Retrospective cohort among women occupational exposed in a biomedical research lab and non-lab workers in universities (n=1629), see Wennborg 2002	Exposure information from employee records and postal questionnaires to the head of department and exposure in 5 year period to several compounds including solvents	Age mother, previous spontaneous abortion, pregnancy number, smoking (including missing data 68%).	Laboratory work (before and during pregnancy) major malformations: OR 1.5 (0.8-2.8) neural crest malform.: OR 1.5 (0.6-4.1) Solvents (long term) major malformations: OR 2.5 (1.0-6.0) neural crest malform.: OR 2.0 (0.4-9.5) *Benzene (longterm)* major malformations: OR 2.1 (0.9-4.9) neural crest malform.: OR 3.5 (1.0-12.0) *Benzene (current)* major malformations: OR 2.3 (0.9-6.2) neural crest malform.: OR 5.3 (0.6-21.1)
Windham 1991	Case-control study, hospital based; 626 cases, 1300 controls	Female exposure; self-reported occupation and solvents and coded by experienced coders. Glycol ethers, adhesives, oil-based paints, paint thinners, paint strippers, TCA, PCE, methylene chloride, trichloroethylene, xylene, (and more), solvents in general	Maternal age, race, education, prior fetal loss, smoking and hours worked	Spontaneous abortion: hospital records. All solvents: 89 cases, 160 controls; OR 1.2 (95% CI 0.87-1.6) Glycol ethers: 7 cases, 9 control; OR 1.6 (95% CI 0.6-4.5) Tetrachloroethylene: (5 cases, 2 controls); crude OR 4.7 (95% CI 1.1-21.1), (4 out of 7 also exposed to trichloroethylene) Trichloroethylene (6 cases, 4 controls); crude OR 3.1 (95% CI 0.92-10.4) Tetra- and trichloroethylene: crude OR 3.4 (95% CI 1.0-12.0) Halogenated solvents: OR 1.0 (95% CI 0.65-1.6) Exposure halogenated solvents >10 hr/week: OR 1.5 (95% CI 0.73-3.0) Methylene chloride: (4 cases, 10 controls); crude OR 0.91 (95% CI 0.49-1.7)
Xu 1998	Retrospective cohort in petrochemical industry, 2853 female workers (20-44 years)	Interview of worker and her spouse, by questionnaire developed by IH. Self-experienced intensity of exposure to solvents. Exposure to: Benzene: 0.86 ppm Toluene: 0.40 ppm Styrene: 0.50 ppm Xylene: 0.03 ppm Gasoline, Acid, Manganese, Lime dust Hydrogen sulfide, Ammonia Exposure low in 1993: Benzene 0.86 ppm (n-38)	Age, education, plant, shift work, standing and kneeling during work, noise, dust, passive smoking and diet	self reported spontaneous abortion: *self-reported exposure to petrochemicals based on interviews:* OR 2.9 (2.0-4.0) *self-reported exposure based on job history:* OR 2.7 (1.8-3.9) self-reported exposure to benzene *OR* 2.5 (1.7-3.7) *self-reported exposure to gasoline* OR 1.8 (1.1-2.9) medium exposure to petrochemicals: *OR* 2.7 (1.9-3.9) high exposure to petrochemicals: OR 3.2 (1.8-5.7) the authors see this as a suggestion for an exposure-response relation (highly debatable)

Zhu 2006	Prospective cohort, Danish National Birth Cohort, 1,025 female laboratory workers vs 8,037 female teachers, see Zhu 2005	Female exposure, interview during pregnancy, job-exposure-matrix, exposure to organic solvents, see Zhu 2005	Maternal age, gravidity, history of spontaneous abortion, prepregnancy CMI, smoking, alcohol, paternal lab work, sex of child, other substances at work, see Zhu 2005	Medical Birth Register, no differences in late fetal loss, multiple births, sex ratio, preterm birth, SGA\; Major malformations: low exposure all solvents OR 3.5 (95% CI 0.9-13.6); high exp all solvents OR 4.5 (95% CI 1.3-16.2)