Health Council of the Netherlands

Ethyleneglycol monomethyl ether (EGME) and ethyleneglycol monomethyl ether acetate (EGMEA)

Health-based recommended occupational exposure limits

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Ethyleneglycol monomethyl ether (EGME) and ethyleneglycol monomethyl ether acetate (EGMEA)*
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Geachte staatssecretaris,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan ethyleenglycol monomethylether (EGME) en ethyleenglycol monomethylether acetaat (EGMEA).

Dit advies maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige advieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Het genoemde advies is opgesteld door de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ethyleenglycol monomethylether (EGME) en ethyleenglycol monomethylether acetaat (EGMEA) werden in het verleden op grote schaal gebruikt. De afgelopen jaren zijn de blootstellingsniveaus in bedrijven aanzienlijk gedaald als gevolg van Europese regelgeving. Tevens is het gebruik van deze stoffen sterk verminderd, doordat ze zijn vervangen door andere, minder schadelijke glycol ethers.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. L.J. Gunning-Schepers,
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Ethyleneglycol monomethyl ether (EGME) and ethyleneglycol monomethyl ether acetate (EGMEA)

Health-based recommended occupational exposure limits

Dutch Expert Committee on Occupational Safety
A Committee of the Health Council of the Netherlands

to:

the State Secretary of Social Affairs and Employment

No. 2011/10, The Hague, June 24, 2011

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Inhoud

Samenvatting

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en Beroepsmatige Blootstelling aan stoffen (GBBS) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in lucht waaraan mensen beroepsmatig blootgesteld kunnen worden. Deze aanbevelingen vormen de basis voor wettelijke grenswaarden, vast te stellen door de minister, waarmee de gezondheid van werknemers beschermd kan worden.

In 1996 heeft de Gezondheidsraad een advies uitgebracht met een evaluatie van de gezondheidsrisico's als gevolg van beroepsmatige blootstelling aan ethyleenglycol-monomethylether (EGME) en ethyleenglycol-monomethyletheracetaat (EGMEA)*. Enkele jaren later heeft ook de Europese SCOEL** een advies uitgebracht over deze stoffen. In het voorliggende rapport, actualiseert de commissie haar advies uit 1996 door gebruik te maken van het SCOEL advies uit 2007 en latere gepubliceerde gegevens.

De conclusies van de commissie berusten op de wetenschappelijke publicaties die vóór maart 2011 zijn verschenen.

* 2-Methoxyethanol (EGME) and 2-Methoxyethyl Acetate (EGMEA).
** SCOEL: Scientific Committee on Occupational Exposure Limits.

Fysische en chemische eigenschappen

EGME en EGMEA zijn zwak geurende kleurloze vloeistoffen, die goed met water mengbaar zijn en met een groot aantal organische oplosmiddelen. EGME en EGMEA worden op uitgebreide schaal gebruikt als oplosmiddelen in olie-water samenstellingen. In 1980 was de productie van de hier beschouwde stoffen in West-Europa en Japan ongeveer 800.000 ton per jaar. De afgelopen jaren zijn de blootstellingsniveaus in Nederlandse bedrijven sterk gedaald als gevolg van regelgeving. Daarnaast is het gebruik van deze stoffen verminderd doordat ze vervangen zijn door andere glycolethers. Recente blootstelingsniveaus zijn echter niet beschikbaar.

Monitoring

Het National Institute for Occupational Safety and Health (NIOSH) van de Verenigde Staten heeft voor het meten van EGME- en EGMEA-concentraties in lucht een methode beschreven die is gebaseerd op gaschromatografische analyse.

Urine concentraties van metabolieten van EGME zouden gebruikt kunnen worden als biologische indicatoren van blootstelling. Een gevalideerde methode voor deze biologische monitoring ontbreekt echter nog.

Huidige grenswaarden

In 1996, adviseerde de Gezondheidsraad een gezondheidskundige advieswaarde van 1 mg/m^3 (0.3 ppm) voor EGME en 1.5 mg/m^3 (0.3 ppm) voor EGMEA. Duitsland kent een grenswaarde voor beroepsmatige blootstelling aan EGME en EGMEA van 1 ppm (respectievelijk 3.2 en 5 milligram per kubieke meter lucht). De ‘treshold limit value’ (TLV) van de ACGIH* in de Verenigde Staten is 0.1 ppm voor beide stoffen (0.3 mg/m^3 (EGME) en 0.5 mg/m^3 (EGMEA)). Het National Institute for Occupational Safety and Health (NIOSH) in de VS heeft dezelfde bovengrens van de gemiddelde blootstelling over een acht-urige werkdag aanbevolen. In alle gevallen is een huidnotatie aanbevolen.

In 2006 adviseerde SCOEL een 8-uur tijdgewogen gemiddelde limietwaarde van 1 ppm voor EGME (3 mg/m^3) en EGMEA (5 mg/m^3).

* ACGIH: American Conference of Industrial Hygienists

Kinetiek

EGME wordt gemakkelijk via de huid opgenomen. De *in vitro* voor de menselijke huid gemeten absorptiesnelheid is ongeveer 2,8 milligram per vierkante centimeter per uur. De opname van EGME in het bloed na inademing bleek in de mens 76% te zijn. De halfwaardetijd voor uitscheiding van de metaboliet 2-MAA was 77 uur.

Bij ratten verspreidde een éénmalige oraal toegediende dosis EGME zich snel door het lichaam. Na 48 uur verscheen 54 tot 70% van de dosis in de urine; 3 tot 12% van de dosis werd binnen 48 uur uitgeademd in de vorm van kooldioxide. Bij ratten is 2-methoxy-azijnzuur (2-MAA) de belangrijkste metaboliet in de urine; het weerspiegelt 80 tot 90% van de in de urine uitgescheiden hoeveelheid EGME. Van EGME dat via het drinkwater aan ratten was toegediend, was de uitscheiding in de vorm van 2-MAA in de urine geringer (34%), terwijl de uitademing in de vorm van kooldioxide hoger was (10 tot 30%). Een andere kwantitatief belangrijke metaboliet in de urine bij ratten was ethyleenglycol (21%). Toediening van EGME aan ratten via de huid gaf een soortgelijk metabolietenpatroon als orale toediening.

EGMEA wordt in het lichaam snel omgezet in EGME. De halfwaardetijd hiervoor in ratten is 12 minuten. Verdere gegevens over de absorbtie en eliminatie van EGMEA ontbreken.

Effecten

Effecten bij de mens

Haematologische afwijkingen zijn waargenomen nadat werknemers zijn blootgesteld aan een gemiddelde concentratie van 4 ppm EGME (13 mg/m^3). Deze effecten verdwenen echter wanneer de blootstelling werd verlaagd naar 2.3 ppm (7 mg/m^3). Verdere humane gegevens zijn beperkt. In 2008 heeft een commissie van de Gezondheidsraad geconcludeerd dat blootstelling aan ethyleen glycol ethers geassocieerd is met spontane abortus. Daarnaast waren er indicaties dat maternale blootstelling het risico op neurale buis effecten en andere afwijkingen verhoogt. Omdat in veel epidemiologische studies blootstelling aan meerdere glycolethers gelijktijdig plaatsvindt, vindt de commissie de bruikbaarheid van deze studies voor het afleiden van een gezondheidskundige advieswaarde voor EGME en EGMEA beperkt.

Proefdiergegevens

Volgens EEC criteria zijn EGME en EGMEA niet irriterend voor huid. De acute toxiciteit is gering.

In Sprague Dawley ratten en New Zealand White konijnen die subchronisch inhalatoir werden blootgesteld aan EGME of EGMEA zijn de relatieve thymus gewichten, de absolute en relatieve testis gewichten, de lever en de lichaamsgewichten verlaagd in beide diersoorten na blootstelling aan de hoogste concentratie van 950 mg/m^3. Bij één van vijf mannelijke konijnen die blootgesteld werden aan de laagste concentratie van 95 mg/m^3 was geringe tubulaire atrofie van de testes waargenomen na inhalatoire blootstelling aan EGME gedurende 13 weken, vijf dagen per week, zes uur per dag. In een vergelijkbaar experiment met ratten bleek deze concentratie een geen-waargenomen-nadelig-effect niveau (NOAEL, No Observed Adverse Effect Level) te zijn. Ook werden deze effecten niet waargenomen toen de studie werd herhaald met konijnen.

Subchronische blootstelling via het drinkwater van 71 mg EGME per kg lichaamsgewicht per dag, gedurende 13 weken, resulteerde bij mannelijke ratten in geringe histopathologische veranderingen in de testes. Bij vrouwelijke ratten leidde een dagelijkse dosis van 70 mg per kg lichaamsgewicht tot een vermindering van het relatieve en het absolute thymusgewicht. Hogere doses induceerden atrofie van de thymus en testes, evenals in hematopoietische weefsels.

EGME en EGMEA zijn niet genotoxisch in *in vitro* en *in vivo* onderzoek.

Langdurige blootstelling aan EGME via verschillende routes verhoogde in ratten testiculaire atrofie en ontwikkelingsstoornissen. Bij muizen, goudhamsters en cavia's werd uitsluitend na orale blootstelling testiculaire toxiciteit waargenomen. De laagste blootstelling waarbij nog testiculaire en reproductieve effecten zijn waargenomen was 50 mg per kg lichaamsgewicht. Bij muizen zijn ontwikkelingsstoornissen gevonden na inhalatoire, en orale blootstelling (53 tot 64 mg per lichaamsgewicht per dag, gedurende 13 weken, te beginnen voor paring en tijdens dracht en lactatieperiode) en zowel na subcutane als intra-peritoneale injectie; Bij konijnen zijn na inhalatoire blootstelling (32 mg per kubieke meter lucht, zes uur per dag van zwangerschapsdag 6 tot 18) effecten op de ontwikkeling gevonden; bij apen na orale toediening (12 mg per kg lichaamsgewicht per dag, op zwangerschapsdag 20 tot 45).

EGMEA gaf testiculaire atrofie bij muizen na orale toediening van 62,5 mg per kg lichaamsgewicht per dag, vijf dagen per week, gedurende vijf weken.

Oraal toegediend kan EGME in ratten en muizen een immunologische effect veroorzaken. De laagste dosis waarbij minimale immunologische effecten optre-

den in ratten was 25 mg/kg lichaamsgewicht per dag gedurende 10 dagen. Bij muizen zijn bij deze dosis geen effecten waargenomen. Bij hogere doseringen (50 mg/kg lichaamsgewicht) waren de effecten duidelijker: afgenomen thymus gewicht, vermindering aantal witte bloedcellen en beenmergcellen.

Evaluatie en advies

Epidemiologische studie studies laten haematologische effecten zien in mannelijke werknemers die worden blootgesteld aan 4 ppm EGME. Deze effecten verdwenen als de blootstelling wordt verlaagd tot 2.3 ppm EGME.

In dierexperimentele studies zijn effecten op de voortplanting van konijnen, muizen en ratten waargenomen als deze subchronisch of langdurig worden blootgesteld aan EGME via verschillende routes. Er is een aantal studies van goede kwaliteit beschikbaar, die als basis voor het afleiden van een gezondheidskundige advieswaarde kunnen dienen. Hiervoor heeft de commissie gebruikt gemaakt van de benchmarkdosis (BMD) software van de Amerikaanse Environmental Protection Agency (US-EPA). Daarmee is het mogelijk met de gegevens een blootstellingsresponsrelatie af te leiden. Dit model wordt vervolgens gebruikt om een blootstellingsniveau af te leiden ($BMDL_{10}$, de onderste concentratie van het 95% betrouwbaarheidsinterval van de bench mark dosis (BMD)), dat als vertrekpunt dient voor het afleiden van de gezondheidskundige advieswaarde.

EGME

De commissie beschikt over een studie waarin drachtige konijnen via de lucht worden blootgesteld aan EGME (0, 3, 10, 50 ppm). Deze studie dient als startpunt voor de BMD analyse. De effecten op de ontwikkeling van nageslacht (nl verhoging van het aantal implantaties en het aantal nesten met resorpties, en vertraagde verbening van het *sternebrae*) beschouwt de commissie als relevant voor de mens. Uit de BMD-analyse werd een $BMDL_{10}$ van 1.3 ppm (4.12 mg/m^3) afgeleid. Deze $BMDL_{10}$ komt overeen met een 10 procent verhoging van het aantal foetussen met vertraagde verbening. Voor het vaststellen van een gezondheidskundige advieswaarde wordt vervolgens rekening gehouden met verschillende onzekerheden. Zo zijn er verschillen tussen diersoorten (dier-mens). De commissie hanteert hiervoor in het algemeen een factor 3. Daarnaast kunnen mensen onderling verschillend op blootstelling reageren. Daarvoor past de commissie nogmaals een factor 3 toe. Op basis van de dierexperimentele gegevens adviseert de commissie daarom een gezondheidskundige advieswaarde van 0.16

ppm EGME (0.5 mg/m^3). De commissie is van mening dat deze advieswaarde ook beschermt tegen de haematologische effecten die in epidemiologische studies zijn beschreven. De commissie vindt te gegevens onvoldoende om een biologische limiet waarde voor te stellen.

EGMEA

Er zijn slechts een beperkt aantal gegevens over de effecten van blootstelling aan EGMEA. De commissie is echter van mening dat het toxiciteitsprofiel van EGMEA vergelijkbaar is met het profiel van EGME. EGMEA wordt in het lichaam snel omgezet tot EGME. Daarom adviseert de commissie een gezondheidskundige advieswaarde die overeenkomt (op molaire basis) met EGME, nl 0.16 ppm EGMEA (0.8 mg/m^s).

Gezondheidskundige advieswaarden

Voor EGME adviseert de commissie een gezondheidskundige advieswaarde van:
- 0.5 mg/m^3 lucht (0,16 ppm).

Voor EGMEA adviseert de commissie een gezondheidskundige advieswaarde van:
- 0,8 mg/m^3 lucht (0,16 ppm).

Voor deze grenswaarden geldt het concentratiegemiddelde over een acht-urige werkdag. De commissie beveelt voor beide stoffen een huidnotatie (H) aan.

Summary

Scope

At request of the Minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, recommends health based occupational exposure limits for airborne substances to which people can be exposed in the air at the workplace. These recommendations serve as a basis in setting legally binding limit values by the minister

In 1996, the Health Council published an advice on the toxicity of ethyleneglycol monomethyl ether and ethyleneglycol monomethyl ether acetate (EGME and EGMEA)*. Several years later, the European Scientific Committee on Occupational Exposure Limits (SCOEL) published an evaluation on the toxicity of both substances as well. In the present advice, the Committee reconsidered the former Health Based Occupational Exposure Limits for both substances based on the report of de SCOEL and the published studies since 2006.

The Committee's conclusions are based on scientific publications from prior to March 2011.

* 2-Methoxyethanol (EGME) and 2-Methoxyethyl Acetate (EGMEA).

Physical and chemical properties

The compounds are colourless liquids with a mild odour, miscible with water and with a large number of organic solvents. The physical and chemical properties are listed in chapter 2 of the report. These substances are extensively used as co-solvents in oil-water compositions. In 1980 the production of these compounds was approximately 800,000 tonnes per year in Western Europe and Japan. 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 EGME) to using other less harmful glycolethers. However, recent exposure data are not available.

Monitoring

The National Institute for Occupational Safety and Health of the United States (NIOSH) has described a method for measuring the concentration of EGME and EGMEA in air at the workplace that is based on gas chromatographic analysis. There is no validated method for biological monitoring.

Urinary concentrations of metabolites of EGME and EGMEA may be useful for biological indicators for occupational exposure. However, a validated method is not yet available.

Current limit values

In 1996, the Health Council of the Netherlands recommended an health based occupational exposure limit of 1 mg/m^3 (0.3 ppm) for EGME and 1.5 mg/m^3 (0.3 ppm) for EGMEA. Germany knows an occupational exposure limit for both EGME en EGMEA of 1 ppm (respectively 3.2 and 5 milligram per cubic meter air). The ‘threshold limit value’ (TLV) of the ACGIH in the United States is 0.1 ppm for both substances (0.3 mg/m^3 (EGME) and 0.5 mg/m^3 (EGMEA)). The National Institute for Occupational Safety and Health (NIOSH) in the United States has recommended comparable occupational exposure limits. All organisations recommended an ‘H’ notation.

Finally, in 2006 the SCOEL* recommended an 8-hour exposure limit value of 1 ppm for EGME (3 mg/m^3) and EGMEA (5 mg/m^3).

* Scientific Committee on Occupational Exposure Limits.

Kinetics

EGME is readily absorbed through the skin. The absorption rate through human skin *in vitro* amounted to approximately 2.8 mg/cm^2 per hour. The uptake of EGME by humans after inhalation during 4 hours was 76%. The elimination half-life of its major metabolite 2-MAA was 77 hours.

A single oral dose of EGME to rats was rapidly distributed within the body. After 48 hours 54% to 70% of the dose was recovered in the urine; 3 to 12% of the dose was exhaled in the form of carbon dioxide within 48 hours. 2-Methoxyacetic acid (2-MAA) was the primary urinary metabolite in rats, accounting for 80% to 90% of the total amount of compound excreted in urine. When EGME was given to rats via the drinking water, less of the compound was eliminated as 2-MAA via the urine (34%) and more was exhaled in the form of carbon dioxide (10%-30%). Another quantitatively important metabolite excreted in the urine was ethyleneglycol (21%). Dermal application of EGME to rats resulted in a metabolite pattern similar to that after oral administration.

EGMEA is hydrolysed to EGME. The half-time for this reaction in rats is 12 minutes. More data on the absorbion and elimination of EGMEA are lacking.

Effects

Human data

Haematological effects were observed in male impregnation workers exposed to an average exposure level of 4 ppm (12.6 mg/m^3) EGME. These effects were not observed in a follow-up study when the exposure was lowered to 2.3 ppm (7.3 mg/m^3). Additional human studies are limited. In 2008, a committee of the Health Council of the Netherlands concluded with respect to the effects on reproduction that the available epidemiological studies indicate an association between maternal exposure to ethylene glycol ethers and the risk of spontaneous abortion. It was also concluded 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. Most epidemiological studies, however, suffer from a lack of reliable exposure data. For this reason and because co-exposure to other chemicals is involved, the Committee is of the opinion that the epidemiological studies can only be of limited use for the derivation of a Health Based Recommended Occupational Exposure Limit.

Animal data

EGME and EGMEA are not irritating to the skin according to EEC criteria. The acute toxicity of the compounds is low.

Subchronic exposure by inhalation to EGME and EGMEA showed decreased relative thymus weight, decreased absolute and relative testis weight and decreased liver and body weight at the highest exposure level (950 mg/m^3) in Sprague Dawley rats or New Zealand White rabbits. Exposure to EGME concentrations of 95 mg/m^3 during 13 weeks, 5 days per week and 6 hours per day, induced minimal tubular atrophy of the testes in one out of five male rabbits. In a comparable experiment with rabbits, however, exposure to a similar concentration appeared to be a no-observed-adverse-effect-level (NOAEL). In addition, no effects were found in rats after exposure to 95 mg/m^3.

Semi-chronic EGME-dosing of male rats with 71 mg/kg bodyweight per day, administered via drinking water during 13 weeks, caused tubular atrophy in the testes. In female rats, a daily dose of 70 mg/kg bodyweight decreased the relative and absolute thymus weight. Higher doses induced thymic and testicular atrophy and histopathological changes in the thymus and haematopoietic tissues.

EGEM and EGMEA have no genotoxic potential in bacteria, several mammalian cell types, Drosophila and in *in vivo* animal studies.

Chronic exposure to EGME induced testicular atrophy and developmental effects in rats via different routes of administration. EGME induced testicular toxicity in mice, golden hamsters and guinea pigs also, but only after oral dosing. In general, oral dosing of 50 mg/kg bodyweight was the threshold level for producing testicular and reproductive effects. Developmental effects were observed in mice after inhalation exposure, oral dosing (53 to 64 mg/kg body weight/day, during 13 weeks premating, gestation and lactation) and subcutaneous and intraperitoneal injection; in rabbits after inhalation exposure (32 mg/m^3 for six hours a day from gestation day 6 to 18) and in monkeys after oral dosing (12 mg/kg bodyweight/day from gestation day 20 to 45).

EGMEA induced testicular atrophy in mice at daily oral doses of 62.5 mg/kg bodyweight, five days a week, for five weeks.

Oral dosing of EGME may elicit an immunological response in rats in mice. The lowest dose with slight immunological effects in rats was 25 mg/kg bw. No effects were observed in mice at this exposure level. Exposure to higher concentrations (50 mg/kg b.w.) resulted in more obvious effects: decreased thymus weight, lowered white blood cell count and bone marrow cells.

Hazard Assessment and recommended occupational exposure limits

EGME

The Committee uses a study in which pregnant rabbits were exposed to EGME (0, 3, 10, 50 ppm) by inhalation as a starting point for the bench mark dose (BMD) analyse. The Committee is of the opinion that effects on reproduction (i.e. increased number of resorbed implantations, increased number of litters with resorptions and increased delayed ossification) are relevant for the human risk assessment. For the assessment the Committee uses the BMD-software of the US-EPA*, which derives doses response relations from the available data. Consequently, the derived dose-response relationship is used to calculate a exposure level corresponding to a specific effect, i.e. BMR (Bench Mark Dose).

From the BMD-analyses using the software of the US-EPA, the Committee derives a BMR of 10% increase in number of fetusses with delayed ossifications. The $BMDL_{10}$ is the 95% lower confidence limit of the BMD_{10} ie. the dose that corresponds with a BMR 10% extra risk (delayed ossification); In this case the $BMDL_{10}$ is 1.3 ppm or 4.1 mg/m^3.

For the assessment of the HBR-OEL, several aspects and uncertainties were considered. Thus, interspecies differences should be taken into account. As a default the Committee uses a factor three. In addition, differences among people should be taken into account as well. The Committee uses an additional factor of three to compensate for this. Using the studies in experimental animals, the Committee recommends a health based occupational exposure limit of 0.16 ppm EGME (0.5 mg/m^3). The Committee is of the opinion that this OEL also protects against the haemotological effects found in the epidemiological studies.

Finally, the Committee considers the available data insufficient for deriving a biological limit value (BLV).

EGMEA

There are only a few data available concerning the effects of eposure to EGMEA. The Committee is of the opinion that the toxicity profile of EGMEA is comparable to the profile of EGME. Moreover, EGMEA is rapidly hydrolised to

* US-EPA: United States Environmental Protection Agency.

EGME. Therefore, the Committee recommends a health based occupational exposure limit for EGMEA identical to that of EGME on a molar basis: 0.8 mg/m^3 (0.16 ppm).

Health based recommended occupational exposure limit

For EGME, the Committee recommends a health based occupational exposure limit of:

- 0.5 mg/m^3 (0.16 ppm).

For EGMEA, the Committee recommends a health based occupational exposure limit of:

- 0.8 mg/m^3 (0.16 ppm).

These health based recommended exposure limits have the form of 8 hours time weighted average concentrations. The Committee recommends a skin notation for both compounds.

Part I

Health based recommended occupational exposure limit for EGME and EGMEA

Chapter 1

Scope

1.1 Background

At the request of the Minister of Social Affairs and Employment (annex A), the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations of the toxicity of substances to which man can be exposed at the workplace. The purpose of these evaluations is to recommend a health-based recommended occupational exposure limit (HBROEL) for the concentration of the substance in air, provided the database allows the derivation of such value.

In 1996, the Health Council published an advice on the toxicity of ethyleneglycol monomethyl ether and ethyleneglycol monomethyl ether acetate (EGME and EGMEA). For both compounds, a health based occupational exposure limit was recommended. Several years later, the European Scientific Committee on Occupational Exposure Limits (SCOEL) published an evaluation on the toxicity of both substances as well.

In the present advice, the Committee reconsiders the former Health Based Occupational Exposure Limits for both substances based on the previous report of the Committee, the advice of the SCOEL published in 2007 and additional published studies from 2006 till March 2011.

1.2 Committee and method of work

The present document contains the re-assessment of the toxicity of ethyleneglycol monomethyl ether and ethyleneglycol monomethyl ether acetate by DECOS. The members of the DECOS are listed in annex B.

In 2011, the DECOS released a draft version of the report for public review. The individuals and organisations that commented on the draft are listed in annex C. DECOS has taken these comments into account in finalising its report.

1.3 Data

In part I of the present document, the Committee evaluates the toxicity of EGME and EGMEA and recommends health based occupational exposure limits for both EGME and EGMEA. This evaluation is based on the data described in Part II of the present report.

Part II of the present document constitutes an update of the Health Council's report* on ethyleneglycol ethers, issued in 1996[1]. For the 1996 report, the literature was surveyed up to and including December 1994. In addition, relevant data were extracted from the 2006 SCOEL report on EGME and EGMEA[2]. Furthermore, additional data were retrieved from the literature published since January 2004 using the online databases Toxline, Medline and Chemical Abstracts (CAPlus), using EGME, ethylene glycol monomethyl ether, methoxyethanol, methylglycol and CAS no 109-86-4 as key words for EGME, and EGMEA, methoxyethylacetate, ethylene glycol monomethyl ether acetate, methyl glycol acetate, methoxyacetic acid and CAS no 110-49-6 as keywords for EGMEA. The last search was performed in March 2011.

* The Health Council's report from 1996 concerned the effects of several glycol ethers. In this report only data concerning the effects of EGME and EGMEA were updated.

Chapter 2

Hazard assessment

This chapter contains a short summary of the relevant data concerning the effects of exposure to EGME or EGMEA, based on the data summarized in Part II of the present report. In addition to evaluating the toxicity of EGME and EGMEA, the Committee recommends health based occupational exposure limits for both compounds.

2.1 Hazard identification

Several epidemiological studies and experimental animal studies have been performed to identify adverse health effects of exposure to ethyleneglycol monomethyl ether (EGME) and ethyleneglycol monomethyl ether acetate (EGMEA). The following paragraphs contain a short evaluation of the relevant toxic effects after single and repeated exposure via food, drinking water, or inhalation.

2.1.1 Human data

In a first survey of Shih *et al*., effects on haematology and reproduction were investigated in impregnation workers exposed to EGME from two factories manufactoring copper clad laminate in Taiwan. Haematological effects* were

* Haematological effects included reduced haemoglobin packed cell volume, and red blood cell count.

seen in male impregnation workers (n=53) exposed to EGME (geometric mean 4 ppm (12.6 mg/m^3), GSD 2.9 ppm). No effects on sperm parameters were observed.[3] In a follow-up study by Shih *et al*., the haematological parameters were followed in impregnation workers (n=29) initially exposed to 9.6 ppm EGME at the start of the study which dropped to 2.3 ppm (7.3 mg/m^3) (geometric mean of 2.3 ppm, GSD 1.8 ppm) 2.5 months later. Results from the haematological examination at the beginning of the study showed effects on haemoglobin levels, packed cell volume, and red blood cell count.[4] However, 2.5 months later when a reduction in exposure was noted, haematological parameters had returned to normal values.

Limited additional human data are available with respect to effects on fertility and development. In 2008, a committee of the Health Council of the Netherlands concluded with respect to the effects on reproduction that the available epidemiological studies indicate an association between maternal exposure to ethylene glycol ethers and the risk of spontaneous abortion[5]. It was also concluded 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. Available epidemiological studies regarding the critical endpoint suffer, however, from a lack of reliable exposure data. For this reason and because co-exposure to other chemicals is involved, these epidemiological studies cannot be used for the derivation of a Health Based Recommended Occupational Exposure Limit.

2.1.2 Animal data

EGME, exposure by inhalation

Exposure of male rats to 79 mg/m^3 (25 ppm) EGME by inhalation during 7 hours per day, 7 days per week for 6 weeks, prior to mating with untreated females, caused neurochemical deviations in the brain of 21-day old offspring[6].

The lowest inhalation concentration of EGME tested, i.e. 9,5 mg/m^3 (3 ppm) (6 h/day), had no effect on mothers and offspring in rats or rabbits after exposure during gestation (see table 1). At the next dose tested, i.e. 32 mg/m^3 (10 ppm), the authors concluded that no effects were observed in rats and mice. However, an increased resorption rate and delayed ossification of *sternebrae* in the foetuses was observed in rabbits at this exposurelevel[7]. The Committee is of the opinion that these effects on development are relevant, and therefore disagrees with the conclusion of SCOEL regarding these effects.

EGME, oral exposure

The no observed adverse effect levels (NOAEL) and lowest observed adverse effect levels (LOAEL) in toxicity studies with *oral* exposure to EGME are listed in Table 1. After uptake, EGME induces effects on blood parameters and the immune system and demonstrates testicular and developmental toxicity.

With respect to effects on fertility and development, a wealth of data concerning oral exposure is available. From studies with rats, mice, golden hamsters and rabbits the Committee concludes that the rabbit is the most sensitive species with respect to testicular toxicity caused by exposure to EGME. In this species, spermatogenesis was affected at oral dose levels ≥ 25 mg/kg bw/day, in a dose related manner. The NOAEL was 12.5 mg/kg bw/day[8,9]. In female rats, oestrus cycle changes were observed at oral dose levels ≥ 30 mg/kg bw/day[10].

Table 1 No effect levels and lowest effect levels observed in toxicity studies of EGME.

dose (mg/m^3 or mg/kg bw)	dosing regimen	species	observations	reference
Inhalation studies (dose in mg/m^3)				
9.5	daily inhalation exposure, 6 h/day on GD 6-18	pregnant rabbits	NOAEL for developmental effects	7
32	daily inhalation exposure, 6 h/day on GD 6-15	pregnant rats, mice	NOAEL for developmental effects	7
32	daily inhalation exposure, 6 h/day on GD 6-18	pregnant rabbits	no maternal effects; increased % age of resorptions, increased % age of litters with resorptions; delayed ossification at *sternebrae*	7
79	daily inhalation exposure, 7 h/day on GD 7-13; test on litter for neuromotor function on days 10-90; brain analysis of litters on day 21	pregnant rats	neither maternal effects, nor effects on the number or wt[a] of live offspring. One of the six behavioural tests revealed significant differences in offspring. Neurochemical deviations in the brainstem and cerebrum	6
95	inhalation exposure for 13 weeks, 5 d/week, 6 h/day	rats	NOAEL for systemic toxicity	11,12
95	inhalation exposure for 13 weeks, 5 d/week, 6 h/day	rabbits	degenerative changes in germinal epithelium of testes (1/10 animals in 2 studies)	11,12
158	daily inhalation exposure, 7 h/day on GD 7-15	pregnant rats	decreased maternal wt gain; increased number of resorptions; increased number of visceral and skeletal malformations	6
158	daily inhalation exposure, 6 h/day on GD 6-15	pregnant mice	decreased maternal wt gain; decreased number of live foetuses; increased incidence of extra ribs and unilateral hypoplastic testicle	7
316	inhalation exposure for 10 days, 6 h/day	rats	NOAEL for systemic toxicity	13
316	inhalation exposure for 13 weeks, 5 d/week, 6 h/day	male rats	NOAEL for effects on fertility and body weight	13
1,244	inhalation exposure for 2 weeks, 5 d/week, 6 h/day	rats	hindlimb paralysis	14

Oral and dermal studies (dose in mg/kg bw)

10	daily oral doses, starting on vaginal metestrus, for 7 days	female rats	NOAEL for effects on oestrus cycle and fertility related hormones	[15]
12	daily oral doses on GD 20-45; Caesarian section on GD 100	monkeys	slight maternal body weight loss during treatment, increased resorptions, no malformations	[16]
12.5	drinking water for 12 weeks, 5 d/week	male rabbits	NOAEL for effects on spermatogenesis	[17,18]
12.5	daily oral doses on GD 6-15; sacrifice on GD 16 and litter on PND 4	pregnant rats	NOAEL for developmental effects (screening test)	[19]
16	daily oral doses on GD 7-18, behavioural tests with offspring on postnatal days 48-65	pregnant rats	increase gestation time, decreased mean pup wt, no behavioural effects in pups	[6]
25	oral doses for 49-51 days (7 weeks)	female rats	NOAEL for effects on body weight, haematology or development	[20]
25	daily oral doses for 10 days	rats	approximate NOAEL for immunotoxicity	[21]
30	daily oral doses for 2 or 4 weeks	female rats	minimal prolonged estrous interval and decreased oestrus frequency, hypertrophy of corpora lutea	[10]
31	daily oral doses on GD 7-18	pregnant rats	malformations in surviving pups	[6]
40	daily dermal applications on GD 7-17	pregnant rats	no maternal effects; no effect on litter size, number of live pups on day 1 and 5 and mean pup wt	[22]
50	oral dose(s) for 1-11 days	male rats	MOAEL for testicular changes and reduced fertility (strain differences)	[23,24,25,21]
50	daily oral doses for 5 days	male mice	reduced fertility at 4 weeks after treatment	[26]
50	daily oral doses on GD 7-13	pregnant rats	maternal reduced body wt gain, apparently due to resorbed foetuses; prolonged gestation period; reduced %age of pregnant dams that delivered, litter size, and pup wt; reduced number of surviving pups; electrocardiographic changes in the survivors	[27]
50	daily oral doses at least for 15 days prior to mating with untreated males, during gestation and for 4 days post partum	female rats	decreased number of live pups born, decreased litter size, increased pup mortality	[20]
62.5	oral doses for 5 weeks, 5 d/week	male mice	decreased testis size and atrophy of the seminiferous epithelium; decrease in white blood cell count	[28]
62.5	oral doses for 5 weeks, 5 d/week	male hamsters	decreased testis size	[28]
70	drinking water administration for 13 weeks	rats	decreased relative and absolute thymus weight in females, minimal testis lesions in males	[29]
70	daily oral dosages for 18 days	male mice	NOAEL for effects on sperm parameters and testis weight	[30]
100	oral doses for 20 days	male rats	decreased relative weights of thymus and testis	[31,32]
100	dermal application (occlusive) for 28 days, 5 d/week	rats	reduced food intake and weight gain	[33]

250	oral doses for 5 weeks, 5 d/week	male guinea pigs	decreased testes wt and white blood cell count; no effect on wt of seminal vesicles and coagulating gland	28
250	single dermal dose on GD 12	pregnant rats	no maternal effects; increased external, visceral, and skeletal malformations in foetuses	34
295	drinking water administration for 13 weeks	male mice	NOAEL for systemic toxicity	29
492	drinking water administration for 13 weeks	female mice	minimal increase in splenic haematopoiesis and mild hypertrophy in the *zona reticularis* of the adrenal gland	29
1,000	dermal application (occlusive) for 13 weeks, 5 d/week, 6 h/day	guinea pigs	decreased body weight and spleen and testes weight, degenerated seminiferous tubules, mild anaemia with increased MCV and lymphopenia with increased neutrophils, increased serum CPK and LDH	35

[a] Wt: weight

EGMEA

Only limited data are available concerning the effects of exposure to EGMEA. The toxicity studies with exposure to EGMEA are listed in Table 2. The few animal data available indicate that EGMEA has a toxicity profile similar to that of EGME.[28,36-38] The hydrolysis of EGMEA into EGME is fairly rapid; half-life times of approximately 12 min[39] or 20-30 min[40] in rat plasma have been observed, as well as a high activity of nasal mucosal carboxylesterase, being higher in mice than in rats.[41]

Table 2 No effect levels and lowest effect levels observed in toxicity studies of EGMEA.

dose (mg/kg)	dosing regimen	species	observations	reference
62.5	daily oral doses for 5 weeks, 5 d/week	mice	testicular atrophy and leukopenia	42; 28
1,225	daily oral doses on GD 7-14	pregnant mice	no maternal effects; 0/31 litters were viable	30,36

2.2 Quantitative assessment of the health risk

2.2.1 Health based recommended occupational exposure limit

For the derivation of an health based recommended occupational exposure limit (HBROEL), DECOS prefers the use of epidemiological data as starting point. The Committee notices that the epidemiological data of Shih *et al.* show effects on blood parameters in male workers exposed to 4 ppm EGME.[3] No effects were observed in an additional study by Shih *et al.* when the exposure was lowered to 2.3 ppm (7.4 mg/m^3).[4] However, both human studies have limitations. The

exposed group was small, only one exposure group was included and the exposure concentration was given as a geometric mean.

Therefore, the Committee decided to evaluate the data obtained from the experimental animals. Taking the whole set of animal data into account, the most evident and relevant effects at the lowest exposure level are found in the study of Hanley *et al.*, concerning reproduction toxic effects in rabbits after inhalation.[7] With data from this study, a dose-response relationship could be assessed for several effects. In deriving a health-based recommended occupational exposure limit (HBROEL), the Committee performed a benchmark dose-analysis (BMD-analysis). The BMD-software of the US-EPA* was used for the analysis.

EGME

For BMD-analysis, the Committee uses data of Hanley *et al.* concerning the effects on reproduction after inhalation exposure to EGME.[7] Female rabbits were exposed to vapour concentrations EGME of 0, 3, 10 or 50 ppm for 6 hours per day on day 6 to day 15 of gestation. The effects on embryonal and fetal development were evaluated.

The effects of exposure to EGME are summarized in Annex D. Exposure of pregnant rabbits to 50 ppm EGME significantly increased the incidence of malformations, minor variations and resorptions of the offspring, as well as decreased fetal body weight. After exposure to lower concentrations of EGME (10 ppm, 32 mg/m^3), the number of implantations resorbed and the number of litters with resorptions increased significantly. Furthermore, a significant increase in delayed ossification of the *sternebrae* was observed in the offspring of rabbits after exposure to 10 ppm (32 mg/m^3) and higher. At the lowest exposure level tested (3 ppm, 9.5 mg/m^3), no effects were observed in the female rabbits or their offspring.

Data on the BMD analysis are shown in Annex D, from which a BMD and BMDL can be derived.

Starting point: The Committee uses a BMR of 10% increase in number of fetusses with delayed ossifications as the starting point for deriving a HBROEL. A BMR of 10% (instead of 5%) is preferred because the severity of the effects on the ossification is mild and not without any debate and the variation in the background control level is more than 5%. The $BMDL_{10}$ is the 95% lower

* US-EPA: United States Environmental Protection Agency.

confidence limit of the BMD_{10} ie. the dose that corresponds with a BMR 10% extra risk (delayed ossification). Moreover, the *lowest* calculated $BMDL_{10}$ is used as starting point in deriving an HBR-OEL; in this case a $BMDL_{10}$ of 1.3 ppm or 4.1 mg/m^3.

Extrapolation to HBROEL: For the establishment of an HBR-OEL, DECOS considered several aspects. The first aspect is the difference between animals and humans. In general, the Committee uses an uncertainty factor of three to compensate for interspecies differences. Moreover, due to possible inter-individual differences among people, the Committee is of the opinion that an (intraspecies) uncertainty factor of three is required as well. Adjusting the $BMDL_{10}$ value of 4.1 mg/m^3 by this factor (3*3), an HBR-OEL for EGME based on data in experimental animals is proposed of 0.5 mg EGME/m^3. The HBR-OEL is based on personal inhalable dust exposure, measured as an eight-hour time weighted average concentration.

Comparison with human data: Eventually, DECOS judges whether the recommended HBROEL based on experimental animal data will also protect against the effects found in human studies. In this case, effects on blood parameters in workers. The Committee is of the opinion that an HBROEL of 0.5 mg/m^3 (0.16 ppm) will also protect against the haematological effects because epidemiological studies do not describe effects after exposure to 7.3 mg EGME/m^3 (2.3 ppm) or above.

Finally, the Committee considers the available data insufficient for deriving a biological limit value (BLV).

EGMEA

The Committee is of the opinion that the toxicity profile of EGMEA is comparable to the profile of EGME. Moreover, EGMEA is rapidly hydrolised to EGME. Therefore, the Committee recommends a health based occupational exposure limit for EGMEA identical to that of EGME on a molar basis: 0.8 mg/m^3 (0.16 ppm).

2.2.2 Local effects

EGME and EGMA exert no local effect on the skin. EGME can act synergistically with other slightly irritant substances that are dissolved in EGME. However, quantitative and qualitative data are lacking. When instilled in the eye EGME and EGMEA are mildly to moderately irritating. EGMEA is not a respiratory irritant.

2.2.3 Skin notation

The Committee recommends a skin notation when dermal absorption adds considerably to the body burden and when the HBROEL is set on the basis of systemic toxicity. A skin notation is considered to be unnecessary when the compound is classified as “not dangerous”, taking into account the whole spectrum of acute and chronic effects.[43]

EGME

The absorption rate of EGME through human skin *in vitro* is approximately 2.8 (mg/cm^2)/h).[44] In human volunteers, the average percutaneous absorption rate of liquid EGME was 2.9 ± 2.0 mg/cm^2/h (range 1.6-5.2 mg/cm^2/h).[45] The percutaneous absorption rate was much lower (1.4-13 µg/cm^2/h) in another human volunteer study in which the forearm was exposed to EGME vapour. [46] Based on the highest dermal absorption measured *in vivo* (5.2 mg/cm^2/h) and assuming that hands and forearms (surface area 2000 cm^2) are exposed during one hour to liquid EGME, the quantity absorbed would amount to 10400 mg. Percutaneous absorption of EGME vapour was estimated to amount 16-55% of the total body burden.[45,46] Via the inhalatory route an amount of approximately 4 mg is absorbed during 8 h exposure to the health based recommended occupational exposure limit of 0.5 mg/m^3, assuming a retention of 0.76. Clearly, skin absorption of EGME adds considerably to the body burden and the Committee deems a skin notation to be necessary.

EGMEA

For EGMEA no data on skin absorption are available. Moreover, data on hydrolysis in or on the skin are also absent. However, because the hydrolysis of EGMEA into EGME is fairly quickly in *in vitro* experiments, the Committee assumes that the skin absorption of EGMEA resembles that of EGME.[39] Therefore, it also recommends a skin notation for EGMEA.

2.3 Groups at extra risk

The Committee emphasizes that the critical effects of exposure to EGME or EGMEA are on reproduction. Therefore, pregnant women should prevent exposure to high levels of EGME and EGMEA. On the other hand, exposure to

levels lower than the HBROEL will protect the progeny from adverse effects as well.

2.4 Health-based recommended occupational exposure limit

The Committee recommends the following health based occupational exposure limits as concentrations in air averaged over 8 hours (8 h TWA):

- ethyleneglycol monomethyl ether (EGME): 0.5 mg/m^3 (0.16 ppm)
- ethyleneglycol monomethyl ether acetate (EGMEA): 0.8 mg/m^3 (0.16 ppm).

A skin notation is recommended for both compounds.

2.5 Recommendations for research

No additional studies are recommended.

2.6 HBROEL DECOS versus SCOEL

In 2006, the European Scientific Committee on Occupational Exposure Limits (SCOEL) recommended an occupational exposure limit of 3 mg/m^3 (1 ppm) for EGME and 5 mg/m^3 (1 ppm) for EGMEA. This recommendation was mainly based on the haematological effects found in epidemiological studies.

DECOS, however, is of the opinion that an OEL of 3 mg/m^3 (1 ppm) for EGME and 5 mg/m^3 (1 ppm) for EGMEA will not protect workers for effects on reproduction. Experimental studies in animals provide information that these effects might occur after occupational exposure to the proposed OELS by the SCOEL.

In additional, the previous recommendation by DECOS from 1996 used the same critical study as the present report. However, the Committee used the $BMDL_{10}$ as a starting point for the derivation of a HBROEL instead of a NOAEL.

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Annexes

Annex **A**

Request for advice

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

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

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

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

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

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

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

Annex **B**

The Committee

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- P.J. Boogaard
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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 inaugural 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 review draft

The following persons or organisations have commented on the public draft:

- NIOSH, Cincinatti, United States of America
- Clariant, Frankfurt am Main, Germany
- INEOS Oxide, Antwerp, Belgium.

Annex **D**

Resuls of the BMD analyses for EGME

D.1 General remarks

Software: US EPA BMDS 2.1.2

- Model type : dichotomous, restricted models
- Risk type : extra risk
- BMR : BMR10 en BMR05
- BMDL : lowest 95% CI of the BMD
- Data source : Hanley et al. 1984
- Exposure :

D.2 Data and results

bred New Zealand white rabbits	0 ppm	3 ppm	10 ppm	50 ppm	BMDL10 lowest values in ppm (model)	BMDL05 lowest value in ppm (model)
pregnant females						
number of implantations resorbed	7/180	14/186	23/210[a]	46/191[a]	15.01 (LogLogistic)	7.11 (LogLogistic)
number of litters with resorptions	5/23	10/24	14/24[a]	16/24[a]	---	---
fetuses						
number of fetuses with delayed ossification of sternebrae	82/173	93/172	123/187[a]	127/145[a]	1.31 (Loglogistic)	0.62 (Loglogistic)

[a] $p<0.05$ (Hanley *et al.* 1984) 1 ppm = 3.16 mg/m^3, 0.62 ppm = 1.96 mg/m^3, 1.31 ppm = 4.14 mg/m^3, 7.11 ppm = 22.47 mg/m^3, 15.01 ppm = 47.43 mg/m^3

D.3 Results curve fitting analyses

Implantations resorbed

model		Gamma	Logistic	Log-logistic	LogProbit	Probit	Weibull	Quantal-Linear	Dichoto-mous-Hill
Log-Likelihood	Calculated difference (LL fitted – LL full model)	0.76	1.86	0.597	3.10	1.72	0.76	0.76	>100
	Difference degrees of freedom (LL fitted – LL full model)	2	2	2	2	2	2	2	4
	Accept model?	yes	yes	yes	no	yes	yes	yes	no
BMR10	BMD (ppm)	22.51	32.21	20.83	-	30.87	22.51	22.51	-
	BMDL (ppm)	16.81	27.46	*15.01*	-	25.95	16.81	16.81	-
BMR05	BMD (ppm)	10.96	19.17	9.87	-	17.89	10.96	10.96	-
	BMDL (ppm)	8.19	16.30	*7.11*	-	15.03	8.18	8.18	-

Values are rounded off by two decimals.
Critical difference log-likelihood at degrees of freedom of *3 is 3.91*, and of *2 it is 3.00*. A model is accepted when the calculated difference in log-likelihoods is lower than the critical difference in log-likelihood at the corresponding degrees of freedom. A model accepted means that it does fit well.

Litters with resorption

model		Gamma	Logistic	Log-logistic	LogProbit	Probit	Weibull	Quantal-Linear	Dichoto-mous-Hill
Log-Likelihood	Calculated difference (LL fitted – LL full model)	20.25	23.88	13.93	28.72	23.89	20.25	20.25	---
	Difference degrees of freedom (LL fitted – LL full model)	2	2	2	2	2	2	2	---
	Accept model?	no	no	no	no	no	no	no	no
BMR10	BMD (ppm)	-	-	-	-	-	-	-	-
	BMDL (ppm)	-	-	-	-	-	-	-	-
BMR05	BMD (ppm)	-	-	-	-	-	-	-	-
	BMDL (ppm)	-	-	-	-	-	-	-	-

Values are rounded off by two decimals.
Critical difference log-likelihood at degrees of freedom of *3 is 3.91*, and of *2 it is 3.00*. A model is accepted when the calculated difference in log-likelihoods is lower than the critical difference in log-likelihood at the corresponding degrees of freedom. A model accepted means that it does fit well.

Fetus delayed ossification of sternebrae

model		Gamma	Logistic	Log-logistic	LogProbit	Probit	Weibull	Quantal-Linear	Dichoto-mous-Hill
Log-Likelihood	Calculated difference (LL fitted – LL full model)	0.66	1.47	0.001	1.69	1.69	0.66	0.66	0.03
	Difference degrees of freedom (LL fitted – LL full model)	2	2	2	2	2	2	2	0
	Accept model?	yes	yes	yes	yes	yes	yes	yes	?
BMR10	BMD (ppm)	3.53	4.86	2.41	6.23	5.22	3.52	3.52	2.80
	BMDL (ppm)	2.76	4.00	*1.31*	4.70	4.38	2.76	2.76	1.18
BMR05	BMD (ppm)	1.71	2.42	1.23	4.33	2.60	1.71	1.71	1.55
	BMDL (ppm)	1.34	1.99	*0.62*	3.27	2.19	1.34	1.34	0.56

Values are rounded off by two decimals.
Critical difference log-likelihood at degrees of freedom of: *3 is 3.91;* of *2 it is 3.00;* and of 1 it is 1.92. A model is accepted when the calculated difference in log-likelihoods is lower than the critical difference in log-likelihood at the corresponding degrees of freedom. A model accepted means that it does fit well.

Part II

Data on EGME and EGMEA

Chapter 1

Identity, properties and monitoring

If not stated otherwise, information in this chapter is retrieved from the previous report of the Health Council on glycol ethers (Gem77, Bro80, Ale82, Row82, ECE82, Amo83, ACG86, Rut86, ECE91, NIO91, NIA92, Lew92, Gin94).*

In this report, the Committee evaluates the toxicity of two glycol ethers, namely: ethyleneglycol monomethyl ether and ethyleneglycol monomethyl ether acetate.

These compounds are extensively used as co-solvents and are marketed under a variety of names. As they are miscible with water and a large number of organic solvents they are especially useful as co-solvents in many oil-water compositions. They are used as solvents for various resins, lacquers, paints, varnishes, dyes, inks, printing pastes, cleaning compositions, liquid soap and cosmetics and as chemical intermediates (ECE82).

* For references 'Gem77' etc see: References Health Council of the Netherlands 1996/01 WGD.

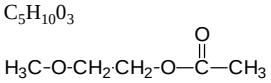
1.1 Identity and physical and chemical properties

1.1.1 Ethyleneglycol monomethyl ether (EGME)

chemical substance prime name	ethanol, 2-methoxy-
synonyms	Dowanol®EM Glycol Ether; EGM; Glycol Ether EM; glycol methyl ether; Jeffersol EM; MECS; methoxyhydroxyethane; Methyl Cellosolve® Solvent; methyl ethoxol; methyl glycol; Methyl Oxitol® Glycol; polysolv EM; PRIST; UN 1188
abbreviation used in this report	EGME
CAS registry number	109-86-4
EINECS nr	203-713-7
EEC nr	603-011-00-4
description	colourless liquid with a mild, agreeable odour
molecular formula	$C_3H_8O_2$
structure	$H_3C\text{-}O\text{-}CH_2\text{-}CH_2OH$
molecular weight	76.10
boiling point (101 kPa)	(101 kPa): 124.2°C (102 kPa): 125°C
melting point	- 85°C
vapour pressure	(101 kPa 25°C): 1290 Pa (101 kPa 20°C): 1100 Pa; 825 Pa (used in this report); 800 Pa; 600 Pa
relative density of the saturated vapour in air (20°C, 101 kPa, air = 1)	1.01
vapour percentage in saturated air (20°C, 101 kPa)	0.81%
flash point	cup?: 39°C open cup: 43.3°C; 46°C closed cup: 39.4°C; 41.7°C
autoignition temperature	285°C
explosion limits	2.5-14 vol% in air 125-140°C: 2.5-19.8 vol% in air 25°C: -24.5 vol% in air (Hig87)
specific gravity	20/4°C: 0.9647; 0.9660 20/20°C: 0.9663 25/4°C: 0.962 25/25°C: 0.963
solubility	in water: completely in acetone, alcohol, benzene, ether: completely
odour threshold	7.3 mg/m^3; 190 mg/m^3 0.288-288 mg/m^3 detection: < 0.3-190 mg/m^3 recognition: 0.7-280 mg/m^3
taste threshold in water at 40°C:	200-400 mg/l in water at 60°C: 80-120 mg/l

log Poct/water	0.61; 0.7
conversion factors (20°C, 101 kPa)	1 ppm = 3.16 mg/m^3 1 mg/m^3 = 0.316 ppm

1.1.2 *Ethyleneglycol monomethylether acetate (EGMEA)*

chemical substance prime name	ethanol, 2-methoxy-, acetate
synonyms	glycol ether EM acetate; MeCsAc; 2-methoxyethyl acetate; Methyl Cellosolve® Acetate; Methyl Glycol Acetate; UN 1189
abbreviation used in this report	EGMEA
CAS registry number	110-49-6
EINECS nr	203-772-9
EEC nr	607-036-00-1 (ECD93)
description	colourless liquid, with a mild, ether-like odour
molecular formula	$C_5H_{10}0_3$
structure	
molecular weight	118.13
boiling point (101 kPa)	144.5°C
melting point	-65°C; -70°C
vapour pressure	(101 kPa 20°C): 266 Pa; 270 Pa (used in this report); 270-490 Pa; 930 Pa
relative density of the saturated vapour in air (20°C, 101 kPa, air = 1)	1.01
vapour percentage in saturated air (20°C, 101 kPa)	0.27%
flash point	44°C; 47°C; 49°C; open cup: 55.6°C; 60°C
autoignition temperature	380°C
explosion limits	1.7-8.2 vol% in air
specific gravity	19/19°C: 1.009 20/20°C: 1.005; 1.0067 25/4°C: 1.007
solubility	in water: completely in common organic solvents: completely
odour threshold	1.632-240 mg/m^3 detection: 1.6 mg/m^3 recognition: 3.1 mg/m^3
log Poct/water	- 0.25 (Mul84)
conversion factors (20°C, 101 kPa)	1 ppm = 4.91 mg/m^3 1 mg/m^3 = 0.204 ppm

1.2 Analytical methods

1.2.1 Environmental monitoring

For EGME and EGMEA validated methods are available.

EGME

For EGME a volume of 1-10 l of air is sampled, the compound is adsorbed on coconut shell charcoal. Desorption is performed with 5% methanol in dichloromethane. The compound is analysed on a gas-liquid chromatograph (GLC) equipped with an FID. Two ranges were studied: 44-160 mg/m^3 and 124-490 mg/m^3. In these ranges the recovery was 92-93% and the measurement precision 0.008-0.009 (NIO84).

EGMEA

For EGMEA a volume of 201 of air is sampled and the compound is adsorbed on charcoal. Desorption is performed with carbon disulfide. The compound is analysed on a gas-liquid chromatography. The method was validated over the range of 51-214 mg/m^3. Under the conditions of sample size (20 l) the probably useful range of this method is 12-360 mg/m^3 (NI074).

Other methods were developed and validated for personal monitoring of exposure to airborne glycol ethers (among which EGME), both short-term and longterm time-weighted-averages (Lan84).

1.2.2 Biological monitoring

No validated method has been described.

Chapter 2

Sources of exposure

If not stated otherwise, information in this chapter is retrieved from the previous report of the Health Council on glycol ethers*.

2.1 Natural occurrence

EGMEA has been found in the plant *Pueraria lobata Ohwi* in a concentration of 4.8% of the volatile constituents of its roots. The compound was identified with GC/MS (Miy88).

2.2 Man-made sources

2.2.1 Production

Glycol ethers are produced by the reaction of alkylene oxides with alcohols or phenols, or of glycols with alkylating agents. The common ethers are derived from ethylene, diethylene, propylene and dipropylene glycols. The ethyleneglycol methyl, ethyl and butyl ethers are the most widely used. In 1980, 260,000 tonnes of these ethers were produced in Western Europe and 529,000 tonnes (all types of glycol ethers) in Japan (ECE82).

* For references 'Gem77' etc see: References: Health Council of the Netherlands 1996/01 WGD.

EGME

The estimated production of EGME in 1981 was $37*10^6$ kg in Western Europe, $3.1*10^6$ kg in Japan and $39*10^6$ kg in the US (IPC90). The use of EGME has declined over the past few years because it has been partially replaced in some countries by less toxic substances (IPC90).

In the years from 1987 to 1992 about $32*10^9$ kg of EGME were produced in the US (Die93). The consumption of EGME in the EC in 1987 was estimated to be $18*10^6$ kg (ECD93).

EGMEA

The acetate EGMEA is produced by standard esterification techniques using EGME, the acid anhydride or chloride and an acid catalyst (IPC90).

2.2.2 *Uses*

EGME

EGME is used as a solvent for many purposes: cellulose esters, dyes, resins, lacquers, varnishes, and stains, as a perfume fixative and as jet fuel deicing additive (ACG86).

In 1989 in the EC about 45% of the total quantity was used as jet fuel, 20% as solvent for electronics and 30% as intermediate for pesticides and other chemicals (ECD93).

EGMEA

EGMEA is used in photographic films, lacquers, textile printing, as a solvent for waxes, oils, various gums and resins, cellulose acetate, and nitro-cellulose (ACG86).

It is a freezing point depressant in explosives formulations (ECD93).

Chapter 3

Environmental levels and human exposure

If not stated otherwise, information in this chapter is retrieved from the previous report of the Health Council on glycol ethers*.

3.1 Environmental levels

The greatest environmental exposure to glycol ethers results from their direct release into the atmosphere when they are used as evaporative solvents. Given the amounts synthesised and transported, there is also a great potential for environmental exposure from accidental releases and the disposal of cleaning products and containers. Discharges of this type result in the transport of these chemicals to soil and water. Because of their water solubility and low vapour pressure, they could build up in water in the absence of degradation. However, biodegradation in surface waters is an important way of disappearance (IPC90).

3.1.1 Water

No data on levels of EGME and EGMEA in water have been found (IPC90), or of the other three glycol ethers.

* For references ‘Gem77’ etc see: References: Health Council of the Netherlands 1996/01 WGD.

3.1.2 *Food*

No data on levels of EGME and EGMEA in food have been found (IPC90), or of the other three glycol ethers.

3.1.3 *Ambient air*

No data on levels of EGME and EGMEA in air have been found (IPC90), or of the other three glycol ethers.

3.2 Human exposure

3.2.1 *General population*

No data have been found for EGME and EGMEA (IPC90).

3.2.2 *Working population*

The major concern for human exposure is the occupational environment. Data have been collected (ECE85, IPC90) and are summarised in Table 1. Data found in other references are summarised in Table 2.

Table 1 Summary of occupational exposures in the US and Europe[a] (ECE85, IPC90).

chemical and job category	range (mg/m^3)	arithmetic mean (mg/m^3)	standard deviations
EGME			
operator, painter, miscellaneous; US	0.31-188.50	6.22-59.28	3.11-8.40
manufacturing, drum filling; Europe	0.60-20.00	29	
51 different plants; US	> 19.30 (peak exposure)		
6 PAS samples, semiconductor industry; US[b]	0.12-3.11	0.68	1.18
4 area samplings, idem	0.093-2.49	0.72	1.18
EGMEA			
51 different plants; US	> 41 (peak exposure	4.40	
miscellaneous, operator, printer, painter; US	0.48-40.57	1.88-12.94	13.04-16.23
16 PAS samples, semiconductor industry; US	N.D.	0.048	0.00
20 area samplings, idem	N.D.	0.048	0.00

[a] all sampling data are TWA 8 h unless stated otherwise
[b] the workers were exposed to other glycol ethers as well
N.D. = not detectable

In the US, it was estimated in 1986 that 28,968 women are occupationally exposed to levels of EGME lower than 790 mg/m^3 (Feu90).

Table 2 Summary of occupational exposures in the US and Europe (other references).

chemical and job category	range (mg/m^3)	arithmetic mean (mg/m^3)	reference
EGME			
9 PAS samples, floor layers[a], Germany[b]	N.D.-150	6.1	Den86
94 air samples, printing, Belgium[a]		N.D.	Veu87
81 air samples, painting, Belgium[a]	5.6-136.9 (found in 4 samples)	31.3	
20 air samples, car repair, Belgium[a]	3.4-15.9 (found in 10 samples)	7.9	
67 air samples, various, Belgiuma		N.D.	
81 PAS samples, shipyard painters, US[c]	0-17.7	2.6 (median: 1.6	Spa88
27 8 h PAS and air samples (of which 8 spray painting, 10 fuel distribution, 9 paperboard manufacturing), US	0-3.3	not given, 17 samples did not contain EGME	Pia90
13 15 min PAS samples (of which 3 spray painting, 5 fuel distribution, 2 paperboard manufacturing and 2 glycolether manufacturing), US	0-21.7	not given, 5 samples did not contain EGME	
EGMEA			
13 10-30 min PAS samples, car spray painting, US[d]	20.5-142		Jay84
94 air samples, printing, Belgium[a]	3.9-4.7 (found in 2 samples)	4.3	Veu87
81 air samples, painting, Belgium[a]		N.D.	
20 air samples, car repair, Belgium[a]	(found in 1 sample)	2.3	
67 air samples, various, Belgium[a]	0.4-143.3 (found in 12 samples)	11.6	

[a] the workers were also exposed to other solvents
[b] during an 8 h shift 8 charcoal tubes were used
[c] the workers were also exposed to ethyleneglycol monoethyl ether and occasionally to lead pigments
[d] the workers were also exposed to other solvents and occasionally to lead and chromium pigments
N.D. = not detectable

Chapter 4

Kinetics

If not stated otherwise, information in this chapter is retrieved from the previous report of the Health Council on glycol ethers*.

4.1 Absorption

4.1.1 EGME

In vivo studies in humans showed rapid absorption of EGME after dermal application of 15 ml of solvent. Two hours after application, blood levels reached 200-300 μg/ml. This rate of absorption was approximately ten times greater than that of methanol, acetone, or methyl acetate (study from 1980, IPC90).

The uptake in human volunteers during a 4 h exposure period to 16 mg EGME/ m^3 was 76% (n = 7). The uptake is equivalent to a dose of only 0.25 mg/kg. During the start of the exposure and up to 120 h thereafter, 2-methoxyacetic acid (2-MAA) was detected in the urine. The elimination half-life of its major metabolite, 2-MMA, was on average 77.1 h. The total amount of 2-MAA excreted was calculated by extrapolation and averaged 85.5% of the inhaled EGME (that means: 85.5% of 76%) (Gro89a).

* For references see: References part I: Health Council of the Netherlands 1996/01WGD.

Absorption through isolated human abdominal skin was measured for several glycolethers. The compounds are listed in order of decreasing steady state absorption rate as follows: EGME > PGME > EGEE = EGEEA > DEGME > EGBE > DEGEE >DEGBE, or, in words: ethyleneglycol monomethylether > propyleneglycol monomethylether > ethyleneglycol monoethylether = ethyleneglycol monoethylether acetate > diethyleneglycol monomethylether > ethyleneglycol monobutylether > diethyleneglycol monoethylether > diethyleneglycol monobutylether. Quantitative data are presented in Table 3. It can be concluded that EGME is readily absorbed through human skin *in vitro*. The possible damaging influence of exposure to the glycolethers was also measured. The damage is expressed as a ratio of permeability of a first and second exposure, both lasting 8 h, separated by a period of recovery in the form of overnight incubation in water. Control damage ratio values for water contact alone lie between 1.0 and 2.0. Thus slight irreversible effects on barrier function occurred for EGME and DEGME.

Table 3 Absorption rate and damage ratio of several glycolethers *in vitro* through human abdominal skin (Dug84).

compound	absorption rate ($mg/cm^2/hr$)	number of determinations	damage ratio	number of determinations
EGME	2.82 ± 2.63	22	3.51 ± 1.47	20
DEGME	0.206 ± 0.156	11	3.16 ± 1.77	10
DEGEE	0.125 ± 0.103	10	1.20 ± 2.62	8
DEGBE	0.035 ± 0.025	9	2.05 ± 1.01	5

Additional data in SCOEL (2006)*

An average absorption rate of 2.9 $mg/cm^2/h$, with large inter-individual variations**, was measured for liquid EGME in an in vivo study with volunteers. Exposure of hands and lower arms to EGME in liquid form was calculated to yield an absorption rate 100 times that of exposure to 5 ppm in the air. The authors also calculated that, with whole-body exposure to EGME vapor, 55% of total uptake occurs via the skin (Kezic *et al.* 1997)SCOEL1.

In another single-arm exposure of seven volunteers to EGME vapours at 25 or 300 ppm, percutaneous absorption rates of 1.4 and 13 $\mu g/cm^2/h$ were obtained (Shih *et al.* 2000b)SCOEL2. Extrapolating these data to whole-body exposure to

* For references 'SCOEL1'etc see: References part II: SCOEL SUM/120, 2006.

** Actually, values for 4 subjects were rather similar: 1.6, 2.1, 3.1, and 5.2 $mg/cm^2/h$. Average: 2.9 ± 2.0 $mg/cm^2/h$.

EGME vapours, 17-20% of the total dose would be absorbed via skin (skin area 1.8 m^2, pulmonary ventilation 10 m^3/8 h).

4.1.2 EGMEA

No data are available.

4.2 Distribution and biotransformation

4.2.1 EGME

Groups of three male Fischer 344 rats received a single oral dose of approximately 1 or 8.7 mmol/kg of ^{14}C-EGME (respectively 76 or 662 mg/kg). After dosing expired air, excreta, and tissues were analysed for ^{14}C, metabolites in urine were isolated and identified. The percentage recovery during a 48-h period is given in Table 4, the tissue distribution of radioactivity is given in Table 5. It can be concluded that EGME is mainly excreted via the urine and that very little compound is distributed in the body (Mil83a).

2-Methoxyacetic acid (2-MAA) was identified as the primary urinary metabolite in rats, accounting for 80 to 90% of the total ^{14}C in urine (Mil83a).

Medinsky *et al.* (Med90) obtained results comparable with those of Miller *et al.* (Mil83a). Dosing of EGME was compared with dosing of 2-ethoxyethanol and 2-butoxyethanol. Groups of male F344/N rats received for 24 h radiolabeled compounds in their drinking water at three dosages. The dosages of EGME were 180, 540 and 1,620 ppm in water, which resulted in an uptake of 160, 344 and 1450 µmol EGME/kg body weight respectively (12.2, 26.2 and 110 mg/kg). The majority of the ^{14}C was eliminated as 2-MAA (34%) in the urine, followed by 10-30% exhalation as CO_2. Ethyleneglycol, a previously unreported metabolite, was excreted in the urine, representing approximately 21% of the dose (Med90).

Foster *et al.* (Fos84) also assayed the tissue distribution of ^{14}C-EGME in rats after a single oral dose. The strain of rats is not mentioned. The data are summarised in Table 6. There was no testis-specific uptake or accumulation of radioactivity (approximately 0.15% of the dose in 24 h).

Table 4 Percentage recovery values during a 48-h period after a single oral dose of ^{14}C-EGME to rats (n = 3) (Mil83a).

compartment	76 mg/kg	662 mg/kg
urine	54.3 ± 5.0	63.2 ± 3.9
faeces	2.7 ± 1.1	2.6 ± 0.9
charcoal	0.4 ± 0.1	0.3 ± 0.9
CO_2	11.8 ± 2.6	11.8 ± 2.0
carcass	18.3 ± 3.0	12.6 ± 1.1
skin	3.5 ± 0.4	3.6 ± 0.2
cage wash	3.8 ± 0.3	1.2 ± 0.3
total	94.8 ± 6.7	95.4 ± 0.7

Table 5 Tissue distribution of radioactivity 48 h after a single oral dose of 662 mg ^{14}C-EGME/kg to rats (n = 3) (Mil83a).

tissue	tissue/blood ratio	percentage dose
body	0.19	9.60 ± 0.89
liver	0.66	1.57 ± 0.32
kidney	0.35	0.20 ± 0.00
blood	= 1.00	0.67 ± 0.21
testes	0.19	0.13 ± 0.06
fat	0.05	not calculated
thymus	0.03	0.02 ± 0.00
spleen	0.29	0.03 ± 0.00
total		12.17 ± 1.43

Table 6 The excretion distribution of ^{14}C-EGME in rats after a single oral dose of 500 mg/kg (Fos84).

tissue	time after dose, h	percentage of administered dose
urine	0-24[a]	58.5 ± 2.3
faeces		1.0 ± 0.2
exhaled		2.3 ± 0.3
urine	24-48[b]	11.5 ± 0.8
faeces		0.5 ± 0.1
exhaled		0.4 ± 0.2
tissues		12.5 ± 0.9
total		86.7 (range: 81.7-94.5)

[a] n = 12
[b] n = 6

The major metabolite found in urine was 2-MAA (73.1%); EGME (14.8%) was also detected together with a third peak (8.1%) that did not chromatograph with any of the standards used (Fos84).

Only 5 minutes after oral administration of a tracer dose of EGME to pregnant mice radioactivity was present throughout the maternal and conceptus compartments. The highest levels were noted in maternal liver, blood, and gastrointestinal tract, and in the placenta, yolk sac, and embryonic structures such as limb buds, somites, and neuroepithelium. Maternal blood levels declined to between 2 and 10% of peak levels after 24 h. At 6 h post administration 69% of the radioactivity in maternal liver and 33% of that in the embryo were acid insoluble. The high levels of covalently bound radioactivity in biosynthetically active tissues, e.g. the liver, bone marrow, and gastric mucosa, is an indication of persistent activity of the compound on cellular components of these tissues. These activities may lead to EGME toxicity (study from 1986, IPC90).

When pregnant CD-1 mice were dosed with a single oral dose of 4.6 mmol ^{14}C-EGME/kg (350 mg/kg) most of the radioactivity administered was excreted by the kidneys, as with rats. Within 12 h after dosing about 35% of the ^{14}C appeared in the urine, and by 24 h that amount had almost doubled. The total radioactivity recovered over 72 h is presented in Table 7.

Table 7 Radioactivity recovered from pregnant mice over 72 h after a single oral dose of 350 mg ^{14}C-EGME/kg on GD 11 (n = 4) (SIe88).

compartment	percentage (range) of total ^{14}C administered
urine	79.8 (68.8-89.8)
expired air	
organic	0.4 (0.3-0.5)
CO_2	5.7 (4.0-7.6)
faeces	2.3 (1.6-3.4)
carcass	2.8 (2.1-3.4)
cage wash	2.3 (1.4-3.3)

Oxidation of EGME to 2-MAA was nearly complete after 1 h when ± 90% of ^{14}C in maternal plasma and conceptus coeluted with authentic ^{14}C-methoxyacetic acid upon HPLC. ^{14}C-MAA levels in embryos were 1.2 times those in plasma one and six h after dosing, although by six hour concentrations had declined to ± 50% of 1-h values. Concomitant administration of ethanol did not affect ^{14}C kinetics as measured in maternal blood after oral ^{14}C-EGME, but retarded EGME conversion to 2-MAA by about two hour. Furthermore, embryo ^{14}C-MAA levels reached only 50% of the peak in embryos from dams dosed with EGME alone, an effect that coincided with less ^{14}C incorporation into macromolecules synthesised by the embryo within six hour (Sle88).

Dermal application of EGME to male F344/N rats resulted in a metabolite pattern which resembles that after oral administration. Three dose levels were used, the amount applied and absorbed was calculated to be 88 ± 15, 383 ± 46 and 866 ± 90 μmol/kg (n = 4), respectively. Approximately 20 to 25% of the dose was absorbed and metabolised, regardless of the applied dose. The majority of the metabolites was excreted in the urine: 67-72% of the total radioactivity. Nine to ten percent was excreted in the faeces. In the carcass 14-16% of the radioactivity was found. Four to eight % was excreted as 'CO'. The ^{14}C not recovered was most likely due to evaporation from the application site during the application and transport to metabolism cages. 2-MAA comprised 62-63% of the total urinary metabolites. Ethyleneglycol was found in a concentration of 10-15%. The glycine conjugate of 2-MAA comprised 9-10% of the urinary metabolites (Sab92 and erratum Sab93).

In a teratogenicity study with Wistar rats the animals received a single ip injection of 400 mg/kg on GD 12. Two, eight or 24 hour later the dams were killed and the concentration assayed in maternal serum, embryo and extraembryonic fluid. There is a timedependent decrease of EGME in all three compartments. The concentration of 2-MAA in the embryo was about twice that of maternal serum at the three time points examined and was closely matched by the concentrations in the extraembryonic fluid. In a preliminary study 2-MAA was still detectable in maternal blood and embryos 69 hour after a single dose of 400 mg/kg on GD 12 (Sco87).

In a teratogenicity study with CD-1 mice the animals received on GD 8 either a single iv bolus dose of 0, 175, 250 or 325 mg EGME/kg or sc constant-rate infusions for 4, 6 or 8 h, totalling 277, 392 or 606 mg EGME/kg. Concentrations of 2-MAA were measured during distribution and elimination in maternal plasma and conceptuses. All *iv* bolus doses resulted in negligible or undetectable concentrations of 2-MAA in maternal plasma and conceptuses at 24 h after injection. Further, the concentrations of 2-MAA in maternal plasma and conceptuses 12 h after removal of the pump were not significantly different from those achieved 12 h after *iv* bolus dosing. The peak concentrations are given in Table 8 (Ter94).

In a teratogenicity study with the monkey *Macaca fascicularis,* groups of females received daily oral doses of 12, 24 or 36 mg EGME/kg on GD 20-45. On days 1, 8, 15 and 22 of treatment, blood samples were taken 2, 4, 7.5 and 24 h after dosing to measure the 2-MAA concentration. Distribution of 2-MAA

indicated a half-life of ca. 20 h, resulting in accumulation of metabolite in maternal serum after repeated dosing. Transplacental studies revealed uniform distribution in the embryo and extraembryonic fluids at a concentration similar to that in maternal serum. Yolk sac, on the other hand, accumulated a very high concentration of 2-MAA, but the embryonic significance of this observation is unknown. Maternal blood levels of 2-MAA correlated only poorly with pregnancy outcome, perhaps related, in part, to the difficulty in discriminating between spontaneous and EGME-induced abortions (see further section 6.2.6) (Sco89).

Table 8 2-MAA peak concentrations (C_{max}) in maternal plasma and conceptuses after a single iv bolus injection or 6 h sc infusion of CD-1 mice of EGME on GD 8 (Ter94).[a]

dose (mg/kg)	maternal plasma Cmax ± SE (mmol/l)	conceptuses Cmax ± SE (mmol/l)
175[b]	3.86 ± 0.09	3.55 ± 0.19
250[b]	5.22 ± 0.25	3.81 ± 0.18
325[b]	8.18 ± 0.66	5.40 ± 0.96
392[c]	5.04 ± 0.39	4.78 ± 0.16[d]

[a] values are derived from the mean of 3-5 litters per time point
[b] based on 12 h 2-MAA kinetic analysis following single bolus iv injection of EGME
[c] based on 18 h 2-MAA kinetic analysis after EGME infused for 6 h
[d] EGME infusion value significantly different from corresponding 250 mg/kg value ($p < 0.01$)

Mebus *et al.* (Meb92) used three different labels of EGME and 2-MAA to investigate further the metabolic pathway of EGME in pregnant mice. The study reveals an elimination pattern quite similar to the one observed by Sleet *et al.* (Sle88). 2-MAA was further metabolised to its glycine conjugate (± 25% of the dose) and an unidentified compound (“peak A”, 12-18% of the dose).

In the same study, whole embryos from CD-1 mice were incubated on GD 11 for four hours with 3 mM 2-MAA and a tracer dose of [1-^{14}C]-2-MAA, [2-^{14}C]-2-MAA or [methoxy-^{14}C]-2-MAA. Monitoring the radioactivity revealed that $^{14}CO_2$ evolved from the former two substrates, while there was none detectable from the latter. The data indicate that dams metabolised [methoxy-^{14}C]-2-MAA to $^{14}CO_2$, while embryos apparently did not.

The production of CO_2 from [2-^{14}C]-EGME suggests that 2-methoxyacetyl-CoA (the precursor for amino acid conjugation with glycine) entered into the tricarboxylic acid cycle. This interpretation is supported by the inhibition of “CO_2 evolution elicited by fluoroacetate (0.1 or 1.0 mM) and sodium acetate (5 mM). It is not yet clear whether entry of 2-methoxyacetyl-CoA as a “false

substrate" in the tricarboxylic acid cycle is of significance for the embryotoxic effects of EGME/2-MAA.

The distribution of ^{14}C-EGME in male mice was studied after iv and oral administration (Ahm94). The administered amount is equivalent to 101.2 µg/mouse or 4 mg/kg body weight, in both routes of administration. In both groups of animals the highest levels of radioactivity were detected in the liver, urinary bladder, bone marrow, kidney, and epididymis, at 1- and 24-h time periods. There was a markedly higher deposition of ^{14}C-EGME and/or its metabolites in various tissues of the orally treated animals than in animals treated intravenously. Data are summarised in Table 9.

EGME can be formed in vitro after cleavage of the central ether linkage of diethyleneglycol dimethylether (referred to as DEGDME). The formation was established using rat and human hepatic microsomes. Pretreatment of the rat with cytochrome P450 inducers increased the conversion of DEGDME into EGME. Also microsomes isolated from a cell line transfected with specific human P450

Table 9 Tissue distribution of ^{14}C-EGME in mice after a single oral dose or iv injection equivalent to 4 mg/kg (Ahm94).

tissue	1 h after oral	1 h after *iv*	24 h after oral	24 h after *iv*
heart-blood/aorta	= 1	= 1	= 1	= 1
eye	0.9	0.8	NI	NI
brain	0.3	0.3	0.6	0.2
spinal cord	0.4	0.3	0.5	NI
salivary gland	0.5	0.8	2.3	1.9
thymus	0.4	0.5	2.0	1.7
adipose tissue	0.6	0.6	2.1	1.4
spleen	0.4	0.6	NI	1.5
adrenal cortex	0.6	0.6	NI	NI
kidney	0.9	1.1	3.8	1.9
lung	0.8	0.7	2.1	1.0
liver	1.5	2.0	4.8	3.1
stomach contents	0.4	0.6	0.7	0.2
stomach mucosa	1.0	0.9	4.0	2.7
intestinal contents	1.1	1.1	1.3	0.6
intestinal mucosa	0.5	0.6	1.9	2.4
urinary bladder	1.7	2.0	3.0	3.8
testis	0.2	0.2	1.0	0.3
epididymis (caput)	0.5	0.7	2.9	1.4
peripheral osseus tissues	1.2	1.4	1.5	2.8
bone marrow	0.5	0.6	2.9	3.2

NI = organ not identified in the analysed autoradiograph

cDNAs increased the conversion of DEGDME into EGME. The rate of conversion by microsomes isolated from untreated rats was 2.7 ± 0.1 nmol EGME /nmol P450 per 30 min incubation period at 37°C. The rate of conversion by microsomes isolated from eight humans, who had died from several causes, was 19.7 ± 3.1 nmol/nmol P450, indicating that human hepatic microsomes are seven times more effective in catalysing the conversion of DEGDME to EGME than rat hepatic microsomes. In addition to EGME, DEGME and an unknown metabolite were also found in human and rat microsomal incubations (Tir93).

4.2.2 EGMEA

The half-life time for the metabolism of EGMEA to EGME in vitro in rat plasma is approximately 12 minutes (ECE85).

4.2.3 EGME, partition coefficient

The partition coefficients of EGME between air and several liquids was determined (Joh88). The data are presented in Table 10.

Table 10 Partition coefficients of EGME between air and several liquids at 37°C (n=5) (Joh88).

system	coefficient
physiological saline/air	35,869 (SE 437)
pooled human blood/air	32,836 (SE 1350)
olive oil/air	529 (SE 11)

4.3 Elimination

4.3.1 EGME

Groups of Sprague Dawley rats were ip injected with 250 mg ^{14}C-EGME/kg. The urine was collected. Radioactivity detected in urine over 48 h after treatment accounted for 55% of the dose. The major urinary metabolite was identified as 2-methoxyacetic acid (2-MAA). Analysis of plasma revealed a rapid conversion of EGME to 2-MAA ($t_{½}$ for disappearance of EGME is 0.6 ± 0.03 h) and gradual clearance of radioactivity ($t_{½}$ is 19.7 ± 2.3 h). When animals were pretreated with a single ip injection of 400 mg pyrazole/kg (an alcohol dehydrogenase inhibitor) 1 h prior to EGME dosing the radioactivity detected in the urine was significantly lower than in the EGME-only group. Analysis of plasma revealed almost complete inhibition of the conversion of EGME to 2-MAA ($t_{½}$ for

disappearance of EGME is 42.6 ± 5.6 h, clearance of radioactivity $t_{½}$ is 51.0 ± 7.8 h) (Mos85).

4.3.2 EGMEA

No data are available.

4.4 Biological monitoring

The characteristics that make glycol ethers ideal for industrial applications make them a challenge for an analytical chemist, that is, their simultaneous lipophilic and hydrophilic properties and low vapour pressures. The hydrophilic nature of glycol ethers prevents their easy isolation from a urine or blood matrix. Moreover, many standard gas chromatographic columns will not resolve simultaneously the diverse glycolethers, and those that do may not resolve them from co-extracted substances. Resolution on porous polymer columns are not sufficient, which precludes direct injection of diluted blood. Low vapour pressures preclude headspace analysis. HPLC is not suitable because these compounds and their metabolites do not absorb significantly in the ultraviolet (Sma84).

4.4.1 EGME

EGME was extracted from human blood with dichloromethane and analysed on a GLC equipped with a flame ionisation detector (FID). The detection limit was 8.8 μg/g. The average recovery was 78% measured over a concentration range of 88-946 μg/g. Extraction of 2-MAA from human urine was performed with dichloromethane. The compound was derivatised by using pentafluoro-benzylation. Analysis was performed on a GLC. The detector is not mentioned. The detection limit was 11.4 μg/ml. The average recovery was 78%, measured over a concentration range of 11.4-1140 g/ml (Sma84).

Groeseneken *et al.* (Gro89b) improved the latter method. Urine samples are adjusted to pH7 and chloropropionic acid is added. The samples are then frozen to -60°C and lyophilised overnight. The dry residue is taken up in methanol, derivatised with pentafluorobenzylbromide and extracted with dichloromethane. The recovery was over 100%. Analysis is performed on a GLC equipped with a FID. The limit of detection was 0.03 mg/l. The standard curve was linear over the range of 0.1 to 200 mg/l.

Johanson also improved the method of Smallwood (Joh89). 2-MAA was extracted from urine into dichloromethane, with tetrabutylammonium acting as counterion, and derivatised with pentafluorobenzylbromide in a single step. After separation of the dichloromethane-phase analysis was performed on a GLC equipped with a FID. The limit of quantitation was estimated to 2 μM (corresponding to an injected amount of 2 μg) of 2-MAA in urine. The detector response was linear up to 80 μM and the formation of derivative at least up to 1 mM. Urine from non-exposed humans (ten females, ten males), did not contain 2-MAA or any interfering material.

Additional data in SCOEL (2006)

Based on the Groeseneken study (Gro89b) a NIOSH report estimated that 8 h exposure to 0.1 ppm EGME would approximate 0.8 mg 2-MAA/g creatinine at end of shift (NIOSH 1991)[SCOEL3].

Using a simple pharmacokinetic one-compartment level and one workweek (5d x 8h) of exposure at 1 ppm, the Groeseneken data would correspond to approximately 6-9 mg/g Cr at Friday end of shift after the first week, 7-11 mg/g Cr after the second week and 8-12 mg/g Cr after several weeks of exposure. Assuming several weeks of exposure the predicted increase from Monday morning before shift to Friday end of shift is 3-5 mg/g Cr.

In a study by Shih *et al.* (2000a)[SCOEL4] in Taiwan, 8-h personal breathing zone samples and urine samples before and after shift were collected from Monday to Saturday from 27 workers exposed to EGME and on Friday from 30 control workers. No urinary 2-MAA was detected in workers in the non-exposed control group. For 18 regular operation workers not using personal protective equipment, a significant correlation ($r = 0.702$, $p = 0.001$) was found between urinary 2-MAA on Friday after shift and weekly mean exposure to EGME. The regression equation indicated that 5 d x 8 h exposure at 1 ppm EGME corresponds to 8 mg 2-MAA/g creatinine. A significant correlation was also found between the weekly increase of urinary 2-MAA (Friday after shift minus Monday before shift) and the weekly mean exposure of EGME. In this case, 1 ppm corresponded to 4 mg 2-MAA/g creatinine.

Similar results were reported by Chang *et al.* (2004)[SCOEL5] in a study primarily aimed to evaluate the effectiveness of gloves during occupational exposure to EGME. In a group of 25 workers involved in special operations (raw material mixing, charging, machine cleaning), with limited use of gloves, the average EGME level in air was 8.1 ppm, while 2-MAA at end of Friday shift was on average 73 mg/g Cr. This corresponds to 9 mg/g Cr at 1 ppm. However, a

group of 49 less exposed workers (average 2.1 ppm) excreted considerably less 2-MAA (average 5.4 mg/g Cr).

The values of Shih *et al.* (2000a)[SCOEL4] and Chang *et al.* (2004)[SCOEL5] of 8 and 9 (end of Friday shift) and 4 (increase during week) mg/g Cr are consistent with those extrapolated from the Groeseneken *et al.* (1989) study of 8-12 and 3-5 mg/g Cr, respectively.

4.4.2 EGMEA

No data are available.

4.5 Summary

EGME is readily absorbed through the skin. Absorption rate through human skin *in vitro* amounted to approximately 2.8 mg/cm^2/hr. In human volunteers, the average percutaneous absorption rate of liquid EGME was 2.9 mg/cm^2/h. The percutaneous absorption rate was much lower (1.4-13 μg/cm^2/h) in another human volunteer study in which the forearm was exposed to EGME vapour. The retention of EGME after inhalation exposure by humans is 0.76. The elimination half-life time of 2-MAA was on average 77.1 h.

Distribution of EGME after a single oral dose to rats is rapid and after 48 h 54-70% of the dose is recovered in the urine. Exhalation in the form of CO_2 amounted to 3-12% of the dose after 48 h. 2-MAA was the primary urinary metabolite in rats, accounting for 80 to 90% of the total ^{14}C in urine.

When EGME is given to rats via the drinking water, somewhat less of the compound is eliminated as 2-MAA via the urine (34%) and more is exhaled as CO_2 (10-30%) compared with oral dosing.

Dermal application of EGME to rats results in a metabolite pattern similar to that after oral administration.

Distribution of EGME after a single oral dose to pregnant mice is rapid and the compound can be found in maternal and conceptus compartments within 5 minutes. EGME is mainly excreted via the urine, as with rats. Within 12 h after dosing about 35% of the ^{14}C appears in the urine, and by 24 h that amount had almost doubled. Exhalation in the form of CO_2 is comparable with that in the rat, and amounted to 6% of the dose after 72 h.

In pregnant mice 2-MAA can be further metabolised to its glycine conjugate and an unidentified compound.

Intravenous injection into mice results in a lower deposition of EGME and/or its metabolites in various tissues than after oral administration.

Both after oral administration and iv injection there is no testis-specific uptake.

Oral dosing of EGME to pregnant monkeys leads to a half-life time of 2-MAA of approximately 20 h. EGME and/or its metabolites is uniformly distributed in the embryo, extraembryonic fluids and maternal serum. The yolk sac accumulated 2-MAA.

EGME can be formed out of diethyleneglycol dimethylether in vitro by rat and human hepatic microsomes.

The half-life time for the enzyme-catalysed hydrolysis of EGMEA to EGME in vitro in rat plasma is approximately 12 min.

For biological monitoring of EGME in urine several not-validated methods are available. For the other compounds no data are available.

Chapter 5

Effects in man

If not stated otherwise, information in this chapter is retrieved from the previous report of the Health Council on glycol ethers*.

5.1 Irritation and sensitization

There is one recorded human eye exposure incident in which complete recovery occurred within 48 h of exposure. There are no quantitative data (Row82).

No data are available concerning the effects of EGMEA.

5.2 Acute toxicity

One fatal case of poisoning is reported in a human in 1946 (Row82). It is believed that the man consumed about 200 ml EGME mixed with rum. He was admitted to the hospital in a comatose condition and died 5 h later without regaining consciousness.

In 1936, two cases of poisoning are reported resulting from inhalation of vapours of EGME encountered in the manufacture of “permanently starched” collars. The symptoms were weakness, sleepiness, headache, gastrointestinal upset, nocturia, loss of weight, burning of the eyes, and a complete change of

* For references see: References part I: Health Council of the Netherlands 1996/01WGD.

personality from one of sharp intelligence to one of stupidity and lethargy. Both patients apparently recovered completely (Row82).

Rowe and Wolf (Row82) present several other cases, with the same symptoms, indicating that the effects are primarily haematotoxic and neurotoxic in nature.

A 32-year old man working in a microfilm manufacturing industry developed reversible subjective central nervous system complaints and asymptomatic haematopoietic effects. Measurement of personal breathing zone chemical exposures revealed time-weighted average EGME levels ranging from 58 to 183 mg/m^3 (the average being approximately 111 mg/m^3), 1-5 ppm methylethylketone and 4.2-12.8 ppm propyleneglycol monomethyl ether (PGME). The fact that the patient also had exposure to PGME may have contributed to toxicity, although the toxicity of PGME is less than that of EGME. Apart from inhalation exposure also skin exposure occurred (Coh84).

No data are available concerning the effects of EGMEA.

5.3 Short and long term exposure

5.3.1 EGME

A group of 40 potentially exposed workers and a group of 25 control persons were investigated for possible haematological and fertility abnormalities. The exposed group worked in a plant producing EGME and related products. The concentration of EGME was measured as PAS and ranged from N.D.* (detection limit not given) via 0.57 mg/m^3 as an 8 h TWA measured in 1976 to 27 mg/m^3 as a 2 h TWA measured in 1980. The control population was drawn from the alkanolamine and salicylic acid plants. With the possible exception of smaller testicular size no gross abnormalities or clinically meaningful differences in haematological or fertility indices were noted. The negative outcome of the study is probably due to the low sample size and the exposure to only low concentrations of EGME (Coo82).

The quality of the semen of 73 painters and 40 controls who worked in a large shipyard was investigated. The painters were not only exposed to EGME with a mean of 2.6 mg/m^3 TWA 8 h, but also to ethyleneglycol monoethyl ether with a

* N.D. Not Detectable.

mean of 9.9 mg/m^3 TWA 8 h. Semen samples were analysed for a large number of parameters. Painters had an increased prevalence of oligospermia and azoospermia and an increased odds ratio for a lower sperm count per ejaculate, while smoking was controlled (Wel88a).

In the same group of workers 94 painters and 55 controls gave a blood sample for haematological analysis. Haemoglobin, haematocrit, red cell indices, total and differential white blood cell counts, and platelet count were measured. Although the means of all variables were comparable between the groups, a significant propo rtion of painters were anaemic (10%) and granulocytopenic (5%); none of the controls were affected. Review of the company records documented that most of these abnormalities were acquired during employment; pre-existing disease and other exposure could not explain the findings (Wel88b).

Three cases with possible haematological effects after EGME exposure were found by Larese *et al.* (Lar92). Three young women were employed in a glasses frame factory. They used a mixture of acetone (70%) and EGME (30%) to glue together cellulose acetate frame components. During a periodic medical examination their white blood cell count was decreased, with a relative lymphocytosis, macrocytosis with red blood cells and haemoglobin at borderline normal values. The parameters returned to normal on stopping exposure. The route of exposure is probably via the skin.

5.3.2 EGMEA

A young woman was exposed to EGMEA by dermal contact and probably by inhalation in the course of two pregnancies. The two boys who were born suffered from hypospadia. Unfortunately the exposure could not be quantified (Bol90).

5.4 Epidemiological studies

Data in SCOEL (2006)*

No significant differences in sperm parameters (volume, sperm count and morphology), except a lower pH in semen, was seen between 14 highly exposed

* For references see: References part II: SCOEL SUM/120, 2006.

(4.0-4.2 ppm) impregnation workers and 13 less exposed (<0.28 ppm) laminate workers at two copper clad laminate factories (Shih *et al.* 2000a)[SCOEL4].

Haematological effects were investigated in 53 impregnation workers from the same two copper clad laminate factories mainly exposed to EGME. Workers were compared with a control group of 121 lamination workers with little, indirect exposure to EGME. The raw material used in the plants included epoxy and phenolic resins, dicyanamide, 2-methylimidazole, antimony, aluminum, titanium and silica oxides, pigments, acetone and EGME. Acetone and EGME were the only volatile substances. The solvent contained 30% acetone and 70% EGME. The average exposures to EGME in the impregnation area were 4.0 ppm (n=55 personal samples) and 4.3 ppm (n=11), respectively, in the two factories. The exposure in the lamination area (n=9) ranged from non-detectable to 0.28 ppm. The corresponding values for 2-MAA in urine were: 20.0 and 20.9 (range 0-66) mg/g Cr in the exposed groups and 1.6 (0-4.2) mg/g Cr in the controls. Haemoglobin, packed cell volume, and red blood cell count in the 47 exposed male workers were significantly lower than in the 93 male controls. Further, the frequency of anaemia in the exposed group (26.1%) was significantly higher than in the control group (3.2%). No differences were found between the 6 exposed female workers and the 27 female controls. Using a multiple regression model with adjustment for sex, body mass index, and duration of employment, red blood cell count was significantly negatively associated with air concentrations of EGME. Haemoglobin, packed cell volume, and red blood cell count were significantly negatively associated with urinary concentrations of 2-MAA (Shih *et al.* 2000a)[SCOEL4].

In a follow-up survey of haematological effects, 29 exposed and 90 non-exposed workers were recruited. Haematological parameters, full-week, full shift personal exposure to EGME, and urinary 2-MAA were repeatedly measured in three consecutive surveys within six months. The mean airborne exposure of EGME in the three surveys dropped from 9.6 to 2.3 ppm after 2.5 months and to 0.34 ppm after 6 months (geometric means). For comparison, the average exposure in the control group was 0.08 (gm, n=9, range n.d-0.8 ppm). In the first exposure survey, haemoglobin, packed cell volume, and red blood cell count were significantly lower in the male exposed workers than those in controls, whereas lymphocyte and platelet counts were increased. The haemoglobin content of the erythrocytes was increased in the exposed (significant increases in MCV, MCH, and MCHC) suggesting disturbed blood formation. The frequency of anaemia was also significantly higher among the exposed (42% versus 3% among controls). The haematological effects were significantly associated with the urinary 2-MAA of exposed workers. The haematological effects had returned

to normal in the first follow up survey and remained normal in the second follow-up. The authors concluded that EGME caused the haematological effects and that the reduction in exposure through both inhalation and potential dermal contact accounted for the recovery (Shih *et al.* 2003)[SCOEL6].

A review of 198 cases of acute myelotic leukaemia (AML) was made in a French case-control study. Blind estimates of exposure to different types of glycol ethers and potential exposure levels were made by an expert panel. No relationship between AML and glycol ethers was seen (Hours *et al.* 1996)[SCOEL7].

There is a report on 44 patients in Matamoros, Mexico, who had a syndrome with characteristic facial distortions and mental retardation. All of them were born in 1971-1977 and were children of mothers who during their pregnancies had worked at a factory making condensers. There are no quantitative data on exposure, but during work the women had dipped their hands into a solution consisting mainly of EGME and ethylene glycol. There was no ventilation, and workers used no protective gloves or face masks. Indications of acute poisoning, with fatigue, dizziness, nausea and vomiting, had occurred during work. A closer examination of 28 of the cases revealed that all of them also had musculoskeletal defects and that about half of them had eye and ear defects as well. There was no familial relationship between the cases and birth defects of this nature had not occurred previously in any of the affected families (Saavedra *et al.* 1997)[SCOEL8].

In a cohort study of 6088 women employed in 14 semiconductor factories, 904 pregnancies and 113 miscarriages were examined. After control for age, smoking habits, ethnic background, education, income, date of pregnancy and stress levels, there was a tendency for women in production work to have a higher proportion of miscarriages than other employees. A significantly higher frequency was seen among women who worked with masking. In this group, the highest risk of miscarriage was associated with etching (Beaumont *et al.* 1995)[SCOEL9]. In a sub-study, the outcomes of 891 pregnancies were sorted according to exposure during the first trimester. Women working with photolithography, who were exposed to ethylene glycol ethers (EGME, EGEE and their acetates), fluorides and other substances, had a significantly higher risk of miscarriage (Swan *et al.* 1995)[SCOEL10].

In a prospective study made at the same companies, 403 women were followed for 6 months by analysis of chorionic gonadotropin in urine. After control for possibility of conceiving, use of contraceptives and age, there was significantly lower fertility in the women working in dipping and the same tendency in those exposed to glycol ethers. In addition, female production workers had a significantly higher risk of spontaneous abortions than those who held other types of jobs. All three pregnancies among the women who were

exposed to ethylene glycol ethers terminated in miscarriages (Eskenazi *et al.* 1995)[SCOEL11]. The same group of women also kept diaries on their menstruations. Prolonged menstrual cycles were seen in women who worked with dipping, and shortened cycles and a greater number of irregular menstruations were seen in the photolithography group (Gold *et al.* 1995)[SCOEL12].

In exposure assessments made in the workplaces at the same time, it is stated that 15-20% of the factories used photo chemicals (negative photo resist), usually containing 3% EGME. All personal monitors registered EGME levels below 10 ppb, average exposure to 2-ethoxyethyl acetate (2EEA, ethylene glycol ethylether acetate, EGEEA) was 22 ppb, and exposure to 1-methoxypropyl acetate was 8 ppb (Hammond *et al.* 1996)[SCOEL13]. Exposures to glycol ethers were strongly correlated to exposures to xylene and n-butyl acetate (Hines *et al.* 1996)[SCOEL14].

In a study of 454 pregnancies among 1368 women employed in the semiconductor industry, risk of spontaneous abortion tended to be higher for those who worked in chip production, and those with chemical exposure outside of chip production, than for unexposed subjects. The proportion of stillbirths also tended to be higher in the two exposed groups. The authors report that chip production involves exposure to glycol ethers and a number of other solvents, which they listed, but exposures were not measured (Pinney and Lemasters, 1996)[SCOEL15].

Another study in the semiconductor industry covered both female employees (561 pregnancies) and the wives of male employees (589 pregnancies). For the female employees, those with the highest likelihood of exposure to ethylene glycol ethers had significantly reduced fertility and elevated risk of spontaneous abortion. No increased risk of miscarriage was found among the wives of employed men, but there was a tendency to lower fertility. No personal monitoring measurements were taken, and only general information on exposures is given. A few measurements yielded glycol ether levels below 0.2 ppm in the highest exposure group. The glycol ethers named in the study are diethylene glycol dimethylether (DEGDME) and EGEEA; EGME was not mentioned. Simultaneous exposure to glycol ethers and hexamethyl disilazane occurred. No increase in frequency of the studied effects was noted with exposure to n-butyl acetate, N-methyl-2-pyrrolidone or xylene, unless there was simultaneous exposure to glycol ether (Correa *et al.* 1996)[SCOEL16].

5.4.1 *Reproductive toxicity*

Data in Health Council (2008)[*5]

Male fertility

In a cross-sectional study, Multigner *et al.* (2007)[HC1] 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. Forty-eight 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 nonexposed 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 significant 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.

* For references see: References part III: Health Council, 2008, 2008/11OSH.

Effects on human development

Fourteen epidemiological studies evaluated the effects of occupational exposure to ethylene glycol ethers on development, including six cohort, six case control and two cross-sectional studies. Of these studies, eleven met the selection criteria of the committee. 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.* (1995)[HC2], Eskenazi *et al.* (1995)[HC3], Schenker *et al.* (1995)[HC4] and Swan *et al.* (1995)[HC5] involved the same cohort of semiconductor workers (Semiconductor Health Study). Cordier *et al.* (1997)[HC6] and Ha *et al.* (1996)[HC7] 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.* (1995), Beaumont *et al.* (1995), and Swan *et al.* (1995) focused on spontaneous abortion in specific subgroups in the retrospective cohort study, whereas Eskenazi *et al.* (1995) 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.* (1995)[HC4] 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.* (1995)[HC2] 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.* 1995 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 cannot be clearly attributed to ethylene glycol ethers.

Finally, Eskenazi *et al.* (1995)[HC3] 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.* 1995[HC8] 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.

Elliott *et al.* (1999)[HC9] 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.* (1991)[HC10] 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.* (1996)[HC7] and Cordier *et al.* (1997)[HC6] 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 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 reported more details of the same multicenter study. For in-depth 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.* (2006)[HC11] 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.

5.5 Conclusion

Very little data are available on eye irritation. A single incident with exposure to EGME resulted in complete recovery.

Exposure to high doses of EGME results in death, preceded by symptoms in the brain, blood and kidneys.

A relationship between exposure to EGME and haematological and fertility abnormalities could not be established, partly because of a small number of exposed persons, partly because the exposure was not to EGME alone. Two cases of hypospadia in the offspring were reported after exposure to an unknown quantity of EGMEA.

Haematologic effects were seen in workers at an EGME exposure level of 4 ppm (Shih *et al.* 2000a)[SCOEL4], but not at 2.3 ppm (Shih *et al.* 2003)[SCOEL6].

The only clear effect on female fertility from epidemiological studies (in the semiconductor industry) was the observation by Correa *et al.* (1996)[HC8] that exposure to ethylene glycol ethers increased the risk for subfertility. Eskenazi *et al.* (1995) and Gold *et al.* (1995)[HC12] 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. A number of other epidemiological studies into an association between ethylene glycol ethers and female fertility were rejected because of methodological deficiencies (insufficient exposure data, no correction for confounders, etc.).

It was concluded that the available data do not allow drawing a conclusion regarding the effects of exposure to ethylene glycol ethers on female fertility.

The studies by Welch *et al.* (1988)[HC13] and Veulemans *et al.* (1993)[HC14] 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.* (2007)[HC15]. Data on other sperm parameters are conflicting. Welch *et al.* (1988) and Shih *et al.* (2000) 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.* (2007)[HC15] observed significant decreases in sperm motility and normal sperm morphology.

It was concluded that the epidemiological data indicate an association between exposure to ethylene glycol monoalkyl ethers and male fertility.

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. Agent-specific analyses performed among women in the semiconductor industry revealed an association between spontaneous abortion and maternal occupational exposure to ethylene-based glycol ethers (Swan *et al.* 1995)[HC5]. In the study by Elliott *et al.* (1999)[HC9], 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. Data on the levels of exposure to ethylene glycol ethers were not available in these studies.

Correa *et al.* (1996) evaluated paternal exposure in the semiconductor industry, but no association was found with the prevalence of spontaneous abortion in their wives.

Overall, it is concluded that the available epidemiological studies indicate an association between female exposure but not paternal exposure to ethylene glycol ethers and the risk of spontaneous abortion.

From the epidemiological data on congenital malformations, Ha *et al.* (1996)[HC7] and Cordier *et al.* (1997)[HC6] 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.* (2006)[HC11] 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.

It was concluded 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.

Chapter 6

Animal experiments

If not stated otherwise, information in this chapter is retrieved from the previous report of the Health Council on glycol ethers*.

6.1 Irritation and sensitisation

6.1.1 EGME

Skin irritation

When in repeated and prolonged contact with skin, EGME does not induce appreciable irritation (ECE82). As an alternative to the skin irritation test on rabbits, the cutaneous blood flow was measured in humans: in a first series of experiments 12 h after application of the pure substance for 48 h and in a second series of experiments 1, 24, 48 and 72 h after application of the 10% diluted EGME for 3 h. Rabbit skin erythema scores were obtained 1, 24, 48 and 72 h after application of undiluted EGME for 4 h. Together with the data of four other chemicals the cutaneous blood flow in humans correlated well with erythema scores obtained in rabbits. However, there was a poor correlation between these *in vivo* data and an *in vitro* test on uridine uptake by a human cell line. EGME was not irritating (Jac89b).

* For references see: References part I: Health Council of the Netherlands 1996/01 WGD.

Skin sensitisation

No satisfactory data on the sensitisation potential of EGME have been reported (IPC90).

Eye irritation

When EGME was introduced into the eyes of rabbits, it produced immediate pain, conjunctival irritation, and slight transitory cloudiness of the cornea, which cleared within 24 h (Gin94).

When the *in vivo* eye irritation of EGME was tested according to the EC directives, this compound was classified as not irritating to the rabbit eye. Results were obtained for erythema, oedema, corneal opacity and corneal swelling (Jac89a, Jac92).

6.1.2 EGMEA

Skin and eye irritation

The compound is mildly irritating to the eyes. It is not significantly irritating to the skin (Gin94).

Respiratory irritation

Muller and Greif (Mul84) determined the log $1/FRD_{50}$* to be -3.44, FRD_{50} being the concentration in air of the test compound (expressed in mg/m^3) which diminishes respiratory frequency in mice by 50%. Therefore it can be calculated that the FRD_{50} for EGMEA in mice is $2.75*10^3$ mg/m^3, implicating that it is not a respiratory irritant.

6.1.3 EGME and EGMEA

Skin irritation

The two compounds were tested on rabbits according to the EC directives. Both were non-irritating in concentrations up to 100%. EGME seems to exert a very

* exposure concentration producing a 50% respiratory rate decrease

strong synergistic effect on other slightly irritant substances, when they are dissolved in EGME. However, no quantitative data are given (Jac87).

6.1.4 Conclusion

EGME and EGMEA are no skin irritants.

EGME is a mild to moderate eye irritant for rabbit eyes *in vivo*. EGMEA is a mild eye irritant. It is not a respiratory irritant in mice *in vivo*.

Table 11 Acute toxicity data of EGME.

species	parameter	dose/concentration	reference
EGME			
rat	single oral, lethal dose	3,400 mg/kg	Gin94
	LD_{50} oral	2,460 mg/kg	
rat, fasted	LD_{50} oral	2,300 mg/kg	
rat, fed	LD_{50} oral	3,900 mg/kg	
rat	LD_{50} iv	2,140 mg/kg	
	LD_{50} iv	2,700 mg/kg[a]	
	LD_{50} iv	2,200 mg/kg[b]	
rat	LC_{50} 7 h inhal.	4,740 mg/m^3	Lew92
rat, Wistar or Sprague-Dawley	inhal., 0/5 animals died after 1-3 h exposure[c]	29,000 mg/m^3	Kli88
rat	LD_{50} ip	2,500 mg/kg	Lew92
mouse	LD_{50} oral	2,800 mg/kg (given in oil)	Gin94
	LD_{50} oral	2,560 mg/kg	Lew92
mouse, fasted	LD_{50} oral	3,900 mg/kg	Gin94
mouse, fed	LD_{50} oral	4,500 mg/kg	
mouse, male ddY[d]	LD_{50} oral	2,449 mg/kg	Tan92
mouse	LC_{50} inhal. 7 h	4,677 mg/m^3	Gin94
mouse	LD_{50} ip	2,147 mg/kg	Lew92
rabbit	single oral, lethal dose	890 mg/kg	Gin94
rabbit	LD_{50} dermal	1,290 mg/kg	
		2,000 mg/kg	
guinea pig	single oral, lethal dose	950 mg/kg	

[a] given as a 25% solution
[b] given undiluted
[c] replicated in six laboratories, each using 5 males and 5 females (OECD test)
[d] when the animals were pretreated with CCl_4 (inhibits hepatic enzymes) the LD_{50} did not change

6.2 Acute effects

6.2.1 General effects

According to the EC directives on dangerous substances and preparations (EC93) EGME and EGMEA must be classified as follows with respect to acute toxicity:

- EGME: harmful by inhalation, in contact with skin and if swallowed. Further classification with R60 (may impair fertility) and R61 (may cause harm to the unborn child) is necessary
- EGMEA: harmful by inhalation, in contact with skin and if swallowed. Further classification with R60-61 is necessary.

Table 12 Acute toxicity data of EGMEA.

species	parameter	dose/concentration	reference
EGMEA			
rat	LD_{50} oral	3,390 mg/kg 3,930 mg/kg	Row82
rat	4 h inhal. 2/6 died	34,370 mg/m^3	
rat	LDlo ip	1,200 mg/kg	Lew92
mouse	3 h inhal. no deaths	ca. 22,360 mg/m^3	Gin94
mouse	LD_{50} sc	4,630 mg/kg 5,040 mg/kg	
rabbit	3 h inhal. no deaths	ca. 22,360 mg/m^3	
rabbit	LD_{50} dermal	5,290 mg/kg	
guinea pig	LD_{50} oral	1,250 mg/kg	
guinea pig	LDlo sc	5,000 mg/kg	Lew92
cat	9 h inhal. LC_{100}	12,275 mg/m^3	Row82
cat	LDlo sc	3,000 mg/kg	Lew92

When given in massive oral doses, the material has a narcotic action, but at lower dosage levels deaths are delayed and are accompanied by lung oedema, slight liver injury, and marked kidney injury. Haematuria may occur from single doses.

A few repeated exposures to 2,520 mg/m^3 or 5,056 mg/m^3 produced serious systemic intoxication, characterised for the most part by irritation of the respiratory tract and lungs, haematuria, albuminuria, cylindrical casts in the urine, and severe glomerulitis. The animal species was not mentioned.

In mice, inhalatory exposure resulted in lung and kidney injury which was generally the cause of death.

When groups of rats were exposed to 980 mg/m^3 for 7 h/day, 5 d/week, there was an increase in the percentage of immature granulocytes in the circulating blood after one week. There were no changes observed in kidneys and lungs (Row82).

6.2.2 *Neurotoxicity*

A partial loss of motor function in the hindlimbs of rats occurred after exposure to 1,244 mg/m^3 for 6 h/day, 5 d/week for 2 weeks. This hindlimb paralysis coincided with the glial cell toxicity noted during the second week of exposure. Recovery was incomplete after 2 weeks post-exposure, the animals receiving the highest dose showing a minor paresis (study from 1980, reported by IPC90).

6.2.3 *Haematology*

Groups of six male F344 rats were dosed *po* with 0, 100 or 500 mg/kg for 4 consecutive days. Animals were killed on days 1, 4, 8 and 22 after the final treatment. EGME produced thymic atrophy, lymphocytopenia and neutropenia. It also abolished splenic extramedullary haemopoiesis which partially recovered on day 4, followed by a marked response on day 8, and return to the moderate control values on day 22. Femoral bone marrow was haemorrhagic one day after treatment which appeared to be associated with sinus endothelial cell damage. By day 4 the histologic appearance of the marrow was normal (Gra85).

Groups of 6 B6C3F$_1$ mice were injected sc with EGME for 5 consecutive days after birth at daily doses of 0, 100, 200 or 400 mg/kg. They were allowed to recover and stressed with 200 rads whole body irradiation at 15 and 21 weeks post-exposure in order to determine a possible residual bone marrow effect. Bone marrow functions were examined. In the groups which were not irradiated the highest dose of EGME induced after 8 weeks a decrease in body weight, and a decrease in cellularity, expressed as cellularity per femur and as the number of colony forming units (CFUs) per 10^5 cells and per femur. Sixteen weeks post-exposure the recovery was complete. Haematological values, including RBC, Hb, Hct and MCV were not significantly affected by EGME after 15 weeks or by irradiation thereafter. In the groups that were irradiated there was a dose-related (EGME-dose) decrease in CFUs per 10^5 cells one week later. The CFUs recovered after 3 and 5 weeks to normal values. After a second irradiation at 21 weeks post exposure there was an even more dramatic difference between the

untreated and EGME exposed mice, suggesting persistent residual damage in bone marrow stem cells and increased sensitivity to a second stress to the haematopoietic system (Hon88a).

6.3 Short-term toxicity

6.3.1 EGME

Oral administration

Male Wistar rats received daily oral doses of 300 mg/kg for 1, 2, 5 or 20 days. The rats were killed and several organs were weighed. After one day feeding no effects were observed in the various relative organ weights. After two days of feeding a decreased relative liver, spleen, thymus, heart and testis weight was observed. After five days of feeding a decreased relative kidney, spleen, thymus, and testis weight was observed. After 20 days of feeding the relative weights of liver, thymus, and testis were decreased. In none of the experiments the relative lung weight was affected.

In a second study, EGME was fed to male Wistar rats in a concentration of 100 or 300 mg/kg, for 20 days. With both dosages the relative thymus and testis weights were decreased. On the other hand, EGME did not change hepatic cytochrome P450, cytochrome b or NADPH-cytochrome c reductase, even after 20 days of feeding the high dose (Kaw90a and b). After five days of feeding the highest dose the activity of gamma-glutamyl transpeptidase was increased in liver, lung and spleen, decreased in pancreas and kidney, whereas in the brain the activity remained unaffected (Kaw91).

Male and female Sprague Dawley rats received daily oral doses of 0, 25, 50 or 100 mg/kg/day for 49-51 days. In males the highest dose decreased the absolute body, liver, kidney, thymus and testis weight. Only the relative thymus weight was decreased. Also were the haematological values decreased at the highest dose. Therefore, the NOAEL for male rats is approximately 50 mg/kg/day (7 weeks). In females no organ weights were measured. Haematological values such as haematocrit percentage, haemoglobin content and the number of platelets were decreased at the two highest dosages. At these two dose levels the body weight gain of pregnant females was decreased and a number of developmental effects were observed (for these data: see section 6.2.6). Therefore, for female rats the NOAEL is 25 mg/kg/day (7 weeks) (Sca92).

Groups of five male and five female F344/N rats and B6C3F$_1$ mice received EGME in the drinking water for 2 weeks. The estimated quantity consumed by the animals is presented in Table 13.

Table 13 Estimated quantity of EGME consumed by male and female F344/N rats and B6C3F$_1$ mice when administered via the drinking water in mg/kg body weight/day (2-week study)(Die93).

study group	male rats	female rats	male mice	female mice
0	0	0	0	0
1	116	113	181	255
2	206	175	380	544
3	273	231	603	971
4	393	297	865	1,094
5	418	326	1,269	1,124

There was a dose-related decrease in mean water consumption in all experimental groups. Therefore, the final compound consumption was lower than the target dose. There were no chemical-related effects on survival. Decreased body weight gains were noted for both male and female rats. Most of the changes in organ weights were sporadic (mice) or related to low final mean body weights (rats), except for thymic atrophy in male and female rats and testicular atrophy in both species (Die93).

In the subsequent 13-week studies ten males and ten females of the same rat and mouse strain were used per dose group. The estimated compound consumption based on water consumption is given in Table 14.

Table 14 Estimated quantity of EGME consumed by male and female F344/N rats and B6C3F$_1$ mice when administered via the drinking water in mg/kg body weight/day (13-week study) (Die93).

study group	male rats	female rats	male mice	female mice
0	0	0	0	0
1	71	70	295	492
2	165	135	529	902
3	324	297	765	1,194
4	715	546	992	1,489
5	806	785	1,367	1,839

Chemical-related mortality occurred in male and female rats of the two highest dose groups. Decreased body weight gains occurred in all dose groups of the rats, except for the lowest dose group. In mice the three highest dose groups had a decreased body weight gain. In rats treatment-related histopathologic changes were observed in the testes, thymus and haematopoietic tissues (spleen, bone marrow and liver). Even at the lowest dose tested the relative and absolute

thymus weight was significantly decreased in the female rat. In the lowest dose group of male rats seven animals out of ten had minimal lesions in the testis.

In mice, EGME had similar effects on the testes, spleen and adrenal gland (females only). There was a dose-related degeneration of the germinal epithelium in seminiferous tubules of the testes in males and an increase in splenic haematopoiesis in females. At the lowest dose tested five out of ten females had a minimal increase in splenic haematopoiesis, all ten females had mild lesions (hypertrophy) in the X-zone (the *zona reticularis)* of the adrenal gland. The lowest dose was a NOAEL for male mice.

In special stop-exposure studies in male rats in which administration of EGME was stopped after 60 days, marked degeneration of the seminiferous tubules was present in rats with 3000 ppm in the drinking water (estimated 324 mg/kg/day), and mild to moderate degeneration was observed in rats treated with 1,500 ppm (estimated 165 mg/kg/day). After 30 and 56 days of recovery from treatment only partial recovery from testicular degeneration was observed. EGME treatment for 13 weeks resulted in a progressive anaemia in rats associated with a cellular depletion of bone marrow and fibrosis of the splenic capsule.

Summarising, it can be concluded that 295 mg/kg/day was a NOAEL for male mice (13 weeks), 492 mg/kg/day was an adverse effect level for female mice (13 weeks), causing minimal splenic haematopoiesis and mild hypertrophy in the adrenal gland. In male rats 71 mg/kg/day (13 weeks) was a MAEL, causing minimal lesions in the testis. In female rats 70 mg/kg/day (13 weeks) decreased the relative and absolute thymus weight (Die93).

Inhalation exposure

Rats exposed for 4 h/day for 7 days to 395 mg/m^3 appeared normal in health but exhibited a decrease in pole climbing response. No tolerance to the exposure was seen; repeated exposure increased the severity of the response. A single exposure of mice to 395 mg/m^3 for 4 h potentiated the hypnotic effects of barbiturates; at 1580 mg/m^3 for 4 h, a decrease in motor activity occurred (study from 1962, reported by Row82).

Groups of five male and five female Fischer 344 rats and B6C3F, mice were exposed to 0, 316, 948 and 3160 mg/m^3, 6 h/day for a total of 9 exposures during an 11-day interval. Approximately 18 h after the final exposure the animals were killed. Each animal was given a complete gross pathologic examination. The high concentration had pronounced adverse effects on body weight gain, peripheral blood counts, bone marrow, testes and lymphoic tissues. Similar but

less pronounced changes also occurred in some animals in the mid-dose group. At the lowest concentration the white blood cell count was reduced in male rats only (Mil81).

Groups of ten male and ten female Sprague Dawley rats and five male and five female New Zealand white rabbits were exposed by inhalation to 0, 95, 316 or 950 mg/m^3 for 13 weeks, 5 d/week, 6 h/day. After 4 and 12 weeks of exposure haematologic analyses were performed. At the end all animals were necropsied. No rats died prior to scheduled sacrifice, but some rabbits in the 316 and 950 mg/m^3 exposure groups died or were sacrificed when moribund during the study. At the top dose the absolute and relative thymus weight was decreased in both species and sexes, the absolute and relative testes weight was decreased in the males. Body and liver weights were decreased in male and female rats. At the mid dose the female rats had a decreased body weight. Haematologic analyses after 12 weeks of exposure revealed in both rats and rabbits exposed to the top dose decreased mean white blood cell counts, platelet counts, packed cell volumes, haemoglobin concentrations and red blood cell count (rabbits only). Haematologic changes of approximately the same severity were also apparent in the same animals when analyses were performed after 4 weeks of exposure to 950 mg/m^3. There were no apparent effects on haematologic parameters of rats and rabbits in the low and mid dose groups at either the 4 week or 12 week intervals. There were no treatment-related changes in differential white blood cell counts for rats and rabbits. Concentrations of total protein, albumin and globulins in serum of rats (but not rabbits) in the 950 mg/m^3 group were lower than for controls. Treatment-related microscopic lesions included degenerative changes in germinal epithelium of testes in all male rats and rabbits in the top dose group, as well as in three of five rabbits in the mid-dose group and in one of five male rabbits in the low dose group. The only effect attributed to exposure to 95 mg/m^3 in this study were slight microscopic changes in testes of one of five male rabbits. Rabbits were apparently more sensitive than rats to EGME vapours. Since haematologic changes were not apparent in either rats or rabbits in the two lower exposure group while some microscopic testicular changes did persist in at least some rabbits, the testicular effects may be a more sensitive index of exposure to EGME in some species than haematologic changes. Testicular changes, however, did not persist in rats at concentrations below those which resulted in haematologic changes. Although exposure to 95 mg/m^3 resulted in testicular effects in one of five rabbits in this study, no effects were found in testes of rabbits exposed to 95 mg/m^3 in a subsequent study

(unpublished report). Hence, exposure of male rabbits to 95 mg/m^3 apparently results in testicular effects in only ten percent of the animals (Mil83b, Mil84).

Dermal application

Doses of 100 or 1000 mg/kg were applied dermally on male Porton-Wistar rats on five consecutive days per week for 28 days. EGME was applied under both occluded and unoccluded conditions. The groups contained eight animals. Weight gain was reduced in the high nonoccluded treated animals and at both doses in the occluded animals. A similar pattern was seen with food intake. The high dose both under occluded and unoccluded conditions changed the testis weight. Only under occluded conditions the high dose induced histological changes in the testis and bone marrow, and haematological effects such as a decrease in WBC, Hb, PCV and MCV and an increase in the number of circulating reticulocytes (Fai89).

Groups of male Harley guinea pigs were dermally exposed to either 0 (n = 7) or 1,000 mg/kg/day (n = 5) for 13 weeks, 5 d/week, 6 h/day. The exposure was occlusive. After necropsy a full (histo)pathological examination was performed. A significant decrease was observed in body weight, spleen and testes weight. The thymus weight was not given. In all animals the seminiferous tubules were degenerated. Haematological changes included mild anaemia with increased erythrocytic mean corpuscular volumes and a lymphopenia with increased neutrophils. The activity of serum creatinine kinase and lactate dehydrogenase was increased. It is concluded that these effects are consistent with the finding of previous studies with rats, rabbits and mice subchronically exposed to EGME via the oral or inhalation route (Hob86).

Two dogs were exposed to 2,370 mg/m^3, 7 h/day, 5 d/week for 12 weeks. The most significant changes were found in the blood. The haemoglobin concentration, cell volume, and the number of erythrocytes were decreased (studies from 1943, reported by Row82).

6.3.2 EGMEA

When mice were fed doses ranging from 62.5 to 4000 mg/kg, 5 d/week for 5 weeks testicular atrophy was observed as well as leukopenia. The intensity of the symptoms was dose-related (study from 1979, reported by Row82).

6.3.3 *Conclusion*

EGME

Subchronic oral dosing of EGME to rats and mice influenced generally the body weight gain and the weight of several organs. Doses up to ca. 1,200 mg/kg/day via the drinking water for 2 weeks decreased sporadically the organ weights in mice and doses up to 300-400 mg/kg/day decreased the body weight in rats, further thymic atrophy in male and female rats and testicular atrophy in males of both species was induced. Thirteen weeks of dosing via the drinking water induced histopathologic changes in testes, thymus and haematopoietic tissues in rats and in testes and spleen in mice and in the adrenal gland in female mice. In stop-exposure studies with male rats given 165 or 324 mg/kg/day there was only partial recovery from testicular degeneration after 30 and 56 days of recovery. After 13 weeks of dosing the following can be concluded from the lowest doses tested: the NOAEL for male mice was 295 mg/kg/day, 492 mg/kg/day caused in female mice minimal splenic haematopoiesis and mild hypertrophy in the adrenal gland. In male rats 71 mg/kg/day caused minimal lesions in the testes. In female rats 70 mg/kg/day decreased the relative and absolute thymus weight.

Inhalation exposure to rats and mice for 9 exposures with concentrations up to 3,160 mg/m^3 induced adverse effects on body weight gain, peripheral blood counts, bone marrow, testes and lymphoic tissues. Thirteen weeks of inhalation exposure of 950 mg/m^3 decreased the thymus weight in male and female rats and rabbits and decreased the testes weight in males. Several haematologic parameters in male and female rats and rabbits were decreased after 4 and 12 weeks of exposure to 950 mg/m^3. Concentrations of 316 or 95 mg/m^3 did not influence the haematology. A small percentage of rabbits showed testicular effects at 95 mg/m^3. For rats this concentration was an NOAEL.

Dermal application of 100 or 1,000 mg/kg reduced the weight gain and testes weight in rats after 28 days. The body weight, spleen and testes weight was decreased in guinea pigs after 13 weeks of dermal exposure to 1,000 mg/kg/day. Testicular degeneration and haematologic changes were also observed in the guinea pigs.

EGMEA

Testicular atrophy and leukopenia were observed in mice after 5 weeks of dosing 62.5 to 4,000 mg/kg.

6.4 Carcinogenicity

No adequate long-term animal studies on EGME and EGMEA have been reported (IPC90).

6.5 Mutagenicity

6.5.1 EGME

EGME does not induce reverse mutations in bacteria, with and without metabolic activation and with alcohol dehydrogenase; it does not induce UDS in mammalian cells *in vitro* with and without metabolic activation; it does not induce point mutations in mammalian cells *in vitro* after metabolic activation; an unclear or unreproducible result has been obtained in the sex-linked recessive lethality test in Drosophila; it induces male sterility in a dominant lethal study in rats; it did not induce forward mutations in Schizosaccharomyces pombe; it did not induce chromosomal aberrations in rats; it induced sperm abnormalities in mice (ECE82, ECE85).

EGME does not induce reverse mutations in bacteria, when tested with four Salmonella strains, with and without rat and hamster liver metabolic activation (Zei92).

EGME does not induce mutations in the HPRT locus of Chinese hamster ovary cells, with or without rat liver metabolic activation (Ma93).

EGME does not induce chromosomal aberrations in mice after oral and iv administration (Au93).

EGME increases the number of chromosomal aberrations and chromatid breaks in human peripheral lymphocytes *in vitro*, after prolonged exposure (24 h) and at high concentrations (from 150 mM). On the other hand, there was no significant difference in proliferation and mitotic indexes (Chi94).

EGME can interrupt intercellular communication in V79 cells (Loc84). The inhibition of intercellular communication in a human embryonal palatal mesenchymal cell line was probably the result of cytotoxicity and poor physical contact between cells (Wel84). However, the dose ranges differed in the two studies: in the first 0.026-0.39 mM was used while in the second study 0.13-0.30 mM was used. Cytotoxicity was also observed in V79 cells at the highest concentration.

Methoxyacetaldehyde, a metabolite of EGME, induces mutations at the GPT locus of Chinese hamster ovary AS52 cells, in a dose-related manner, without rat liver metabolic activation. It does not induce mutations at the HPRT locus of Chinese hamster ovary K1-BH4 cells with or without rat liver metabolic activation (Ma93).

It does not induce chromosomal aberrations in mice after oral administration (Au93).

It increases the percentage chromosomal aberrations and the number of chromatid breaks in human peripheral lymphocytes *in vitro* at 40 mM for 1 h exposure, and at 2.5 mM after 24 h of exposure. After 24 h the proliferation and the mitotic index are also increased (Chi94).

It induces SCE in human peripheral lymphocytes at toxic concentrations (Chi94).

It induces an increase in the percentage of abnormal cells and of chromatid exchanges, an increase in the percentage of chromatid breaks. The mitotic index increased in two CHO cell lines (Chi94).

Additional data in SCOEL (2006)

Increased numbers of micronuclei and mitotic irregularities were seen in vitro at 65 mM EGME, 0.12 mM methoxyaldehyde and 3.2 mM 2-MAA. With methoxyaldehyde, an elevated frequency of mutations was seen at 1-10 mM, of sister chromatid exchanges and chromosome aberrations at 0.1-1 mM, and of morphological transformations at 0.1-0.3 mM. The authors regard the results as weakly positive for EGME and 2-MAA and clearly positive for methoxyaldehyde (Elias *et al.* 1996)[SCOEL17].

6.5.2 *EGMEA*

EGMEA did not induce reverse mutations in bacteria when tested in five Salmonella strains, with and without rat and hamster liver metabolic activation (Zei92).

EGMEA induces aneuploidy in Saccharomyces cerevisiae; it does not induce point mutations or recombination in S. cerevisiae; it does not induce micronuclei in Chinese hamsters (IPC90).

It induces aneuploidy in S. cerevisiae (Whi89, Osg91).

It accelerates pig brain tubulin assembly (Gro85) and Drosophila tubulin assembly (Seh90).

It induces SCE in CHO cells with and without rat liver metabolic activation; it did not induce chromosomal abe rrations in CHO cells without metabolic activation, however chromosomal aberrations were found in CHO cells after metabolic activation (Lov90).

6.5.3 Conclusion

EGME and EGMEA have very little mutagenic or genotoxic potential. The compounds were negative in the Ames assay.

EGME does not induce mutations in mammalian cells *in vitro*. In human peripheral lymphocytes EGME and methoxyacetaldehyde increase the number of chromosomal aberrations and chromatid breaks at high concentrations.

EGME induces male sterility in rats and sperm abnormalities in mice. EGMEA does not induce micronuclei in Chinese hamsters.

6.6 Reproduction toxicity

6.6.1 EGME

Male reproductive effects were reported for EGME as early as 1938 (Nel89).

It is the general idea that the effects are caused by metabolites of EGME. For instance, when Sertoli cells of Sprague Dawley rats are incubated with EGME no morphological evidence of toxicity is observed, at concentrations up to 50 mM and incubation times up to 72 h. HPLC analysis of the 24-h medium confirmed the lack of metabolism: no 2-MAA or other metabolites were detected. However, when Sertoli cells are incubated with 2-MAA degeneration of the pachytene and dividing spermatocytes was observed at 2 mM for 24 h (Gray85).

A test with Sertoli cell cultures prepared from Alpk/Ap rats incubated with 2-methoxyacetaldehyde (MALD) showed that MALD produces specific cellular toxicity to pachytene spermatocytes as evidenced by morphological changes to these cells, an increase in germ-cell detachment and leakage of the marker enzyme lactate dehydrogenase-X. These effects occurred at concentrations where the known testicular toxicant 2-MAA was without effect (0.2 and 0.5 mM) (Fos86).

In vivo data are presented in Table 15 (testicular effects in males), Table 16 (exposure of pregnant females, developmental effects in offspring) and Table 17 (exposure of males and females, effects in offspring).

Other studies, which cannot be presented in the table form are described hereunder.

In an attempt to correlate histopathological and biochemical changes induced in the rat testes after oral dosing of EGME, male Alpk:APfSD rats were studied in a dose response and a time-dose response course. At doses which clearly induced changes in early and late pachytene and dividing spermatocytes no changes in either testicular or plasma sorbitol dehydrogenase or leucine aminotransferase were found. Similarly no effects were observed for plasma luteinising hormone or testosterone. Lactate dehydrogenase isozyme C, (LDH-C,) was reduced in testes and increased in plasma and plasma LDH-C, remained elevated up to 96 h after dosing. Also androgen binding protein (ABP) levels in plasma were increased. A reduction in testicular testosterone levels was recorded and plasma follicle-stimulating hormone concentrations were elevated. It is suggested that LDH-C, activity and ABP may be of diagnostic values in acute testicular toxicity. Increases in plasma LDH-C, precede noticeable histological findings (Rea91). It is not mentioned whether these markers are also present in human plasma. However, the presence of LDH had been assayed in human spermatozoa (Gol63) and in human liver (War69, Bla66).

Additional data in SCOEL (2006)

Rabbits were given EGME in drinking water, 12.5-50 mg/kg/day, 5 days/week for 12 weeks: there were dose-dependent declines in several parameters of sperm quality. The effects were significant at 37.5 and 50 mg/kg, and the most marked effect was reduced number of sperm per ejaculate. Histological examination revealed that spermatogenesis (number of round spermatids per Sertoli cell) was somewhat reduced at 25 and severely disrupted at 37.5 mg/kg. At the highest dose, 50 mg/kg, spermatogenesis ceased almost completely in 5 of 7 rabbits. No effects were seen on the libido or fertility of the rabbits that still had functional sperm production, and no other pathological or histopathological effects were observed. The authors concluded that spermatogenesis in rabbits is about 10 times more sensitive to EGME than that in rats or mice (Foote *et al.* 1995[SCOEL18], Berndtson and Foote 1997[SCOEL19]).

EGME given to female rats in doses of 300 mg/kg/day completely eliminated the oestrus cycle: inhibition of ovulation, luteal body hypertrophy, permanently elevated progesterone levels and permanently low levels of estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin. Addition of 2-MAA to luteal cells in vitro resulted in elevated progesterone levels in the

cultivation medium at all levels; 1 mM was the lowest tested concentration (Davis *et al.* 1997)[SCOEL20]. 2-MAA was also tested in an in vitro system with luteinized granulose cells from humans. Incubation with 0-5 mM of 2-MAA for 6 to 48 h yielded a duration- and concentration-dependent increase of progesterone. The effect was significant at 1 mM, but a tendency was also apparent at 0.1 and 0.5 mM. The authors associate these observations with the effects on menstrual cycle and ovarian function in humans (Almekinder *et al.* 1997)[SCOEL21].

Additional information

EGME was orally administered to female Sprague-Dawley rats (10/group) at dose levels of 0, 30, 100 and 300 mg/kg bw/day for 2 or 4 weeks. At dose levels ≥ 100 mg/kg, estrous interval was prolonged and oestrus frequency was decreased. At the same dose levels, hypertrophy of corpora lutea was observed upon histopathological examination, characterized by round to polygonal luteal cells and abundant vacuolated cytoplasm. Thymic lymphoid depletion, bone marrow hypocellularity and splenic capsular fibrosis were also observed at ≥ 100 mg/kg in animals treated for 2 or 4 weeks. Adrenal weights were reduced at dose levels ≥ 30 mg/kg after 4 weeks of treatment, without a histopathological correlation.

In the second part of this study, female rats received the same dose levels of EGME for 2 weeks, and were subsequently cohabited with non-treated males for up to 2 weeks. At necropsy, the numbers of corpora lutea of pregnancy, implantations and live and dead embryos were counted, and conception and pregnancy rates were calculated. In addition, vaginal smears of all females were collected during the pre-mating period to evaluate estrous stages. At all dose levels of EGME, continuous dioestrus was frequently observed. In association with the continuous dioestrus, cases of prolonged estrous cycle, irregular estrous cycle and decreased frequency of oestrus were observed at 30 mg/kg, though these estrous cycle changes were small and not statistically significant at 30 mg/kg (Dodo *et al.* 2009).[1]

The effect of route of administration on the teratogenic potential of EGME was tested by Doe (Doe84). No details are given, but probably the strain used was the Alpk/Ap rat, exposed on GD 6-20. The following routes of administration were used: ip, po, sc, dermal and by inhalation. Group size was 15 animals for the first four routes and 20 animals for the control and inhalation routes. Two hundred to

250 mg/kg caused complete loss of litters by the ip, po and sc route; 948 mg/m^3 by inhalation, which corresponds to an approximate dose of 350 mg/kg assuming 100% retention, caused a similar result. At lower doses EGME causes a loss of approximately half the litters, with reduced litter size and survivability in the pups. This is shown by 40 mg/kg sc and 316 mg/m^3 by inhalation (which corresponds to an approximate dose of 115 mg/kg assuming 100% retention). Interestingly, a similar dose administered dermally, ie, 40 mg/kg, had no effect on the developing foetus. This result indicates that the skin has some barrier function with regard to EGME and that the sc route has a larger effect than inhalatory exposure.

Table 15 Testicular toxicity and reproduction performance studies of EGME and metabolites.

species	dosing regimen	results	NOAEL	reference
CD rats (n= 10, control n=10)	single oral dose of 0, 500, 750, 1,000 or 1,500 mg/kg; kills after 3, 4, 5, 6, 7 and 8 weeks	after 3, 4 and 5 weeks: reduced testes wt; after 6 weeks: highest dose group with reduced testes wt; after 4, 5, 6 and 7 weeks: reduced sperm count; after 6 weeks: highest incidence of abnormal sperm; after 8 weeks: no abnormal sperm except for the highest dose group	<500 mg/kg	And87
idem	single oral dose of 592 or 1,184 mg 2-MAA/kg	after 6 weeks: both dosages: normal testes wt; decreased total sperm count, increased %age abnormal sperm and separated heads	-	
rats (n=6, control n=6) (strain not mentioned)	daily oral doses of 500 mg/kg for 4 days; kills after 0, 2, 4 or 8 weeks	after 0 weeks: marked decreased relative liver and testes wt; after 2 weeks: liver recovered; after 8 weeks: testes recovered; testicular histology showed marked damage after 0 and 2 weeks; after 4 weeks recovery progressed	-	Fos84
Sprague-Dawley rats (n=6, control n=6)	daily oral doses of 592 mg 2-MAA/kg for 4 days	decreased relative liver and testes wt; extensive depletion of early pachytenes, moderate depletion and degeneration of late pachytenes	-	Fos84, Gray85
idem	idem EGME 500 mg/kg, pretreatment with pyrazole (inhibitor of alcohol dehydrogenase)	no abnormalities detected	-	Fos84
idem	idem 592 mg 2-MAA/kg, pretreatment with sulfiram or pargyline (inhibitors of aldehyde dehydrogenase)	similar testicular damage as without pretreatment	-	Fos84
Sprague-Dawley rats (n=3)	single ip inj of 250 mg/kg	tubular lesions; extensive degeneration of early and late pachytenes	-	Mos85
idem	idem, pretreatment with a single ip inj of pyrazole (an alcohol dehydrogenase inhibitor)	no effects on testes		

Alpk/Ap rats (n=5) idem	single oral dose of 195 or 974 mg methoxyacetaldehyde/kg single ip inj of 97 or 487 mg methoxyacetaldehyde/kg	both treatments: no decreased testes wt; similar target cells and stages of spermatogenesis are affected as with 2-MAA but with less overall damage	-	
idem	single oral dose or ip inj of 500 mg 2-MAA/kg	decreased testes wt in both cases	-	
rats (n=6, control n=6) (strain not mentioned)	daily oral doses of 0, 50, 100, 250 or 500 mg/kg for 1, 2, 4, 7 and 11 days; kills 6 and 24 h after a single dose and further after 2, 4, 7 and 11 days	after 6 h: no effects; at other time points: decreased relative liver and testes wt; at 50 mg/kg; normal testicular histology; with increasing doses and time: increasing testicular damage	50 mg/kg	Fos84
CD rats (n= 10, control n=10) each	single oral dose of 0, 500, 750, 1,000 or 1,500 mg/kg; after 5 and 6 weeks male was mated with 2 females	on GD 20 the females were killed. In the 5 wk group the mean total implants were decreased dose-related. In the 6 wk group there were no pregnancies, except for the low dose group; in the few pups available no gross abnormalities were observed. No induction dominant lethal mutations	<500 mg/kg	And87
F344 rats (n=9, control n=9)	daily oral doses of 0, 50, 100 or 200 mg/kg for 5 days; kills at 1, 2, 3, 4, 5, 6 and 7 weeks	low dose group: very mild testes changes; mid and high dose group: widespread damage and cell death, with some recovery; no treatment-related effect on seminal vesicle or prostate wt	MAEL 50 mg/kg	Cha85b
idem	idem, each male is mated to 2 females/week for 8 weeks	two weeks after removal from the male the females are killed. Low dose: no change in pregnancy rate, number of live pups; mid dose: decreased number of litters sired at week 5; high dose: male fertility declined at week 4 and remained low; mid and high dose groups: time- and dose-related decreases in sperm concentration and motility and increased sperm abnormality	50 mg/kg	Cha85a
AP/Alpk rats (n=1-2, control n=1-3)	single oral dose of 0, 50, 100 or 200 mg/kg; kills twice weekly at weeks 1-8 after treatment; spermatozoa were collected and incubated with oocytes from immature female rats	low dose reduced fertility at 5 weeks; mid dose: reduced fertility at 3.5, 4.5, 5, 6 and 6.5 weeks; high dose: reduced fertility at 2, 3, 4.5-6 and 7 weeks	< 50 mg/kg	Hol90
Sprague-Dawley rats (n=4-6, control n=4-6)	single oral dose of 250 mg/kg; kills 2 and 14 days later	after 2 days: decreased prostate wt; missing spermatocytes in stages XII to mid-VII and degenerating zygotenes in stages XIII, XIV. After 14 days: maturation depletion of spermatids and early pachytenes	-	Lin92
rats (n=?, control n=?)	single oral dose of 0 or 500 mg/kg or equimolar dose of 2-MAA; kills 3, 6, 12 and 24 h later	after 6 h: early effects in late spermatocytes; after 12 h: effects in early spermatocytes; after 24 h: degeneration or cell loss in pachytene and secondary spermatocytes	-	Bla85
F344 rats (n=8, control n=6)	daily oral dosing with 0 or 150 mg/kg, 5 d/week; kills after I, 2, 4, 7 and 10 days	target cells affected are both early and late spermatocytes in the testes; Sertoli cells are relatively unaffected	< 150 mg/kg	Cha84

Sprague-Dawley rats (n=10, control n=9-10)	single ip inj of 0, 250 or 750 mg/kg, collection of urine, kills 2 days later	both dosages: increased ratio of creatine/ creatinine excretion; decreased absolute and relative testes wt; dose-related increase in testicular atrophy; the high dose induced diuretic effect on day 2	-	Nah93
Sprague-Dawley rats (n=6-9, control n=12)	single ip inj of 0, 250, 350, 500 or 700 mg/kg; collection of urine; kills 2 days later	from 350 mg/kg upward: decreased relative testes wt; from 250 mg/kg upward: increased ratio of creatine/creatinine excretion; the high dose induced a diuretic effect after 24 hr	-	Raw89
Alpk/Ap (n=?, control n=?)	single 3 h exposure to saturated vapour 24,000 mg/m^3	15 days later: decreased relative testes wt; no haematuria	-	Doe84
idem (n=20, control n=?)	single 4 h exposure to 0, 474, 948, 1,975, 3,950, 7,900 or 15,800 mg/m^3	14 days later: at 1,975 mg/m^3 damage to maturing spermatids; at 3,950 mg/m^3 and higher dose-related decreased body wt gain and testes wt; testicular atrophy	948 mg/m^3	
idem (n=10, control n=10)	single 4 h exposure to 3160 or 7,900 mg/m^3; kills I, 2, 3, 4, 5, 8, 10, 15 and 19 days later	both concentrations: 2-19 days later: decreased testes wt and testicular atrophy. At 7,900 mg/ m^3: on several time points decreased cauda epididymal wt and decreased accessory gland wt; at 7,900 mg/m^3 at day 4: increased epididymal wt probably reflecting the passage of l arge numbers of immature germ cells shed from the testes	-	
Alpk/Ap rats (n=10, control n=?)	exposure to 0, 316 or 948 mg/ m^3, 6 h/day for 10 consecutive days	at 316 mg/m^3 no effects; at 948 mg/m^3 reduced body wt and body wt gain; decreased thymus and testes M; reduced white and red blood cell count, and haemoglobin content; testicular atrophy	316 mg/m^3	Doe84
Sprague-Dawley rats (n=14-15, control: male n=?, female n=15-18)	79 mg/m^3, 7 hr/day, 7 d/week for 6 weeks; then mating with untreated females; test on litter for neuromotor function on days 10-90; brain analysis of litters on day 21	no effect on paternal or maternal animals, nor on the number or wt of live offspring. No effect on behaviour. Neurochemical deviations were numerous in the brainstem and cerebrum, but fewer in the cerebellum and midbrain	< 79 mg/m^3	Nel84b
Crl:CD® (SD)BR rats (n=20, control n=20, that is: 5 at each time point)	exposure to 0 or 948 mg/m^3, 6 h/day, 5 d/week for 2 weeks; kills after 10 d of exposure and 14, 42 and 84 days post exposure (PE)	after 10 d of exposure decreased body wt, regained normal after 42 d PE; decreased testes wt at all time points; decreased epididymis wt at all PE time points; decreased prostate wt at 10 d of exposure and 14 d PE; testicular atrophy at 14 d PE; most but not all testes had normal morphology at 84 d PE	-	Lee89
Sprague-Dawley rats (n=?, control n=?)	exposure to 0, 95, 316 or 948 mg/m^3, 6 h/day, 5 d/week for 13 weeks; exposed males were mated with unexposed females	at low and mid-dose: no effects; at high dose: decreased body wt and fertility index: no females delivered pups	316 mg/m^3	Han84b
Sprague-Dawley rats (n=20, control n=20, that is: 5 at each time point)	dermally: 0, 625, 1,250 or 2,500 mg/kg on occluded sites for 7 days	all dose groups: soft stool and perineal staining; from 1,250 mg/kg: decreased activity, tremors, strangulated penis and death	< 625 mg/kg	Feu89

idem	idem, 0, 1,250, 2,500 or 5,000 mg/kg on unoccluded sites for 7 days both treatments: the number and morphology of caudal epididymal sperm, number of testicular spermatids, and wts of reproductive organs were determined on weeks 4, 7, 10 and 15; fertility was assessed on weeks -1, 4,7,10 and 14	at 5,000 mg/kg: soft stool; from 2500 mg/kg: decreased testes wt both treatments: the effects were dose-related and included a decline in epididymal sperm count and testicular spermatid count, a reduction in wts of testes and epididymides, an increase in the number of sperm with abnormal morphology, and a reduction in fertility. The effects were more severe and recovery proceeded at a slower rate when the skin sites were covered		
CD-1 mice (n=10, control n=10)	single oral dose of 0, 500, 750, 1,000 or 1,500 mg/kg; after 4 and 5 weeks each male was mated with 2 females. On GD 18 the females were killed	no effect on mean total implants or early deaths, gross abnormalities. No induction of dominant lethal mutations	1500 mg/kg	And87
CD-1 mice (n=10, control n=10)	single oral dose of 0, 500, 750, 1,000 or 1,500 mg/kg; kills after 2, 3, 4, 5, 7 and 8 weeks	after 2-5 weeks: decreased testes wt, returning to normal after 8 weeks; decreased total sperm count only in the 750 mg/kg group in week 3 and the 500 and 1,000 mg/kg groups in week 4; dose-related increase in %age abnormal sperm at week 4, returning to normal in weeks 7-8	< 500 mg/kg	And87
(C57Bl/Cne x C3H/Cne)F1 mice (n=5, control n=20)	single oral dose of 0, 50, 100, 300, 600 or 900 mg 2-MAA/kg; kills after 2, 7, 14, 28 and 45 days	cytotoxic damage on primary spermatocytes; Sertoli and Leydig cells appeared unaffected; at 2 days: reduced testes wt from 100 mg/kg; at 7, 14 and 28 d: reduced testes wt from 300 mg/kg; at 7-14 d: complete recovery for 50 and 100 mg/kg; at 45 d: almost complete recovery for all dosages	50 mg 2-MAA/kg	Spa91
CrI:CD mice (n=10, control n=10)	daily oral dosages of 0, 70, 250 or 700 mg/kg for 18 days	top dose: decreased number of sperm/g cauda, decreased %age motile sperm; two highest dosages: decreased testes wt	70 mg/kg	Har92
ICR mice (n=12 or 16, control n=16 or 22)	daily oral doses of 0, 50, 200, 750 or 1,500 mg/kg for 5 days followed by weekly mating with superovulated virgin females for 7 weeks; at 26 h after mating the females were killed and the 2-cell and 4-cell embryos isolated. The embryos were aggregated into chimera pairs: either from two different control females mated to control males, or from one control female mated to a control male with a control female mated to a treated male; the proliferation ratio was assessed (the cellular contribution from each partner embryo)	three low-dose groups: proliferation ratios significantly decreased at week 4, which corresponds to the pachytene spermatocyte stage of spermatogenesis; top dose: proliferation ratio significantly decreased at week 5; in week 4: transient infertility	< 50 mg/kg	Oud93

ICL-ICR mice (n=5, control n=5)	daily oral doses of 0, 62.5, 125, 250, 500, 1,000 or 2000 mg/kg, 5 d/week, for 5 weeks	effects on testes: dose-related decrease in size and atrophy of the seminiferous epithelium; normal Sertoli and Leydig cells. Dose-related decrease in white blood cell count; at higher dose levels: decrease in red cell count, packed cell volume and/or haemoglobin content	< 62.5 mg/kg	Nag84
Syrian golden hamsters (n=4, control n=4)	daily oral doses of 0, 62.5, 125, 250 or 500 mg/kg, 5 d/week, for 5 weeks	effects on testes: dose-related decrease in size; no effect on wt of seminal vesicles and coagulating gland nor white blood cell count	< 62.5 mg/kg	
guinea pigs (n=3, control n=3)	daily oral doses of 0, 250 or 500 mg/kg, 5 d/week, for 5 weeks	at both regimens the same decrease in testes wt and white blood cell count; no effect on wt of seminal vesicles and coagulating gland	< 250 mg/kg	
Additional data in SCOEL (2006)				
male Dutch rabbits (n=6, control n=6)	0, 12.5, 25.0, 37.5 or 50.0 mg/kg bw/day, in drinking water, 5 d/week for 12 weeks	dose-related depression of spermatogenesis	12.5 mg/kg	Foote *et al.* (1995) SCOEL18, Berndtson and Foote (1997)SCOEL19
female Sprague–Dawley rats (n=6-9, control n=6-9, 2 exp.)	daily oral doses of 10, 100 or 300 mg/kg bw/day, starting on vaginal metestrus, for 7 days	within 3 to 8 days, suppression of cyclicity, inhibition of ovulation, and hypertrophy of corpora lutea, permanently elevated progesterone levels and permanently low levels of estradiol, FSH, LH and prolactin, at 100 mg/kg without systemic toxicity	10 mg/kg	Davis *et al.* (1997)SCOEL20
Additional information				
female Sprague-Dawley rats (n=10, control n=10)	daily oral doses of 0, 30, 100 or 300 mg/kg bw/day, for 2 or 4 weeks; part of the animals were treated for 2 weeks and subsequently cohabited with non-treated males for up to 2 weeks	prolonged estrous interval and decreased oestrus frequency, hypertrophy of corpora lutea, minimal and statistically not significant at 30 mg/kg; decreased numbers of corpora lutea of pregnancy, implantations, and live embryos, and increased incidence of post-implantation loss at 100 mg/kg; no pregnancies at 300 mg/kg, although 7/10 females copulated	≤ 30 mg/kg	Dodo *et al.* (2009)[1]

Table 16 Developmental toxicity studies of EGME and metabolites.

species	dosing regimen	results	NOAEL	reference
? rats (rats (n=?)	daily oral dosages at least with one dose of 41 mg/kg on GD 6-15	60% of the surviving foetuses are malformed	< 41 mg/kg	unpubl. Sco89
Sprague-Dawley rats (n=30, control n=25)	daily oral doses of 0, 50 or 75 mg/kg on GD 7-13	maternal reduced body wt gain, apparently due to resorbed foetuses; prolonged gestation period; reduced %age of pregnant dams that delivered, litter size, and pup wt; at 75 mg/kg: no survivors beyond 3 days of age; at 50 mg/kg: reduced number of survivors; electrocardiographic changes in the survivors	< 50 mg/kg	Tor88

Fischer 344 rats (n=20-21, control n=19)	daily oral doses of 0, 12.5, 25, 50 or 100 mg/kg on GD 6-15; sacrifice on GD 16 and litter on postnatal day (PND) 4	top dose: decreased maternal wt gain and gravid uterine wt; on GD 16: number of pregnant dams: 9 (control), 8 (low dose), 8, 6, 6 (top dose); dose related increase in early resorptions/litter; on PND 4: number of litters: 7 (control), 10 (low dose), 9, 7, 13 (top dose, includes 12 dams with 100% resorptions); dose-related decrease of live pups/litter; low dose: increased adjusted pup body wt on PND 1, but not on PND 4	12.5 mg/kg	Mor89b
Sprague-Dawley rats (n=26, control n=27)	single oral dose of 0 or 150 mg/kg on GD 13	no maternal effects; 15/26 litters and 50/357 foetuses had paw malformations	< 150 mg/kg	Nel91
idem	radiofrequency radiation sufficient to elevate rectal temperature to 42.0°C for 30 min on GD 13	no maternal effects; 10/18 litters and 67/222 foetuses had paw malformations		
idem (n=24)	both treatments together	no maternal effects; 18/18 litters and 191/250 foetuses had paw malformations	synergism	
Sprague-Dawley rats (n=9-11, control n=10)	daily oral doses of 0, 16, 31, 73, 140, 198, 290 or 620 mg/kg on GD 7-18	at the four top doses the maternal wt gain was decreased and 100% of the litters was resorbed; at 31 mg/kg maternal wt gain was increased; the mean pup wt decreased at the 3 lowest dosages; at 73 mg/kg 40% of the survivors was malformed; at 31 mg/kg 4% of the survivors was malformed	< 16 mg/kg	Nel89
idem (n=8, resp. 4)	daily oral doses of 39 or 79 mg 2-MAA/kg on GD 7-18	top dose: reduced maternal wt gain and 100% resorption; low dose: 58% resorptions, reduced pup wt and 15% of the survivors malformed	< 39 mg 2-MAA/kg	
idem (n=8, resp. 10)	daily oral doses of 44 or 81 mg EGME/kg + 35% ethanol-derived calories on GD 7-18, as a competitor for alcohol dehydrogenase	reduced maternal wt gain and pup wt; top dose: 89% resorptions and 25% of the survivors malformed		
idem (n=9, resp. 7)	daily oral doses of 59 mg EGME/kg or 59 EGME/kg + 35% ethanol derived calories (= 16,000 mg/kg/ day) on GD 7-18 (pair feeding)	reduced maternal wt gain in both treatments; increase in resorptions (31%) after EGME alone, slight increase in resorptions after EGME + ethanol; both treatments: no external malformations in pups, decreased pup wt, visceral malformations	no reduction in terato-genicity	
idem (n=9, resp. 5)	daily oral doses of 54 EGME/ kg + 4.0 mg amiloride (increases the teratogenicity of other acidic compounds) or 4.0 mg amiloride/kg on GD 7-18 (pair feeding)	both treatments: decreased maternal wt gain and reduced pup wt; EGME + amiloride: increase in resorptions (23%); amiloride alone: no increase; EMGE + amiloride: 11/54 foetuses malformed; amiloride alone: 0/61 foetuses malformed; the EGME + amiloride produced an incidence of cardiovascular malformations in foetuses twice that of EGME alone	increase in terato-genicity	
idem (n=11-12, control n=12)	daily oral doses of 0, 16, 31 or ± 35 mg/kg on GD 7-18, behavioural tests with offspring on postnatal days 48-65	mid-dose: approx. 50% mortality in the offspring and increased number of errors in 1/4 tests[a]; no behavioral effects at either dose; low dose: no decrease in pup wt; all doses: delayed delivery (= increased gestation time)	< 16 mg/kg	

Sprague-Dawley rats (n=8, control n=8)	daily oral doses of 0, 25, 50 or 75 mg/kg on GD 6-12; dams were sacrificed on GD 9, 11, 13 or 15 and the last group was allowed to deliver or killed on GD 25 if they did not deliver; assay of ornithine decarboxylase (ODC) activity in whole embryo	no change in maternal wt gain; top dose: no litters; mid dose: 50% with litters; dose-related increase in gestation time; only on GD 11 a dose-related decrease of ODC activity	25 mg/kg	Tor86
Sprague-Dawley rats (n=10, control n=10)	daily oral doses of 0, 25, 50 or 100 mg/kg at least for 15 days prior to mating, during gestation and for 4 days post partum	mating with untreated males: from 50 mg/kg and higher: decreased number of live pups born, decreased litter size, increased mortality during the first 4 days post partum	25 mg/kg	Sca92
Alpk/AP rats (n=15, control n=15)	daily oral doses of 0 or 200 mg/kg on GD 7-17	[b] decreased maternal wt gain; no viable litters	< 200 mg/kg	Wic87
idem	daily ip inj of 0 or 50 mg/kg on GD 7-17	decreased maternal wt gain; no viable litters	< 50 mg/kg	
idem	daily dermal applications of 0 or 40 mg/kg on GD 7-17	no maternal effects; no effect on litter size, number of live pups on day 1 and 5 and mean pup wt	40 mg/kg	
idem	daily sc inj of 0 or 40 mg/kg on GD 7-17	no maternal effects, decreased number of viable litters and of live pups on days I and 5	<40 mg/kg	
Sprague-Dawley rats (n=16-18, control n=15-18)	daily inhalation exposure to 0 or 79 mg/m^3, 7 h/day on GD 7-13 or 14-20; test on litter for neuromotor function on days 10-90; brain analysis of litters on day 21	no maternal effects, nor on the number or wt of live offspring. One of the six behavioral tests revealed significant differences in offspring of mothers exposed on GD 7-13. Neurochemical deviations were numerous in the brainstem and cerebrum, but fewer in the cerebellum and midbrain	< 79 mg/m^3	Nel84b
Sprague-Dawley rats (n=14, 34, 8, resp., control n=34)	daily inhalation exposure to 0, 158, 316 or 632 mg/m^3, 7 h/day on GD 7-15	decreased maternal wt gain; at 158 and 316 mg/m^3: increased number of resorptions; at 632 mg/m^3: 100% resorptions; dose-related decrease of live foetal wt; at 316 mg/m^3: increased number of visceral malformations; at 158 and 316 mg/m^3: increased number of skeletal malformations	< 158 mg/m^3	Nel84a
Alpk/Ap rats (n=20, control n=20)	daily inhalation exposure to 0, 316 or 948 mg/m^3, 6 h/day on GD 6-17	at 316 mg/m^3: decreased number of litters; decreased proportion of live pups per litter initially and 3 days postpartum; increased gestation period; at 948 mg/m^3 : decreased maternal body wt gain; no litters	< 316 mg/m^3	Doe84
Fischer 344 rats (n=28 -30, control n=30)	daily inhalation exposure of 0, 9.5, 32 or 158 mg/m^3, 6 h/day on GD 6-15	all dose levels: no maternal effects; at 158 mg/m^3 : increased number of lumbar spurs, delayed ossification	32 mg/m^3	Han84a
CF-1 mice (n=30-32, control n=31)	daily inhalation exposure of 0, 32 or 158 mg/m^3, 6 h/day, on GD 6-15	at 158 mg/m^3 : decreased maternal wt gain on GD 12-15 and GD 6-15 not on GD 6-17; decreased number of live foetuses; increased incidence of extra ribs and unilateral hypoplastic testicle	32 mg/m^3	

New Zealand white rabbits (n= 29-30, control n=30)	daily inhalation exposure of 0, 9.5, 32 or 158 mg/m^3, 6 h/day on GD 6-18	at 9.5 and 32 mg/m^3 : no maternal effects; at 158 mg/m^3 : decreased maternal wt gain; at 32 and 158 mg/m^3 : increased % age of resorptions, increased %age of litters with resorptions; delayed ossification at sternebrae; at 158 mg/m^3 : decreased foetal body wt; increased total malformations	9.5 mg/m^3 [c]	
Alpk/Ap rats (n=15, control n=15)	daily sc inj. of 0, 40 or 250 µl/kg on GD 6-20	at low dose: decreased number of litters, decreased number of live pups per litter initially and 5 days post partum; at high dose: some dams with piloerection and vaginal bleeding; reduced maternal body gain; no litters	< 40 µl/kg	Doe84
Sprague-Dawley rats (n=8-10, control n=10)	single dermal dose of 0, 250, 500, 1,000 or 2,000 mg/kg on GD 12, or of 2,000 mg/kg on GD 10, 11, 12, 13 or 14	no maternal effects; from 500 mg/kg upward: increased external, visceral, and skeletal malformations in foetuses; at 2000 mg/kg foetal malformations at all GD of administration	250 mg/kg	Feu90
F344 rats (n=8 and 25, resp., control n=8 and 25, resp.)	daily dermal applications of 0, 420, 840 or 1,260 mg/kg on GD 6-15	at the two highest doses: decrease of %age of animals with viable implants; at highest dose: decrease in maternal body wt gain; at mid dose: decreased foetal body wt; external and visceral malformations, and 54 skeletal malformations; 100% of the foetuses had soft tissue and skeletal variations	420 mg/kg	Tyl92
Wistar rats (n=?)	single ip inj. of 380 mg/kg on GD 12, embryos were removed 1-72 h after inj. and on GD 20	altered limb bud development in all cases; at GD 20: 86/87 foetuses had fore- or hindlimb malformations	-	Sco87
Wistar rats (n=6-7, control n=13)	single oral dose of 0, 158 or 315 mg/kg on GD 12; single ip inj of 0, 158 or 315 mg/kg on GD 12; termination on GD 20	both routes were not significantly different from each other; dose-related increase in %age dead and resorbed foetuses, and in survivors malformed	-	Rit85
idem (n=8, control n=13)	idem with equimolar doses of 2-MAA (oral dosing)	in quantitative and qualitative respect the same results as with EGME	-	
idem (n=7, control n=13)	single ip inj of 315 mg EGME + 100 mg 4-methylpyrazole/kg (alcohol dehydrogenase inhibitor) on GD 12	significant protection against embryotoxicity: from 100% to 16.8%	-	
Wistar-Porton rats (n=8-10, control n=5-7)	single ip inj of 223 mg 2-MAA/kg on GD 8, 10, 12 or 14	increased %age of resorptions: 93% on GD 8, 2.8% on GD14; increased %age of malformations: 42% GD 8, 82% on GD 14 (mainly skeletal, hydrocephalus and urogenital)	-	Bro84
idem (n=5, control n=5)	single ip inj of 9-223 mg 2-MAA/kg on GD 10 or 12	on GD 12: dose-related increase in foetal death, abnormal live foetuses, skeletal malformations and hydrocephalus; on GD 10: no clear-cut dose-response relationship	-	
Alpk/AP rats (n=10, control n=10)	daily dermal applications of 0, 3, 10, 30 or 100% on GD 6-17	[d] top dose: all dams died; 30% dose: all foetuses died; 10% dose: decreased incidence of pregnancy, decreased number of viable litters; 3% dose: no effects	3%	Wic86

idem (n=9-12, control n=11)	daily oral doses of 250 mg/kg on GD 7-9; 8-10; 9-11; 7-8; 9-10 or 10-11	decreased number of live foetuses on GD 7-9; increased %age of resorptions at all treatments; decreased foetal wt at all treatments; no effect on maternal wt; digit anomalies, fused vertebrae and exencephaly at two or three treatments	-	
idem (n=9-11, control n=16)	single oral dose of 0, 100, 175, 250, 300, 350, 400 or 450 mg/kg on GD 11	no effect on number of live foetuses or %age of resorptions; decreased foetal body wt at 250 mg/kg and higher; no maternal toxicity; digit anomalies were not induced by 100 mg/kg, but occurred at 175 mg/kg and increased in a dose-related manner	100 mg/kg	
Crl:CD-1 ICR BR mice (n=9- 12, control n=16)	single oral dose of 0 or 500 mg/kg on GD 9, 10, 11, 12 or 13	decreased number of live foetuses on GD 9 or 10; increased %age of resorptions on GD 9, 10 or 11; decreased foetal body wt on GD 9, 10, 11, 12 or 13; no effect on maternal wt	-	Hor85
ICL-ICR mice (n=21-24, control n=22)	daily oral doses of 0, 32, 62.5, 125, 250, 500 or 1,000 mg/kg on GD 7-14	[e] no maternal effects; at all dose levels: skeletal variations and delayed ossification; at 62.5 mg/kg and higher: skeletal malformations; at 125 mg/kg and higher: reduction in foetal body wt; at 250 mg/kg: increased incidence of dead foetuses; gross abnormalities; at 500 mg/kg: all foetuses except one were dead; at 1000 mg/kg: all foetuses dead	< 32 mg/kg	Nag84
CD-1 mice (n=49, control n=50)	daily oral doses of 0 or 1,400 mg/kg on GD 7-14	7 dams died (14%); 0/30 litters were viable (0%)	-	Sch84
CD-1 mice (n=16, control n=14)	single oral dose of 0 or 304 mg/kg on GD 11	no maternal effects; increased incidence of paw defects (68.5% of the foetuses); no effects on litter size, live pups born and foetal body wt	< 304 mg/kg	Har87
Crl:CD-1 mice (n=11- 12, control n=10)	daily oral dosages of 0, 70, 250 or 700 mg/kg on GD 8-14	top dose: no viable litters (0/11); two highest dosages: decreased number of live neonates on postnatal days 0, 1 and 4	70 mg/kg	Har92
CD-1 mice (n=10-18 litters, control n=16 litters)	single oral dose of 0, 100, 175, 250, 300, 350, 400, 450 or 500 mg/kg on GD 11; kills on GD 18	from 175 mg/kg: dose-related increase in % age foetuses with forepaw malformations	100 mg/kg	Gre87
idem (n=3-4, control n=3)	single oral dose of 0, 100, 250 or 350 mg/kg on GD 11; kills 24 h later	at low dose: slight increase in cell death; mid and high dose: progressive cell necrosis in the fore-limb buds	< 100 mg/kg	
CD-1 mice (n=5, control n=5) idem (n=5, control n=5)	single ip inj. of 0 or 250 mg/kg on GD 11; kills after 48 h single oral dose of 0, 250 or 500 mg 2-MAA/kg on GD 11; kills after 48 h	both compounds: serious effects on the two main maternal (placental) vessels (congestion, dilatation, haemorrhages, necrosis)	< 250 mg/kg	Khe93
Crl:CD-1 ICR BR mice (n=3-7, control n=3-7)	single sc inj of 0, 100, 175 or 250 mg/kg on GD 11 or via osmotic minipump: 34.7 or 69.4 mg/kg/h for up to 12 h on GD 11 (total dose: 139-833 mg/kg)	in all cases: decreased live foetus wt; no effect on % age dead, % age malformations, or incidence of digit malformations	-	Cla92

CrI:CD-1 ICR BR mice (n=8, control n=10)	single iv inj of 0 or 250 mg/kg on GD 7 and 8 or on GD 7	increased maternal wt gain; %age of foetuses with exencephaly: 3% on GD 7, 22% on GD 7+8, 11% on GD 8, 0% on GD 9, after inj of 250 mg/kg;	-	Ter94
idem (n=7-10, control n=10)	single iv inj of 0, 175, 250, 325 mg/kg on GD 8	idem: %age of litters affected with exencephaly and/or nonviable embryo/foetuses: resp.: 58, 100, 73 and 22%		
idem (n=9, control n=10)	single iv inj of 0 or 250 GD 9; kills on GD 18			
idem (n=10-14, control n=11)	sc infusion via osmotic minipump: for up to 8 h on GD 8 (total dose: 277-606 mg/kg)	decreased maternal wt gain after 8 h infusion; dose-related increased %age of litters with dead implants; increased %age of exencephaly	-	
Macaca fascicularis (n=14, 11 and 8, resp., control n=6)	daily oral doses of 0, 12, 24 or 36 mg/kg on GD 20-45; Caesarian section on GD 100	dose-related maternal body wt loss (anorexia) during treatment; after treatment: regain of appetite; after treatment: no consistent haematological effects, but tendency to reduced red blood cell count; embryos: resp. 0/6, 4/14, 4/11 and 8/8 resorptions (resp. 3/13, 3/10 and 8/8 resorptions were considered treatment-related); all foetuses that survived to 100 days of gestation were free from malformations	< 12 mg/kg	Sco89

[a] considering the unusual nature of the maze effect and the lack of significant differences from controls in the other behavioral tests some uncertainty exists concerning the meaning of this finding

[b] in this study no statistical calculations were made and the teratogenicity was not assayed

[c] The Committee notes that the authors attributed the increased resorption seen in rabbits at 32 mg/m^3 to the low control value and not related to EGME. The delayed ossification in sternebrae at the same exposure level was considered a reflection of the normal variability within this species. Historical control data provide support for the increased resorption rate but historical control data on skeletal variations are not provided.

[d] the mean no. of live pups was assayed on PND 1 and 5; the teratogenicity was not assayed

[e] viscera were not examined, but kidney and heart are two primary target organs

Table 17 Effects of maternal and paternal exposure to EGME on the offspring.

species	dosing regimen	results	NOAEL	reference
Sprague-Dawley rats, males and female, continuously cohabitating (n=40 pairs, control n=40 pairs)	daily oral dosing via the drinking water: either 0, 0.01, 0.03 or 0.1% for approx. 6 weeks (until 2 litters were produced), or 0, 0.006, 0.012 or 0.024 % for approx. 18 weeks (until 5 litters were produced) (resp. L2 and L5 experiment)	L2 experiment: control: 36/40 pairs produced litters, with 2-16 pups/litter; L5 experiment: control: 28/40 pairs produced litter with 3-16 pups/litter. L2 experiment: top dose: one litter delivered, the other pairs were infertile, mid dose: decreased number of live pups per litter and proportion of pups born alive; L5 experiment: top dose: 14% decrease in live pup number	0.012% in drinking water	Gul91
Crl:CD-1 mice (n=10 pairs, control n=10 pairs)	daily oral doses of 0, 70, 250 or 700 mg/kg on days 0-20 for the females and days 3-20 for the males; mating on days 8-12; males were killed on day 20, females on day 21	results of the males: see Table 15; results of the females: top dose: decreased number of pregnancies; no effect on number of live implants or total implants/female	250 mg/kg	Har92

When EGME or 2-MAA were administered to pregnant CD-1 mice they were equipotent in causing teratogenicity. This was measured as the incidence of digit malformation in the offspring. Alcohol dehydrogenase (ADH) catalyses the initial rate-limiting oxidation that leads to embryotoxicity. The ADH-inhibitor 4-methylpyrazole (0.12 or 1.2 mmol/kg) or ethanol (43.3 mg/kg, single dose concomitant with EGME or additional ethanol 5 and 10 h later) reduced the incidence of malformations 60-100%, depending on the dosing regimen (Sle88).

In order to assess the possible strain differences in susceptibility to the reproductive toxicity of EGME three different mouse strains were used: Swiss CD-1, C57B1/6 and C3H, respectively with high, intermediate and low fecundity. Per study group 30 pairs were used to increase statistical power and to accommodate the expected decline in fertility. At 11 weeks of age, mice were exposed to EGME-containing water as their only drinking solution for 1 week, whereupon they were randomly assigned an opposite-sex cage-mate of the same treatment group and cohabited for 14 weeks. EGME was provided as the drinking solution from the start of the study until necropsy. The concentrations of EGME were 0.03, 0.1 and 0.3% w/v. Water consumption was measured at weeks 1, 2, 6, 10 and 14 during cohabitation. Immediately after each litter was produced, the neonates were removed from the dam, counted, sexed, weighed, and humanely euthanatised. In this 14-week cohabitation design, pairs could produce up to 4 or 5 litters. At 14 weeks the Fo pairs were separated, and any litters born during the next 3-week period were reared for assessment of toxicity in the second generation. Dams continued to receive EGME-dosed water during gestation and nursing. At weaning, pups were given drinking water containing the same concentration of EGME as consumed by their parents. After F, mice were weaned, the Fo animals were killed by asphyxiation and necropsied. The fertility of the second generation was assessed in a 1-week mating trial when the F, animals were 74 ± 10 days of age. After the week of cohabitation, the F, mice were housed separately under continued exposure to EGME-containing water until any pups were delivered and assessed, and then the F, and F2 mice were euthanatised by CO_2 asphyxiation and necropsied. No thymus weight was recorded.

There were no consistent dose-related decreases in water consumption in any of the strains during the study. For the Swiss, C57 and C3H mice, respectively, the low dose pairs consumed a mean of 60, 53 and 64 mg EGME/kg/day, while the middle dose groups consumed a mean of 198, 170 and 219 mg EGME/kg/day, and the high dose mice consumed 540, 505 and 636 mg EGME/kg/day. Thus, the calculations suggest that the C57 mice received slightly less EGME per

day than did the other strains, while the C3H are thought to have received slightly more EGME than the other strains at all dose levels. The data on overall fertility, reproductive performance, mean live pups/litter and adjusted mean live pup weight suggest that the more fertile strain tended to be more resistant to the effects of EGME, although these differences were not confirmed statistically. The testes weight in the high dose groups was decreased to 83, 67 and 48% of control weights in the Swiss CD-1, C57 and C3H strain, respectively. Sperm motility was decreased in the same ranking order: 12, 34 and 72% of the control value in the respective strains.

Differences among the strains in EGME response were generally less clear when considering the organ weight and other necropsy data.

The assay for the reproductive performance of the second generation was weakened by the fact that there were insufficient C3H mice to form 20 nonsibling pairs for this mating. Nevertheless, the same trends in numbers of pups per litter and adjusted live pup weight are seen across strains in the control F, mating as were seen in the Fo mating. Since there were no pups from the high-dose pairs during continuous breeding, there were no high-dose pairs for the F, mating. At the mid dose (0.10%) seven pairs of Swiss CD-1 mice produced an average of 7 pups/litter, while in the C57 and C3H strains, no live pups were produced in 6 and 5 litters, respectively. There were no biologically significant effects seen at the low dose.

It can be concluded that the most fecund strain (Swiss) was affected the least by exposure to EGME, while the least fecund strain (C3H) suffered the greatest decline in fertility. The study shows that the primary difference between the strains is quantitative, not qualitative; for equal response in the different strains in most of the endpoints examined above, there was an approximately threefold difference in dose. Thus, this compound would be identified as a reproductive hazard even in a robust strain, although the NOAEL might be different.

The lowest dose tested, 0.03% EGME in drinking water, affected one or more parameters in all three strains. Moreover, the thymus weights were not recorded, a possible target organ for EGME toxicity.

In *Swiss CD-1 mice* 60 mg EGME/kg/day increased the absolute kidney weight in males and females and increased the sperm density in the F_0 generation; in the F_1 generation the percentage of pups born alive was decreased.

In *C57Bl/6 mice* 53 mg EGME/kg/day increased the adjusted mean live pup weight, while there were no other effects observed in the F_0 generation; in the F_1 generation no effect on fertility or reproductive performance was found.

In *C3H mice* 64 mg EGME/kg/day increased the absolute and relative liver weight in females. In the F_1 generation the relative kidney weight was increased in the males (the relative organ weights are published by Gul88, Gul89) (Cha93).

In a continuous breeding protocol COBS Crl:CD-1 (ICR) BR outbred Swiss albino mice were used. The mice were exposed to EGME for a 7-day premating period and a 98-day period of cohabitation. During the next 21 days any final litters were delivered and kept for at least 21 days. To assess the reproductive function of the offspring the mother was dosed through weaning and F, mice were dosed until mated at 74 ± 10 days of age. In the crossover mating trial the high-dose animals of each sex were mated to control mice of the opposite sex. In all cases there were 20 animals/sex/treatment group. EGME was administered via the food or the drinking water, it is unclear which route was used. In the continuous breeding phase six levels of EGME were used, corresponding to an estimated daily dose of 0.16, 0.34, 0.62, 0.78, 1.27 and 1.91 g/kg body weight. The three highest dosages produced infe rtile pairs. The next dose produced insufficient data for statistical analysis: parameters like fertility index, mean number of litters per pair, mean number of live pups per litter were decreased. At 340 mg/kg the same parameters were decreased, except for the fertility index. At 160 mg/kg the mean number of live pups per pair was decreased (females only), the proportion of pups born alive was decreased and the mean live male pup weight per litter was increased.

The reproductive function of the offspring was tested with only one EGME dose level, corresponding to an estimated daily dose of 210 mg/kg body weight. It reduced the mating and fertility index, and the proportion of pups born alive: there were no pups.

In the crossover mating trial the estimated daily doses were 360 and 1050 mg/kg body weight. Three breeding pair combinations were used. The highest dose increased the mating index in females but decreased the fertility index in males and females: for both treated sexes the pairs were infertile, after mating with a control animal of the opposite sex. The low dose treatment of males increased the mean number of live female pups per litter. The low dose treatment of females decreased the mean number of live pups per litter (males only), the proportion of pups born alive and the adjusted mean live pup weight per litter (females only).

It can be concluded that the lowest dose tested, 160 mg/kg, is not an NOAEL in CD-1 mice in a continuous breeding protocol (Mor89a).

Several parameters were also measured in the crossover mating trial. At the high dose the weight of the epididymis and testis was decreased, sperm motility

and concentration were decreased and the percentage of sperm abnormalities was increased. The female cycle length was increased. At the low dose only the female cycle length was increased (Mor88).

Nine-day old conceptuses of female rats of the Wistar-Porton strain were cultured *in vitro* in the presence of 5 mM EGME or 1, 2, 3 or 5 mM 2-MAA. After 46 h in culture several developmental parameters were tested. The control group grew and developed normally. No significant effects of EGME on embryonic growth and development were observed at 5 mM. This can be explained by the fact that the rat foetus *in vitro* has little or no ability to convert EGME and, at the organogenesis phase, lacks alcohol dehydrogenase activity. 2-MAA, however, induced a dose-related decrease in yolk sac diameter, crown-rump length of the embryo, head length, and number and regularity of somites. The predominant morphological abnormality was irregular fusion of the neural tube, also at the lowest concentration. At higher concentrations open neural tubes and stunted telencephalic hemispheres were observed (Yon84).

Nine-day old foetuses of Crl-CD rats were cultured for 48 h in a medium containing 0, 25, 50, 75, 100 or 200 mM EGME or 0, 0.1, 0.2, 0.4 or 0.8 mM 2-MAA. The highest concentration of EGME caused 100% embryolethality. A dysmorphogenic effect was observed for concentrations of 50 mM or above. The NOAEL was 25 mM. For 2-MAA there was a dose-related reduction of the somite number, general score for morphological alterations and mean protein content per embryo. The NOAEL was 0.1 mM (Gia93).

Nine-day old foetuses of rats of an unspecified strain and five-day old chicken embryos were cultered in a range of concentrations of EGME and 2-MAA. EGME at very high concentrations reduced the growth of rat embryos but did not induce specific malformations. In the chick hypoplasia of the nervous system was observed at 10^{-2} mol/litre only. 2-MAA produced characteristic abnormalities of the optic and otic vesicles, branchial region and somites. They were observed at 10^{-4}-10^{-3} mol/litre. It is concluded that EGME has not, but 2-MAA has teratogenic properties. Both compounds had relatively low toxicity in a test with aggregating brain cells obtained from foetal rats (Kuc93).

The teratogenic potential of EGME was also assessed in three strains of *Drosophila melanogaster*. Two strains, Adh^{71K} K and Adh^{F} had an alcohol dehydrogenase activity of about equal potency, the third strain, $bAdh^{n4}$ had no alcohol dehydrogenase activity. When eggs of all three strains were laid on EGME-supplemented media, EGME induced teratogenic effects in the adults at all concentrations used (20-115 mM), which can be deduced from the bristle

number increase per fly. Also the period of development from larvae to adults was sensitive to the teratogenic activity of EGME. However, toxicity was also observed in the form of a low survival in both developmental periods. Therefore, a limited number of larvae was available for evaluation. Higher detoxification occurs in the strain with increased ADH activity. EGME is much more toxic than its oxidation product 2-MAA at the level of adult eclosion. Based on the fact that teratogenic effects were also observed in the ADH-negative strain it is concluded that EGME is apparently a teratogenic compound by itself (Eis89).

6.6.2 EGMEA

The testicular and developmental effects of EGMEA are summarised in Table 18.

Table 18 Testicular and developmental effects of EGMEA in *in vivo* studies.

species	dosing regimen	results	NOAEL	reference
male ICL-ICR mice (n=5, control n=5)	daily oral doses of 0, 62.5, 125, 250, 500, 1,000 or 2,000 mg/kg, 5 d/week for 5 weeks	effects on testes: dose-related decrease in size and atrophy of the seminiferous epithelium; normal Sertoli and Leydig cells; dose-related decrease in white blood cell count; at higher dose levels: decrease in red cell count, packed cell volume and/or haemoglobin content.	< 62.5 mg/kg	Nag84
female CD-1 mice (n=49, control n=50)	daily oral doses of 0 or 1,225 mg/kg on GD 7-14	no maternal effects; 0/31 litters were viable	< 1225 mg/kg	Har87

6.6.3 Conclusion

EGME, male animals

After administration of EGME testicular atrophy as well as a decrease in testes weight was induced in a variety of species: rat, mouse, golden hamster, guinea pig and rabbit. In rats EGME produced testicular damage after oral dosing, ip injection, inhalation exposure, and dermal application. In the other species only the oral route was studied. EGME affected the reproductive performance of male rats after oral dosing and inhalation exposure.

The reproductive performance of male mice was not affected after a single oral dose up to 1,500 mg/kg, but five daily oral doses of 50 mg/kg produced after mating with virgin females a decrease in cell proliferation of preimplantation embryos.

The oxidation of EGME into 2-methoxyacetaldehyde and further into 2-MAA plays an important role in the induction of testicular effects. When rats are pretreated with an inhibitor of alcohol dehydrogenase oral dosing of EGME

does not induce any testicular effects. Equimolar doses of EGME and 2-MAA induce the same effects in rats and mice. This was also proven in studies *in vitro*: rat embryos, which lack the possibility to convert EGME into 2-MAA, were relatively insensitive to incubation with EGME (NOAEL 25 mM), in contrast with incubation with 2-MAA (NOAEL 0.1 mM). Also Sertoli cells from rats did not change their morphology in the presence of 50 mM EGME, whereas 2 mM 2-MAA degenerated spermatocytes.

In search for possible species differences in sensitivity the lowest doses tested orally are compared: 50 mg/kg as a single oral dose produces minimal effects in reproductive performance in AP/Alpk rats in one study, but the same dose administered for 5 days is a NOAEL for the reproductive performance of F344 rats. Five days of oral dosing of 50 mg/kg produces minimal testicular effects in F344 rats, eleven days of administering this dose to rats of an unknown strain produces no testicular effects. Therefore, oral dosing of 50 mg/kg is the threshold level for producing testicular and reproductive effects in male rats. The same dose administered to male mice for 5 days decreased marginally the reproductive performance. A dose of 62.5 mg/kg for 25 days produced minimal testicular effects and 70 mg/kg for 18 days did not induce testicular toxicity. Therefore, also in the mouse, oral dosing of 50 mg/kg is the threshold level for producing testicular and reproductive effects. The single study which uses golden hamsters indicates that 62.5 mg/kg decreased the testes weight. The rabbit appears to be the most sensitive species with respect to testicular toxicity from EGME. In a 12-wk drinking water study, spermatogenesis was affected at oral dose levels $\geq$ 25 mg/kg bw/day, in a dose related manner. No effects on sperm parameters were observed at 12.5 mg/kg bw/day.

The most relevant route of administration for the occupational setting, inhalation, was only used with rats. In a 13-week study with intermittent exposure 316 mg/m^3 had no effects on body weight or fertility index. A 6-week study with intermittent exposure found neurochemical deviations in the brain of offspring at 79 mg/m^3.

EGME, female animals

Administration of EGME to pregnant rats during organogenesis can induce a reduction in maternal weight gain, prolonged gestation period, reduced percentage of pregnant dams that deliver, an increase in resorptions, a decrease in litter size, pup weight, number of survivors after several postnatal days and an increase in skeletal, visceral and cardiovascular malformations in the foetuses.

One or more of these effects can be observed after oral dosing, inhalation exposure, sc injection, ip injection, and dermal application to rats, inhalation exposure, oral dosing, sc and ip injection to mice, inhalation exposure to rabbits and oral dosing to monkeys. Teratogenic effects have been observed in Drosophila melanogaster, where EGME does not need to be metabolised into 2-MAA.

Equimolar doses of the metabolite 2-MAA can cause similar effects as EGME after oral dosing to rats and mice.

It is remarkable that the developmental effects are observed at low dosages: a no adverse effect level is found in the Fischer 344 rat at 12.5 mg/kg after oral dosing on GD 6-15, but oral dosing of 16 mg/kg to Sprague-Dawley rats on GD 7-18 decreased the mean pup weight and increased gestation time. Inhalation exposure of Sprague-Dawley rats to 79 mg/m^3, 7 h/day on GD 7-13 induced behavioural effects in the offspring, when the rats were exposed on GD 14-20 no behavioural effects were observed; however, both dosing regimens induced neurochemical deviations in the brain of 21-day old pups. A concentration of 32 mg/m^3, 6 h/day on GD 6-15 had no effect on the litter of Fischer 344 rats. The lowest dose tested in ICL -ICR mice, 32 mg/kg given as oral dosages on GD 7-14, induced skeletal variations and delayed ossification in the pups. Inhalation exposure to 32 mg/m^3, 6 h/day on GD 6-15 was a no-adverse effect level for CF-1 mice, the next concentration tested, 158 mg/m^3, induced maternal, embryotoxic and teratogenic effects.

For rabbits, exposure to 9.5 mg/m^3, 6 h/day on GD 6-18 was a no-adverse effect level. The next concentration tested, 32 mg/m^3, increased the percentage of resorptions and delayed the ossification in the foetuses.

In the monkey Macaca fascicularis daily oral doses of 12 mg/kg on GD 20-45 induced slight maternal anorexia and an increase in resorptions. In mothers there was a tendency to a reduced red blood cell count.

EGME, male and female animals

The fertility of pairs of mice was very sensitive to the effects of EGME. Doses of 53-64 mg/kg/day, administered via the drinking water for 7 premating days and 98 days of cohabitation increased the sperm density or the adjusted mean live pup weight or liver or kidney weight in the F_0 generation, depending on the strain of mice used. In the F_1 generation the percentage of pups born alive was decreased or the relative kidney weight was increased in the males. These doses, therefore, induce marginal effects as far as fertility is concerned in mice.

EGMEA produced testicular atrophy in mice after oral dosing. The lowest dose tested, 62.5 mg/kg, was not a no-adverse effect level. The only dose tested in female mice, 1,225 mg/kg, did not produce viable litters. Due to lack of data species specific differences in sensitivity cannot be established.

6.7 Other studies

6.7.1 EGME, cytotoxicity

Several tests on cytotoxicity have been performed and are summarised in Table 19.

Table 19 Several *in vitro* toxicity tests with EGME and 2-MAA.

concentration	cell type	effect	reference
EGME			
8.47 mM	C6 glioma cell line	ED50 for cell viability	Tan92
9.27 mM	NI8TG-2 neuroblastoma cell line	ED50 for cell viability	
>40mM	neural retina cells of chick embryos	no effect on aggregation, differentiation of growth, probably because of the absence of a metabolising system	Das91
88.7 mM	rabbit cornea	highest tolerated dose for morphological alterations	Sho85
177 mM	Balb/c 3T3 cells	highest tolerated dose for morphological alterations	Bor85
177.4 mM	mouse RAW264.7 cells	highest tolerated dose for morphological alterations	
190 mM	Chinese hamster V79 cells	highest tolerated dose for morphological alterations	
210 mM	Balb/c 3T3 cells	reduces the neutral red concentration in the cells to 90% of the control value	
228 mM	human HepG2 cells	50% inhibition of uridine uptake	Die86
253.4 mM	human HepG2 cells	highest tolerated dose for morphological alterations	Bor85
399 mM	human KB cells, derived from an oral epidermoid carcinoma	50% inhibition of uridine uptake	Jac89a
456 mM	Balb/c 3T3 cells	50% inhibition of uridine uptake	Sho85
490 mM	cells CHO cells	50% reduction in cloning efficiency	Jac85
900 mM	rat peripheral blood lymphocytes	8-fold increase in mitotic index in the presence of Concanavalin A	Guz89
5-12,000 mM	human erythrocytes	no effect on osmotic fragility, membrane acetylcholinesterase activity or membrane ATP-ase activity	Mori89
2-MAA			
0.951 mM	human erythrocytes	50% increase in osmotic fragility	
1.36 mM	human erythrocytes	50% inhibition of membrane ATP-ase activity	
4 mM	rat blood	no effect on haematocryt value or haemoglobin content	Gha89
5 mM	GD 11 CD-1 mouse embryos cultured in serum-free medium	50% reduction of 3H-thymidine incorporation	Ste89
5.59 mM	human erythrocytes	50% inhibition of membrane acetylcholinesterase activity	Mori89

10 mM	neural retina cells of chick embryos	lowest observed effect concentration (LOEC) for aggregation	Das91
20 mM	neural retina cells of chick embryos	LOEC for differentiation	
30 mM	neural retina cells of chick embryos	LOEC for growth	
50 mM	CHO cells	50% reduction in cloning efficiency	Jac85
50 mM	GD 11 CD-I mouse embryos cultured in serum-containing medium	50% reduction in 3H-thymidine incorporation	Ste89

6.7.2 *EGME, immunotoxicity*

Immunological studies have been performed by several authors. The results are summarised in Table 20.

The immunosuppressive potency of EGME was assessed after implantation of leukaemia cells in mice. Groups of 6-8 female allogeneic $B6C3F_1$ mice (C57Bl/6 x C3H) and syngeneic $CD2F_1$ mice (Balb/c x DBA/2) were used. Allogeneic mice will not die when challenged with leukaemia cells unless they have been immunosuppressed, while in syngeneic mice the tumour will grow and kill the animals unless there is a direct cytotoxic effect as a result of chemical treatment on the tumour cells. In this study the mouse lymphoid leukaemia L1210 was utilised. This ascited tumour is propagated serially by ip implantation in DBA/2 inbred mice. The $B6C3F_1$ mice were given oral doses of 300, 600 or 1200 mg EGME/kg on days -12 through -8 or a single ip injection of 180 mg cyclophosphamide (Cy)/kg on day -1. Untreated controls were given oral doses of water on days -12 through -8 and -5 through -1. On day 0, the mice were challenged with $1*10^2$, $3*10^3$, $1*10^5$ or $3*10^6$ L1210 cells by the ip route. The $CD2F_1$ mice were challenged with $1*10^5$ L1210 cells on day 0 and were treated on days 1 to 5 and 8 to 12 with the same dosages of EGME used for the $B6C3F_1$ mice. Water treated syngeneic mice died with a median survival time (MST) of 8.0 days. There was no effect on the MST of syngeneic mice treated with EGME, indicating no direct antitumour effect of the compounds. All allogeneic mice receiving either water or cyclophosphamide and challenged with $3*10^6$ tumour cells, died with ascites. However, when mice were treated with EGME and challenged with $3*10^6$ tumour cells, no more than one animal per group died. This would indicate that there was a prophylactic action of EGME or that the immune system was stimulated. Blood smears of allogeneic mice were made for differential counts the last day of drug dosing, the day of death where possible, and on survivors at day 43 post-tumour implantation. Differential counts showed evidence of monocytosis, considered to be indicative of monocytic leukaemia, in those mice not surviving until the day of sacrifice. All surviving allogeneic mice

Table 20 Immunological studies with EGME in several laboratory animals.

species	dosing regimen	results	NOAEL	reference
male and female Fischer 344 rats (n=6-8, control n=6-8)	10 oral gavage dosages on consecutive days of 0, 25, 50, 100, 200 or 400 mg/kg	25 mg/kg: no effect on plaque-forming cell response to trinitrophenyllipopolysaccharide (TNPLPS) (males and females, no other parameters measured); 50 mg/kg and higher: a dose-related response in one or more of the following parameters: reduced thymus wt; decreased lymphoproliferative response of splenic lymphocytes in the presence of Concanavalin-A; decreased plaque-forming cell response to TNP-LPS; decreased production of interleukine-2 by splenocytes; a reduction in the expulsion of adult worms (Trichinella spiralis) (male studies); no effects were observed at all dosages on body wt, spleen wt, natural killer cell activity, plaque-forming cell response to sheep red blood cells, relative quantity of subsets of splenic lymphocyte, seminal vesicle wt, epididimides wt, cauda epididimydes wt, sperm count (male studies); the testes wt was decreased at the highest dose	± 25 mg/kg	Smi91
idem (n=6-8, control n=6-8)	idem, 0, 25, 50, 100 or 200 mg 2-MAA/kg	dose-related decrease in plaque-forming cell response to TNP-LPS (male studies)	± 25 mg/kg	
male Fischer 344 rat (n=6, control n=6)	two oral doses on consecutive days equimolar concentration, 2.64 mmol/kg, of EGME, 2-methoxyacetaldehyde or 2-MAA	equivalent suppression of the antibody response (plaque-forming cell response to TNP-LPS)		Smi93
idem, pretreated with aldehyde dehydrogenase inhibitors	idem, with EGME, methoxyacetaldehyde or EGMEA	no effect on antibody response (PFC-TNP-LPS)		
idem, pretreated with alcohol dehydrogenase inhibitor	idem, with EGME or EGMEA	suppression of PFC response to TNP-LPS		Smi93, Smi92
Sprague-Dawley rats (n=6 pairs, control idem)	daily doses in the drinking water for 21 days, resulting in 0, 161 or 486 mg/kg/day for males and 0, 200 or 531 mg/kg/day for females	males and females: both dosages: reduced absolute and relative thymus wt; increased natural killer (NK) cell cytotoxic activity; decreased production of specific antibodies; males: both dosages: decreased production of interferon; high dose: decreased production of splenocytes; females: both dosages: decreased production of splenocytes; high dose: decreased production of interleukin-2 and of interferon	< 161 mg/kg	Exo91

male and female B6C3F1 mice (n=7-5 pairs, control pairs n=5-7	4 oral doses on consecutive days of 0, 50, 100 or 250 mg/kg; assays 1, 5 and 14 days after the last treatment	males and females: no change in body wt, liver, spleen, kidney or thymus wt; females: at two highest doses: decreased red blood cell count and haematocrit values, decreased bone marrow cellularity and colony forming units, decreased erythropoiesis in bone marrow. Males: high dose: decreased testes wt; all doses: decreased white blood cell count; decreased bone marrow cellularity 1 day after exposure, which recovered after 14 days; dose-related decrease of colony forming units, which did not recover within 14 days	< 50 mg/kg	Hon88b
female B6C3F1 mice (n=6-10, control n=6-10)	10 oral doses in 2 weeks of a total of 250, 500 or 1000 mg/kg EGME or 2-MAA	at 500 and 1,000 mg/kg: a reduced thymus wt; no effect on immunopathology, humoral immunity, cell-mediated immunity, macrophage function, and host resistance to Listeria monocytogenes	250 mg/kg, total dose	Hou85
male C3H/HeN mice (n=6, control n=5)	daily oral doses of 0 or 1000 mg/kg for 5 days	decreased relative wt of spleen, thymus, liver and testes and body wt gain; no effect on red blood cell and thrombocyte and lymphocyte count; 30% decrease in white blood cell count	< 1,000 mg/kg	Kay91
idem (n=5, control n=5)	daily oral doses of 0, 500 or 1000 mg/kg for 10 days	top dose: decreased relative thymus wt; both doses: decreased thymic cellularity; no effect on spleen parameters; low dose: increased response of thymocytes to Concanavalin-A; the thymus has a markedly atrophic cortex and almost intact medulla; decrease in CD4+/ CD8+, Thy-1+, PNA+ immature thymocytes, increase in CD4-/CD8-, H2+ mature thymocytes	< 500 mg/kg	
rats: Lewis, Fischer 344, Wistar/Furth, Sprague-Dawley (n=?)	daily oral doses of 0.33-2.64 mM EGME or 2-MAA/kg/day for 10 days (25-200 mg EGME)	top dose: suppression of the PFC response in all strains of rats; Lewis rats were the most sensitive	< 0.66 mM/kg	Smi94
mice: C3H, C57BL/6J, B6C3F1, CD-1 (n=?)	daily oral doses of 0.66-5.28 mM EGME or 2-MAA/kg/day for 10 days (50-400 mg EGME); rats and mice were immunised with TNP-LPS on day 9 of dosing and PFC responses evaluated 3 days later	no suppression of PFC response in any of the mouse strains	> 5.28 mM/kg	

were sacrificed and autopsied on day 43. Cholecystitis was observed in 58% of the animals. The data suggest that EGME may exert an antitumour effect through increased immunological competence or immunomodulation (Hou84).

6.7.3 *EGME, EGMEA, immunotoxicity*

The immunological response of EGME and EGMEA to Fischer 344 rats was studied (Smi92). Rats were immunised with trinitrophenyl-lipopolysaccharide (TNP-LPS) and then 4 and 24 h later exposed to 50, 100, 200 or 400 mg/kg of

EGME, EGMEA or 2-MAA. Forty eight h later the animals were killed and the spleens removed. EGME and EGMEA decreased the plaque-forming cell (PFC) response per 10^6 cells and the number of PFC per spleen at all dose levels. 2-MAA decreased the PFC response per 10^6 cells at the two highest dose levels. At the three highest dose levels the number of PFC per spleen was diminished. The following NOAELs can be derived from the study:

- EGME: < 50 mg/kg (rat, single oral dose, immunological effects)
- EGMEA: < 50 mg/kg (idem).

Groups of 8-10 male F344/N rats received a sc injection with 0 or $20*10^6$ leukaemic cells. Simultaneously drinking water was given ad libitum containing 0, 0.25, 1.0, 2.5 or 5.0 mg EGME/ml. Based on measurements of water consumption a concentration of 2.5 mg/ml, for instance, corresponded to an EGME dose of about 100 mg/kg. When the drinking water did not contain a chemical, the leukaemia cell injection resulted after 60 days in a 10-fold increase in relative spleen weights, a 100-fold increase in white blood cell counts, and a 50% reduction in red blood cell (RBC) indices and platelet counts. At this interval, EGME given at a dose of 2.5 mg/ml in the drinking water completely eliminated all clinical, morphological, and histopathological evidence of leukaemia. A minimal effective dose for a 50% reduction in the leukaemic responses was 0.25 mg EGME/ml in the drinking water (15 mg/kg body weight). In addition, the *in vitro* exposure of a leukaemic spleen mononuclear cell culture to EGME caused a dose- and time-dependent reduction in the number of leukaemia cells after a single exposure to 1-100 μM concentrations, whereas the EGME metabolite 2-MAA was only half as effective. Based on a favourable efficacy-to-toxicity ratio EGME should be considered for further development as chemotherapeutic agent (Die90). However, the authors evaluated only part of the studies available on the reproductive and developmental toxicity of EGME, therefore the efficacy-to-toxicity ratio of EGME is not as favourable as they make believe.

6.7.4 EGMEA, carboxylesterase

The lesions caused by several glycolether acetates in the olfactory neuroepithelium of exposed rats and/or mice consist of a specific degeneration. Should enzymatic hydrolysis of absorbed glycolether acetate occur in the nasal mucosa then acidic metabolites may be partially or solely responsible for producing the observed lesions. Moreover, to examine potential species differences in nasal mucosal carboxylesterase activity, nasal tissue preparations

from mice, rats, rabbits, and dogs were assayed for carboxylesterase activity using EGMEA as the substrate. The results are presented in Table 21. The activities of carboxylesterase in the different tissues of the mouse is given in Table 22. The V_{max} for nasal mucosal carboxylesterase of mice using EGMEA as a substrate was 0.870 mM/min. The K_m was 13.4 mM. The relatively high V_{max} and V_{max}/K_m value indicate a high rate of hydrolysis (Sto85).

Additional data in SCOEL (2006)

Addition of 10 μM 2-MAA, but not 1 μM, reduced the proliferative capacity of foetal mouse liver cells in vitro, observed as reduced incorporation of tritium-labelled thymidine. However, no effect on survival of the cells was observed (Holladay *et al.* 1994)[SCOEL22].

Studies with bone marrow cells from a leukaemia patient without bone marrow involvement showed 50% inhibition after 24-h treatment with 3 mM MALD or 3.9 mM 2-MAA. Similar results were obtained with a human leukaemia cell line (HL60). Caspase-3 enzyme activity, an effector of apoptosis, was greatly enhanced by MALD, and inhibition of caspase-3 attenuated MALD and 2-MAA-induced cell death (Takagi *et al.* 2002)[SCOEL23].

6.7.5 *Conclusion*

EGME

EGME is not typically cytotoxic to several mammalian cell types and does not exert a specific effect. 2-MAA is more cytotoxic than EGME, indicating that EGME has to be metabolised before it can exert effects like a decrease in aggregation, differentiation and growth.

EGME has immunotoxic activity. After oral dosing to rats it can reduce the thymus weight, increase the natural killer cell activity and decrease the production of specific antibodies, the lymphoproliferative response of splenic lymphocytes in the presence of Concanavalin-A and decrease the plaque-forming cell response to trinitrophenyl-lipopolysaccharide. At the lowest dose tested, 25 mg/kg for 10 days, not all parameters were assayed, but this dose is probably a minimal adverse effect level, based on the severity of the effects found at the next dose, 50 mg/kg.

Mice are less sensitive to the immunotoxic effects of EGME than rats. The lowest dose tested, 25 mg/kg for 10 days, was a no-adverse effect level. The next dose, 50 mg/kg decreased the thymus weight when administered for 10 days, but

not when ad ministered for 4 days. This dose decreased the white blood cell count and bone marrow cellularity when given for 4 days, but no immunotoxic effect were observed when given for 10 days. EGME has some immunosuppressive activity.

EGMEA

A single oral dose of 50 mg/kg to rats decreased the plaque-forming cell (PFC) response per 10^6 cells and the number of PFCs per spleen.

EGMEA can be rapidly hydrolysed into EGME in the presence of carboxylesterase. The activity of nasal mucosal carboxylesterase varied in different species, where mouse and dog have the highest activity, rat somewhat lower and rabbit a 7-fold lower activity.

Table 21 Comparison of nasal mucosal carboxylesterase activity by sex and species (Sto85).

species	sex	N	activity (nmol EGME/mg protein/min)
mouse	male	12	560 ± 79.9
	female	12	561 ± 99.5
rat	male	8	456 ± 86.7[a]
rabbit	male	4	88.1 ± 23.0[a]
	female	4	82.1 ± 18.7[a]
dog	female	2	635 ± 112

[a] statistically significantly different from mice ($p < 0.05$)

Table 22 Comparison of mouse tissue carboxylesterase activity using EGMEA as a substrate (n = 4 animals) (Sto85).

tissue	activity (nmol EGME/mg protein/ min)
nasal mucos[a]	667 ± 66.0
liver	677 ± 64.9
kidney	266 ± 76.6[a]
lung	177 ± 25.5[a]
blood	83.6 ± 8.4[a]

[a] statistically significantly different from mice ($p < 0.05$)

6.8 Summary

Irritation and sensitisation

EGME and EGMEA are no skin irritants. EGME is a mild to moderate eye irritant, EGMEA is a mild eye irritant, it is not a respiratory irritant in mice.

Acute toxicity

EGME and EGMEA are not very acutely toxic. According to the EC classification EGME and EGMEA are harmful by inhalation, in contact with skin and if swallowed. Also these compounds may impair fertility and may cause harm to the unborn child.

Short-term toxicity

EGME
Subchronic oral dosing of EGME to rats and mice influenced generally the body weight gain and the weight of several organs. Doses up to ca. 1,200 mg/kg/day via the drinking water for 2 weeks decreased sporadically the organ weights in mice and doses up to 300-400 mg/kg/day decreased the body weight in rats, further thymic atrophy in male and female rats and testicular atrophy in males of both species was induced. Thirteen weeks of dosing via the drinking water induced histopathologic changes in testes, thymus and haematopoietic tissues in rats and in testes and spleen in mice and in the adrenal gland in female mice. In stop-exposure studies with male rats given 165 or 324 mg/kg/day there was only partial recovery from testicular degeneration after 30 and 56 days of recovery. After 13 weeks of dosing the following can be concluded from the lowest doses tested: the NOAEL for male mice was 295 mg/kg/day, 492 mg/kg/day caused in female mice minimal splenic haematopoiesis and mild hypertrophy in the adrenal gland. In male rats 71 mg/kg/day caused minimal lesions in the testes. In female rats 70 mg/kg/day decreased the relative and absolute thymus weight.

Inhalation exposure to rats and mice for 9 exposures with concentrations up to 3,160 mg/m^3 induced adverse effects on body weight gain, peripheral blood counts, bone marrow, testes and lymphoic tissues. Thirteen weeks of inhalation exposure of 950 mg/m^3 decreased the thymus weight in male and female rats and rabbits and decreased the testes weight in males. Several haematologic

parameters in male and female rats and rabbits were decreased after 4 and 12 weeks of exposure to 950 mg/m^3. Concentrations of 316 or 95 mg/m^3 did not influence the haematology. A small percentage of rabbits showed testicular effects at 95 mg/m^3. For rats this concentration was an NOAEL.

Dermal application of 100 or 1,000 mg/kg reduced the weight gain and testes weight in rats after 28 days. The body weight, spleen and testes weight was decreased in guinea pigs after 13 weeks of dermal exposure to 1,000 mg/kg/day. Testicular degeneration and haematologic changes were also observed in the guinea pigs.

EGMEA
Testicular atrophy and leukopenia were observed in mice after 5 weeks of dosing 62.5 to 4,000 mg/kg.

Long-term toxicity

There are no adequate long-term studies on EGME and EGMEA have been reported.

Mutagenicity/genotoxicity

EGME and EGMEA have very little mutagenic or genotoxic potential. The compounds were negative in the Ames assay.

EGME does not induce mutations in mammalian cells *in vitro*. In human peripheral lymphocytes EGME and methoxyacetaldehyde increase the number of chromosomal aberrations and chromatid breaks at high concentrations.

EGME induces male sterility in rats and sperm abnormalities in mice. EGMEA does not induce micronuclei in Chinese hamsters.

Reproduction toxicity

EGME can induce testicular atrophy in rats after oral dosing, ip injection, inhalation exposure and dermal application. After oral dosing EGME induces testicular toxicity in mice, golden hamsters, guinea pigs and rabbits. It affected the reproductive performance of male rats after oral dosing and inhalation exposure. In the most sensitive species, rabbits, an oral dose of 12.5 mg/kg represents the no-adverse-effect level with respect to effects on male fertility. In female rats, oestrus cycle was affected by EGME at oral dose levels $\geq$ 30 mg/kg

bw/day, although the changes at the lowest dose level were small and not statistically significant.

EGME can induce developmental effects in rats after oral dosing, inhalation exposure, sc and ip injection and dermal application. In mice developmental effects have been observed after inhalation exposure, oral dosing and sc and ip injection, in rabbits after inhalation exposure and in monkeys after oral dosing. In the monkey Macaca fascicularis daily oral doses of 12 mg/kg on GD 20-45 induce slight maternal anorexia and an increase in resorptions. In mothers there was a tendency to reduced blood cell count. Low EGME doses (53-64 mg/kg/day), decreased marginally the fertility in mice.

EGMEA induces testicular atrophy in mice at the lowest dose tested: 62.5 mg/kg.

Immunotoxicity

Oral dosing of EGME can elicit an immunological response in rats and mice. Rats are more sensitive than mice in this respect. For rats 25 mg/kg is probably a minimal adverse effect level, based on the effects found at the next dose, 50 mg/kg (a decrease in thymus weight, in lymphoproliferative response of splenic lymphocytes in the presence of Concanavalin-A, in plaque-forming cell response to trinitrophenyllipopolysaccharide, in the production of interleukine-2 and in the expulsion of adult worms). For mice 50 mg/kg is the threshold level for producing a decrease in white blood cell count, bone marrow cellularity and thymus weight.

EGMEA increases the plaque-forming cell (PFC) response per 10^6 cells and the number of PFCs per spleen in rats at 50 mg/kg, whereas DEGME does not influence these parameters up to 200 mg/kg. EGME has some immunosuppressive activity.

Immune response was studied in rats and mice that had been given EGME, EGMEA, MALD or 2-MAA in oral doses of 50 to 400 mg/kg/day for 10 days. In the rats, the four substances yielded similar immunosuppression, expressed as reduced thymus and spleen weights and reduced antibody plaque-forming cell (PFC) response. The effects were significant at the lowest dose level, and equimolar doses of the four substances produced equivalent immunosuppression. Pre-treatment with 4-methylpyrazole caused these effects to disappear, which indicates that metabolic activation is required. This immunosuppression was observed in all of the rat strains but in none of the mouse strains. Nor did 2-MAA in subcutaneous doses of up to 1920 mg/kg/day produce immunosuppression in

the mice, which indicates that the difference between the species cannot be explained by differences in bioavailability or metabolic rate.

Atrophy, dose-dependent reduction in cellularity, and changes in thymocyte patterns indicating disturbances in thymocyte maturation were observed in thymus glands from the young of mice given EGME in doses of 100-200 mg/kg/day on days 10 to 17 of gestation (Holladay *et al.* 1994)[SCOEL22].

Chapter 7

Existing guidelines, standards and evaluations

7.1 General population

The concentration considered immediately dangerous to life or health (IDLH) is 6,320 mg/m^3 for EGME and 22,095 mg/m^3 for EGMEA (Sit85).

7.2 Working population

Table 23 (NSB93, ACG94, HSE95, Ano93, DFG95, I-SZW95, RTE94).

chemical and country	TLV 8 h TWA		STEL		remarks
EGME					
Health Council[a]	1 mg/m^3	0.3 ppm			
SCOEL	3.1 mg/m^3	1 ppm			[c]
US/ACGIH	0.3 mg/m^3	0.1 ppm			[c]
OSHA	80 mg/m^3	25 ppm			[c], permissible
NIOSH	0.3 mg/m^3	0.1 ppm			[c], recommended
Germany	3.2 mg/m^3	1 ppm	[b]		[c,d]
Great Britain[f]	16 mg/m^3	5 ppm			[c]
Sweden	19 mg/m^3	5 ppm	40 mg/m^3	10 ppm	[c,e]
EGMEA					
Health Council	1.5 mg/m^3	0.3 ppm			
SCOEL	4.9 mg/m^3	1 ppm			[c]
US/ACGIH	0.5 mg/m^3	0.1 ppm			[c]

OSHA	120 mg/m³	25 ppm			[c], permissible
NIOSH	0.5 mg/m³	0.1 ppm			[c], recommended
Germany	5 mg/m³	1 ppm	[b]		[c,d]
Great Britain	25 mg/m³	5 ppm			[c]
Sweden	25 mg/m³	5 ppm	50 mg/m³	10 ppm	[c,e]

[a] HBROEL: Health Based Recommended Occupational Exposure Limit

[b] peak exposure limited to 2*MAK for a period of 30 min, average value, with a maximum of 4 times per shift

[c] skin notation, the compound can be absorbed through the skin

[d] pregnancy group B: according to the currently available information, a risk of damage to the developing embryo or foetus must be considered to be probable. Damage to the developing organism cannot be excluded when pregnant women are exposed even when MAK and BAT values are observed

[e] the scientific basis for the limit values of EGME and EGMEA is that they have reproduction disturbing effects in animals. When determining occupational exposure to mixtures of glycol ethers and other solvents the following figures shall be used:
EGME: level limit value (= LLV) = 80 mg/m³ (25 ppm), short-term value (= STV) = 160 mg/m³ (50 ppm)
EGMEA: level limit value = 120 mg/m³ (25 ppm), short-term value = 250 mg/m³ = 50 ppm

[f] WEL: Workplace Exposure Limit

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