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# Cyclic acid anhydrides

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Health-based recommended occupational exposure limit

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Aan de minister van Sociale Zaken en Werkgelegenheid

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Onderwerp : Aanbieding advies *Cyclische zuuranhydriden*  
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Geachte minister,

Graag bied ik u hierbij het advies aan over de beroepsmatige blootstelling aan cyclische zuuranhydriden.

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

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport en aan de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Met vriendelijke groet,

prof. dr. J.A. Knottnerus

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# **Cyclic acid anhydrides**

Health-based recommended occupational exposure limit

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Dutch expert Committee on Occupational Safety  
a Committee of the Health Council of the Netherlands  
in cooperation with the Nordic Expert Group for  
Criteria Documentation of Health Risks from Chemicals

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to:

the Minister of Social Affairs and Employment

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No. 2010/02OSH, The Hague, January 21, 2010

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# Samenvatting

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## Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (Commissie GBBS; één van de vaste commissies van de Gezondheidsraad) gezondheidkundige advieswaarden af voor stoffen in lucht waaraan mensen blootgesteld kunnen worden tijdens hun beroepsuitoefening. Deze advieswaarden vormen vervolgens de basis voor grenswaarden waarmee de gezondheid van werknemers beschermd kan worden.

In dit advies bespreekt de commissie de gevolgen van blootstelling aan cyclische zuuranhydriden\* en stelt zij gezondheidkundige advieswaarden vast.

In een eerste stap is de toxiciteit van de zuuranhydriden geëvalueerd. Dat is gedaan in samenwerking met de *Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals*. De resultaten van deze evaluatie zijn eerder gepubliceerd in 2004 (deze publicatie is integraal opgenomen in deel 2 van dit advies); vervolgens heeft de Commissie GBBS op basis van deze evaluatie én nieuwe relevante informatie een onderbouwing gegeven van haar advies (opge-

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\* De in dit advies beoordeelde cyclische zuuranhydriden zijn: chlorendic anhydride (CA); dodeceny succinaatanhydride (DSA); himicanhydride (HA); hexahydroftaalzuuranhydride (HHPA); maleïnezuuranhydride (MA); methylhexahydroftaalzuuranhydride (MHHPA); methyltetrahydroftaalzuuranhydride (MTHPA); ftaalzuuranhydride (PA); pyromellietzuurdianhydride (PMDA); succinaatanhydride (SA); tetrabroomftaalzuuranhydride (TBPA); tetrachloorftaalzuuranhydride (TCPA); tetrahydroftaalzuuranhydride (THPA); trimellietzuuranhydride (TMA).

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nomen in het eerste deel van dit advies). De conclusies zijn gebaseerd op wetenschappelijke gegevens die voor december 2009 zijn verschenen.

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### **Fysisch-chemische eigenschappen en gebruik**

Cyclische zuuranhydriden komen voornamelijk voor als poeder of kristallen, en de methylsubstituten van cyclische zuuranhydriden als olieachtige vloeistoffen. De meeste cyclische zuuranhydriden lossen op in organische oplosmiddelen, zoals alcohol en benzeen. In alcohol worden ze dan snel omgezet – met behulp van water – in carboxylzuren. Van in ieder geval ftaalzuur- en maleïnezuuranhydride is bekend dat ze ook in water oplossen.

Cyclische zuuranhydriden kennen een brede toepassing in de chemische industrie, vooral bij de productie van polyesters, plastics en harsen. Van nature komen ze niet voor, al kunnen ze wel als verontreiniging in het milieu worden aangetroffen.

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### **Monitoring**

Monsternamen van cyclische zuuranhydriden in de lucht vindt in de regel plaats met speciale absorptiebuisjes, Teflon- of PVC-filters of een combinatie van beide. Om de hoeveelheid bemonsterde cyclische zuuranhydriden te kunnen bepalen zijn er vervolgens verschillende geschikte analysemethoden, waaronder gaschromatografische gecombineerd met vlamionisatie, massaspectrometrische en *electron capture* detectiemethoden.

Daarnaast bestaan er ook analysemethoden om gehalten aan bepaalde dicarboxylzuren in urine (van de corresponderende cyclische zuuranhydriden) en in plasma (voor HHPA en MHHPA) te bepalen.

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### **Huidige grenswaarden**

In Nederland bestaan op dit moment geen wettelijke grenswaarden voor cyclische zuuranhydriden. Elders zijn er voor sommige cyclische zuuranhydriden wel grenswaarden vastgesteld; deze variëren van 200 tot 12 000  $\mu\text{g}/\text{m}^3$  (microgram per kubieke meter lucht) voor ftaalzuuranhydride, van 400 tot 1 200  $\mu\text{g}/\text{m}^3$  voor maleïnezuuranhydride en voor trimellietzuuranhydride op 40  $\mu\text{g}/\text{m}^3$ . Het gaat hierbij om zowel tijdgewogen gemiddelden van 8 uur als van 15 minuten, en om plafondwaarden.

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PA, TMA, MA, HHPA, MHHPA, MTHPA, THPA, TCPA, HA en PMDA zijn door de Europese Unie geclassificeerd als inhalatie- en huidsensibiliserend (R42 en R43).

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### **Opname, verdeling en uitscheiding**

Uit onderzoek met mensen blijkt dat hexahydroftaalzuuranhydride na inademing efficiënt door het lichaam wordt opgenomen, terwijl opname via de huid minimaal is. Met betrekking tot andere cyclische zuuranhydriden zijn geen gegevens over opname bekend, evenmin over opname via andere blootstellingsroutes.

In het bloed binden cyclische zuuranhydriden aan hemoglobine in rode bloedcellen en aan plasma-eiwitten, zoals albumine. Er zijn aanwijzingen dat deze zuuranhydriden ook kunnen binden aan eiwitten in organen, bijvoorbeeld in de luchtwegen, het maag-darmkanaalstelsel en de nieren.

Ongeveer vijftientig procent van de ingeademde hexahydroftaalzuuranhydride verlaat het lichaam uiteindelijk via de urine in de vorm van dicarboxy-zuren. De halfwaardetijd (de tijd die het lichaam nodig heeft om de concentratie van de gevormde dicarboxy-zuren in het bloedplasma te halveren) hangt af van het type cyclische zuuranhydride, en varieert van twee tot veertien uur.

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### **Het toxisch werkingsmechanisme**

De meest in het oog springende schadelijke gezondheidseffecten door blootstelling aan cyclische zuuranhydriden zijn irritatie en allergische overgevoeligheid. De irritatie kan ontstaan door zowel cyclische zuuranhydriden als de hydrolyse-producten.

De allergische overgevoeligheid treedt op door zogenaamde IgE-gemedieerde immuunresponsen, ook wel aangeduid als het '*onmiddellijke type*'. Dit is bekend doordat zowel bij mensen als dieren specifieke antilichamen van het type IgE in het bloed aangetroffen worden. Deze antilichamen zijn betrokken bij allergische processen; de aanwezigheid ervan duidt op allergische sensibilisatie. Sensibilisatie is een cruciale en noodzakelijke stap bij de ontwikkeling van allergieën. Blijft de blootstelling voortduren, dan kan dat leiden tot aandoeningen als netelroos (urticaria), allergische neus- en oogontstekingen (rhinitis, rhinoconjunctivitis) en allergische astma.

Tenslotte zijn er aanwijzingen dat cyclische zuuranhydriden ook andere typen immuunresponsen kunnen opwekken, bijvoorbeeld stimulatie van de productie van andere typen specifieke antilichamen (IgG) of stimulatie van cellulaire immuunresponsen.

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## Gegevens over effecten

### Waarnemingen bij mensen

Huidcontact kan leiden tot allergische netelroos.

Korte blootstelling via inademing kan tot acute effecten leiden, bijvoorbeeld ontstekingen van de slijmvliezen in de bovenste en onderste luchtwegen. Dit kan het gevolg zijn van irritatie.

Van sommige cyclische zuuranhydriden zijn gegevens verzameld over de allergische overgevoeligheid en luchtwegaandoeningen als gevolg van beroepsmatige blootstelling. Niet in alle gevallen konden de symptomen met een allergie geassocieerd worden; dit komt doordat allergische en irritatiereacties vaak leiden tot vergelijkbare klachten. Bovendien is het mogelijk dat in sommige gevallen sprake was van zowel irritatie als van allergie.

Schattingen van het percentage gevallen van beroepsastma door blootstelling aan cyclische zuuranhydriden variëren van 8 tot 18% voor PA, 2 tot 12% voor TMA, 15% voor HHPA, 11% voor MHHPA, en 3 tot 39% voor TCPA.

Gezellen van allergische sensibilisatie door beroepsmatige blootstelling zijn beschreven voor in principe de meeste cyclische zuuranhydriden die in dit advies zijn geëvalueerd. Niet voor alle verbindingen kon dit echter gecorreleerd worden aan een bepaalde hoogte van blootstelling. Bij de zuuranhydriden waarvoor dat wel kon, liepen de laagst gemeten blootstellingen die leidden tot allergische sensibilisatie uiteen van onder de  $1 \mu\text{g}/\text{m}^3$  (TMA, HHPA en MHHPA) en rond de  $15 \mu\text{g}/\text{m}^3$  (MTHPA), tot variabele blootstellingen in de honderden microgrammen per kubieke meter (PA en TCPA). Deze blootstellingen gingen in alle gevallen gepaard met (allergische) luchtwegklachten, rhinitis en astma bijvoorbeeld. Gegevens van nog lagere blootstellingen zijn, behalve voor TMA, niet bekend. Het is dus mogelijk dat zelfs onder de laagst gemeten blootstellingsniveaus nog gevallen voorkomen van allergische sensibilisatie en luchtwegklachten.

Tot slot zijn in het onderzoek met mensen gevallen beschreven van allergische keelontsteking, ontsteking van longblaasjes en een vorm van bloedarmoede. Er zijn geen gegevens bekend over effecten in andere organen.

### Waarnemingen bij dieren

Uit enkele onderzoeken blijkt dat bepaalde cyclische zuuranhydriden door direct contact kunnen leiden tot huid- en oogirritatie. Er is echter een verschil in potentie tussen de verschillende cyclische zuuranhydriden; zo veroorzaakte ftaalzuur-

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anhydride in de ogen van konijnen slechts lichte irritatie, terwijl maleïnezuur- en trimellietzuuranhydride daarentegen tot forse irritatie leidden.

Er is een beperkt aantal dieronderzoeken uitgevoerd – met cavia's, ratten en muizen – waarin het optreden van allergische sensibilisatie door cyclische zuuranhydriden (PA, TMA, HHPA, MHHPA, en MTHPA) is onderzocht. In de meeste gevallen betrof het bifasisch onderzoek: dieren ondergingen twee fasen van blootstelling (een sensibilisatie- en een *challenge*-fase) soms via andere blootstellingsroutes en met andere blootstellingsniveaus.

Bij dieren traden vergelijkbare effecten op als bij de mens. Zo werden na huid- en inhalatieblootstelling in het bloed specifieke IgE-antilichamen gevonden (wat duidt op allergische sensibilisatie) en werden afwijkingen in longweefsel waargenomen.

Wat betreft onderzoek waarbij dieren alleen via inademing zijn blootgesteld, is de laagste blootstelling waarbij onderzoekers nog specifieke IgE-antilichamen tegen TMA in het bloed aantreffen  $400 \mu\text{g}/\text{m}^3$ ; in hetzelfde onderzoek werden bij  $40 \mu\text{g}/\text{m}^3$  echter geen effecten aangetroffen. Het aantal dieren in dit onderzoek was bijzonder klein en de blootstellingduur was kort, zodat moeilijk een definitieve conclusie te trekken valt. Bij 30 tot  $40 \mu\text{g}/\text{m}^3$  traden wel weefselafwijkingen in de longen op. In een ander dieronderzoek werd al bij  $10 \mu\text{g TMA}/\text{m}^3$  een verhoogde antilichaamrespons tegen TMA waargenomen. Tevens bleek de toename van de respons sterk te correleren met een toename van weefselafwijkingen in de longen en longgewicht.

In ratten, hamsters en apen die een half jaar aan maleïnezuuranhydride ( $1\ 100$  tot  $9\ 800 \mu\text{g}/\text{m}^3$ ) blootgesteld werden, werden er in de longen, lever, milt, beenmerg en nieren geen afwijkingen gevonden. Het neusslijmvlies van de knaagdieren was wel geïrriteerd. Dit was echter omkeerbaar en niet gerelateerd aan de hoogte van de blootstelling.

Met betrekking tot langdurige blootstelling aan andere cyclische zuuranhydriden ontbreken op dit moment de gegevens.

Er is ook een gebrek aan gegevens over mogelijke kankerverwekkendheid. Uit een beperkt aantal onderzoeken valt op te maken dat cyclische zuuranhydriden geen schade aan het genetisch materiaal toebrengen.

Er zijn verder geen aanwijzingen gevonden voor effecten op de vruchtbaarheid en het nageslacht. Maar ook hiervoor geldt dat er nauwelijks gegevens zijn.

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## Werkwijze bij het bepalen van advieswaarden voor allergenen

### De keuze van het kritische effect

De commissie beschouwt het optreden van allergische sensibilisatie als het kritische effect waarop advieswaarden voor cyclische zuuranhydriden gebaseerd dienen te zijn. De reden daarvoor is dat sensibilisatie in de meeste gevallen tot een blijvende allergische overgevoeligheid leidt. Door sensibilisatie te voorkomen wordt tevens voorkomen dat de betreffende persoon bij voortdurende blootstelling allergische luchtwegklachten ontwikkelt. De commissie verwacht dat zodoende ook het optreden van irritatie aan de luchtwegen voorkomen wordt.

De commissie geeft verder de voorkeur aan gegevens over IgE-gemedieerde sensibilisatie, omdat IgG-gemedieerde immuunresponsen volgens de huidige inzichten een minder sterke associatie met allergische klachten hebben.

### Keuze voor gezondheidkundige advieswaarden of referentiewaarden

In de wetenschappelijke literatuur wordt al langer gediscussieerd over de vraag of voor allergene stoffen een blootstellingsconcentratie bestaat, en dus een drempelwaarde, waaronder niet te verwachten is dat er allergische sensibilisatie en luchtwegklachten optreden.

De Gezondheidsraad heeft daarover recent een advies uitgebracht, waarin geconcludeerd wordt dat – gezien de huidige wetenschappelijke kennis – zo'n drempelwaarde voor ingeademde allergenen in theorie wel bestaat. Dit betekent dat een gezondheidkundige advieswaarde afgeleid kan worden volgens de methoden die ook gelden voor alle andere niet-kankerverwekkende stoffen.

Voor de meeste allergene stoffen verwacht de Gezondheidsraad echter dat het afleiden van een betrouwbare gezondheidkundige advieswaarde in de praktijk niet mogelijk is: de drempelwaarde ligt dan zo laag dat die met de huidige beschikbare technieken niet meetbaar is. Voor die allergenen beveelt de raad aan om zogenaamde referentiewaarden af te leiden: concentratieniveaus die corresponderen met een vooraf gedefinieerde kans op het optreden van allergische sensibilisatie.

Bovenstaande werkwijze is ook gevolgd om advieswaarden voor cyclische zuuranhydriden af te leiden. Gegevens afkomstig van epidemiologisch onderzoek op de werkplek dienden hierbij als basis. Vervolgens heeft de commissie met behulp van de benchmarksoftware (BMD-software van de Amerikaanse *Environmental*

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*Protection Agency*), de gegevens geanalyseerd om de best passende beschrijving van de blootstellingsresponsrelatie vast te kunnen stellen. De gegevens zijn vervolgens gebruikt om een blootstellingsniveau af te leiden, dat als vertrekpunt dient voor het schatten van referentiewaarden.

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## Evaluatie en aanbevelingen

De uitkomsten van de afzonderlijke cyclisch zuuranhydriden staan hieronder weergegeven, voorzien van een onderbouwing.

### Trimellietzuuranhydride (TMA)

Er zijn gegevens beschikbaar over de hoogte van beroepsmatige blootstelling en het optreden van TMA-geïnduceerde sensibilisatie bij werknemers. Gebruikmakend van de best passende dosisresponsrelatie, concludeert de commissie dat bij een beroepsmatige blootstelling aan  $18 \mu\text{g}/\text{m}^3$ , het extra risico op specifieke TMA geïnduceerde sensibilisatie 10% bedraagt. De commissie heeft dit blootstellingsniveau gebruikt als uitgangspunt voor het afleiden van lagere risico's op sensibilisatie, namelijk van 0,1% and 1,0%. Voor deze lagere extra risico's is gekozen, omdat een eenmaal verkregen overgevoeligheid voor een specifiek allergeen niet meer over gaat en verder de kans op TMA geïnduceerde sensibilisatie in de algemene bevolking vrijwel nihil is. De corresponderende blootstellingsniveaus (referentiewaarden) bij deze risico's zijn berekend met behulp van een lineair model, en komen uit op:

- 0,18 microgram TMA per kubieke meter als een gemiddelde concentratie over een achturige werkdag, die correspondeert met een extra risico van 0,1% door beroepsmatige blootstelling
- 1,8 microgram TMA per kubieke meter als een gemiddelde concentratie over een achturige werkdag, die correspondeert met een extra risico van 1,0% door beroepsmatige blootstelling.

De referentiewaarden zijn gegeven als illustratie; ook waarden gebaseerd op andere risiconiveaus kunnen hier worden gekozen; in Nederland zijn namelijk nog geen beslissingen genomen over welke risiconiveau voor allergische sensibilisatie nog geaccepteerd wordt.

## HHPA

Gebruikmakend van de best passende dosisresponsrelatie, concludeert de commissie dat bij een beroepsmatige blootstelling aan  $0,73 \mu\text{g HHPA}/\text{m}^3$ , het extra risico op specifieke HHPA geïnduceerde sensibilisatie 10% bedraagt. De commissie heeft dit blootstellingsniveau gebruikt als uitgangspunt voor het afleiden van lagere risico's op sensibilisatie, namelijk van 0,1% and 1,0%. Voor deze lagere extra risico's is gekozen, omdat een eenmaal verkregen overgevoeligheid voor een specifiek allergeen niet meer over gaat en verder de kans op HHPA geïnduceerde sensibilisatie in de algemene bevolking vrijwel nihil is. De corresponderende blootstellingsniveaus (referentiewaarden) bij deze risico's zijn berekend met behulp van een lineair model, en komen uit op:

- 0,007 microgram HHPA per kubieke meter als een gemiddelde concentratie over een achturige werkdag, die correspondeert met een extra risico van 0,1% door beroepsmatige blootstelling
- 0,07 microgram HHPA per kubieke meter als een gemiddelde concentratie over een achturige werkdag, die correspondeert met een extra risico van 1,0% door beroepsmatige blootstelling.

De referentiewaarden zijn gegeven als illustratie; ook waarden gebaseerd op andere risiconiveaus kunnen hier worden gekozen; in Nederland zijn namelijk nog geen beslissingen gemaakt ten aanzien van welke risiconiveau voor allergische sensibilisatie nog aanvaardbaar is.

## CA, DSA, HA, MA, MTHPA, PA, PMDA, SA, TBPA, TCPA, THPA

Vanwege een gebrek aan goede gegevens om een advieswaarde te kunnen afleiden, onthoudt de Commissie GBBS zich van een aanbeveling voor de volgende cyclische zuuranhydriden:

- chlorendic anhydride (CA)
  - dodeceny succinaatanhydride (DSA)
  - himicanhydride (HA)
  - maleïnezuuranhydride (MA)
  - methylhexahydroftaalzuuranhydride (MHHPA)
  - methyltetrahydroftaalzuuranhydride (MTHPA)
  - ftaalzuuranhydride (PA)
  - pyromellietzuurdianhydride (PMDA)
  - succinaatanhydride (SA)
  - tetrabroomftaalzuuranhydride (TBPA)
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- tetrachloorftaalzuuranhydride (TCPA)
- tetrahydroftaalzuuranhydride (THPA).

Als aanvullende overweging voegt de commissie toe dat het verstandig is blootstelling aan deze cyclische zuuranhydriden zo goed mogelijk te vermijden; hierbij gaat het in het bijzonder om die omstandigheden waar het blootstellingniveau boven die van de bekendere cyclische zuuranhydriden uitkomt (TMA en HHPA) en waarvan bekend is dat zij bij een bepaald blootstellingniveau allergische sensibilisatie veroorzaken.



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# Executive summary

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## Scope

At request of the Minister of Social Affairs and Employment, the Dutch expert Committee on Occupational Exposure Safety (DECOS), one of the permanent committees of experts of the Health Council, proposes health-based recommended occupational exposure limits for chemical substances in the air in the workplace. These recommendations serve as basis in setting legally binding occupational exposure limits by the minister. In this advisory report, the committee evaluates the consequences of exposure to cyclic acid anhydrides\*, and derives a health-based recommended occupational exposure limit (HBR-OEL).

In a first step the toxicity of the compounds was evaluated. This is done in cooperation with the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. The results of the evaluation, which are published in 2004, are included in Part II of this advisory report. In the first part, the DECOS describes the most relevant data, supplied by newly presented data, and advices on an HBR-OEL. The conclusions of the DECOS are based on scientific papers published before December 2009.

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\* Cyclic acid anhydrides evaluated in this report: chlorendic anhydride (CA); dodecenylsuccinic anhydride (DSA); himic anhydride (HA); maleïc anhydride (MA); hexahydrophthalic anhydride (HHPA); methyl hexahydrophthalic anhydride (MHHPA); methyl tetrahydrophthalic anhydride (MTHPA); phthalic anhydride (PA); pyromellitic dianhydride (PMDA); succinic anhydride (SA); tetrabromophthalic anhydride (TBPA); tetrachlorophthalic anhydride (TCPA); tetrahydrophthalic anhydride (THPA); trimellitic anhydride (TMA).

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### Physical and chemical properties

Cyclic acid anhydrides are mainly powders or crystals; methyl derivatives of the cyclic acid anhydrides are oily liquids. Most cyclic acid anhydrides dissolve in organic solvents, such as in alcohol and benzene. With alcohol they are quickly hydrolyzed by water to form carboxylic acids. At least phthalic and maleic anhydride also dissolve in water.

Cyclic acid anhydrides are broadly used in the chemical industry, in particular in the production of polyesters, plastics and resins. They do not occur naturally, but may be found as contaminants in the environment.

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### Monitoring

In general, samples of cyclic acid anhydrides in the air are taken by the use of special absorption tubes, Teflon or PVF filters, or a combination of these possibilities. Various analysis methods are suitable to determine the concentration of acid anhydrides in the samples. These include gas chromatography combined with flame ionisation detection, mass spectrometric detection, and electron capture detection methods.

Analysis methods are available for analyzing in urine the dicarboxylic acids formed from the corresponding cyclic acid anhydrides, and for measuring in plasma the dicarboxylic acids formed from HHPA and MHHPA.

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### Current limit values

In the Netherlands, no legally binding standards exist for cyclic acid anhydrides. Elsewhere, for some acid anhydrides occupational exposure limits are set. These vary between 200 and 12,000  $\mu\text{g}/\text{m}^3$  for phthalic anhydride, 40  $\mu\text{g}/\text{m}^3$  for trimellitic anhydride, and between 400 to 1,200  $\mu\text{g}/\text{m}^3$  for maleic anhydride, and include eight-hour and 15-minute time weighted average concentrations, and ceiling values.

PA, TMA, MA, HHPA, MHHPA, MTHPA, THPA, TCPA, HA, and PMDA are classified by the European Union as sensitizers by inhalation and skin contact (R42 and R42).

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## **Kinetics**

Investigations in humans reveal that hexahydrophthalic anhydride is efficiently taken up by the body after inhalation, whereas uptake via the skin is minimal. Regarding other cyclic acid anhydrides, no data on uptake after inhalation, or via other routes of exposure is known.

In blood, cyclic acid anhydrides bind to hemoglobin, which is present in red blood cells, and to proteins in plasma, such as albumin. They likely bind also to proteins in organs, such as those of the respiratory and gastrointestinal tract, and the kidneys.

Approximately 85% of the inhaled hexahydrophthalic anhydride is released from the body via the urine in the form of dicarboxy acids. The half-life, *i.e.*, the time the body needs to half the dose of dicarboxy acids in plasma, depends on the type of acid anhydride, and varies from two to fourteen hours.

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## **Mechanism of toxicity**

The most marked adverse health effects due to exposure to cyclic acid anhydrides are irritation and allergic hypersensitivity. Irritation may be caused by both cyclic acid anhydrides and their hydrolysis products.

Allergic hypersensitivity by cyclic acid anhydrides is caused by induction of an IgE mediated specific immune response (immediate type). This is known because in both humans and animals specific antibodies of the IgE type are found in the blood. These antibodies are involved in allergic processes, and the presence of them points to allergic sensitisation. Sensitisation is a crucial and necessary step in the development of allergies. Continuing exposure after being sensitised may lead to disorders, such as contact urticaria, allergic rhinitis and rhinoconjunctivitis, and allergic asthma.

There are indications that cyclic acid anhydrides might also induce other types of immune responses, involving induction of specific IgG antibodies, and delayed-type of responsiveness.

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## **Effects**

### Observations in humans

Skin contact may lead to contact urticaria.

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Short-term inhalation may lead to acute effects, such as inflammation of the mucous membranes in the upper and lower respiratory tract. These types of effects might well be caused by irritation.

Of some cyclic acid anhydrides data are collected on the allergic hypersensitivity and respiratory disorders, due to occupational exposure. However, not in all cases respiratory disorders could be associated with allergic hypersensitivity and thus with allergy. This is caused by the fact that irritation gives comparable symptoms. Furthermore, it is possible that in some cases irritation and allergy appeared at the same time in the same person.

Estimations on the prevalences of occupational asthma due to exposure to cyclic acid anhydrides vary between 8 and 18% for PA, 2 to 12% for TMA, 15% for HHPA, 11% for MHHPA, and 3 to 39% for TCPA.

In principle, for most cyclic acid anhydrides evaluated in this advisory report, cases of allergic sensitisation have been reported due to occupational exposure. However, not for all acid anhydrides this could be clearly correlated with a certain exposure level. For those for which data on exposure levels were available, the lowest levels at which still cases of allergic sensitisation were reported varied from less than 1  $\mu\text{g}/\text{m}^3$  (TMA, HHPA and MHHPA), and 15  $\mu\text{g}/\text{m}^3$  (MTHPA), to exposure levels of hundreds of micrograms per cubic meter (PA and TCPA). In all cases, at these exposure levels also (allergic) respiratory symptoms were reported, such as rhinitis and asthma. Data on lower exposure levels of the respective compounds are not available, except for TMA, thus it is possible that below the lowest measured levels, still allergic sensitisation and symptoms may be induced.

Finally, there are cases reported on hemorrhagic alveolitis and anaemia, and allergic alveolitis and laryngitis. No data are available on adverse effects in other organs than the respiratory tract.

### Observation in animals

A few studies indicate that certain cyclic acid anhydrides can lead to skin and eye irritation by direct contact. There are, however, potency differences among the different acid anhydrides. For instance, phthalic anhydride caused slight irritation in the eyes of rabbits, but maleic and trimellitic anhydride caused moderate to severe eye irritation.

A limited number of animal studies has been performed, using guinea pigs, rats and mice, in which the induction of allergic sensitisation of cyclic acid anhydrides (PA, TMA, HHPA, MHHPA, and MTHPA) was investigated. The majority of the studies had a so-called biphasic design. This means that animals

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underwent two phases of exposure, a sensitisation phase and a challenge phase. Sometimes different exposure routes and exposure levels were chosen for each phase.

Similar adverse health effects were observed in animals as in man. For instance, after dermal and inhalation exposure, specific IgE antibodies were found in blood, an indication of allergic sensitisation. Also pathological changes in lung tissue were observed.

Regarding investigations on inhalation exposure, the lowest concentration at which still elevated levels of TMA-specific IgE was found was 400 µg/m<sup>3</sup>, whereas no elevated levels were found after exposure to 40 µg/m<sup>3</sup>. However, the low number of animals and the short duration of exposure hamper a final conclusion on these outcomes. Concentrations of 30 to 40 µg/m<sup>3</sup> caused histopathological changes in the lungs. In another animal study, increased levels of antibody response were observed at 10 µg TMA /m<sup>3</sup> and higher. Furthermore, the increase in response correlated strongly with an increase in hemorrhagic lung foci and lung weight.

In rats, hamsters, and monkeys, which were exposed to maleic anhydride (1,100 to 9,800 µg/m<sup>3</sup>) for a half year, no abnormalities were found in the lungs, liver, pancreas, bone marrow, and kidneys. In rodents, however, irritation of the nasal mucous membranes was reported, but this was reversible and could not be correlated to the level of exposure.

Animal data on long-term exposure to other cyclic acid anhydrides is lacking.

There is also a lack of data on possible carcinogenic properties. The few available data on genotoxicity are negative.

Furthermore, no indications for effects on reproduction and progeny have been found. However, hardly any data on reproductive toxicity is available.

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## **Procedure by assessing health-based occupational exposure limits for allergens**

### The choice of critical effect

DECOS considers the induction of allergic sensitisation as the critical adverse health effect against which workers should be protected. The main reason being that allergic sensitisation is an irreversible change, that is associated with a higher risk in developing allergic reactions. By preventing allergic sensitisation, DECOS expects that also the development of an allergy due to continued exposure will be prevented. Furthermore, it expects that by preventing sensitisation also induction of irritation of the respiratory tract will be prevented.

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The committee prefers the use of data on anhydride-specific IgE mediated sensitisation, because according to the current scientific views, specific IgG mediated sensitisation is less associated with allergic symptoms.

#### Choice of HBR-OELs or reference values

In the scientific literature it is discussed whether for allergens a threshold level exists, below which no cases of allergic sensitisation or allergy symptoms are to be expected.

Based on the current knowledge, the Health Council concluded in its recently published advisory report that based on current knowledge, in theory a threshold level exists for allergic sensitisation by inhaled allergens. This implies that a health-based recommended occupational exposure limit can be calculated, using the same procedures and methods as those for other non-carcinogenic substances.

However, in the same report, the Health Council emphasizes that for most allergens, in practice it will not be possible to calculate a reliable health-based recommended occupational exposure limit. The reason being that, in most cases, the threshold level will be too low to discern using the techniques presently available.

For those allergens, the Health Council proposed an alternative approach, involving determination of reference values, *i.e.* concentration levels that correspond to predefined accepted levels of risk of allergic sensitisation.

The procedure outlined is followed by DECOS to derive advisory values for cyclic acid anhydrides. For this purpose epidemiological data served as basis. Furthermore, for the recommendations, DECOS used the benchmark dose software of the US Environmental Protection Agency to assess the best fitting shape of the exposure-response relationship. The relationship was then used to derive an exposure level that could serve as point of departure in estimating reference values.

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#### Evaluation and recommendations

The outcomes and considerations of the individual cyclic acid anhydrides are given below.

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## TMA

Exposure and response data were available from an observational study with a cohort design (Grammer *et al.* (1999)). From the fitted dose response curve, an exposure level was calculated at which 10% of the occupationally exposed population will get specifically sensitized to TMA. This levels corresponded to 18  $\mu\text{g TMA}/\text{m}^3$ . This level was used as a starting point for calculating exposure levels with lower sensitizing risks, *i.e.* 0.1% and 1%.

Using a linear extrapolation model, the exposure levels (reference values) corresponding to an additional risk of 0.1% and 1% amount to:

- 0.18  $\mu\text{g TMA}/\text{m}^3$ , which corresponds to an additional risk of 0.1% due to occupational exposure, as an 8-hour time weighted average concentration
- 1.8  $\mu\text{g TMA}/\text{m}^3$ , which corresponds to an additional risk of 1% due to occupational exposure, as an 8-hour time weighted average concentration.

The predefined additional risks are extra risks caused by occupational exposure that comes on top of the risk of getting sensitised to TMA in the general population. Furthermore, the reference values serve as examples, since also policy and social considerations should be taken into account in deciding on the level of the predefined additional risk levels. In the Netherlands, no decisions have yet been made about accepted additional response levels for allergic sensitisation of inhaled allergens.

## HHPA

Exposure and response data were available from an observational study (Rosqvist *et al.* (2003)). From the fitted dose response curve, an exposure level was calculated at which 10% of the occupationally exposed population will get specifically sensitized to HHPA. This levels corresponded to 0.73  $\mu\text{g HHPA}/\text{m}^3$ . This level was used as a starting point for calculating exposure levels with lower sensitizing risks, *i.e.* 0.1% and 1%.

Using a linear extrapolation model, the exposure levels (reference values) corresponding to an additional risk of 0.1% and 1% amount to:

- 0.007  $\mu\text{g HHPA}/\text{m}^3$ , which corresponds to an additional risk of 0.1% due to occupational exposure, as an 8-hour time weighted average concentration
  - 0.07  $\mu\text{g HHPA}/\text{m}^3$ , which corresponds to an additional risk of 1% due to occupational exposure, as an 8-hour time weighted average concentration.
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The predefined additional risks are extra risks caused by occupational exposure that comes on top of the risk of getting sensitised to HHPA in the general population. Furthermore, the reference values serve as examples, since also policy and social considerations should be taken into account in deciding on the level of the predefined additional risk levels. In the Netherlands, no decisions have yet been made about accepted additional response levels for allergic sensitisation of inhaled allergens

CA, DSA, HA, MA, MHHPA, MTHPA, PA, PMDA, SA, TBPA, TCPA, THPA

The DECOS abstains from making recommendations for the following cyclic acid anhydrides, because of a lack of adequate data on allergic sensitisation and respiratory symptoms to derive an HBR-OEL of reference values:

- chlorendic anhydride (CA)
- dodecenylsuccinic anhydride (DSA)
- himic anhydride (HA)
- maleïc anhydride (MA)
- methyl hexahydrophthalic anhydride (MHHPA)
- methyl tetrahydrophthalic anhydride (MTHPA)
- phthalic anhydride (PA)
- pyromellitic dianhydride (PMDA)
- succinic anhydride (SA)
- tetrabromophthalic anhydride (TBPA)
- tetrachlorophthalic anhydride (TCPA)
- tetrahydrophthalic anhydride (THPA).

As an additional consideration, the DECOS notes that it would be prudent to avoid respiratory exposure to these cyclic acid anhydrides in as much as possible, in particular in those conditions where exposure exceeds concentrations in which better studied anhydrides, such as TMA and HHPA, are known to cause sensitization.

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Dutch Expert Committee on Occupational Safety (DECOS)  
Basis for quantitative hazard assessment

Part

**I**

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## **Health Council of the Netherlands: Cyclic acid anhydrides**



# Scope

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## 1.1 Background

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, performs scientific evaluations on the toxicity of chemical substances that are used in the workplace (Annex A). The purpose of these evaluations is to recommend health-based occupational exposure limits, which specify levels of exposure to airborne substances, at or below which it may be reasonably expected that there is no risk of adverse health effects.

In this advisory report, such an evaluation and recommendation is made for cyclic acid anhydrides:

- Chlorendic anhydride (CA)
  - Dodecenylsuccinic anhydride (DSA)
  - Himic anhydride (HA)
  - Hexahydrophthalic anhydride (HHPA)
  - Methylhexahydrophthalic anhydride (MHHPA)
  - Maleic anhydride (MA)
  - Methyltetrahydrophthalic anhydride (MTHPA)
  - Phthalic anhydride (PA)
  - Pyromellitic dianhydride (PMDA)
  - Succinic anhydride (SA)
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- Tetrabromophthalic anhydride (TBPA)
- Tetrachlorophthalic anhydride (TCPA)
- Tetrahydrophthalic anhydride (THPA)
- Trimellitic anhydride (TMA).

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## 1.2 Committees and procedures

The evaluation is a co-production of the DECOS and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). It is a result of an agreement between both groups to prepare jointly scientific criteria documents, which can be used by the national regulatory authorities in the Netherlands and the Scandinavian countries for establishing exposure limits. The members of the DECOS and NEG are listed in Annex B.

The joint draft evaluation has been prepared by Dr. H. Keskinen from the Finnish Institute of Occupational Health, Helsinki, Finland. In addition, the draft was reviewed first by the NEG and subsequently by the DECOS. In 2004, the final evaluation was published by the Swedish National Institute of Occupational Health (in the Arbete och Hälsa series). It is included in Part II of this advisory report.<sup>17</sup>

On the basis of the evaluation and additional data, the DECOS judged the toxicity of cyclic acid anhydrides in recommending a health-based occupational exposure limit. This judgment and recommendation are described in Part 1 of this report.

In 2009, the president of the Health Council released a draft of this advisory report for public review. The individuals and organisations that commented on this draft are listed in Annex C. The DECOS has taken these comments into account in deciding on the final version of the report.

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## 1.3 Data

In the sections below, a summary of the findings from the joint NEG/DECOS report on cyclic acid anhydrides (part II)\* is presented first under ‘Summary of data presented in Arbete och Hälsa’, while any additional information from the literature searches is subsequently included in the paragraph ‘additional information’.

Additional literature was retrieved from the on-line databases Medline and Toxline starting from 1999. The final search has been carried out in December

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\* references from part II are referred to as ‘NEGxx’

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2009. The searches were performed using the following search profiles:  
CAS 85-44-9 OR phthalic anhydride; CAS 552-30-7 OR trimellitic anhydride;  
CAS 108-31-6 OR maleic anhydride; CAS 85-42-7 OR hexahydrophthalic anhy-  
dride OR HHPA; CAS 25550-51-0 OR methyl hexahydrophthalic anhydride  
OR MHHPA; CAS 26590-20-5 OR methyl tetrahydrophthalic anhydride  
OR MTHPA; CAS 85-43-8 OR tetrahydrophthalic anhydride OR THPA;  
CAS 117-08-8 OR tetrachlorophthalic anhydride OR TCPA.

In Annex F a list of abbreviations is given of the individual cyclic acid anhy-  
drides evaluated in this report.





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# Effects

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In the sections below, the first part summarizes the most relevant findings from the co-production by the NEG and the DECOS (see Part II for the complete evaluation and references). Additional information on toxicity, with special emphasis on exposure-response relationships, is summarized in the second part of the sections.

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## 2.1 Observations in humans

Observations in humans are limited to effects in the skin, eyes, airways and lungs. No data are available on effects on other organ systems.

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### 2.1.1 Irritation

Summary of the data presented in *Arbete och Hälsa*  
(for more details see Part II)

Conjunctival, nasal, and bronchial irritation is a common immediate feature following exposure to cyclic acid anhydrides. On mucous membranes and on sweating skin, the substances are hydrolysed to acids and can cause irritation, reddening, corneal damage, caustic dermatitis, and burns.

In one study, sixty workers were exposed to mean levels of 400 µg PA/m<sup>3</sup>, with peak levels of 6,600 µg/m<sup>3</sup> during loading of reactors. Of the heavily exposed workers 14% had asthma and 69% work-related symptoms of rhinitis and conjunctivitis. Since a minority was positive for specific IgE,

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the authors concluded that the adverse health effects were mainly caused by irritation. The findings could not be reproduced in a follow-up study among loaders, in that none of them experienced exposure-related respiratory effects.

In another study, nine of the twenty workers with work-related nasal symptoms and exposed to HHPA, were reported to be caused by irritation, because they did not show allergic sensitisation (by skin prick test and serological tests).

#### Additional information

No new data were found.

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### 2.1.2 *Allergic dermatitis*

#### Summary of the data presented in Arbete och Hälsa (for more details see Part II)

Allergic dermatitis due to cyclic acid anhydride exposure is rare. There are only two case-reports on delayed-type contact allergy after DSA and MHHPA exposure. IgE-mediated contact urticaria is more commonly induced by contact or sometimes even airborne exposure to MA, MHHPA, MTHPA, HHPA, and CA.

#### Additional information

No new data were found.

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### 2.1.3 *Respiratory allergies*

Allergic respiratory manifestations are well known effects of occupational exposure to cyclic acid anhydrides. Respiratory diseases that could be related to allergy include rhinitis, rhinoconjunctivitis and occupational asthma. Urticaria (skin) and allergic rhinoconjunctivitis often precede asthma. Also cases of hemorrhagic alveolitis, hemorrhagic anaemia, allergic alveolitis, and allergic laryngitis have been reported.<sup>17</sup> Allergic rhinitis and asthma are associated with IgE-mediated (immediate type) immune responses; hemorrhagic disorders with late type immune responses.<sup>13</sup>

## Summary of the data presented in Arbete och Hälsa (for more details see Part II)

Symptoms of respiratory allergies have been those typical of occupational asthma and rhinitis. After a symptom-free latency period, the worker is likely to experience symptoms after renewed exposure to cyclic acid anhydrides. Diagnosis of respiratory allergies has been based on data on exposure and symptoms, which were obtained by physical examination, questionnaires and immunological or provocation tests.

Reports on occupational asthma are presented in Table 10 of Part II. Summarised, it is estimated that the percentage of occupational asthma cases due to exposure to different cyclic acid anhydrides varies between 8-18% in those exposed to PA, 2-11% for TMA, 15% for HHPA, 11% for MHPA, and 3-39% for TCPA.

There is convincing proof that cyclic acid anhydrides cause respiratory allergies by IgE-mediated sensitisation. For instance, specific IgE antibodies are found against PA, TMA, MA, TCPA, HHPA, HA, MHPA, MHPA and CA. Also cross-reactivity has been reported between some cyclic acid anhydrides. Furthermore, specific IgG antibodies are found for some anhydrides, such as for TMA and PA.

A summary of the findings on respiratory allergies and sensitisation of the individual cyclic acid anhydrides is given below.

### **Trimellitic anhydride (TMA)**

TMA has been reported to cause IgE mediated respiratory allergies.

In a cross-sectional study by Zeiss *et al.* (1992), 474 workers were divided in five groups on the basis of job history and personal monitoring results. The group mainly consisted of new enrollees (n=321). Of all workers, 62% had no work-related symptoms or elevated specific IgE blood levels, whereas 32% experienced respiratory irritant effects, and 7% could be related to a respiratory allergy, because of elevated specific IgE blood levels. Regarding new enrollees, respiratory symptoms were concentrated into the three highest exposure groups, whereas specific IgE-levels were only significantly elevated in the highest exposure group (geometric mean, 170  $\mu\text{g}/\text{m}^3$ ; negative with exposure lower than 87  $\mu\text{g}/\text{m}^3$ , geometric mean).<sup>NEG213</sup>

No TMA-related respiratory effects were observed in eleven workers from a factory preparing epoxy resins, who were exposed to less than 180  $\mu\text{g}/\text{m}^3$  after improvements in work hygiene. The study population was small; therefore it is difficult to make a final conclusion on these findings.

Also a few cases of disease-anaemia syndrome due to TMA fume exposure have been reported, and one case of allergic alveolitis. However, no data on exposure levels have been reported.

### **Hexahydrophthalic and methylhexahydrophthalic anhydride (HHPA and MHHPA)**

Data on HHPA and MHHPA mainly concern co-exposure. It is furthermore known that there is a strong cross-reactivity between the two compounds. Therefore, it is difficult to differentiate effects and exposure between them.

HHPA caused work-related nasal symptoms and occupational asthma. For instance, in one study 74% of the twenty-seven workers experienced rhinitis and/or conjunctivitis, and about 15% had asthma. All asthma cases showed elevated levels of specific IgE, as were elevated levels found in six cases of rhinitis. Average exposure levels ranged from 1,900 to 3,800  $\mu\text{g}/\text{m}^3$ .

One rare case of specific MHHPA-induced laryngitis has been reported. In that case, specific IgE antibodies to MHHPA were found in the blood. However, no data on exposure level was reported.

### **Chlorendic anhydride (CA)**

One case of work-related urticaria and asthmatic symptoms was reported, due to exposure to the thermal degradation fumes from a polyester paint. A skin prick test with conjugate of CA was positive. The worker was simultaneously exposed to other agents and no data on exposure levels were presented. No other data were found.

### **Himic anhydride (HA)**

In a manufacture of a synthetic flame retardant, seven of the twenty workers developed work-related respiratory symptoms after exposure to HA powder. Three of them had also specific IgE antibodies against conjugates of HA. No data on exposure level was presented, nor were there any other studies on HA exposure available.

### **Maleic anhydride (MA)**

There is limited information on respiratory allergies induced by MA alone. In all cases interaction with predisposing factors, such as smoking and atopy, a genetic predisposition, could have influenced the outcomes. One case of MA-related asthmatic symptoms was described, in which a worker also developed severe haemolytic anaemia. He had high levels of specific IgE antibodies, but not IgG antibodies. No data on exposure level was presented.

### **Methyltetrahydrophthalic anhydride (MTHPA)**

Yokota *et al.* (1996, 1997, 1998) published various papers on the adverse health effects of MTHPA in plants where condensers for electric appliances were manufactured, and the compound was used as a hardener in the epoxy resin insulation.<sup>NEG199,NEG200,NEG201,NEG204,NEG205</sup> Work-related respiratory

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allergies were observed at minimum exposure levels of 15 to 22  $\mu\text{g}/\text{m}^3$ . Furthermore, 66% of the 148 workers, who participated in the studies, had specific IgE antibodies in their blood. Work-related respiratory allergies and the presence of specific IgE in the blood were also reported in a group of workers in a plant producing barrels for grenade firearms. The authors reported airborne concentrations of MTHPA under 150  $\mu\text{g}/\text{m}^3$ .

### **Phthalic anhydride (PA)**

Among 118 workers in four plants producing alkyd or unsaturated polyester resins, 24% had work-related rhinitis, 18% asthma, and 11% chronic bronchitis. In half of the asthmatics, rhinitis preceded the asthmatic symptoms. The latency period before the onset of symptoms varied between one month and sixteen years. The average full day TWA was 400  $\mu\text{g PA}/\text{m}^3$ , with peak levels of up to 13,000  $\mu\text{g}/\text{m}^3$  during loading of reactors and handling of bags. No other data were available.

### **Pyromellitic dianhydride (PMDA)**

Data on PMDA exposure mainly concern mixed exposure to other cyclic acid anhydrides. Furthermore, no data were available on exposure levels. However, in some of the workers having work-related respiratory also specific IgE against PMDA were observed. Symptoms were associated with dyspnoea, bronchial obstruction, hemorrhagic rhinitis, and one case of acute hemorrhagic alveolitis associated with anaemia. In the latter case specific IgG antibodies were found, but no specific IgE antibodies.

### **Tetrachlorophthalic anhydride (TCPA)**

Two independent studies reported on respiratory allergic disorders and allergic sensitisation in workers who used TCPA as an epoxy-resin curing agent. In the first, no data were available on workplace exposure levels, but inhalation challenge tests with controlled TCPA concentrations from 1.3 to approximately 960  $\mu\text{g}/\text{m}^3$  induced respiratory effects in seven cases of diagnosed occupational asthma. All these persons showed immediate and late immunological responses. In the second study, the prevalence of work-related respiratory symptoms was 27 to 39%. This included asthma and rhinitis. Thirty-one percent had specific IgE, and 39% had specific IgG against conjugated TCPA. The highest prevalences were found among jobs with highest exposure. The mean airborne TCPA concentration varied between 210 and 3,990  $\mu\text{g}/\text{m}^3$ . The exposure was reduced by improved ventilation to less than 110  $\mu\text{g}/\text{m}^3$ . Since then symptoms decreased and no new cases of occupational asthma were found, at least in a period of about one year after ventilation improvements.

## Other cyclic acid anhydrides

No data were available on tetrahydrophthalic anhydride (THPA), succinic anhydride (SA), dodecylsuccinic anhydride (DSA), and tetrabromophthalic anhydride (TBPA).

## Predisposing factors

In several studies on different cyclic acid anhydrides, it was shown that certain risk factors, other than exposure, increased the risk in developing respiratory symptoms and allergic sensitisation. These include smoking and atopy, a genetic predisposition, and maybe other types of genetic predispositions. It is however unclear to what extent these factors and maybe yet other unknown factors may have influenced exposure-response relationships in a quantitative way.

## Additional information

### TMA

In a study by Grammer *et al.* (1999), 286 workers were annually investigated for the appearance of TMA-induced allergic respiratory disease, for three consecutive years.<sup>15</sup> The workers were assigned to five exposure classes by type of job; exposure levels of the classes were based on some industrial hygiene measurements (personal monitoring). None of the 24 workers in the lowest exposure class (class 5; mean  $<0.5 \mu\text{g TMA/m}^3$ ) developed allergic sensitisation or respiratory symptoms during the observation period. Of the 98 workers in class 4 (mean  $0.5 \mu\text{g TMA/m}^3$ ) nine were positive for the presence of specific IgG. No other effects were observed. In the three highest classes with mean exposure of 2 to up to  $130 \mu\text{g TMA/m}^3$ , part of the workers had specific IgG or IgE against conjugates of TMA in their blood, and part of them also reported respiratory symptoms. The authors reported that the strongest exposure-response-related effect was the increase of percentage of workers positive for specific IgE. Based on their findings the authors suggested furthermore that workers exposed to less than  $2 \mu\text{g TMA/m}^3$  are at low risk of developing TMA-induced allergic respiratory disorders.

Later, the same authors reported on disease status of 42 workers who developed allergic lung disorders and were transferred to low-exposure jobs.<sup>14</sup> In 35 individuals, symptoms disappeared, pulmonary functions improved, and specific IgG and IgE levels decreased. In a separate study, 25 workers with occupational asthma were questioned about disease development.<sup>12</sup> Most of them reported

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also symptoms associated with rhinitis and conjunctivitis. They also reported that these symptoms preceded asthma symptoms.

Blomqvist *et al.* (2005) studied the prevalence of respiratory symptoms and immunological responses among 119 powder painters, working in six different shops.<sup>8</sup> They also described exposure levels of TMA. In these paints, resins are used as binding agent, and for the manufacturing of these resins cyclic acid anhydrides are used. Part of the acids may remain in the paint. Data on exposure was limited to one shop. In that shop personal exposure measurements revealed airborne concentrations of between 6 and 180  $\mu\text{g TMA}/\text{m}^3$ , and stationary samples had concentrations between 15 and 1,040  $\mu\text{g TMA}/\text{m}^3$ . In a spray booth the exposure was 200  $\mu\text{g TMA}/\text{m}^3$ , whereas outside the spray booth it was less than 3  $\mu\text{g TMA}/\text{m}^3$ . Medical examination and lung function testing revealed work-related symptoms, such as eye irritation, nasal discharge, blockage and nose bleeding, sneezing, sore and dry throat, cough and asthma symptoms. In some of these workers also specific IgG in the blood could be detected, but in none of the workers specific IgE was found. Overall, the authors stated that the study population may have been not representative of powder sprayers in general. They also suggested that respiratory irritation may have been caused by the dust of the powder, rather than TMA itself. The committee noted the poor quality of the exposure measurements.

#### HHPA and MHHPA

Two independent cross-sectional studies were performed by the same research group to investigate the association between HHPA and MHHPA exposure and the existence of allergic sensitisation and respiratory symptoms.<sup>24,29</sup>

In the first, Nielsen *et al.* (2001) assigned 211 workers of an epoxy-resin factory in four exposure categories (HHPA and MHHPA combined, personal breathing zone sampling): no exposure, <10  $\mu\text{g}/\text{m}^3$ , 10 to 50  $\mu\text{g}/\text{m}^3$ , and >50  $\mu\text{g}/\text{m}^3$ .<sup>24</sup> For the statistical analyses, the concentrations were assumed to be 0, 5, 30 and 70  $\mu\text{g}/\text{m}^3$ , respectively. None of the 57 referents (no exposure) was positive for allergic sensitisation, as measured by the skin prick test and serologic testing on specific IgE and IgG levels. However, cases of specific IgE and IgG-mediated sensitisation were reported in all exposed categories. Tests for trend showed that the increase of sensitisation was significantly exposure-related. Cases of work-related eye, airway and respiratory symptoms were reported in all categories, including the referents. Although the percentage of workers with symptoms was highest in the two highest exposure-categories, test for trend did not reveal a significant trend in height of exposure and increase in prevalences. The authors sug-

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gested that their data indicate that an exposure level of 10 to 20  $\mu\text{g}/\text{m}^3$  could prevent symptoms, but not allergic sensitisation.

From the same research group, a prospective study demonstrated an exposure-response relationship, in which the risk of specific IgE-mediated sensitisation to HHPA, MHHPA and MTHPA (combined) increased with increasing exposure.<sup>33</sup> This study contained data from three different epoxy resin factories, including the one of the study by Nielsen *et al.* (2001). No data were available on sensitisation of the individual acid anhydrides. Mean exposure levels in all plants were 6.6, 6.1 and 2.7  $\mu\text{g}/\text{m}^3$  for MTHPA, HHPA and MHHPA, respectively. Thirteen percent of the participating workers (21/163) had a positive specific IgE (RAST) for one of these three acid anhydrides. All these sensitized workers, but one, scored also positive in the skin prick test. For exposure-response relationship analyses, workers were divided into four exposure groups: 0-5, >5-10, >10-15, and >15  $\mu\text{g}/\text{m}^3$  (combined exposure levels of all three acid anhydrides). Although the incidence increased with increasing exposure, this only became statistically significant at the highest exposure group.

In the second cross-sectional study, Rosqvist *et al.* (2003) assigned 139 workers of a plant producing electrical capacitors in four exposure categories (based on total plasma protein adducts): HHPA, < 1  $\mu\text{g}/\text{m}^3$ , 1 to 3  $\mu\text{g}/\text{m}^3$ , 3 to 9  $\mu\text{g}/\text{m}^3$ , and > 9  $\mu\text{g}/\text{m}^3$ ; MHHPA, < 1  $\mu\text{g}/\text{m}^3$ , 1 to 3  $\mu\text{g}/\text{m}^3$ , 3 to 15  $\mu\text{g}/\text{m}^3$ , and > 15  $\mu\text{g}/\text{m}^3$ .<sup>28,29</sup> In all exposure categories, cases of specific IgE- and IgG-mediated sensitisation were observed. The prevalences tended to increase with increasing exposure, however a clear association was found only for HHPA in the two highest exposure categories. When all workers were considered together, 17 to 19% were found to be positive for allergic sensitisation. Regarding work-related symptoms, about 27% of the workers had symptoms of the nose, 21% of the eyes, 11% of the lower airways, and 8% had nose bleedings. A clear positive association was found only between HHPA exposure and eye symptoms. The authors suggested that their findings indicated that even at the lowest exposure levels measured (about 1  $\mu\text{g}/\text{m}^3$ ), there is a risk in developing allergic sensitisation and symptoms due to occupational exposure to HHPA and MHHPA.

In another study by Nielsen *et al.* (2006), they reported the occurrence of new cases of work-related ocular and airway symptoms among 146 new employees in three plants handling acid anhydrides, who were followed for up to 8.5 years.<sup>23</sup> Symptoms were found frequently at mean exposure levels of less than 10  $\mu\text{g}/\text{m}^3$  (HHPA, MHHPA, and MTHPA combined). Exposures varied between 6 and 39  $\mu\text{g}/\text{m}^3$ . Eye symptoms and nose blockage were positively associated with specific IgE sensitisation, smoking and atopy, and also for exposure regarding nose blockage and lower airway symptoms.

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Yokota *et al.* (2002) performed a cross-sectional study on 32 workers from three workplaces in a plant using epoxy resin with mixtures of HHPA and MHHPA.<sup>35</sup> Twenty-four workers had no signs of allergic sensitisation and respiratory symptoms. Of the remaining eight, all were positive for specific IgE in serum and five of them also experienced symptoms of the eyes and nose during work. Air sampling revealed exposure levels of 3.8 to 33.0  $\mu\text{g}/\text{m}^3$  for HHPA, and 2.3 to about 16  $\mu\text{g}/\text{m}^3$  for MHHPA. The authors noted that peak exposure to unknown levels has occurred during study. They also suggested handling interpretation of their results carefully, since exposure in the past was not known and a healthy-worker effect may have occurred.

## MTHPA

More recently, Yokota *et al.* (2004) investigated 147 workers from two condenser plants using epoxy resin with MTHPA, on the presence of allergic sensitisation, airway symptoms, and risk factors.<sup>34</sup> Of the currently exposed workers 65% had positive specific IgE in serum. In particular sensitised workers reported eye and nose symptoms. None of the exposed workers had symptoms of work-related asthma. Exposure levels ranged from about 5 to 68.5  $\mu\text{g}/\text{m}^3$  (geometric mean, air samples), depending on the working zone in the workshops. The investigations did not allow for exposure-response analysis, because only the total number of sensitised workers were mentioned, and no distinction was made among the different working zones. The authors suggested that atopic subjects and smokers are at increased risk for allergic sensitisation by MTHPA.

Drexler *et al.* (2000) studied the prevalence of allergic sensitisation and occupational exposure to acid anhydrides in three different manufacturing plants.<sup>10</sup> In one plant at the time of investigation, workers were exposed to MTHPA alone. However a few years earlier workers were also exposed to HHPA. Among the 86 workers who participated in the study, 21% showed IgE-mediated sensitisation or were positive using the skin prick test. Since workers were earlier exposed also to HHPA, and the authors did not make a distinction between MTHPA- and HHPA-induced specific sensitisation, it is unclear whether the positive cases on sensitisation were due to MTHPA exposure alone. No data on work-related symptoms were presented. Air monitoring in the plant revealed MTHPA levels of 2.3  $\mu\text{g}/\text{m}^3$  (median, stationary air sampling, range < 0.5-26.2  $\mu\text{g}/\text{m}^3$ ), and 13  $\mu\text{g}/\text{m}^3$  (median, personal air sampling, range < 4.7-35.7  $\mu\text{g}/\text{m}^3$ ). No quantitative exposure-response relationship analysis was performed.

## Other cyclic acid anhydrides

No additional information on exposure-response relationships was found for CA, DSA, HA, MA, PA, PMDA, TCPA, THPA, SA, and TBPA.

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### 2.1.4 *Carcinogenesis and genotoxicity*

No data available.

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### 2.1.5 *Reproductive and developmental toxicity*

No data available.

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## 2.2 **Observations in animals**

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### 2.2.1 *Irritation*

Summary of data presented in Arbete och Hälsa  
(for more details see Part II)

The number of data on skin and eye irritation is limited. At least, MA and TMA have been shown to be extremely irritating to the eyes of rabbits; PA gave only a slight irritation. PA furthermore did not irritate rabbit ears after dermal application of a 0.5% solution. Based on data using the Buehler test and the mouse ear swelling test, PA is considered a moderate skin sensitiser.

#### Additional information

Arts *et al.* (2001) investigated the irritating properties of TMA in an animal inhalation model using female Brown Norway and Wistar rats.<sup>2</sup> Groups of four animals were exposed to approximately 0, 15, 30, 50, 100, 200, or 250 mg TMA/m<sup>3</sup> for 30 minutes. Before, during, and after exposure respiration was monitored. The day after exposure, all animals were killed and examined grossly for pathological changes in the airways and lungs. Changes in breathing patterns and frequencies were observed at 29 mg/m<sup>3</sup> and 60 mg/m<sup>3</sup> in Brown Norway rats, respectively, and at 34 mg/m<sup>3</sup> in Wistar rats. These changes were also observed at higher exposure levels. The authors suggested that the changes were reversible and caused by irritation of the lower airways rather than of the upper airways. No concentration-related lung weight changes or pathologies were observed. The authors did not investigate allergic sensitisation.

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## 2.2.2 Allergic sensitisation and respiratory symptoms

The committee emphasizes that at the moment there is a lack of validated inhalatory animal models suitable for quantitative risk analysis on respiratory allergic disorders. However, data have been published using animal studies.

Many of these animal studies on allergic sensitisation and allergy development concern biphasic exposure designs (sensitisation *versus* challenge phase), in which sometimes dermal or other routes of exposure are followed by inhalatory exposure, or different airborne exposure levels were used for the different phases. These types of animal studies give valuable information on the sensitising and allergic potency of the cyclic acid anhydrides. But care should be taken in using data from these studies to derive 'safe' exposure limits, in particular when allergic sensitisation was initiated by other routes of exposure than inhalation. Furthermore, the committee noted that in many of these animal studies a low number of animals was used, and that the exposure duration was relatively short. This might have influenced the outcomes, since it is for instance known that in humans allergic sensitisation still may occur years after first exposure.

The foregoing points are taken into account by the committee in presenting and interpreting data in this section.

### Summary of data presented in Arbete och Hälsa (for more details see Part II)

In the literature, animal studies were mainly performed on TMA, and to a much lesser extent to PA, HHPA, MHHPA, and MTHHPA. No data are available on other cyclic acid anhydrides. In summary, cyclic acid anhydride-specific IgE antibodies were found in the blood in various animal species (rats, mice, guinea pigs) after dermal and inhalation exposure. Also pathological changes in lung tissue were observed. Furthermore, changes in lung function and hyperresponsiveness were noted, and some investigators found elevated levels of specific IgG antibodies in the blood.

Regarding PA, immediate respiratory reactions in guinea pigs were induced at PA dust levels of 500 up to 5,000  $\mu\text{g}/\text{m}^3$  followed with an inhalatory challenge of 2,000 or 5,000  $\mu\text{g}/\text{m}^3$ . Exposure was for 3 hours per day for five consecutive days; the challenge followed two weeks after the final exposure. The animals were also positive for IgG antibodies against conjugated PA. Effects were observed at all exposure levels.

Regarding TMA, groups of male and female rats were exposed to 0, 10, 30, 100 or 300  $\mu\text{g}/\text{m}^3$ , 6 hours per day for five days, for two weeks. The investigators reported on antibody responses to rat serum albumin-TMA conjugates at 10  $\mu\text{g}/\text{m}^3$  and higher after 10 days of TMA exposure. They also

found a significant dose-related correlation between antibody level and hemorrhagic lung foci (in male rats), and lung weight (in male and female rats).

In a mouse inhalation study, specific IgG and IgE antibodies against conjugated TMA were found after exposure to 5,000  $\mu\text{g TMA}/\text{m}^3$  for one hour per day for three days.

In another animal study, rats inhaled 500  $\mu\text{g TMA powder}/\text{m}^3$  for 6 hours per day on days 1, 5, and 10; at day 29 or 22 they were challenged by a six-hour exposure to 300, 500 or 540  $\mu\text{g TMA}/\text{m}^3$ . At all exposure levels hemorrhagic lung foci were found, and IgG antibodies were detected. Fewer effects were seen in the low-exposure groups.

There are no data on HHPA, MHHPA and MTHPA induced effects, which were induced by inhalatory exposure. But data indicate that after dermal sensitisation and inhalatory challenge, respiratory and immunological effects are induced. These include anaphylactic bronchoconstriction in guinea pigs, and specific IgE- and IgG-mediated immune responses.

Finally, animal data on sensitising potency differences among cyclic acid anhydrides by dermal sensitisation showed considerable variation among the acid anhydrides.

### Additional information

Only new data were found on TMA.

Zhang *et al.* (2006) used female Brown Norway rats. In this study, groups of four or eight animals were exposed to 0, 40, 400, 4,000, or 40,000  $\mu\text{g TMA aerosol}/\text{m}^3$  for 10 minutes, once a week for a total of 10 weeks.<sup>38</sup> All groups, except those with the highest exposure, were re-challenged to 40,000  $\mu\text{g}/\text{m}^3$ , two weeks after the last TMA exposure. A day after the last exposure animals were killed for pathologic analyses of the lungs. TMA-specific IgE was detected in groups exposed to 400  $\mu\text{g}/\text{m}^3$  and higher. The highest levels were found in the highest exposure group. The authors also noted a decrease in specific IgE levels during the experiment. Early and late airway responses, using enhanced pause as an index of airway obstruction, were observed in all animals at 40,000  $\mu\text{g}/\text{m}^3$ , in all animals after 4,000  $\mu\text{g}/\text{m}^3$  followed by re-challenge, and in one animal only after 400  $\mu\text{g}/\text{m}^3$  followed by re-challenge. The authors reported furthermore, exposure-dependent histopathological changes, the principal changes being eosinophilic granulomatous interstitial pneumonia, eosinophilic perivascular infiltrates, peribronchiolar plasma cell infiltrates, and hyperplasia of bronchus-associated lymphoid tissue. The latter type of lesion was observed in two of the four animals of the lowest exposure group (40  $\mu\text{g}/\text{m}^3$ ).

Warbrick *et al.* (2002) investigated the stimulation of TMA-specific IgE antibodies following TMA exposure in female Brown Norway rats.<sup>31</sup> Groups of five animals inhaled (nose only) about 5,000  $\mu\text{g TMA}/\text{m}^3$  for one hour for 1, 3 or 5

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consecutive days. TMA-specific IgE levels in serum were determined before the first exposure, and 28 and 35 days after the last exposure. Exposure resulted in a transient elevation of specific IgE levels in a minority of the animals, which reached statistical significance only at the group, which was exposed for 5 consecutive days.

A number of biphasic animal studies have been performed in which sensitisation by TMA was induced by topical application, followed by a challenge via inhalation or intratracheal application, and using rats, mice and guinea pigs.<sup>1,3,7,9,19,20,25,26</sup> These studies revealed that TMA induced changes in airway responses, such as changes in hyperresponsiveness, breathing pattern and frequency; and, stimulated TMA-specific IgE production. Responses were observed after inhalation challenge at levels of as low as 1,000 µg TMA/m<sup>3</sup> in Brown Norway rats.<sup>39</sup> No changes in airway responses was found in the same animal species after inhalation challenge to 200 µg/m<sup>3</sup>.<sup>1</sup> Part of the airway changes was associated with irritation.

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### 2.2.3 *Other adverse health effects after single and repeated exposure*

#### Summary of data presented in Arbete och Hälsa (for more details see Part II)

LD<sub>50</sub> values after single oral exposure varies between around 400 mg/kg bw (MA, rats and guinea pigs) and more than 15,800 mg/kg bw (TCPA, rats). No data on single inhalatory exposure were found.

After 2 weeks of exposure to HHPA vapours (137,300 µg/m<sup>3</sup>), acetylcholinesterase activity was below control level in rats. Levels had normalised after 11 weeks.

When rats, hamsters, and monkeys were exposed to vapours of MA at concentrations of 1,100 to 9,800 µg/m<sup>3</sup> for six months, no exposure-related effects were found in histopathological investigations of the lungs, liver, spleen, bone marrow and kidneys. In rodents, however, nasal irritation (hyperplasia and metaplasia) was observed, and in all species also nasal inflammatory changes were noted. The effects were observed at all exposure levels, and the incidence increased by increasing exposure. The authors remarked that all effects were reversible. No data on allergic sensitisation was presented.

#### Additional information

No new and relevant data were found.

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

Summary of the data presented in Arbete och Hälsa  
(for more details see Part II)

No evidence for carcinogenic properties of cyclic acid anhydrides was found.

Additional information

No new data were found.

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#### 2.2.5 *Genotoxicity*

Summary of the data presented in Arbete och Hälsa  
(for more details see Part II)

In *in vitro* studies, PA, TCPA and TBPA did not induce point mutations or chromosomal abnormalities. No data are available on other cyclic acid anhydrides. Overall the amount of data on genotoxicity is limited.

Additional information

No new data were found.

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#### 2.2.6 *Reproductive and developmental toxicity*

Summary of data presented in Arbete och Hälsa  
(for more details see Part II)

There is no evidence of reproductive and developmental effects of cyclic acid anhydrides at exposure levels below maternal toxicity. However, the set of data is limited and only a few cyclic acid anhydrides have been tested.

Additional information

No new data were found.

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## Hazard assessment

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### 3.1 Hazard identification

In humans and animals, exposure to certain cyclic acid anhydrides is strongly associated with irritation and allergic disorders of the skin and respiratory tract. More specifically, the predominant effect observed in humans after skin contact is allergic urticaria. The predominant effects after inhalation are rhinitis, rhinoconjunctivitis, and asthma. Other effects observed are contact dermatitis, hemorrhagic alveolitis and anaemia, and allergic alveolitis and laryngitis.

The airway and respiratory symptoms can be caused by irritation, allergic hypersensitivity, or a combination of both. The distinction between these two types of effects can be made by immunological testing on the presence of cyclic acid anhydride-specific sensitisation. However, not in all epidemiological and animal studies such testing was performed, and thus in these studies symptoms could not be associated with certainty to the irritating or to the sensitizing properties of these compounds.

Regarding allergic sensitisation, the induction of specific sensitisation by allergens is a first step in allergic hypersensitivity and allergy development. It is a crucial and necessary step, in which – in case of cyclic acid anhydrides – mainly specific IgE antibodies are produced. At renewed exposure, these antibodies can subsequently trigger immune responses, which result into inflammation of the mucous membranes of the respiratory tract. Although after ending exposure the presence of specific antibodies and symptoms may disappear, the

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acquired allergic hypersensitivity is generally irreversible and incurable. This means that a hypersensitive person, who is currently not exposed anymore, may develop allergic symptoms at the moment of renewed exposure in the future. This is an important difference with irritating effects for which no life-long vulnerability is developed.

In humans and animals, other potential adverse health effects induced by cyclic acid anhydrides, than those mentioned in the foregoing, have not been sufficiently investigated. Overall, there is a lack of information on carcinogenic, reproductive and developmental effects of cyclic acid anhydrides.

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### 3.1.1 *Findings from observational studies*

Epidemiological data presented in Part II of this advisory report and additional information (see Part 1) on exposure-response relationships showed that the lowest exposure levels at which work-related allergic IgE-mediated sensitisation due to cyclic acid anhydride exposure was observed, varied between 1 to 50  $\mu\text{g}/\text{m}^3$  for TMA, HHPA, MHHPA and MTHPA, and hundreds of  $\mu\text{g}/\text{m}^3$  for TCPA and PA. At all these levels also the presence of respiratory disorders, such as rhinitis and asthma, has been reported. Data on lower exposure levels of the respective compounds are not available, except for TMA, thus it is possible that below the lowest measured levels, still allergic sensitisation and symptoms may be induced.

In some studies peak exposures to high levels were reported. It is likely that these may have increased the risk in developing irritation, allergic sensitisation and respiratory symptoms. Furthermore, it is likely that in case of allergic sensitisation cumulative exposure is one of the determining factors in developing allergy. However, to what extent these factors may have influenced the outcomes of the studies is unknown. This is also the case for the presence of other risk factors, such as genetic predisposition and smoking habits; the influence of these risk factors on exposure-response relationships is insufficiently investigated.

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### 3.1.2 *Findings from animal studies*

A limited number of animal studies has been performed, using guinea pigs, rats and mice, in which the induction of allergic sensitisation of cyclic acid anhydrides (PA, TMA, HHPA, MHHPA, and MTHPA) was investigated. The majority of the studies had a so-called biphasic design. This means that animals underwent two phases of exposure, a sensitisation phase and a challenge phase.

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Sometimes different exposure routes and exposure levels were chosen for each phase.

Similar adverse health effects were observed in animals as in man. For instance, after dermal and inhalation exposure, specific IgE antibodies were found in blood, an indication of allergic sensitisation. Also specific IgG and pathological changes in lung tissue were observed.

Regarding investigations on inhalation exposure, the lowest concentration at which still elevated levels of TMA-specific IgE were found was 400 µg/m<sup>3</sup>, whereas no elevated levels were found after exposure to 40 µg/m<sup>3</sup>. However, the low number of animals and the short duration of exposure hamper a final conclusion on these outcomes. Concentrations of 30 to 40 µg/m<sup>3</sup> resulted in histopathological changes in the lungs. In another animal study increased levels of antibody response was observed at 10 µg TMA /m<sup>3</sup> and higher. Furthermore, the increase in response correlated strongly with an increase in hemorrhagic lung foci and lung weight.

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### **3.2 The critical effect**

Based on data presented in this report, DECOS considers the induction of allergic sensitisation the critical adverse health effect against which workers should be protected. The main reason being that allergic sensitisation is an irreversible change, that is associated with a higher risk in developing allergic reactions.

The committee did not find clear data suggesting that irritation occurred below exposure levels at which no allergic hypersensitivity have been reported. Therefore, the committee expects that by preventing hypersensitivity also irritation to the airways and lungs will be prevented.

The choice of effect parameter is allergic IgE-mediated sensitisation. This is based on a recently published advisory report of the Health Council on work-related respiratory allergies and recommended occupational exposure levels.<sup>16</sup> In that report the Health Council stated that in case of allergy, data on allergic sensitisation is the best basis to derive health-based recommended occupational exposure limits (OELs).

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### **3.3 Procedure to derive health-based recommended OELs for allergens**

In the scientific literature on quantitative risk analyses of allergens, it is discussed whether for these group of substances a threshold level exists, below which no cases of allergic sensitisation or allergy symptoms are to be expected. Based on the current scientific knowledge, the Health Council concluded in its

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advisory report on work-related respiratory allergies that a threshold level does exist for allergic sensitisation by inhaled allergens. This implies that a health-based recommended occupational exposure limit can be calculated, using the same procedures and methods as those for other non-carcinogenic substances.

However, in the same report, the Health Council emphasizes that for most allergens, it will not be possible to calculate a reliable health-based recommended occupational exposure limit. The reason being that, in most cases, the threshold level will be too low to discern using the techniques presently available. For those allergens, the Health Council proposed an alternative approach, involving determination of reference values, *i.e.* concentration levels that correspond to predefined accepted levels of risk of allergic sensitisation.

The Health Council furthermore recommended, that where sufficient data are not available to determine reference values either, the first step should be to undertake additional research in order to acquire response data for the lowest exposure levels. In the interim, an exposure limit could be based on data concerning the development of allergic respiratory symptoms. The Health Council emphasizes, however, that from a health perspective this is not ideal.

The foregoing strategic proposal is followed by DECOS in recommending health-based occupational exposure limits or reference values for cyclic acid anhydrides. For that purpose human data were used as basis, since observations made in humans represent occupational exposure situations more closely than animal studies. In addition, data on animal studies are of limited use, because of the complex exposure designs, the low number of animals used, and the short exposure and observation periods.

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### **3.4 Recommendations and additional considerations**

#### Trimellitic anhydride (TMA)

Two epidemiological studies on the relationship between exposure to TMA and the occurrence of allergic sensitisation and respiratory symptoms have been reported. These are the studies by Grammer *et al.* (1999), and by Zeiss *et al.* (1992), both of the same research group, but with different study populations (see Table 3.1).<sup>15,37</sup>

Table 3.1 Human data on TMA-induced IgE-mediated sensitisation.

Study design	Exposure category	TMA-specific IgE-mediated sensitisation (elevated IgE antibody)
Grammer <i>et al.</i> (1999). <sup>15</sup> Three-year clinical and immunologic survey, USA; TMA manufacturing facility; 286 employees. None of the employees had been diagnosed as having an immunological mediated disease by TMA.	Mean (range) in $\mu\text{g}/\text{m}^3$ 1: 130 (31,700) 2: 36 (2.3-1,900) 3: 2 (0.1-120) 4: 0.5 (0.2-2.4) 5: <0.5 (<0.5-<0.6)	No. of positive employees: 1: 7/28 (=25%) 2: 6/57 (=11%) 3: 5/79 (= 6%) 4: 0/98 (= 0%) 5: 0/24 (= 0%) Relationship (weak but positive) between IgE antibody and exposure class ( $p < 0.0002$ ; effect strength = 28.2 (values less than 40 are considered relatively weak)).
Zeiss <i>et al.</i> (1992). <sup>37</sup> One-year cross-sectional survey, USA; TMA manufacturing factory; 321 new enrollees; period 1988-1989.	Geometric mean (range) in $\mu\text{g}/\text{m}^3$ : 1: 170 (<0.5-6,500) 2: 87 (5.8-970) 3: <0.55 (-) 4: <0.41 (<0.3-<0.6) 5: <0.53 (<0.5-<0.6)	No. of positive cases after one year of exposure: 1: 2/8 (=25%) 2: 0/39 (= 0%) 3: 0/98 (= 0%) 4: 0/123 (= 0%) 5: 0/42 (= 0%)

First, the committee emphasizes that the observation period in both studies was rather short. Since it is known that new cases of allergic sensitisation can occur, even after years of occupational exposure, it is most likely that new cases would have been reported when the observation period had been longer. Furthermore, it should be taken into account that variations in exposure levels have occurred during work, although its significance on quantitative risk analysis cannot be assessed. In addition, workers were assigned in an exposure class by type of job; exposure levels of the classes were based on some industrial hygiene measurements (personal monitoring). These factors may have weakened the strength of the exposure and response data.

Regarding the study of choice, according to DECOS, quantitative risk assessment is best done by using data of the Grammer-study, because of the cohort design. In epidemiology, in controlling selection bias, cohort designs are preferred above cross-sectional designs. Furthermore, the Grammer-study had a more extended set of data on adverse health effects, other than IgE-mediated sensitisation, than the Zeiss-study.

Subsequently, the committee used the curving fitting program of the Bench Mark software of the US-EPA for the fitting the data of the Grammer study. In Annex E, details and the outcome of the curve fitting analyses are shown.

Regarding the data, no cases were reported by Grammer *et al.* (1999) of IgE-mediated allergic sensitization in a group of workers exposed to 0.5  $\text{mg}/\text{m}^3$  and lower. This might indicate the presence of a safe threshold level. However, the

committee doubts whether from this study a clear threshold level can be derived. In the first place, taking all the data from the curve-fitting analysis into account, the committee noted a steep concentration-response at the lower-exposure range (see Annex E). Secondly, the observation period was rather short. Since it is known that new cases of allergic sensitization can occur, even after years of exposure, it is most likely that more cases would have been reported when the observation period had been longer. Taking these considerations into account, the committee decided that no safe threshold level can be derived for TMA, and thus that predefined additional risk levels need to be calculated.

Preferably, the committee uses the fitted dose response curve. However, in case of TMA, the variability in the exposure levels corresponding to the lower sensitization risks is high. Therefore, the committee decided to use that concentration as starting point which has a limited variability. From this exposure level, the committee extrapolates linearly to the exposure levels corresponding to the lower additional sensitisation risks. The committee determined an exposure level at which 10% of the occupationally exposed population will get specifically sensitised to TMA as the starting point. This level corresponds to 18  $\mu\text{g TMA}/\text{m}^3$ .

The committee uses this level as a starting point for calculating exposure levels at lower additional sensitization risks. The committee takes into account that it is important to protect workers against allergen exposure, because of the everlasting higher risk in developing allergy when sensitised to a specific allergen and because the background incidence in the general population is virtually zero. Therefore, DECOS calculates additional sensitization risks in humans under workplace exposure conditions of 0.1% to 1%.

Using the exposure level of 18  $\mu\text{g TMA}/\text{m}^3$  with an additional risk of sensitisation of 10% as point of departure, the exposure levels (reference values) corresponding to an additional risk of 0.1% and 1% amount to:

- 0.18  $\mu\text{g TMA}/\text{m}^3$ , which corresponds to an additional risk of 0.1% due to occupational exposure, as an 8-hour time weighted average concentration
- 1.8  $\mu\text{g TMA}/\text{m}^3$ , which corresponds to an additional risk of 1% due to occupational exposure, as an 8-hour time weighted average concentration.

The predefined additional risks are extra risks caused by occupational exposure that comes on top of the risk of getting sensitised to TMA in the general population. Furthermore, the reference values serve as examples, since also policy and social considerations should be taken into account in deciding on the level of the predefined additional risk levels. In the Netherlands, no decisions have yet been made about accepted additional response levels for allergic sensitisation of inhaled allergens.

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## Hexahydrophthalic anhydride (HHPA)

Two studies on the relationship between exposure to HHPA and specific IgE sensitisation have been published that could provide a basis for deriving an occupational exposure limit.<sup>24,29</sup> These are the studies by Nielsen *et al.* (2001) and by Rosqvist *et al.* (2003) (see Table 3.2). It concerns two different study populations from the same research group, with combined exposure to MHHPA, but with data separated for allergic IgE-mediated sensitisation and exposure levels. To begin with, the committee judged the quality of the studies. As both studies have a cross-sectional design, selection bias or the healthy-worker effect might have occurred. Furthermore both study populations were rather limited, indicating that small effects might have been missed. Regarding the exposure measurements in the Nielsen-study, the committee noticed that workers who were assigned in the highest exposure category used protective respiratory devices, and thus actual exposure might have been lower. In the Rosqvist-study, airborne exposure was estimated by correlating exposure data, which were obtained earlier, with measured levels of plasma protein adducts of HHPA and MHHPA. Notwithstanding the foregoing remarks, cases of allergic sensitisation to HHPA and MHHPA are found at the lowest measured exposure levels, taking into account the small study

Table 3.2 Human data on HHPA- and MHHPA-induced IgE-mediated sensitisation.

Study design	Exposure category	HHPA and MHHPA specific IgE-mediated sensitisation (elevated IgE antibody)
Nielsen <i>et al.</i> (2001). <sup>24</sup> Cross-sectional study; 154 exposed workers and 57 referents;	In $\mu\text{g}/\text{m}^3$ for HHPA and MHHPA combined (value used for statistical analysis) 1: 0 (0) 2: < 10 (5) 3: 10-50 (30) 4: > 50 (70) Based on personal sampling in breathing zone.	Percentage positive: HHPAMHHPA 1: 0                    0 2: 13                   15 3: 26                   26 4: 21                   17 Test for trend, $p < 0.001$
Rosqvist <i>et al.</i> (2003). <sup>29</sup> Cross-sectional study; 139 exposed workers, no referents.	Estimated air levels in $\mu\text{g}/\text{m}^3$ : HHPA                    MHHPA 1: < 1                    < 1 2: 1-3                    1-3 3: 3-9                    3-15 4: > 9                    > 15 Air levels are calculated from data on total plasma protein adducts of HHPA and MHHPA, respectively.	No. of positive cases: HHPA                    MHHPA 1: 2/35                   8/33 2: 7/37                   3/33 3: 8/32                   7/35 4: 10/35                   9/38 Corresponding prevalence odds ratio (95% CI): HHPA                    MHHPA 1: 1.0 (-)                   1.0 (-) 2: 3.8 (0.7-20.0)           0.3 (0.1-1.3) 3: 5.5 (1.1-28.2)           0.8 (0.2-2.5) 4: 6.6 (1.3-32.8)           1.0 (0.3-2.9)

populations, and that background incidences are expected to be near zero. Moreover, the results of the two independently performed studies were rather consistent.

In Annex E, details and the outcome of the curve fitting analyses are shown.

Next, the committee has to establish exposure levels corresponding to pre-defined additional risks on sensitization. Again, the committee prefers to use the fitted dose response curve. However, also in case of HHPA, the variability in the exposure levels corresponding to the lower sensitization risks is high. Therefore, the committee decided to use that concentration as starting point which has a limited variability. From this exposure level, the committee extrapolates linearly to the exposure levels corresponding to the lower additional sensitization risks. The committee determined an exposure level at which 10% of the occupationally exposed population will get specifically sensitized to HHPA as the starting point. This level corresponds to  $0.73 \mu\text{g HHPA}/\text{m}^3$ .

The committee takes this level as a starting point for calculating exposure levels corresponding to lower additional sensitization risks. The committee considers it important to protect workers against allergen exposure, because of the everlasting higher risk in developing allergy when sensitised to a specific allergen, and because in the general population the background incidence is virtually zero. Therefore, DECOS calculates additional sensitization risks in humans under workplace exposure conditions of 0.1% to 1%.

In general, the DECOS is of the opinion that it is justified to use a linear model to estimate exposure levels at other additional risk levels, unless data indicate that a linear model is not appropriate. The linear model was applied for HHPA, because data that would indicate otherwise are limited.

Using the exposure level of  $0.73 \mu\text{g HHPA}/\text{m}^3$  with an additional risk of sensitization of 10% as point of departure, the exposure levels (reference values) corresponding to an additional risk of 0.1% and 1% amount to:

- $0.007 \mu\text{g HHPA}/\text{m}^3$ , which corresponds to an additional risk of 0.1% due to occupational exposure, as an 8-hour time weighted average concentration
- $0.07 \mu\text{g HHPA}/\text{m}^3$ , which corresponds to an additional risk of 1% due to occupational exposure, as an 8-hour time weighted average concentration.

The predefined additional risks are extra risks caused by occupational exposure that comes on top of the risk of getting sensitised to HHPA in the general population. Furthermore, the reference values serve as examples, since also policy and social considerations should be taken into account in deciding on the level of the predefined additional risk levels. In the Netherlands, no decisions have yet been

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made about accepted additional response levels for allergic sensitisation of inhaled allergens.

### Methylhexahydrophthalic anhydride (MHHPA)

Data on MHHPA exposure and IgE-mediated sensitisation are obtained from the same two studies as for HHPA (see Table 3.2).<sup>24,29</sup>

Regarding the Rosqvist-study, the DECOS noticed that in the lowest exposure category more positive cases of allergic sensitisation were reported than in higher exposure categories. In addition, the increase in incidence of allergic sensitisation was not statistically significant. Therefore, the committee concluded that this study was not suitable for assessing an occupational exposure limit for MHHPA.

Regarding the Nielsen-study, details and the outcome of the curve fitting analysis are shown in Annex E. The outcome indicates that the data cannot be fitted accurately. Based on this finding, DECOS concludes that data of the Nielsen-study are insufficient for assessing a health-based recommended occupational exposure limit or reference values for MHHPA in a reliable way, or to give an additional consideration as well.

### Phthalic anhydride (PA)

Work-related respiratory symptoms were reported at average full-day exposure to 400 µg/m<sup>3</sup> combined with peak exposures to up to 13,000 µg/m<sup>3</sup>.<sup>17</sup> Due to the variation in exposure levels, it is uncertain what the average exposure during a full shift working day was, and, therefore, the committee considers the available data not suitable for deriving a health-based recommended OEL or reference value. In conclusion, the committee abstains from giving a recommendation.

### Methyltetrahydrophthalic anhydride (MTHPA)

In a few cross-sectional studies, positive cases of MTHPA-specific IgE-mediated sensitisation and respiratory symptoms were found at exposure levels around 2 to 5 µg/m<sup>3</sup> and higher.<sup>17</sup> No levels were reported at which no cases of allergic sensitisation and respiratory symptoms were observed. However, data are too limited for establishing a reliable exposure-response relationship by quantitative risk analysis, because of mixed exposure and lack of classification in different exposure groups. Therefore, the committee abstains from recommending a health-based OEL or reference value.

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#### Tetrachlorophthalic anhydride (TCPA)

Work-related specific IgE mediated sensitisation and respiratory symptoms were reported in a cross-sectional study by Liss *et al.* (1993), at personal airborne exposure levels that varied during the working days between 210 and 3,990  $\mu\text{g}/\text{m}^3$  (geometric mean).<sup>21</sup> The committee noted the small number of participants in study, and the considerable variation in exposure levels during the day. This limits the suitability for quantitative risk analyses. Therefore, the committee abstains from giving a recommendation.

#### Chlorendic anhydride (CA)

No human or animal data on exposure and response were available. Therefore, the committee abstains from giving a health-based recommended OEL or reference value.

#### Dodecenylsuccinic anhydride (DSA)

No human or animal data on exposure and response were available. Therefore, the committee abstains from giving a health-based recommended OEL or reference value.

#### Himic anhydride (HA)

No human or animal data on exposure and response were available. Therefore, the committee abstains from giving a health-based recommended OEL or reference value.

#### Maleic anhydride (MA)

No human or animal data on exposure and response were available. Therefore, the committee abstains from giving a health-based recommended OEL or reference value.

#### Pyromellitic dianhydride (PMDA)

No human or animal data on exposure and response were available. Therefore, the committee abstains from giving a health-based recommended OEL or reference value.

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### Succinic anhydride (SA)

No human or animal data on exposure and response were available. Therefore, the committee abstains from giving a health-based recommended OEL or reference value.

### Tetrabromophthalic anhydride (TBPA)

No human or animal data on exposure and response were available. Therefore, the committee abstains from giving a health-based recommended OEL or reference value.

### Tetrahydrophthalic anhydride (THPA)

No human or animal data on exposure and response were available. Therefore, the committee abstains from giving a health-based recommended OEL or reference value.

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## 3.5 Chemical and immunological resemblance

Ten of the fourteen cyclic acid anhydrides, which are evaluated in this report, have been shown to have allergic sensitizing properties. For the other four anhydrides (DSA, THPA, SA and TBPA) no experimental or observational data are available, but based on chemical resemblance the committee considers it likely that these possess also sensitizing properties.

The committee is concerned that only for a few of the cyclic acid anhydrides (TMA and HHPA) sufficient data on exposure levels, cyclic acid anhydride-induced specific sensitisation, and allergic respiratory symptoms are available to allow an HBR-OEL or reference values. Since the DECOS considers it important that workers are protected against the sensitizing properties, it has explored the possibility of recommending an HBR-OEL for those cyclic acid anhydrides for which insufficient exposure-response data were available, based on the recommendations made for TMA and HHPA. This is done by exploring resemblances in chemical composition and reactivity, and in immunological reactivity or potency.

The sensitizing potential of a substance is related to its ability to react with proteins to form covalently linked conjugates, and recognition of these by the immune system. This binding to proteins is mainly dependent on the electrophilic reactivity of the substance. The committee noted that certain cyclic acid

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anhydrides have similar electrophilic reactivity, such as HHPA, MHPA, MTHPA, and THPA (see annex D). These data confirm the concept that all cyclic acid anhydrides are likely to have sensitizing potential. However, chemical resemblance does not automatically have a predictive value for the immunological reactivity, since small changes in molecular structure may have a considerable impact on sensitizing potency.

Regarding sensitizing potency, currently, QSAR approaches for indicating relative sensitizing potencies have not been developed. However, Welinder *et al.* (1995) and Zhang *et al.* (1997) have attempted to rank cyclic anhydrides according to the induction of specific IgG antibodies upon one single intradermal exposure, at a standardized concentration.<sup>32,41</sup> They found considerable differences in the magnitude of the response. Yet, the study did not take differences in kinetics of the antibody production into consideration, and therefore the outcome of these studies cannot be taken as a direct indication of differential potency of the chemicals tested.

An alternative way to evaluate relative potency of sensitizers is the murine local lymph node assay (LLNA).<sup>6,18,30</sup> The assay is developed for skin sensitizing substances, but so far all respiratory sensitizers tested were also positive. The LLNA is based upon measurement of the induction of the T lymphocyte proliferative response in skin draining lymph nodes following topical exposure of mice to chemicals. T lymphocyte proliferative responses are necessary for the acquisition of sensitization caused by skin contact and inhalation.

The dose-response curve in the LLNA can be interpolated to provide a dose that fails to induce a positive response. Furthermore, the so-called EC3 value, that is the dose estimated to cause a three-fold increase in LLNA proliferative activity compared to concurrent vehicle controls, may be indicative of the relative potencies of the chemicals tested.<sup>6,18,30</sup> The relative ranking of different skin sensitizers established according this scheme appear to correlate well with the information on potency coming from experience in humans.<sup>5,11</sup> However, whether or not this would also apply to respiratory sensitizers remains to be established. In a preliminary study using model respiratory sensitizers, the vigor of responses after skin sensitization, as done in the LLNA, differed somewhat as compared to the vigor of the responses after respiratory sensitization (Arts *et al.* 2008).<sup>42</sup>

Regarding cyclic acid anhydrides, up to now EC3 values have only been obtained for TMA and PA (TMA, 0.22%; PA, 0.36%; estimated concentration in percentage required for a three-fold increase in LLNA proliferative activity).<sup>30</sup> The values indicate that both anhydrides have at least a strong skin sensitizing

potential. There are also LLNA assays performed on MA and HHPA, but from these data no EC3 values can be derived.<sup>27</sup>

Finally, cross-reactivity in humans between some anhydrides has been proposed (using skin prick tests, specific IgE determination, and cross-inhibition tests), such as between MTHPA, HHPA and MHHPA.<sup>17,32</sup> A cross-reaction occurs when a specific antibody attaches an allergen or part of an allergen other than its target allergen. It usually involves allergens that are structurally very similar. Also, it is suggested that TMA-sensitized animals react immunologically to TCPA-, PA- and MA-protein conjugates.<sup>17,22,40</sup> However, for PA-exposed human subjects the cross-reactivity with other anhydrides was less pronounced. The committee noted that the findings depended partly on the choice of the immunological tests used. Furthermore, the committee emphasizes that if an antibody of allergen A cross-reacts with allergen B, this does not necessarily mean that antibody of allergen B cross-reacts also with allergen A. Moreover, cross-reactivity does not indicate whether allergen A induces sensitization at the same exposure level as allergen B.

In conclusion, the committee is of the opinion that it is likely that all cyclic acid anhydrides evaluated in this report have sensitizing properties. However data on chemical composition and reactivity, immunological reactivity, and cross-reactivity are insufficient. Therefore, the committee cannot make reliable predictions on whether an HBR-OEL or reference value for one cyclic acid anhydride will also protect against the sensitizing properties of other cyclic acid anhydrides. The committee notes that it would be prudent to avoid respiratory exposure to cyclic acid anhydrides as much as possible, in particular in those conditions where exposure exceeds concentrations in which the better studied anhydrides, such as TMA and HHPA, are known to cause sensitization.

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### 3.6 Groups at risk

It should be taken into account that persons who smoke or have a genetic predisposition (atopy) may be at higher risk in developing allergic sensitisation and airway symptoms when exposed to cyclic acid anhydrides.<sup>4,23,24,33,34,36</sup> To what extent this may increase the risk is however unknown.

Furthermore, it should be taken into account that workers, who are sensitized to one of the cyclic acid anhydrides, are likely to be at increased risk in becoming sensitized for other cyclic acid anhydrides.

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### 3.7 Research needs

There is an urgent need for information on exposure-response relationships after inhalatory exposure to individual cyclic acid anhydrides at low levels. Also a better view should be given to the influence of additional risk factors (*i.e.*, genetic predisposition, exposure pattern) on the exposure-response relationships, and on relative potency differences among the cyclic acid anhydrides. Long-term animal studies should be performed to evaluate long-term carcinogenic and non-carcinogenic effects.

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- A Request for advice
- 
- B The committees
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- C Comments from public review draft
- 
- D Physical and chemical properties
- 
- E Results of the curve fitting analysis for TMA, HHPA and MHPA
- 
- F Abbreviations

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## **Annexes**



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## **Request for advice**

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

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

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

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

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

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in 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.

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## The committees

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### The Dutch expert Committee on Occupational Safety (DECOS)

- G.J. Mulder, *chairman*  
emeritus professor of toxicology, Leiden University, Leiden
  - R.B. Beems  
toxicologic pathologist, formerly employed at the National Institute for Public Health and the Environment, Bilthoven
  - P.J. Boogaard  
toxicologist, Shell International BV, The Hague
  - J.J.A.M. Brokamp, *advisor*  
Social and Economic Council, The Hague
  - D.J.J. Heederik  
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  - R. Houba  
occupational hygienist, The Netherlands Expertise Centre for Occupational Respiratory Disorders, Utrecht
  - H. van Loveren  
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Professor of translational toxicology, Wageningen University, Wageningen, and TNO Quality of Life, Zeist
- P.B. Wulp  
occupational physician, Labour Inspectorate, Groningen
- J.M. Rijnkels, *scientific secretary*  
Health Council of the Netherlands, The Hague
- A.S.A.M van der Burght, *scientific secretary*  
Health Council of the Netherlands, The Hague

#### The Health Council and interests

Members of Health Council Committees – which also include the members of the Advisory Council on Health Research (RGO) since 1 February 2008 – 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

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they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

#### The Nordic Expert Group (NEG)

- G. Johanson, *chairman*  
professor of occupational toxicology, Karolinska Institutet, formerly the National Institute for Working Life (Sweden)
- V. Kristjansson  
organic chemist, Administration of Occupational Safety and Health (Iceland)
- K. Savolainen  
professor of toxicology, Finnish Institute of Occupational Health (Finland)
- V. Skaug  
toxicologist, occupational physician, National Institute of Occupational health (Norway)
- K. Sørig Hougaard  
toxicologist, National Research Centre for the Working Environment, formerly the Institute of Occupational Health (Denmark)
- J. Järnberg, *scientific secretary*  
Swedish Work Environment Authority, formerly the National Institute for Working Life (Sweden)





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## **Comments from public review draft**

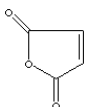
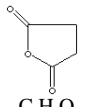
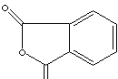

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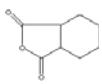
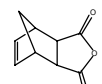
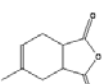

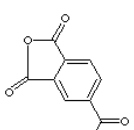
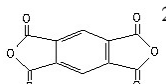

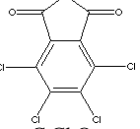
A draft of this advisory report was released in 2009 for public review. The following organisations and persons have commented on the draft:

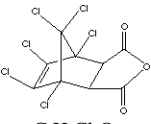
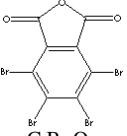
- A.G.M. van Oostrum, Ministry of Social Affairs and Employment, The Netherlands.
- R. Zumwalde, National Institute for Occupational Safety and Health, the USA.



## Physical and chemical properties

Name cyclic acid anhydride Cas No.	Structural formula	Mw	Melting point (°C)	Boiling point (°C)	Solubility in water EST/EXP	Log P <sub>ow</sub> EST/EXP	Van der Waals volume (Å) <sup>3</sup>	Electrophilicity (eV)
Maleic anhydride (MA) 108-31-6	 <chem>C4H2O3</chem>	98.06	51-53	202 (decomposes)	4,910 mg/L (25°C) EST	1.62 EST	87.89	-1.6186
Succinic anhydride (SA) 108-30-5	 <chem>C4H4O3</chem>	100.07	119-120	261	23,800 mg/L (25°C) EST	0.81 EST	91.68	0.11291
Phthalic anhydride (PA) 85-44-9	 <chem>C8H4O3</chem>	148.2	130-131	284-295	6,200 mg/L (25°C) EXP	1.60 EXP	139.05	-1.4580
Tetrahydrophthalic anhydride (THPA) 85-43-8	 <chem>C8H8O3</chem>	152.16	102	195 (6.7 kPa)	1,580 mg/L (25°C) EST	1.96 EST	147.25	0.15527

Hexahydrophthalic anhydride (HHPA) 85-42-7		154.17	3 0	296	1,760 mg/L (25°C) EST	2.17 EST	151.63	0.2624
Himic anhydride (HA) 826-62-0		164.16	158-164	N.D.	N.D.	1.85	156.78	0.24708
Methyl tetrahydrophthalic anhydride (MTHPA) 26590-20-5		166.18	N.D.	N.D.	465 mg/L (25°C) EST	2.51 EST	165.40	0.16852
Methyl hexahydrophthalic anhydride (MHHPA) 25550-51-0		168.19	-29	120 (130 Pa)	36,000 mg/L (20°C)	2.59	169.70	0.26825
Trimellitic anhydride (TMA) 552-30-7		192.13	161-164	390	1,040 mg/L (25°C) EST	1.95 EST	166.96	1.9950
Pyromellitic dianhydride (PMDA) 89-32-7		218.1	152	397-400	59.7 mg/L (25°C) EST	2.14 EST	178.63	-2.8549
Dodecenyloxy-succinic anhydride (DSA) 25377-73-5		266.38	-30	190-215	0.064 mg/L (25°C) EST	6.41 EST	307.46	0.17964
Tetrachlorophthalic anhydride (TCPA) 117-08-8		285.88	55-257	371	0.048 mg/L (25°C) EST	4.65 EST	191.46	-2.1143

Chlorendic anhydride (CA) 115-27-5		370.83	235-239	N.D.	0.098 mg/L (25°C) EST	4.37 EST	225.96	-0.5513
	$C_9H_2Cl_6O_3$							
Tetrabromophthalic anhydride (TBPA) 632-79-1		463.72	280	N.D.	0.019 mg/L (25°C) EST	5.63 EST	209.53	-2.19665
	$C_8Br_4O_3$							

N.D., no data available; EST, estimated by the Physical Properties Database (PHYSPROP); EXP, based on experimental data.



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## **Results of the curve fitting analysis for TMA, HHPA and MHHPA**

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The BenchMark Dose (BMD) software of the US-EPA is used to analyze the data on the effects of a chemical on animal or human health in order to determine, as accurately as possible, the relationship between a given exposure level and the likelihood of its detrimental effects, the so-called responses. The statistical uncertainty to which this data is invariably subject is incorporated into the calculations.

Regarding cyclic acid anhydrides, the critical effect considered to be harmful is IgE-mediated sensitisation. DECOS calculates a BMD corresponding to 0.1% and 1.0% (or 10%) additional sensitization due to occupational exposure to these substances. In addition, also the 95% (or 90%) lower limit of the BMD, *i.e.* BMDL was determined. The BMDL gives an indication about the uncertainty in the curve fitting.

Below are shown the data and the outcomes of the curve fitting analyses of TMA, HHPA and MHHPA. The analyses have been performed using the BenchMark Software of the US EPA (Version 2.0), and the log-likelihood ratio test for model fitting.

## Trimelletic anhydride: Grammer-study

### Data

Exposure group (in µg TMA/m <sup>3</sup> ) <sup>a</sup>	Concentration used for BMD-analysis	Number of persons in group	Number of persons posi- tive for TMA-induced IgE-mediated sensitiza- tion
<0.5	0.25	24	0
0.5	0.5	98	0
2	2	79	5
36	36	57	6
130	130	28	7

<sup>a</sup> Source Grammer *et al.* (1999).

### Results BMD-analysis

Model tested	Log-likelihood			Calculated difference log-likeli- hoods <sup>a</sup>	Accept model	BMR 1%		BMR 10%	
	Fitted model	Full model	d.f.			BMD	BMDL <sup>b</sup>	BMD	BMDL <sup>b</sup>
Gamma	57.95	53.56	3	4.39	No	4.35	2.66	45.64	27.89
Log-logistic	57.86	53.56	3	4.30	No	3.82	2.17	42.06	23.91
Log-probit <sup>c</sup>	56.36	53.56	3	2.80	Yes	0.37	0.04	17.83	8.22
Multi-stage	57.95	53.56	3	4.39	No	4.35	2.66	45.64	27.88
Weibull	57.95	53.56	3	4.39	No	4.35	2.66	45.64	27.88

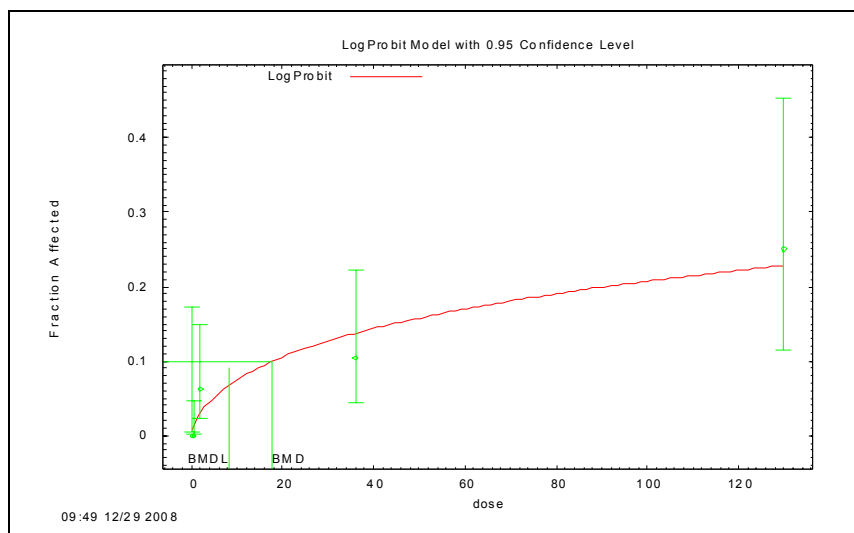
Values are rounded off by two decimals. BMD and BMDL in µg /m<sup>3</sup>.

<sup>a</sup> Critical difference log-likelihood at d.f. (degrees of freedom) 3 is 3.91. 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.

<sup>b</sup> Data shown are based on the 95% confidence interval for the BMD.

<sup>c</sup> The BMDS software of EPA contains a constraint in the log-probit model. By switching off the mark at 'restrict slope >=1' the problem is solved. Presented data: mark is put off.





### Evaluation and conclusion DECOS

Of the models tested only the log-probit model passes the log-likelihood ratio test. This means that only the BMD calculation from this model can be used as point of departure in deriving a health-based OEL or reference values for TMA.

Next, the committee has to establish exposure levels corresponding to pre-defined additional risks on sensitization. The committee prefers to use the fitted dose response curve. However, in case of TMA, the variability in the exposure levels corresponding to the lower sensitization risks is high. The committee notes that at a BMR of 1% the corresponding BMDL of the log-probit is rather high. This implies that the power of discernment at BMR1% is too low, and the model uncertainty too high. The same outcome was obtained at BMR values up to 10% (data not shown). Only at a BMR10%, a reliable BMDL was derived.

Therefore, the committee decided to use that concentration as starting point which has a limited variability. From this exposure level, the committee extrapolates linearly to the exposure levels corresponding to the lower additional sensitization risks. The committee determined an exposure level at which 10% of the occupationally exposed population will get specifically sensitised to TMA as the starting point. This level corresponds to 18  $\mu\text{g TMA}/\text{m}^3$ .

The outcome of the analysis implies furthermore that no reliable threshold level can be discerned from the available data.

From the foregoing, the DECOS concludes that for TMA reference values should be derived, and that the BMD 17.83  $\mu\text{g}/\text{m}^3$ , which corresponds with a BMR of 10%, is the best point of departure in deriving such values.

### Hexahydrophthalic anhydride (HHPA): Rosqvist-study and Nielsen-study

#### Data Rosqvist-study

Exposure group (in $\mu\text{g}$ HHPA/ $\text{m}^3$ ) <sup>a</sup>	Concentration used for BMD-analysis	Number of persons in group	Number of persons posi- tive for TMA-induced IgE-mediated sensitisa- tion
<1	0.5	35	2
1-3	2	37	7
3-9	6	32	8
>9	18	35	10

<sup>a</sup> Source Rosqvist *et al.* (2003).

#### Results curve fitting analysis Rosqvist-study

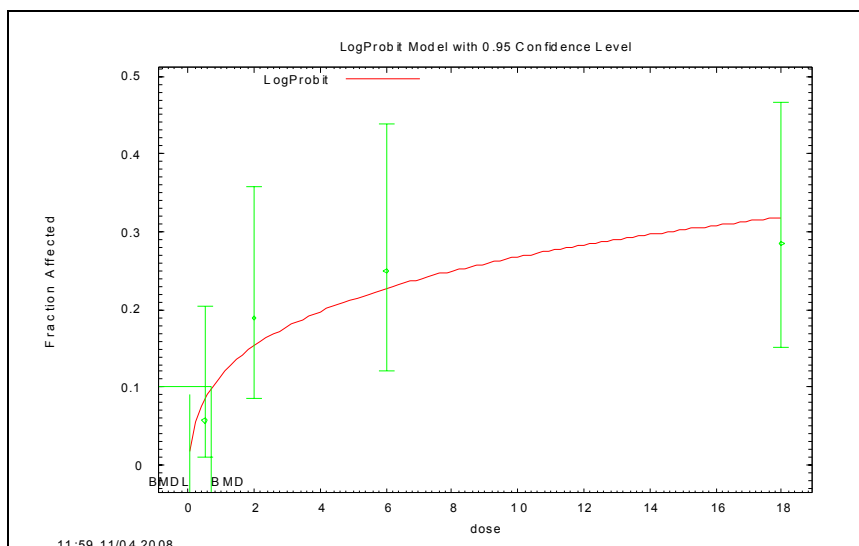
Model tested	Log-likelihood			Calculated difference log-likeli- hoods <sup>a</sup>	Accept model	BMR 1%		BMR 10%	
	Fitted model	Full model	d.f.			BMD	BMDL <sup>b</sup>	BMD	BMDL <sup>b</sup>
Gamma	66.15	64.55	2	1.60	Yes	0.67	0.34	6.99	3.58
Log-logistic	66.03	64.55	2	1.48	Yes	0.54	0.24	5.94	2.68
<i>Log-probit</i>	65.05	64.55	2	0.50	<i>Yes</i>	0.01	$3 \times 10^{-7}$	0.73	0.03
Multi-stage	66.15	64.55	2	1.60	Yes	0.67	0.34	6.99	3.58
Weibull	66.15	64.55	2	1.60	Yes	0.67	0.34	6.99	3.58

Values are rounded off by two decimals. BMD and BMDL in  $\mu\text{g}/\text{m}^3$ .

<sup>a</sup> Critical difference log-likelihood at d.f. (degrees of freedom) 2 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.

<sup>b</sup> Data shown are based on the 95% confidence interval for the BMD.

<sup>c</sup> The BMDS software of EPA contains a constraint in the log-probit model. By switching off the mark at 'restrict slope  $\geq 1$ ' the problem is solved. Presented data: mark is put off.



### Data Nielsen-study

Exposure group (in $\mu\text{g HHPA}/\text{m}^3$ ) <sup>a</sup>	Concentration used for BMD-analysis <sup>b</sup>	No. of persons in group	IgE-mediated sensitisation	
			No. of positive <sup>c</sup>	% of positive
0	0	57	(0)	0
< 10	5	53	(7)	13
10-50	30	72	(19)	26
> 50	70	29	(6)	21

<sup>a</sup> Source Nielsen *et al.* (2001).

<sup>b</sup> The concentrations used for BMD-analysis are the same as used by the investigators for their analyses.

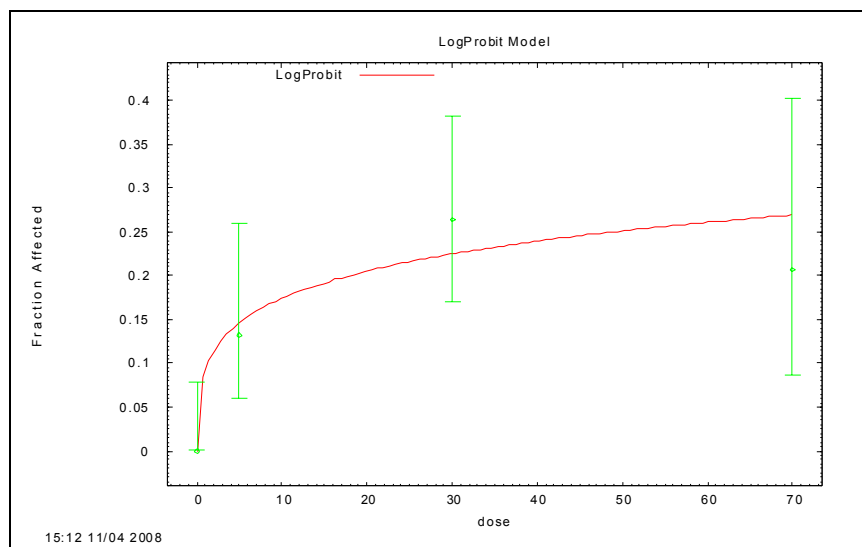
<sup>c</sup> Data between brackets indicates number of positive cases, based on data on percentage of positive cases.

## Results curve fitting analysis Nielsen-study

Model tested	Log-likelihood			Calculated difference log-likelihoods <sup>a</sup>	Accept model?	BMR 1%		BMR 10%	
	Fitted model	Full model	d.f.			BMD	BMDL <sup>b</sup>	BMD	BMDL <sup>b</sup>
Gamma	83.40	77.02	2	6.38	No	1.64	1.06	17.24	11.12
Log-logistic	82.55	77.02	2	5.53	No	1.18	0.71	13.03	7.89
Log-probit	77.67	77.02	2	0.65	Yes	0.002	≈0.00	1.23	≈0.00
Multi-stage	83.40	77.02	2	6.38	No	1.64	1.06	17.24	11.12
Weibull	83.40	77.02	2	6.38	No	1.64	1.06	17.24	11.12

Values are rounded off by two decimals. BMD and BMDL in  $\mu\text{g}/\text{m}^3$ .

- <sup>a</sup> Critical difference log-likelihood at d.f. (degrees of freedom) 2 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.
- <sup>b</sup> Data shown are based on the 95% confidence interval for the BMD.
- <sup>c</sup> The BMDS software of EPA contains a constraint in the log-probit model. By switching off the mark at 'restrict slope >=1' the problem is solved. Presented data: mark is put off.



## Evaluation and conclusion DECOS

In the study of Rosqvist all models passes the log-likelihood test. In the study of Nielsen only the log-probit model passes the log-likelihood ratio test. As for TMA the log-probit model was used, the committee prefers to use this models for the other anhydrides as well. This means that the BMD of this model was be

used as point of departure in deriving health-based OEL or reference values for HHPA.

Next, the committee has to establish exposure levels corresponding to pre-defined additional risks on sensitization. The committee prefers to use the fitted dose response curve. However, in case of HHPA, the variability in the exposure levels corresponding to the lower sensitization risks is high. The committee notes that at a BMR of 1% the corresponding BMDL of both the log-probit models (Nielsen and Rosqvist) is rather high. This implies that the power of discernment at BMR1% is too low, and the model uncertainty too high. The same outcome was obtained at BMR values up to 10% (data not shown). Only at a BMR10%, a reliable BMDL was derived in the study of Rosqvist.

The outcome of the analysis implies furthermore that no reliable threshold level can be discerned from the available data.

From the foregoing, the DECOS concludes that for HHPA reference values should be derived, and that the BMD of 0.73  $\mu\text{g}/\text{m}^3$ , which corresponds with a BMR of 10%, is the best point of departure in deriving such values.

---

### Methylhexahydrophthalic anhydride (MHHPA): Nielsen-study

#### Data

Exposure group (in $\mu\text{g}$ MHHPA/ $\text{m}^3$ ) <sup>a</sup>	Concentration used for BMD-analysis <sup>b</sup>	No. of persons in group	IgE-mediated sensitisation	
			No. of positive <sup>c</sup>	% of positive
0	0	57	(0)	0
< 10	5	53	(8)	15
10-50	30	72	(19)	26
> 50	70	29	(5)	17

<sup>a</sup> Source Nielsen *et al.* (2001).

<sup>b</sup> The concentrations used for BMD-analysis are the same as used by the investigators for their analyses.

<sup>c</sup> Data between brackets indicates number of positive cases, based on data on percentage of positive cases.

## Results BMD analysis

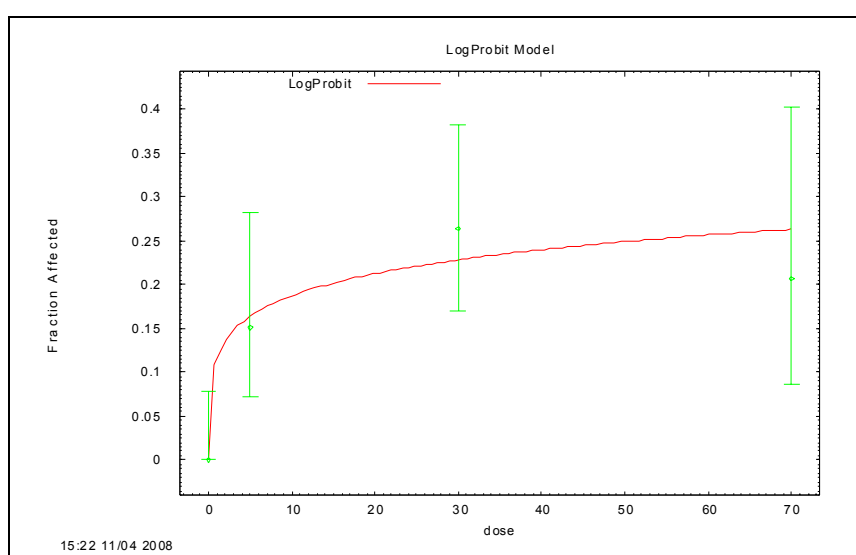
Model tested	Log-likelihood			Calculated difference log-likelihoods <sup>a</sup>	Accept model?	BMR 1%		BMR 10%	
	Fitted model	Full model	d.f.			BMD	BMDL <sup>b</sup>	BMD	BMDL <sup>b</sup>
Gamma	85.33	77.37	2	7.96	No	1.93	1.18	20.28	12.36
Log-logistic	84.64	77.37	2	7.27	No	1.41	0.78	15.53	8.62
<i>Log-probit</i>	78.29	77.37	3	0.92	Yes	n.d.	≈0.00	0.22	1x10 <sup>-15</sup>
Multi-stage	85.33	77.37	2	7.96	No	1.93	1.18	20.28	12.36
Weibull	85.33	77.37	2	7.96	No	1.93	1.18	20.28	12.36

Values are rounded off by two decimals. BMD and BMDL in  $\mu\text{g}/\text{m}^3$ .

<sup>a</sup> Critical difference log-likelihood at d.f. (degrees of freedom) 2 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.

<sup>b</sup> Data shown are based on the 95% confidence interval for the BMD.

<sup>c</sup> The BMDS software of EPA contains a constraint in the log-probit model. By switching off the mark at 'restrict slope >=1' the problem is solved. Presented data: mark is put off.



## Evaluation and conclusion DECOS

Of the models tested only the log-probit model passed the log-likelihood ration test.

Next, the committee has to establish exposure levels corresponding to pre-defined additional risks on sensitization. The committee prefers to use the fitted dose response curve. However, in case of MHHPA, the variability in the exposure levels corresponding to the lower sensitization risks is high. The committee notes that at a BMR of 1% and BMR 10% the corresponding BMDLs of the log-probit are very low. This implies that the power of discernment at BMR1% and BMR10% is too low, and the model uncertainty too high.

The outcome of the analysis implies furthermore that no reliable threshold level can be discerned from the available data. From the foregoing, the DECOS concludes that for MHHPA reference values should be derived. However, data are insufficient to estimate a reliable BMDL.





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## Abbreviations

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### Cyclic acid anhydrides

<i>CA</i>	chlorendic anhydride
<i>DSA</i>	dodecenylsuccinic anhydride
<i>HA</i>	himic anhydride
<i>HHPA</i>	hexahydrophthalic anhydride
<i>MA</i>	maleic anhydride
<i>MHHPA</i>	methyl hexahydrophthalic anhydride
<i>MTHPA</i>	methyl tetrahydrophthalic anhydride
<i>PA</i>	phthalic anhydride
<i>PMDA</i>	pyromellitic dianhydride
<i>SA</i>	succinic anhydride
<i>TBPA</i>	tetrabromophthalic anhydride
<i>TCPA</i>	tetrachlorophthalic anhydride
<i>THPA</i>	tetrahydrophthalic anhydride
<i>TMA</i>	trimellitic anhydride

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

nr 2004:1517

Helena Keskinen

Part

**II**

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## **Arbete och Hälsa: Cyclic acid anhydrides**



NR 2004:I 5

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

## 136. Cyclic acid anhydrides

*Helena Keskinen*



Nordic Council of Ministers

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# Preface

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

The document on health effects of cyclic acid anhydrides was written by Dr. Helena Keskinen at the Finnish Institute of Occupational Health, Helsinki, Finland and has been reviewed by DECOS as well as by NEG.

Editorial work and technical editing was performed by Anna-Karin Alexandrie, and Jill Järnberg, NEG's scientific secretaries at the National Institute for Working Life in Sweden.

All criteria document produced by the Nordic Expert Group may be downloaded from [www.nordicexpertgroup.org](http://www.nordicexpertgroup.org).

We acknowledge the Nordic Council for its financial support of this project.

G.J. Mulder  
Chairman  
DECOS

G. Johanson  
Chairman  
NEG

## Abbreviations

### *Cyclic acid anhydrides*

CA	chlorendic anhydride
DSA	dodecenylsuccinic anhydride
HA	himic anhydride
HHPA	hexahydrophthalic anhydride
MA	maleic anhydride
MHHPA	methyl hexahydrophthalic anhydride
MMA	methyl maleic anhydride
MPA	methyl phthalic anhydride
MTHPA	methyl tetrahydrophthalic anhydride
PA	phthalic anhydride
PMDA	pyromellitic dianhydride
SA	succinic anhydride
TBPA	tetrabromophthalic anhydride
TCPA	tetrachlorophthalic anhydride
THPA	tetrahydrophthalic anhydride
TMA	trimellitic anhydride

### *Other abbreviations*

BAL	bronchoalveolar lavage
ECD	electron capture detector
FID	flame ionisation detector
GC	gas chromatography
GPSA	guinea pig serum albumin
HPLC	high performance liquid chromatography
HSA	human serum albumin
MS	mass spectrometry
NIOSH	National Institute of Occupational Safety and Health
PVC	polyvinyl chloride
RSA	rat serum albumin
TLV	threshold limit value
TWA	time weighted average
UV	ultraviolet



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## 1. Introduction

Cyclic acid anhydrides are widely used in the chemical industry, especially in the manufacture of polyester and alkyd resins and plasticizers for thermoplastic polymers. The anhydrides are also used as hardeners for epoxy resins and chain cross-linkers for thermoplastic polymers. Workers are exposed to acid anhydrides in powder form during various manufacturing processes, such as during synthesis or when the acid anhydrides are used as starting agents for thermosetting products. Workers are also exposed to anhydride fumes in hot processes, such as when epoxy resins are hardened, polyester paints are cured, alkyd or polyester painted metal surfaces are welded or when the paints are burned from surfaces.

Acid anhydrides are irritants and are especially potent as occupational sensitizers.

Phthalic anhydride was the first anhydride to be reported as a sensitizer, as early as 1939. Later several new derivatives were also found to be capable of inducing allergies.

This document deals with the following cyclic organic acid anhydrides: phthalic anhydride (PA), trimellitic anhydride (TMA), maleic anhydride (MA), hexahydrophthalic anhydride (HHPA), methyl hexahydrophthalic anhydride (MHHPA), methyl tetrahydrophthalic anhydride (MTHPA), tetrahydrophthalic anhydride (THPA), and tetrachlorophthalic anhydride (TCPA). Other anhydrides to be mentioned whenever data are available are pyromellitic dianhydride (PMDA), himic anhydride (HA), succinic anhydride (SA), dodecenylsuccinic anhydride (DSA), chlorendic anhydride (CA), and tetrabromophthalic anhydride (TBPA).

This document updates the earlier one given by the Nordic Expert Group in 1991 (101).

## 2. Substance identification

Data on the substance identification of the cyclic acid anhydrides dealt with in this document are given in Table 1 and Figure 1.

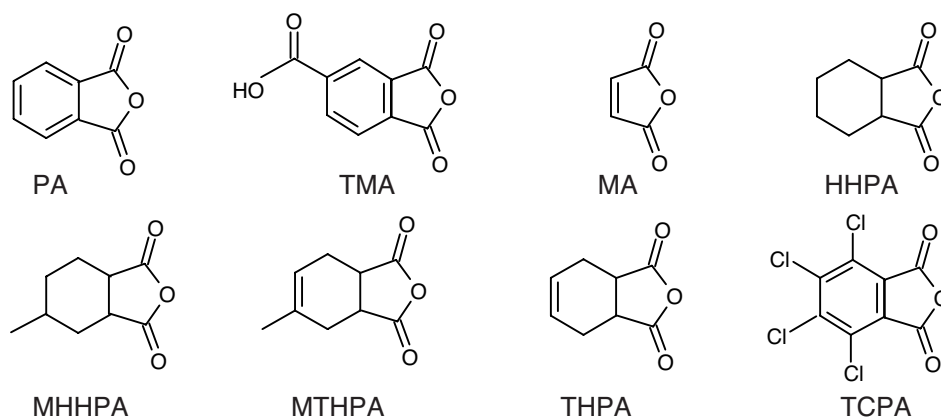
## 3. Physical and chemical properties

Data on the physical and chemical properties are presented in Tables 2a and 2b. Cyclic anhydrides are mainly powders or crystals. Methyl substitution converts them to oily liquids. A halogen – chlorine or bromine – in the molecule endows flame retardant properties (178).

**Table 1.** Substance identification of cyclic acid anhydrides (3, 24, 42, 139, 167).

Acid anhydride	CAS No	Synonyms	Molecular formula	Molecular weight
PA	85-44-9	phthalic anhydride 1,3-isobenzofurandione, 1,2-benzenedicarboxylic acid anhydride, phthalic acid anhydride, 1,3-dioxophthalan, 1,3-phthalandione	C <sub>8</sub> H <sub>4</sub> O <sub>3</sub>	148.12
TMA	552-30-7	trimellitic anhydride 1,3-dihydro-1,3-dioxo-5-isobenzofuran- carboxylic acid anhydride, trimellitic acid 1,2-anhydride	C <sub>9</sub> H <sub>4</sub> O <sub>5</sub>	192.13
MA	108-31-6	maleic anhydride 2,5-furandione, cis-butanedioic anhydride, toxilic anhydride	C <sub>4</sub> H <sub>2</sub> O <sub>3</sub>	98.06
HHPA	85-42-7	hexahydrophthalic anhydride 1,2-cyclohexanedicarboxylic anhydride	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	154.17
MHHPA	25550-51-0	methyl hexahydrophthalic anhydride hexahydromethyl-1,3-isobenzofurandione, 4-methylhexahydrophthalic anhydride	C <sub>9</sub> H <sub>12</sub> O <sub>3</sub>	168.19
MTHPA <sub>44</sub> <sup>a</sup>	26590-20-5	methyl tetrahydrophthalic anhydride 1,2,3,6-tetrahydromethylphthalic anhydride, 3a,4,7,7a-tetrahydromethyl-1,3-isobenzofurane, 4-methyl-delta 4-tetrahydrophthalic anhydride	C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>	166.19
THPA	85-43-8	tetrahydrophthalic anhydride 4-cyclohexene-1,2-dicarboxylic anhydride, 3a,4,7,7a-tetrahydro-1,3-isobenzofurandione, 1,2,3,6-tetrahydrophthalic anhydride	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	152.16
TCPA	117-08-8	tetrachlorophthalic anhydride 4,5,6,7-tetrachloro-1,3-isobenzofurandione	C <sub>8</sub> Cl <sub>4</sub> O <sub>3</sub>	285.88

<sup>a</sup>4-methyl-delta 4-tetrahydrophthalic anhydride. Commercial products contain also isomers 3-methyl-delta 4-tetrahydrophthalic anhydride (MTHPA<sub>34</sub>) and 4-methyl-delta 3-tetrahydrophthalic anhydride (MTHPA<sub>43</sub>) (112).

**Figure 1.** Structural formulas of cyclic acid anhydrides.

**Table 2a.** Physical and chemical properties of cyclic acid anhydrides (3, 24, 42, 139, 167).

	PA	TMA	MA	HHPA
Description	White crystalline needles	Crystals or needles	Colourless or white crystals, pungent odour	Clear, colourless, viscous liquid
Melting point	130.8°C	161-163.5°C	53°C	Becomes a glassy solid 35-36°C
Boiling point	284°C (sublimes)	240-245°C	202°C (sublimes)	158°C (2.3 kPa)
Vapour pressure (volatility)	<6.6 Pa at 20°C	<10 Pa at 25°C	25 Pa at 20°C	
Vapour density (air=1)	5.1		3.4	
Specific gravity	1.53		1.43 at 20°C	
Density (water=1)	1.53		1.5	1.19 (4°C)
Solubility in water	0.62 g/100 ml		40 g/100ml	
Solubility in organic solvents	Alcohol, ether	Acetone, ethyl acetate, dimethylformamide	Acetone, ethyl acetate, chloroform and benzene	Miscible with benzene, toluene, acetone, carbon tetrachloride, chloroform, ethanol and ethyl acetate. Slightly soluble in petroleum ether
Partition coefficient (octanol/water)	$\log P_{ow}$ : -0.62			
Odour threshold	0.32 mg/m <sup>3</sup>		1.23 mg/m <sup>3</sup>	
Conversion factors in air (25°C, 101.3 kPa)	1 ppm = 6.046 mg/m <sup>3</sup> 1 mg /m <sup>3</sup> = 0.165 ppm	1 ppm = 7.842 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.128 ppm	1 ppm = 4.002 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.250 ppm	1 ppm = 6.293 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.159 ppm

**Table 2b.** Physical and chemical properties of cyclic acid anhydrides (3, 24, 42, 139, 167).

	MHHPA	MTHPA	THPA	TCPA
Description	Oily liquid	Oily liquid	White crystalline powder	White, odourless, free-flowing non hygroscopic powder
Melting point	-29°C		101.9°C	254-255°C
Boiling point	120°C (130 Pa)		195°C (6.7 kPa)	371°C (sublimes)
Vapour pressure (volatility)			1.3 Pa at 20°C	
Vapour density (air=1)			5.25	
Flash point	350°C		157°C	
Density			1.375 (25/20°C)	
Solubility in organic solvents			Slightly soluble in petroleum ether and ethyl ether, soluble in benzene	
Conversion factors in air (25°C, 101.3 kPa)	1 ppm = 6.865 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.146 ppm	1 ppm = 6.783 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.147 ppm	1 ppm = 6.783 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.147 ppm	1 ppm = 11.669 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.086 ppm

## 4. Occurrence, production and use

### 4.1 Occurrence

Organic acid anhydrides are man-made chemicals commercially available at high purity as liquids or crystals, depending on the type of anhydride. They are not found in nature but may be found as environmental contaminants (183).

### 4.2 Production

The annual world production of PA has been about 2 200 000 tonnes during the past decade, the European share being about 820 000 tonnes. The main producers in Europe are Belgium, the United Kingdom, the Russian Federation, Italy, and Germany. Of the Scandinavian countries, Sweden has production of PA, about 30 000 tonnes annually. PA production in 1996 was about 830 000 in Asia, about 420 000 in North America and 150 000 tonnes in South America. According to annual export statistics Belgium, the United States and Italy are the main countries to produce MA. The exported amounts were 58 000, 44 000, and 25 000 tonnes, respectively, in 1997 (179).

### 4.3 Production processes

Industrial processes used in the production of cyclic acid anhydrides are shown in Table 3. Technical anhydride products may contain other related cyclic anhydrides as impurities or they can be mixtures of different isomers. For example, PA contains 0.03% MA and MHHPA contains 4.2% MTHPA (150, 178). The technical product of MTHPA is reported to contain the three isomers 4-methyl-delta 4-tetrahydrophthalic anhydride, 3-methyl-delta 4-tetrahydrophthalic anhydride, and 4-methyl-delta 3-tetrahydrophthalic anhydride (112).

**Table 3.** Industrial processes used in the production of cyclic acid anhydrides (24, 178, 217).

Cyclic acid anhydride	Production process
PA	In 1872 by oxidation of naphthalene. After 1960 by oxidation of <i>o</i> -xylene. The technical grade product contains 99.9% PA, 0.03% MA, and 0.03% benzoic acid.
TMA	Sublimation of trimellitic acid above its melting point or by heating crude trimellitic acid with vanadium pentoxide.
MA	Catalytic oxidation of benzene or C <sub>4</sub> hydrocarbons.
HHPA	Hydrogenation of THPA.
MHHPA	Hydrogenation of MTHPA.
MTHPA	Diels-Alder reaction between isoprene and MA.
THPA	Diels-Alder reaction between MA and butadiene.
TCPA	Chlorination of PA.

**Table 4.** Annual import (in tonnes) of acid anhydrides in the Nordic countries and in the Netherlands in 1997 (179).

Acid anhydride	Denmark	Finland	Norway	Sweden	Iceland	The Netherlands
PA	1 263	10 457	– <sup>a</sup>	2 784	– <sup>a</sup>	31 706
MA	– <sup>a</sup>	2 644	5 722	539	– <sup>a</sup>	7 177

<sup>a</sup>Information lacking.

#### 4.4 Use

The information available on the annual import of acid anhydrides in the Nordic countries and in The Netherlands is shown in Table 4. In the trade reports the cyclic acid anhydrides are mainly grouped with related chemicals.

The cyclic acid anhydrides are mainly used in the manufacture of polyester and alkyd resins and plasticizers and as epoxy resin hardeners. The different types of uses for acid anhydrides are presented in Table 5.

## 5. Occupational exposure data

The concentrations of organic acid anhydrides measured in workplace air are presented in Table 6.

Early measurements of PA showed very high exposure levels (320-17 400  $\mu\text{g}/\text{m}^3$ ) especially when process difficulties and in loading of reactors occurred (136, 147). When alkyd and unsaturated polyester resins were produced, MA and TMA were used in addition to PA (136).

In a more recent study, in which both particles and vapours were sampled, the full-shift personal samples taken during the production of alkyd resins gave 10-100 fold lower concentrations of PA than in the previous studies. The task-specific PA concentrations were higher, up to 1 860  $\mu\text{g}/\text{m}^3$  during charging, giving better information of the peak exposures during the working day. The results of 3 factories were given. In 2 of the factories (factory 1 and 3), TMA and MA were intermittently used in addition to PA and were only detectable in half of the samples (182).

The PA concentrations during polyvinyl chloride (PVC) processing were low, but measurable (180).

Exposure measurements of TMA were carried out during the manufacture of cushioned flooring. The highest exposure levels were in charging (150-20 433  $\mu\text{g}/\text{m}^3$ ), when both particles and vapours were sampled. Otherwise only few values were above the occupational exposure limit 40  $\mu\text{g}/\text{m}^3$  but the results were based on few samples, 1-4 per task (182).

The exposure levels of MA have been low even in charging in the production of alkyd resins (182).

In two plants for epoxy resin isolation, the highest HHPA concentrations were found in casting (130-500  $\mu\text{g}/\text{m}^3$ ) based on several personal samples. In one plant



**Table 5.** Use of organic acid anhydrides (24, 42, 80, 140, 167, 178, 192, 195).

Acid anhydride	Use
PA	Manufacture of phthalate plasticizers, phthaleins, unsaturated polyester resins, alkyd resins, halogenated phthalic anhydrides, and phthalocyanide dyes. Preparation of benzoic acid. Hardener in epoxy resins.
TMA	Manufacture of plasticizers with high thermal resistance and of unsaturated polyester resins. Hardener in epoxy resins.
MA	Manufacture of unsaturated polyesters, alkyd resins, lacquers, plasticizers, copolymers, lubricants, pesticides, pharmaceuticals, and permanent-press resins (textiles). Synthesis of some organic acid anhydrides. Diels-Alder reaction.
HHPA	Manufacture of alkyd resins, plasticizers, insect repellents, and rust inhibitors. Hardener in epoxy resins.
MHHPA	Hardener in epoxy resins.
MTHPA	Hardener in epoxy resins.
THPA	Production of unsaturated polyester resins and alkyd resins with increased resistance to water and solvents. Starting agent for light coloured alkyds, polyesters, and plasticizers used in adhesives. Intermediate for pesticides. Hardener of epoxy resins.
TCPA	Flame retardant in unsaturated polyester resins, polyurethane foams and surface coatings and plasticizers. Intermediate in the production of dyes and pharmaceuticals. Hardener in epoxy resins.
PMDA	Production of polyimide resins. Polyimide resins are high heat resistant polymers with good electrical and physical properties. They are used for films, fibres, moulding compounds, varnishes, and wire coatings.
HA	Production of fire retardants.
SA	Production of adhesives, dyes, and elastomers. It is used as a cross-linking agent for epoxy resins and as a chemical intermediate for paints containing drying oils, succinylated monoglyceride food emulsifiers, silver haloid photographic emulsions, pharmaceuticals, alkyd resins, and plasticizers.
DSA	DSA is used in alkyd, epoxy and other resins, anticorrosive agents, plasticizers, and wetting agents for bituminous compounds.
CA	Flame retardant in polyester resins and plasticizers.
TBPA	Flame retardant in unsaturated polyester resins and moulding products.

(plant A) also MHPA was used and exposure levels up to  $403 \mu\text{g}/\text{m}^3$  of MHPA were found in casting (195). In casting operations in, for example, the electronics industry, solid or semi-solid anhydride curing agents (MHPA, HHPA and MHPA) are heated, and the compounds are vaporised (sublimates). The major exposure in these industries may derive from leakages from ovens during the subsequent curing step (195).

When epoxy resin was handled in the wet part of the process in the manufacture of barrels, MHPA concentrations of  $380 \mu\text{g}/\text{m}^3$  were measured, but exposure levels up to  $3\,000 \mu\text{g}/\text{m}^3$  of MHPA were found close to the heated, wet material before curing (192).

In the manufacture of condensers 5 area samples were collected. The exposure levels of MHPA in assembling and hardening were between  $36.5\text{-}695 \mu\text{g}/\text{m}^3$  (geometric mean). Earlier, before worsening of the work hygiene, 10-fold lower concentrations of MHPA had been measured (82).

TCPA exposure has been followed in the manufacture of solenoid coils when TCPA cured epoxy resin was used. Exposure levels of  $140\text{-}590 \mu\text{g}/\text{m}^3$  were measured in the moulding. After improvements in work hygiene the exposure levels were decreased to  $<10\text{-}110 \mu\text{g}/\text{m}^3$  (118).

When products containing rest monomers or esters of cyclic *ortho*-dicarboxylic acids are heated, anhydrides tend to be released and sublimate into the ambient air. This problem occurs in several work processes, e.g. in the curing of polyester powder paints containing unsaturated polyesters at elevated temperatures. PA has been detected when diethylhexyl phthalate, an ester plasticizer, is heated (147). Cyclic anhydrides have also been detected in welding fumes from painted steel (73, 102).

All cyclic anhydrides react with water, especially if warmed, and the corresponding acid is formed.

When chlorinated anhydrides are heated to the point of decomposition, chlorine is released and toxic vapours are emitted (111).

To conclude, the data on exposure measurements from the workplaces are limited and the measurements have been prompted by work-related health problems at the work place. Mostly the number of samples is small. When filters are not used in the sampling, exposure in particulate form may be missed.

The highest exposure levels have been found in flaking, sacking, loading of reactors and charging with anhydrides in solid form, especially with PA, TCPA, and TMA. The exposure levels from the last decade have generally been lower than the earlier ones pointing to the awareness of the harmful effects and to improved occupational hygiene. Anhydride vapours and sublimates are found in the work atmosphere when products containing anhydrides are heated. Often several anhydrides as well as other sensitising or irritating agents are included in the processes. This makes the exposure more difficult to assess. It is not possible to group cyclic acid anhydrides according to vapour pressure because of lack of data.

**Table 6.** Exposure measurements of cyclic anhydrides in workplaces.

Acid anhydride Industry	Processing method/job	No. of samples	Sampling type/time	Results ( $\mu\text{g}/\text{m}^3$ )	Method	Reference
<b>PA</b> Production of PA and unsaturated polyester resin	Flaking	6	Breathing zone/60 min	AM (range) 1 490 (1 260-1 620)	Membrane filter, TENAX tube, GC-ECD	(147)
	Sacking	6		520 (320-720)		
	Flaking (process difficulties)	4		2 950 (2 340-3 560)		
	Sacking ( " " )	4		1 180 (980-1 380)		
Production of alkyd and/or unsaturated polyester resins	<i>Plant A</i>		Personal, hours	TWA (range)	Glass-fibre filter, HPLC	(136)
	Loading of reactors	6	1.9	6 100 (1 800-14 900)		
	Other work	6	12	<0.1		
	<i>Plant B</i>		6.0	6 800 (1 500-17 400)		
PVC processing (incl. di-ethylhexyl phthalate)	Extrusion	16	Stationary/1.5-3 hours	AM $\pm$ SD	Membrane filter, TENAX tube, GC-ECD	(180)
	Catendering	8		0.3 $\pm$ 0.5		
	Welding	4		0.2 $\pm$ 0.1		
	Injection moulding	2		5.0 $\pm$ 2.0		
	Thermofoming	2		<0.02		
	Spread coating	4		0.1 $\pm$ 0.05 1.2 $\pm$ 0.2		

**Table 6.** Cont.

Acid anhydride Industry	Processing method/job	No. of samples	Sampling type/time	Results ( $\mu\text{g}/\text{m}^3$ )	Method	Reference
<b>PA</b> Production of alkyd resins	<i>Factory 1</i>		Personal/full-shift	AM	Glass-fibre filter, TENAX tube, HPLC-UV	(182)
	Resin operator	9		25.1		
	Filter operator	9		1.5		
	Warehouseman	1		1.0		
	<i>Factory 3</i>					
	Resin operator	12		137.7		
	Filter operator	3		19.4		
	Warehouseman	4		4.7		
	Maintenance	3		3.4		
	Lab. worker R&D resin	3		15.1		
	Lab. worker QC resin	4		2.5		
	<i>Factory 4</i>					
	Resin operator	13		20.1		
	Pilot plant operator resin	3		10.4		
Lab. worker QC resin	10		1.8			
Production of alkyd resins	Handling bags	2	Task-specific, min	AM (range)	Glass-fibre filter, TENAX tube, HPLC-UV	(182)
	Charging	8	87.0	33.0 (21.9-44.0)		
	Sampling/testing	18	58.6	363.9 (53.8-1 862.6)		
	Resin finishing	2	78.3	300.3 (8.8-1 276.6)		
	Laboratory work	2	37.0	103.9 (10.6-197.1)		
	Opening system	7	91.0	17.5 (5.5-29.5)		
	Delivery of liquid PA	6	15.3	179.6 (6.0-389.3)		
		38.8	25.0 (6.5-51.4)			

**Table 6.** Cont.

Acid anhydride Industry	Processing method/job	No. of samples	Sampling type/time	Results ( $\mu\text{g}/\text{m}^3$ )	Method	Reference
<b>TMA</b> Manufacture of cushioned flooring	Printer	16	Personal/full-shift	AM 31.2	Glass-fibre filter, TENAX tube, HPLC-UV	(182)
	Ink mixer	8		15.4		
	Pilot plant operator flooring	8		3.6		
	De-reel, reel-up operator	10		21.8		
	Floor processing operator	4		5.2		
Manufacture of cushioned flooring	Handling bags	1	Task-specific, min	AM (range) 99.7	Glass-fibre filter, TENAX tube, HPLC-UV	(182)
	Charging	4	6.0	6 340.3 (150.5-20 433)		
	Sampling/testing	2	15.3	4.1 (2.5-5.7)		
	Finishing resin	2	65.7	5.5 (5.1-5.9)		
	Opening system	1	37.0	19.6		
	Clean workplace	1	50.0	60.2		
	Mixing batch	2	44.0	34.0 (16.2-51.7)		
	Mixing inks	3	21.5	19.9 (14.6-26.8)		
	Loading inks	2	50.7	25.0 (17.1-33.0)		
	Printing	2	26.5	12.3 (5.0-19.7)		
	Cleaning equipment	2	135.5	14.3 (11.1-17.5)		
		2	26.3			

**Table 6.** Cont.

Acid anhydride Industry	Processing method/job	No. of samples	Sampling type/time	Results ( $\mu\text{g}/\text{m}^3$ )	Method	Reference
<b>MIA</b> Production of alkyl resins	Handling bags	1	Task-specific, min	AM (range) 6.9	Glass-fibre filter, TENAX tubes, HPLC-UV	(182)
	Charging	3	30.0	17.3 (10.1-28.6)		
	Sampling/testing	2	13.0	5.5 (1.4-9.7)		
	Finishing resin	2	83.6 37.0	10.8 (5.9-15.7)		
<b>HHPA</b> Production of components (A) or capacitors (B), epoxy resin isolation	<i>Plant A</i>		Personal/sampling rate	Mean (range)	XAD-2 tubes, GC-FID	(195)
	Casting department 1	21	0.2-1.0 l/min, 4-55 l air	33 (14-131)		
	Casting department 2	18		23 (2-98)		
	<i>Plant B</i>					
	Casting department 1	58		140 (3-470)		
	Casting department 2	13		35 (2-210)		
	Mixed work	7		15 (7-27)		
	Mounting	26		4 (2-9)		
<b>MHHPA</b> Production of components (A)	<i>Plant A</i>		Personal/sampling rate	Mean (range)	XAD-2 tubes, GC-FID	(195)
	Casting department 1	21	0.2-0.1 l/min, 4-55 l air	48 (6-403)		
	Casting department 2	18		9 (2-30)		
<b>MTHPA</b> Manufacture of barrels			Personal, hours	GM (range)	XAD-2-tubes, GC-FID	(192)
	Zone I: handling epoxy resin	40	148	85 (7-380)		
	Wet part	32	117	100 (20-380)		
	Dry part	8	31	15 (7-30)		
	Zone II: adj. departments	12	57/personal+area	14 (<1-30)		
	Zone III: other departments	4	17/area	10 (7-14)		

**Table 6.** Cont.

Acid anhydride Industry	Processing method/job	No. of samples	Sampling type/time	Results ( $\mu\text{g}/\text{m}^3$ )	Method	Reference
<b>MTHPA</b> Manufacture of condensers	<i>Plant A</i>			GM (GSD)	Silica-gel-tubes (Davisil™646), GC-ECD	(82)
	Assembly 1	5	Area/0-60 min, sampling rate 1 l/min.	36.5 (9.25)		
	Hardening 1	5		129 (3.44)		
	Inspection 1	5		34.2 (6.22)		
	Assembly 2	5		254 (3.06)		
	Hardening 2	5		695 (4.53)		
	<i>Plant B</i>					
	Hardening	5		373 (1.91)		
	Coating	5		185 (3.75)		
	Cutting	5		42.5 (3.80)		
Finishing	5		4.58 (2.40)			
<b>TCPA</b> Manufacturing solenoid coils, TCPA cured epoxy resin	<i>1989</i>			GM (range)	Teflon filters, TENAX tubes, GC	(118)
	Preformer machine	1	Sampling rate 1 l/min	230		
	Moulding	3	Area	210 (140-340)		
	Moulding	3	Personal	320 (170-590)		
	<i>1990</i>					
	Preformer machine	1	Area	30		
	Moulding	6	Personal	21 (<10-110)		
	Moulding	3	Area	9 (<10-10)		
	Leak testing	1	Area	<10		
	Winding	1	Area	10		
Front office	1	Area	<10			

AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation.

## 6. Measurements and analysis of workplace exposure

### 6.1 Measurements and analysis of workplace exposure

Solid sorbent tubes are used for the collection of samples of vapours (Tenax, XAD-2). A bubbler or impinger method is also possible. However, either device will sample the anhydride as the corresponding acid. Therefore a derivatisation step is needed in the analytical procedure. The impinger or bubbler method is also efficient for the sampling of particles but not for small particles formed by, for example, condensed vapour. Another possibility for sampling particles is to use PVC or Teflon filters in series with solid sorbent tubes. To recover both particles and vapours, sampling with both methods is recommended in studies of plants at which the state of the exposure is unknown (87, 93). For analysis, gas chromatography (GC) with flame ionisation detection (FID), electron capture detection (ECD) or mass-spectrometric (MS) detection has been used. Acetic anhydride may be added to the eluting solutions to increase the stability of the samples in the elution and analysis steps (87, 93).

#### 6.1.1 Phthalic anhydride

Pfäffli sampled PA from air with Tenax polymer tubes and analysed PA by GC utilising a  $^{63}\text{Ni}$ -ECD. The limit of detection was  $0.4 \mu\text{g}/\text{m}^3$  (0.00007 ppm) with an air sample of 12 l (147, 149).

PA can also be analysed as the corresponding phthalic acid by reversed phase high performance liquid chromatography (HPLC), as described by Nielsen *et al.* (136).

#### 6.1.2 Trimellitic anhydride

The *Manual of Analytical Methods* published by the National Institute for Occupational Safety and Health (NIOSH) in the United States (US) gives method 5036 for measuring TMA. The air contaminants are sampled on a PVC copolymer membrane filter. After treatment with methanol and boron trifluoride TMA is analysed as its trimethyl ester by GC-FID. The detection limit is  $2 \mu\text{g}$  per sample (400 l air). The method does not differentiate between TMA and trimellitic acid (138).

Geyer *et al.* collected samples on glass fibre filters and converted TMA to the corresponding acid with a 0.05 M NaOH solution. The analysis was carried out with HPLC with an ultraviolet (UV) detector. The minimum quantifiable amount was  $1 \mu\text{g}$  on a filter sample (sample size not given) (55).

Pfäffli modified the NIOSH method using a glass-fibre filter in series with a Tenax tube. The analysis was carried out by GC-ECD. The detection limit was  $0.6 \mu\text{g}/\text{m}^3$  (12 l of air and a sampling rate of 0.2 l/min) (148).

#### 6.1.3 Maleic anhydride

The US NIOSH *Manual of Analytical Methods* gives method 3512 for measuring MA. A known volume of air is drawn through a midget bubbler containing 15 ml



of distilled water. Maleic acid is analysed by HPLC with an UV detector. The limit of detection is estimated to  $15 \mu\text{g}/\text{m}^3$  per sample. The method does not distinguish between MA and maleic acid, and it has limited sample stability (138).

Geyer and Saunders used a similar method with 0.1% phosphoric acid in distilled water as the absorbing solution and as the mobile phase. The minimum quantifiable amount of MA was  $100 \mu\text{g}/\text{m}^3$  of MA using a 100 l air sample (56).

The US Occupational Health and Safety Administration (OSHA) has described an HPLC method for sampling MA, in which sampling is performed on *p*-anisidine-treated XAD-2. Determination of the sampled anhydride using an ECD gave a detection limit of  $0.1 \mu\text{g}/\text{m}^3$  with a 12 l sample volume of air (87).

#### 6.1.4 Hexahydrophthalic anhydride

Jönsson *et al.* reported a method where HHPA was sampled with XAD-2 or a Tenax tube. The analysis was carried out with GC-FID. The detection limit was  $0.1 \mu\text{g}/\text{ml}$  of desorption solution (87, 88).

HHPA has also been sampled with bubblers containing aqueous sodium hydroxide (NaOH) and detected using GC with FID or electron ionisation MS after derivatisation to dimethyl esters. The detection limit using electron ionisation MS was  $0.01 \mu\text{g}/\text{sample}$  (60 l of air) (87, 88, 93).

Glass-fibre filter sampling (eluted with aqueous NaOH, derivatisation with pentafluorobenzyl bromide and determination by GC-MS in the negative-ion chemical ionisation mode gave results comparable with those of the Tenax method (93).

A Fourier transform infrared spectrometer has been tested for the direct measurement of peak levels of HHPA. The limit of detection was  $120 \mu\text{g}/\text{m}^3$  (117).

#### 6.1.5 Methyl hexahydrophthalic anhydride

MHHPA has been sampled with Tenax tubes and analysed with GC-ECD (151). When the sampling was carried out with XAD-2 tubes and analysed with GC-FID, the detection limit was  $0.1 \mu\text{g}/\text{sample}$  (sample volume not given). The detection limit was equal for the *cis* and *trans* isomers of MHHPA (93).

#### 6.1.6 Methyl tetrahydrophthalic anhydride

MTHPA has been sampled using Amberlite XAD-2 solid sorbent tubes and analysed by GC-FID. The detection limit for air samples was  $10 \mu\text{g}/\text{m}^3$  for a 20 l sampling volume (189). The sensitivity was equal for the isomers in technical quality of MTHPA (112).

Johyama *et al.* used silica-gel tubes for the sampling and GC-ECD for the analysis. MTHPA concentrations  $>1.0 \mu\text{g}/\text{m}^3$  were quantified at 20 minutes sampling with a sampling rate of 1 l/min (82).

#### 6.1.7 Tetrahydrophthalic anhydride

THPA has been sampled with XAD-2 tubes and analysed using GC-FID. The detection limit was  $0.1 \mu\text{g}/\text{sample}$  (sample volume not given) (93).

### 6.1.8 Tetrachlorophthalic anhydride

Liss *et al.* sampled TCPA with Teflon filters connected to Tenax tubes and made the analysis using GC. No detection limits were given (118).

## 6.2 Measurements of dicarboxylic acids from samples of urine and plasma

Pfäffli reported a method for determining the dicarboxylic acids of PA, HHPA, MHPA, and THPA in urine. The acids were esterified with 2,2,2-trichloroethanol and analysed by GC-MS. The detection limits were 2-4 ng/ml urine for aliphatic and alicyclic acids, and 15 ng/ml for phthalic acid (151). In another study by Pfäffli urine samples were collected from PA exposed workers pre-shift, on-shift, post-shift, in the evening, and on the following morning. The samples were esterified with boron trifluoride and methanol and analysed with GC-ECD. The detection limit was 0.05  $\mu\text{mol/l}$  (10 ml urine samples). A significant correlation was found between the phthalic acid concentration in urine samples and the atmospheric PA concentrations. When the exposure level was about 30% of the hygienic reference value at that time 6 000  $\mu\text{g/m}^3$ , a body-burden was caused which was not eliminated overnight (146).

Jönsson *et al.* used esterification with methanol and boron trifluoride and GC-MS for the analysis. For HHPA-exposed workers a correlation was found between time-weighted levels of HHPA in air and hexahydrophthalic acid in post-shift urine ( $r_s=0.93$ ;  $p<0.023$ ). The detection limit in urine was 20 ng/ml. Because the urine analysis of one worker exposed to a HHPA time weighted average (TWA) concentration of 30  $\mu\text{g/m}^3$  showed that more than 85% of the inhaled amount was excreted in the urine as hexahydrophthalic acid, it was estimated that it is possible to monitor HHPA air concentrations of approximately 1-2  $\mu\text{g/m}^3$  with this method (86, 89).

Lindh *et al.* developed the method further for the analysis of methyl tetrahydrophthalic acid in urine. The commercial MTHPA used was composed of three major isomers. The overall detection limit for the three isomers was <6 ng/ml (112).

The method was further developed to be less labour-intensive in biological monitoring and applicable for the determination of hexahydrophthalic acid and methyl hexahydrophthalic acid. The detection limits in urine were 11 ng/ml and 17 ng/ml, respectively (84).

A method has been developed to measure hexahydrophthalic acid and methyl hexahydrophthalic acid simultaneously in plasma. Pentafluorobenzyl bromide was used as the derivatisation agent and the pentafluorobenzyl esters formed were analysed by GC-MS. The detection limit was 0.4 ng/ml for hexahydrophthalic acid and 0.3 ng/ml for methyl hexahydrophthalic acid (113).

## 6.3 Conclusions

Sensitive methods are available to measure air levels of exposure to cyclic acid anhydrides at the workplace. It is important to choose sampling method according

to the type of exposure. There are also sensitive methods for analysing the corresponding dicarboxylic acids to PA, HHPA, MHHPA, MTHPA, and THPA in urine, and dicarboxylic acids in plasma originating from HHPA and MHHPA.

## 7. Toxicokinetics

### 7.1 Uptake

When 5 healthy volunteers were exposed to gaseous HHPA at  $80 \mu\text{g}/\text{m}^3$  for 8 hours, 1-4% was found in exhaled air during exposure. The concentration of the metabolite hexahydrophthalic acid in plasma rose rapidly during exposure (90). One worker was exposed to an 8 hour TWA concentration of HHPA of  $30 \mu\text{g}/\text{m}^3$  and urine was collected during 24 hours. More than 85% of the inhaled dose was excreted in urine as hexahydrophthalic acid (86).

HHPA (1 400  $\mu\text{g}$ ) in petrolatum was applied with the epicutaneous skin test technique (4 Finn Chambers) to the back skin of 3 volunteers for 48 hours. The volunteers' urine was collected for 72 hours. The excreted amounts of hexahydrophthalic acid, as a fraction of the totally applied amount of HHPA, were within intervals between 1.4-4.5%, 0.2-1.3% and 0-0.4%, respectively for the 3 subjects. This indicated that the percutaneous absorption of HHPA was minimal. The person with the highest absorption had pale erythema after the removal of the mixture from the skin, a possible higher absorption through inflamed skin was suggested (91).

There are no data on absorption via the gastrointestinal system.

In conclusion, absorption of HHPA via inhalation is efficient whereas dermal absorption is low.

### 7.2 Distribution

Lindh *et al.* studied the distribution of HHPA by autoradiography after exposing guinea pigs and rats to ( $^3\text{H}_2$ )HHPA via inhalation for 3-8 hours. Medium to high levels of radioactivity were found in the mucosa of the nasal region and trachea, whereas negligible levels were observed in lung tissue. Tissue-bound radioactivity was also present in the gastrointestinal tract and conjunctiva. A low level of tissue-bound radioactivity was found in the cortex of the kidneys in rat, but not in guinea pigs. The radioactivity persisted for at least 7 days after the end of the exposure. The HHPA-derived radioactivity could only partially be extracted by organic solvents and water, suggesting a covalent binding to tissue macromolecules. However, in the lung, the little radioactive HHPA that was found could also be extracted. The radioactivity in dialysed plasma was mainly found in the same fractions as albumin (115).

### 7.3 Biotransformation and excretion

Acid anhydrides are used to change the properties of proteins to separate them from their matrix (141). The anhydride group reacts readily with amino acids and this reaction explains their conjugation with human serum albumin (HSA), which takes place in the hapten formation of acid anhydrides (175, 214). TMA was conjugated with HSA rapidly *in vitro* at 37°C. The reaction was essentially completed in 1 minute (214).

Plasma protein and albumin adducts have been measured in sera from HHPA- and MHPA-exposed workers. The adduct levels correlated well with the exposure. The half-time of the adducts *in vivo* was about 20 days (161).

MHPA was mainly bound to lysine in the collagen of guinea pig lung in both *in vitro* and *in vivo* exposure tests (92). When human erythrocytes were exposed to HHPA and MHPA, conjugation with haemoglobin was found. The major amino acid binding HHPA was also lysine (114).

Acid anhydrides are excreted in urine as the corresponding acids (dicarboxylic acids).

Both in an animal study and when MHPA-exposed workers were investigated, it was shown that the hydrolysis of the anhydride in the body takes time (150, 166). MHPA concentrations from 3.4-10.7 nmol/l were detectable after the work-shift in the blood samples of workers exposed to MHPA at levels between 140-310  $\mu\text{g}/\text{m}^3$ . The MHPA found had the same *cis* form as in the air samples. No free acids were found (150).

Pfäffli followed the excretion of phthalic acid in workers exposed to PA by taking urine samples pre-shift, on-shift, post-shift, in the evening, and on the following morning. At low atmospheric exposure to PA (150 (range 30-330)  $\mu\text{g}/\text{m}^3$ ) the pre-shift phthalic acid concentrations were on the same level as those found in the urine samples of occupationally unexposed people (0.34 (range 0.02-0.89)  $\mu\text{mol}/\text{mmol}$  creatinine). In workers exposed to higher concentrations (1 630 (standard deviation (SD) 130)  $\mu\text{g}/\text{m}^3$  of PA) an accumulation of phthalic acid in urine was found. The pre-shift phthalic acid excretion was 1.02 (SD 0.25)  $\mu\text{mol}/\text{mmol}$  creatinine. When the exposure was high, 10 500  $\mu\text{g}/\text{m}^3$ , the pre-shift urinary concentration was 4.8  $\mu\text{mol}/\text{mmol}$  creatinine, about 14 times that seen in workers with low exposure. No conjugation of phthalic acid to glucuronide was observed (146).

The urine analysis of one worker exposed to a HHPA concentration of 30  $\mu\text{g}/\text{m}^3$  (TWA) showed that more than 85% of the inhaled amount was excreted in the urine as hexahydrophthalic acid (86).

The half-time of phthalic acid in urine of PA-exposed workers was shown to be about 14 hours (146). The assumed half-time of the dicarboxylic acids in the urine of workers with low exposure to MHPA was about 7 hours, and for HHPA and THPA the corresponding value was about 14 hours. After 4 hours of exposure to an MHPA concentration of 116  $\mu\text{g}/\text{m}^3$  in air, an input-output equilibrium for the anhydride and the urinary acid developed (151). In another study the half-time of hexahydrophthalic acid in urine was 2-3 hours in HHPA-exposed workers (86).

The half-times of hexahydrophthalic acid in plasma of 2 male healthy volunteers were 1.7-1.8 hour after exposure to  $80 \mu\text{g}/\text{m}^3$  HHPA for 8 hours (90). Urine analysis indicated half-times of 3, 3 and 6 hours for the three isomers 3-methyl-delta 4-tetrahydrophthalic anhydride, 4-methyl-delta 4-tetrahydrophthalic anhydride, and 4-methyl-delta 3-tetrahydrophthalic anhydride, respectively in a worker exposed to commercial MTHPA (112).

To conclude, cyclic acid anhydrides bind to plasma proteins and haemoglobin. The main binding amino acid seems to be lysine. The half-time of MHHPA-adducts has been shown to be 20 days. Cyclic acid anhydrides are after hydrolysis to corresponding dicarboxylic acids effectively excreted in urine. As much as 85% of the inhaled dose of HHPA has been recovered in urine. The half-time for the dicarboxylic acid of PA in urine was 14 hours, whereas the corresponding half-times of HHPA, MHHPA and MTHPA were in general shorter, between 2 and 7 hours.

## 8. Biological monitoring

Pfäffli analysed phthalic acid in urine of workers exposed to PA levels of 30-10 500  $\mu\text{g}/\text{m}^3$  (TWA) and found concentrations between 0.3-14.0  $\mu\text{mol}/\text{mmol}$  creatinine. At exposure levels of 2 000  $\mu\text{g}/\text{m}^3$  of PA, phthalic acid could still be detected in urine the next day (146).

Hexahydrophthalic acid in plasma and in urine of experimentally exposed volunteers showed good correlation ( $r > 0.90$ ) with the air level of HHPA (90).

In another experiment the researchers studied 27 workers exposed to a mean MHHPA concentration of 15 (range 5-60)  $\mu\text{g}/\text{m}^3$ , mainly in casting and in leaks from curing ovens in a plant manufacturing electrical capacitors. Urine was collected during the last 4 hours of the shift and from 8 workers before the start of the work shift and then at 4-hour intervals (7 hours during the night) for 24 hours. Plasma was sampled at the end of the 8-hour work shift. The elimination half-time in urine varied between 4 and 10 hours. Workers exposed to less than 10  $\mu\text{g}/\text{m}^3$  had urinary levels below the quantification limit before the next shift. A correlation ( $r = 0.94$ ) was found between the TWA air levels of MHHPA and the creatinine-adjusted methyl hexahydrophthalic acid levels in urine samples collected during the last 4 hours of the exposure and likewise between the exposure and the plasma concentration of methyl hexahydrophthalic acid. An exposure to 20  $\mu\text{g}/\text{m}^3$  corresponded to a methyl hexahydrophthalic acid concentration of about 140 nmol/mmol creatinine in urine and about 40 nmol/l in plasma (116).

When protein adducts were measured in the plasma of HHPA and MHHPA exposed workers, the concentrations correlated with the exposure levels. It was shown that air levels even below 1  $\mu\text{g}/\text{m}^3$  of HHPA and MHHPA were possible to monitor (161).

In cross-sectional studies the proportion of persons with immunoglobulin (Ig)G specific for PA, HHPA and MTHPA increased as exposure increased (136, 192,

195). However, as 50% or more of the subjects were negative even in the groups with the highest exposure intensity, the value of IgG as a biomarker of exposure seems limited. This limitation is emphasised by the results of Jönsson *et al.* who measured both haemoglobin adducts and HHPA-specific IgG antibodies in serum in HHPA exposed workers. The exposure was determined from the analysis of urinary carboxylic acid measurements. The authors found a correlation ( $r=0.87$ ) between the urinary hexahydrophthalic acid level and the number of haemoglobin-HHPA adducts. However, there was no significant correlation between the exposure and the HHPA-specific IgG (85).

In conclusion, measurement of the corresponding dicarboxylic acids in the urine of exposed workers is a sensitive non-invasive method for biological monitoring of some cyclic acid anhydrides. Measurement in plasma samples is also possible. However, biomonitoring data and methods to measure the corresponding acids of TMA, MA and TCPA are lacking.

The determination of specific IgG antibodies from blood samples does not give information of the exposure level according to studies on HHPA-exposed workers. Results obtained with determination of protein adducts (HHPA and MHPA) in plasma are more promising.

## 9. Mechanism of toxicity

### 9.1 Irritation

Cyclic acid anhydrides react easily with water, and the corresponding acids are formed. The formation of acids explains the irritating effects on the skin and the mucous membranes of the eyes and the respiratory organs (11, 15, 23, 45, 47, 122, 136, 171).

### 9.2 Allergic contact dermatitis (Type IV)

In animal studies PA has been classified as a moderate sensitizer causing allergic contact dermatitis of type IV allergy (48). When the allergenicity of chemicals was more recently studied using cytokine stimulation, PA, TMA, MA, HHPA and MHPA were not considered as contact allergens (31-33). The small number of case reports also suggests that the potency of cyclic acid anhydrides to induce allergic contact dermatitis is low (94, 96).

### 9.3 Contact urticaria (Type I)

IgE-mediated contact urticaria due to cyclic acid anhydrides is more usual. MHPA, MHPA, HHPA, CA, and MA have induced contact urticaria in exposed workers with specific IgE antibodies and positive results in skin prick tests and in open tests with the anhydride (83, 94, 95, 102, 174). In some cases airborne exposure without skin contact has resulted in contact urticaria (95, 174).

#### 9.4 Respiratory sensitisation

Allergic asthma, often preceded by rhinoconjunctivitis, is a well documented disease of workers exposed to cyclic acid anhydrides, and its occurrence has stimulated several works concerning the mechanism of the sensitisation. In case reports and industrial surveys, IgE mediated sensitisation has been verified by positive reactions in skin prick tests with conjugates of the anhydrides and HSA, and by specific IgE. In exposed workers, bronchial hyperresponsiveness, a typical finding in asthma, has been correlated to the specific sensitisation (14). Immediate, dual, or late bronchial reactions have been found in challenge tests with PA, MA, HHPA, MTHPA, TCPA, and PMDA (16, 17, 26, 36, 37, 108, 168, 184, 186, 196).

The formation of protein adducts *in vivo* is believed to be the first step in the sensitisation process. This has been shown when total protein and albumin adducts of HHPA and MHPA were measured in the plasma of exposed workers (161).

Also in sensitised animals the formation of anhydride-specific IgE and IgG antibodies has been shown (4, 10, 68, 71, 217, 219, 220). In studies with PA, TMA, and HHPA, an obstructive bronchial reaction has followed the challenge tests of sensitised animals (10, 28, 68, 71, 165, 218).

There are some findings of mediator release in acid anhydride sensitivity. When basophilic leukocytes were challenged *in vitro* with PA or TCPA-HSA conjugates, there was a release of histamine, a mediator of allergic reaction. The *in vitro* histamine assay was claimed to be useful in the identification of subjects with allergic responses to anhydrides, even without evidence of IgE-mediated reaction (44).

In animal studies using pretreatment with different blocking agents, the mediators histamine and thromboxane A<sub>2</sub> have been shown to be mainly responsible for the early and late bronchoconstriction response to TMA. Leukotrienes and histamine were found to mediate airway plasma exudation to some extent (6, 8, 69, 71). In sensitised guinea pigs, pretreatment with budesonide significantly inhibited the increase in airway responsiveness, but not the eosinophilic inflammation, induced by exposure to TMA dust (67).

Rats pretreated with the immunosuppressant cyclophosphamide showed no lung lesions and no antibody reaction after exposure to 95 µg/m<sup>3</sup> TMA 6 hours/day, 5 days/week for 2 weeks. Thus, the elimination of T- and B-lymphocyte function could prevent the TMA-induced lesions (106).

Oral pretreatment with cyclosporin A inhibited the immunisation process caused by TMA in guinea pigs, whereas betamethasone and azelastine had no significant effect (5). In another study with brown Norway rats both betamethasone and cyclosporin A given over the time of sensitisation inhibited the development of TMA specific IgE and IgG (155).

Activation of inducible nitric oxide synthase has been demonstrated in bronchial tissue after TMA-guinea pig serum albumin (GPSA) challenge in sensitised guinea pigs (198).

The pulmonary disease-anaemia syndrome described due to fumes from TMA-cured epoxy resin, is a rare disease with haemorrhagic alveolitis and specific IgG antibodies. In animal studies similar reactions have been found (25, 107).

## 9.5 Conclusion

Cyclic acid anhydrides are irritants because of formation of corresponding acids in wet surroundings. They rarely induce contact allergy of the skin but more easily induce IgE-mediated contact urticaria. The mechanism of respiratory sensitisation is mainly IgE mediated allergy both in animal studies and when exposed workers have been investigated. In the challenge tests bronchial obstruction has been verified, as well as development of inflammation. The determination of specific antibodies and mediators of allergies and allergic inflammation in the studies with cyclic acid anhydrides has given new information on allergic reactions in general.

## 10. Effects in animals and *in vitro* studies

### 10.1 Irritation and sensitisation

#### 10.1.1 Irritation

In animal studies PA has not been found to be as irritating as MA and TMA (Table 7).

A PA solution (50%) in oil did not irritate rabbit ears after 20 hours of exposure (34). PA (0.5 g/patch) did not cause skin irritation on rabbits when applied by the semi-occlusive or occlusive method over a period of 1 or 4 hours. The results were assessed at 1, 24, 48 and 72 hours, or 7 days later (154).

One drop of PA (5%) in polyethylene glycol 400 was slightly irritating to rabbit eyes, while a 0.5% solution was not irritating (34). In an experiment with rabbits, the irritant effects of PA on the skin and eyes correlated with each other. PA was found to be a mild skin irritant, but a moderate eye irritant (47).

TMA (50%) caused dermatitis in mice and rats after a single or repeated application to the skin for 2 hours. The effects were slight and reversible (15).

MA and TMA have been shown to be extremely irritating to eyes in animal experiments. There was cloudiness of the cornea and hyperaemia of the conjunctiva a few minutes after the application of 1% MA to the eyes of rabbits. The next morning the eyes were normal. A 5% solution of MA induced more intense irritation, which lasted 1 week. A minute amount of MA powder caused long-lasting damage with vascularisation of the cornea of the rabbit (197). The application of 50 mg of TMA powder to rabbit eyes produced reversible hyperaemia of the conjunctiva, and lacrimation and blepharospasms (15).

In a 6-month inhalation study on MA, rats, hamsters and monkeys were exposed to concentrations of 0, 1 100, 3 300, and 9 800  $\mu\text{g}/\text{m}^3$ , 6 hours/day for 5 days/week. Dose-related ocular and nasal irritative signs were present at all



**Table 7.** Irritative and sensitising effects of acid anhydrides on different animal species.

Anhydride/ Species	Route of administration	Exposure data	Effect	Reference
<b>PA</b>				
Rabbit	Eye application	50 mg	Moderate irritation	(139)
Rabbit	Dermal (patch)	500 mg (1 or 4 h)	No skin irritation	(154)
Rat, Brown Norway	Intradermal	0.1 ml 0.2 M PA <sup>a</sup>	Specific IgE and specific IgG antibodies	(220)
<b>TMA</b>				
Rabbit	Eye application	50 mg	Conjunctival hyperemia, lacrimation	(15)
Guinea pig	Intradermal	0.1 ml of 30% TMA	Specific IgG <sub>1</sub> and IgE antibodies	(22)

<sup>a</sup> Specific IgE and IgG antibodies have been induced in similar studies with TMA, MA, HHPA, MHPA and MTHPA (220).

exposure levels. A histopathological examination of nasal tissue (turbinate sections immediately posterior to the upper incisors) revealed irritation (hyperplasia, metaplasia) in rodent species and inflammatory changes in all species. All changes were judged to be reversible (171).

#### 10.1.2 Allergic contact dermatitis

The potency of PA to induce allergic contact dermatitis has been investigated with the Buehler test (closed patch test in guinea pigs) and the mouse ear swelling test (MEST). According to both tests PA was classified as a moderate sensitizer (48). The guinea pig maximisation test has not been carried out with acid anhydrides.

In other studies investigating the patterns in cytokine production following topical sensitisation, PA, TMA, MA, HHPA and MTHPA were not found to be contact allergens (31-33).

#### 10.1.3 Respiratory sensitisation

Table 8 contains data from the respiratory sensitisation studies. Sensitisation with the production of specific antibodies is essential for the development of an allergic respiratory disease. Antibody response has been induced by both bronchial, subcutaneous, intradermal and parenteral sensitisation routes.

When monkeys were exposed parenterally to PA-monkey serum albumin (MSA), PA dissolved in ethanol saline, MSA, or ethanol-saline alone, sensitisation was observed only with PA-MSA. The presence of new antigenic determinants formed by PA on protein carriers was essential for the parenteral sensitisation (21).

Guinea pigs were sensitised by inhalation of PA dust at 500, 1 000, 5 000  $\mu\text{g}/\text{m}^3$ , for 3 hours/day for 5 consecutive days. A PA-guinea pig serum albumin (GPSA) challenge after 2 weeks elicited an immediate onset of respiratory reactions, determined by plethysmography, in animals exposed to all 3 levels of dust. The inhalation challenge with PA dust (5 000  $\mu\text{g}/\text{m}^3$ ) did not cause an

immediate response, but the animals had significant number of haemorrhagic lung foci. No foci were seen in the lungs of PA-GPSA challenged animals. IgG-PA-GPSA antibodies were detected in sera of all the PA-exposed animals, and the dose-response relationship was highly significant (165).

In a Japanese study rabbits were sensitised subcutaneously to PA-rat serum albumin (RSA). High titres of IgG against PA-RSA were found, but also against PA-HSA and HSA. IgG-PA-HSA antibodies had cross-reactivity with HHPA-HSA, MHPA-HSA, and MTHPA-HSA. After purification of specific IgG-PA, the levels of specific IgG to other conjugates were unchanged. Two types of IgG antibody production were suspected, one to PA hapten alone and the other to new antigenic determinants on HSA (66).

Dykewicz *et al.* sensitised 2 rhesus monkeys intrabronchially with serum from a worker with TMA asthma and high titres of IgE, IgG, and IgA to TMA-HSA. The monkeys were challenged with TMA-HSA aerosol and bronchospasm appeared. After 1 week the challenge was negative. Passive cutaneous anaphylaxis was also found with the Prausnitz-Küstner test (38).

In an inhalation experiment rats were exposed 3 hours/day for 5 days to 0, 10, 30, 100 or 300  $\mu\text{g}/\text{m}^3$  of TMA dust. Haemorrhagic lung foci were found in relation to exposure concentrations of 30-300  $\mu\text{g}/\text{m}^3$ . The serum antibody binding of trimellitic-RSA correlated with exposure concentration, presence of haemorrhagic lung foci and lung weight. The lung lesions had healed 12 days after the exposure, but returned soon after a repeated exposure (211). A histological examination of the lung lesions indicated extensive cellular infiltration, primarily macrophages, alveolar haemorrhage, and pneumonitis. These effects increased in proportion to the concentration. The lungs were the only organs affected (107).

Chandler *et al.* exposed rats to TMA powder (100  $\mu\text{g}/\text{m}^3$ ) for 6 hours/day, 5 days/week for 2 weeks. Haemorrhagic foci were observed on the surface of the lungs at autopsy. The authors found higher total antibody concentrations in the fluid of bronchoalveolar lavage (BAL) than in serum. IgG, IgA, and IgM antibodies to TMA-RSA were detected. Inhibition studies showed that both TMA-RSA and TMA-HSA conjugates cause complete inhibition of the rat IgG binding, whereas the human IgG was inhibited only by TMA-HSA. The early antibody response in the rat was directed towards new antigenic determinants common to TMA-modified albumins (25). The immune response to inhaled TMA has been found to occur in parallel with the development of lung lesions. The antibody levels in BAL and serum were highly correlated with the lung injury (209).

After rats had inhaled TMA powder (500  $\mu\text{g}/\text{m}^3$  or 330  $\mu\text{g}/\text{m}^3$ ) on days 1, 5 and 10 for 6 hours/day they were challenged with TMA (540  $\mu\text{g}/\text{m}^3$  or 300  $\mu\text{g}/\text{m}^3$ ), on day 29 or 22, respectively. In the high exposure group, IgM and IgA antibodies to TMA-RSA started to increase from day 5 and peaked at day 20. IgG antibodies appeared on day 7 and peaked at day 20. A mean of 216 haemorrhagic lung foci was found. In the low exposure group animals that were not rechallenged had fewer lung foci than the rechallenged animals. In the rechallenged group there was a correlation between all the antibody measures and lung injury. A subgroup of animals was exposed to a TMA level of 500  $\mu\text{g}/\text{m}^3$  only on days 1 and 5 and

challenged with  $500 \mu\text{g}/\text{m}^3$ , on day 29. A mean of 112 haemorrhagic lung foci was found, and there was a good correlation between the antibody response and the lung injury (208).

Hayes *et al.* developed a guinea pig model for TMA-induced airway hyper-sensitivity responses by sensitising animals intradermally with 0.1 ml of 0.3% free TMA in corn oil. Control animals were given 0.1 ml corn oil. An increase in the level of specific serum IgG<sub>1</sub> antibodies was found in all sensitised animals, and IgE antibodies were detected in 6 of 8 sensitised animals. On days 21 to 28 a tracheal challenge (50  $\mu\text{l}$ ) with 1% TMA-GPSA gave increased lung resistance in sensitised animals compared with non-sensitised animals. Airway microvascular leakage was also seen in sensitised animals when tested with Evans blue (70). When challenged by inhalation through the nose ( $12\,000 \mu\text{g}/\text{m}^3$  TMA, 30 minutes), the animals showed a significant increase in bronchial reactivity 8 hours after the exposure, and the increase was accompanied by an eosinophilic inflammatory exudate (68).

Arakawa *et al.* investigated the time course of immune and airway responses after sensitising guinea pigs through two intradermal injections (0.1 ml of 0.3% TMA in corn oil). They challenged the animals after 1, 2, 3, 5 and 8 weeks with 50  $\mu\text{l}$  of 0.5% TMA-GPSA intratracheally. The challenge induced a significant increase in lung resistance, reaching a maximum at 2.5 minutes in the 1-week group and between 5 and 6 minutes in the other sensitised animals. A significant extravasation was also found that increased up to 8 weeks. Specific IgG<sub>1</sub> antibodies were detected in all the animals in the 3-, 5-, and 8-week groups; this result correlated with the extravasation but not with the increase in the resistance (7).

Brown Norway rats were intradermally sensitised with TMA and then challenged once or seven times with TMA-RSA conjugate. High levels of TMA-specific IgE and IgG were found in all the sensitised rats when they were compared with controls. A single allergen challenge did not cause bronchial hyperreactivity but repeated challenge produced significant bronchial hyperreactivity in sensitised rats. Repeated, low-dose challenges produced more hyperreactivity than a 10 times higher single dose. Bronchial eosinophilia was found in the sensitised and single-challenged groups, but not in the non-sensitised non-challenged and sensitised re-challenged groups (28).

Inhibiting complement activation prevented inflammatory cell infiltration in TMA-induced asthma. This phenomenon was studied by pretreatment of guinea pigs with a cobra venom that reduced the complement component C<sub>3</sub> in bronchoalveolar lavage fluid after TMA-GPSA challenge. The immediate bronchoconstriction was not affected, nor was the microvascular leakage. The TMA-induced increase in mononuclear cells, total white blood cells and red blood cells, and the erythrocyte peroxidase activity was reduced (46).

When sensitised brown Norway rats were challenged, TMA induced an immediate bronchoconstriction. Eosinophilic aggregates and goblet cell hyperplasia and hypertrophy were seen in the lungs and also induction of haemorrhages in

sensitised animals. A less marked eosinophilic infiltration of the lungs was seen also after the challenge tests of the non-sensitised animals (10).

Zhang *et al.* studied the mechanism of allergy by developing a regime for the intradermal sensitisation of guinea pigs to HHPA. The animals were immunised by an intradermal injection with single and booster injections of 0.1 ml of 0.02%, 0.1%, 0.5%, 5%, and 10% mixtures of HHPA in olive oil. Single injections of <0.5% produced no positive findings of specific IgE or IgG antibodies. A single injection induced optimal levels of IgG after 14 days at a dose of 5% HHPA. The IgE titres were low and only positive in 40-50% of the animals at injections of 0.5-10% HHPA. For IgE induction, booster injections were needed (217). The authors also studied the relationship between specific IgG<sub>1</sub> levels and airway responses to predict the sensitising potential of acid anhydrides. They concluded that allergen challenge in HHPA-sensitised guinea pigs results in both airway obstruction and plasma extravasation, and that responses are related to the serum levels of specific IgG<sub>1</sub> (218, 219).

Anaphylactic bronchoconstriction has been induced in guinea pigs sensitised with HHPA or MTHPA and challenged by inhalation or intravenously with the corresponding GPSA conjugate. A steep dose-response relationship was found. The critical dose was accordingly established to be approximately 40 µg/kg (221).

A model to differentiate chemicals for different types of allergenicity has been developed. Mice were sensitised topically, by applying the test material dissolved in 4:1 acetone:olive oil, to a shaved flank under an occluded patch for 48 hours. After 5 days the ear thickness was measured, and then the dorsum of both ears was treated with 25 µl of the tested chemicals, TMA, and 2,4-dinitrochlorobenzene, the later being a potent contact allergen without respiratory sensitisation properties. When the levels of activation (cell proliferation) in lymph nodes draining the site of application were similar, comparable levels of contact sensitisation and IgG anti-hapten antibodies were induced by these chemicals, but only TMA increased the IgE production. Furthermore, while TMA induced IgG<sub>2b</sub> rather than IgG<sub>2a</sub> antibodies, the reverse pattern was observed with the contact allergen. The results pointed to a different type of T lymphocyte (Th<sub>1</sub> and Th<sub>2</sub>) response to these chemicals (31). A similar response has been found with PA, MA, HHPA, and MTHPA (32, 33). Arts *et al.* used brown Norway rats in a very similar setting with TMA, dinitrochlorobenzene, formaldehyde, and methyl salicylate. They also found a significant increase in the serum IgE concentration after exposure to TMA but not after exposure to the other chemicals, skin sensitisers, or irritants (9).

Welinder *et al.* studied the relationships between chemical structure and immunogenicity for 13 dicarboxylic acid anhydrides in guinea pigs intradermally sensitised with SA, MA, methyl maleic anhydride (MMA), *cis*-HHPA, *trans*-HHPA, MHHPA, *cis*-1,2,3,6-tetrahydrophthalic anhydride (THPA<sub>1236</sub>), *cis*-3,4,5,6-tetrahydrophthalic anhydride (THPA<sub>3456</sub>), MTHPA<sub>34</sub>, MTHPA<sub>44</sub>, PA, 4-methyl phthalic anhydride (4-MPA) and TMA. Specific IgG was significantly increased in all animals except those immunised with THPA<sub>3456</sub> and SA, which sensitised some but not all the animals. The titres of specific IgG<sub>1</sub> and IgG<sub>2</sub> were

increased in IgG-positive animals. Passive cutaneous anaphylaxis test was used for determining specific IgE. Specific IgE was positive in all the animals immunised with MA, MHHPA, MTHPA (both isomers), and 4-MPA and in 6/9 and 5/9 guinea pigs immunised with TMA and MMA, respectively. The test results were contradictory for PA and HHPA. The sensitising potentials of different organic acid anhydrides showed a considerable variation. The substitution of hydrogen in the C<sub>4</sub> position by a methyl group or adding a double-bond to the same position enhanced the immunogenicity of the anhydrides (194, 219). In brown Norway rats sensitised intradermally with 14 different acid anhydrides (0.1 ml of 0.2 M) or 3 anhydride conjugates (1 400 µg of RSA conjugate) specific IgE antibodies were measured after 4 weeks. The titres (median) obtained after sensitisation with free MPA, PA, *cis*-HHPA, 4-MHHPA and TMA were 1 600-3 200 (range 800-6 400) and after sensitisation with MA, THPA<sub>1236</sub> and both isomers of MTHPA the titre was 800 (range 200-3 200). SA as free was negative, but in conjugate form, positive titres of specific IgE were found as well as with MA and *cis*-HHPA conjugates (220).

The specificity of the antibodies was studied with inhibition tests. The anhydrides with methyl group or double-bond similar to the sensitising anhydride caused higher inhibition effect when compared to the anhydrides with different structures (217, 219, 220).

In conclusion, specific IgE and specific IgG antibodies (PA, TMA, HHPA, MHHPA, and MTHPA) have been measured as a marker of sensitisation in animals after immunisation. In guinea pig studies methylation and the presence of a double bonding in the C<sub>4</sub> position of the anhydride molecule enhanced the sensitising potential. However, the results of the studies with brown Norway rats were partly different. In inhibition studies cross-reactivity was shown between anhydrides with similar structures. In challenge studies of sensitised animals, bronchial responses similar to bronchial asthma in humans have been seen. TMA dust evokes a lung reaction in animals that is similar to the pulmonary disease-anaemia syndrome.

**Table 8.** Effects of acid anhydrides on animals in short-term exposure studies concerning sensitisation.

Anhydride/ Species	Route of administration	Exposure data	Effect	Reference
<b>PA</b> Guinea pig	Inhalation	500, 1 000, 5 000 $\mu\text{g}/\text{m}^3$ PA dust 3 h/day for 5 days, challenged with PA-GPSA 2 000 $\mu\text{g}/\text{m}^3$ or PA dust 5 000 $\mu\text{g}/\text{m}^3$	IgG antibodies in lowest exposure greater than in air exposure ( $p<0.05$ ). Dose-response in IgG antibodies ( $p<0.001$ ). Haemorrhagic lung foci in highest exposure after PA dust challenge.	(165)
Rabbit	Subcutaneous	PA-RSA 0.25 ml weekly/12 weeks	High titre IgG to PA-RSA and PA-HSA, cross-reactivity with HHPA-, MHPA- and MTHPA-HSA conjugates.	(66)
Mouse	Cutaneous	PA 4:1 in acetone:olive oil 50 $\mu\text{l}$ on both flanks and after 7 days 1:1 dilution 25 $\mu\text{l}$ x 2 on both ears	Marked total IgE elevation maximal after 14 days. IgE anti-hapten antibodies in PCA test. IgG <sub>2b</sub> antihapten antibody production.	(32)
<b>TMA</b> Guinea pig	Intravenous immunisation, inhalation challenge	After 21-28 days tracheal challenge with 50 $\mu\text{l}$ of 1% TMA-GPSA	Specific IgG <sub>1</sub> and IgE antibodies, increased lung resistance and airway microvascular leakage.	(70)
Rat	Inhalation	30-300 $\mu\text{g}/\text{m}^3$ TMA dust 6 h/day for 5 or 10 days	Haemorrhagic lung foci and TM-MSA antibodies after 10 days.	(211)
Rat	Inhalation	10 $\mu\text{g}/\text{m}^3$ TMA dust 6 h/day for 5 or 10 days	No effect on lungs.	(211)

**Table 8.** Cont.

<b>Anhydride/ Species</b>	<b>Route of administration</b>	<b>Exposure data</b>	<b>Effect</b>	<b>Reference</b>
<b>TMA</b> Rat	Inhalation	500 µg/m <sup>3</sup> TMA powder 6 h/day on days 1, 5 and 10. On day 29 challenged 6 h to 540 µg/m <sup>3</sup>	IgG-, IgM- and IgA-TMA-RSA antibodies, haemorrhagic foci mean 216/lung.	(208)
Rat	Inhalation	500 µg/m <sup>3</sup> TMA powder 6 h/day on days 1 and 5. On day 29 challenged 6 h to 500 µg/m <sup>3</sup>	Haemorrhagic foci mean 112/lung, good correlation with antibody activity (p=0.027).	(208)
Rat	Intravenous immunisation, inhalation challenge	After 3 weeks challenged with 0.003 or 0.03% TMA-RSA (15 min) in 1 or 7 days	High levels of specific IgE and IgG (p<0.001), significant rise in bronchial hyperreactivity after repeated challenges (p<0.05), more often in low-dose challenge (p<0.05). Slight damage in airway epithelium in repeat-challenged groups.	(28)
Mouse	Inhalation	5 000 µg/m <sup>3</sup> TMA dust 1 h/day for 3 days	IgG-TMA-BSA antibodies after 1 week, IgE-TMA-BSA antibodies after 2 weeks.	(30)
<b>HHPA and MTHPA</b> Guinea pig	Intravenous immunisation, inhalation challenge	After 4 weeks HHPA- or MTHPA-GPSA 3 ml (nebulizer) or 0.01-1 000 µg/kg (iv)	Decrease in static compliance and arterial oxygen level and increase in inspiratory resistance after 10 minutes. Critical dose 40 µg/kg.	(221)

**Table 9.** Acute lethality data for organic acid anhydrides.

Anhydride	Species	Route of administration	LD <sub>50</sub> (mg/kg bw)	Reference
PA	Cat	Oral	800	(139)
	Rat	Oral	1 530	(139)
	Mouse	Oral	1 500	(139)
	Mouse	Intraperitoneal	75.5	(41)
TMA	Mouse	Oral	1 900	(15)
	Rabbit	Oral	5 600	(139)
MA	Rat	Intraperitoneal	97	(139)
	Rat	Oral	400	(139)
	Guinea pig	Oral	390	(139)
	Mouse	Oral	465	(139)
	Rabbit	Oral	875	(139)
	Rabbit	Dermal	2 620	(187)
MTHPA	Rat	Oral	2 140	(173)
THPA	Rat	Oral	5 410	(167)
TCPA	Rat	Oral	>15 800	(167)
	Rabbit	Dermal	>5 000	(167)

### 10.2 Effects of single and short-term exposure

Table 9 presents compiled data on the lethal dose for 50% of the exposed animals at single administration (LD<sub>50</sub>) of organic acid anhydrides. PA and MA have the lowest LD<sub>50</sub>-values in animal studies. In rats the oral LD<sub>50</sub> values are 400 and 1 530 mg/kg, respectively. TCPA is the least acutely toxic anhydride with LD<sub>50</sub> value >15 800 mg/kg orally in rats.

Oral TCPA (7 days, at 25 000, 100 000, 250 000 or 500 000  $\mu\text{g}/\text{kg}$  in corn oil) has been shown to be a weak wide-spectrum inducer of microsomal enzymes in rats. A similar effect was not observed for mice (159).

### 10.3 Effects of long-term exposure and carcinogenicity

When rats, hamsters and monkeys were exposed to MA vapours (1 100-9 800  $\mu\text{g}/\text{m}^3$ ) for 6 months, no exposure-related effects were found in histopathological investigations of lungs, liver, spleen, bone marrow or kidneys. A histopathological examination of nasal tissue revealed irritation (hyperplasia, metaplasia) in rodent species and inflammatory changes in all species. All changes were judged to be reversible. In the metaplastic changes the cuboidal to low columnar epithelium became hyperplastic and pseudostratified. Transformation to nonkeratinising squamous-type epithelium was seen. Metaplastic changes were present at all dose levels with a nonlinear increase in incidence (171).

When rats were exposed to HHPA vapours at 34 300, 68 600 or 137 300  $\mu\text{g}/\text{m}^3$  (6 hours/day, 5 days/week for 2-11 weeks), their cerebral and cerebellar acetylcholinesterase activity was below the control range at 137 300  $\mu\text{g}/\text{m}^3$  after 2



weeks ( $p < 0.01$ ). After 11 weeks the activities had normalised to control levels. The creatine kinase activity increased in the cerebellar tissue after 11 weeks of exposure ( $p < 0.01$ ) (166).

Information on carcinogenicity of acid anhydrides is scarce. No evidence of carcinogenicity was found for PA in long-term feeding studies with rodents (65, 103, 104, 169).

When 6 rats were injected subcutaneously twice weekly with 2 000  $\mu\text{g}$  of SA in 0.5 ml of arachis oil for 65 weeks, subcutaneous sarcomas developed at the injection site in the 3 rats that had survived 93-106 weeks. No tumours occurred in the 24 controls injected with arachis oil alone. The controls survived 45-106 weeks (77).

#### **10.4 Mutagenicity and genotoxicity**

No mutagenic activity has been found with PA, TCPA, or TBPA in *Salmonella typhimurium* in Ames test (121, 207). No effect of PA or TCPA was found on chromosomal aberrations neither in cells derived from Chinese hamster ovary cells nor in rat liver cells *in vitro* (49, 152).

Furthermore, when Chinese hamster ovary cells were tested for the induction of sister chromatid exchanges, PA and TCPA produced negative results (49). In a later chromosome aberration study using a higher and cytotoxic PA concentration (10 mM), an 18.5% increase in aberrations was seen compared to 3% in the control test (75).

There are no data on the mutagenicity or genotoxicity of other cyclic anhydrides.

#### **10.5 Reproductive and developmental studies**

Fabro *et al.* studied the teratogenicity of PA and SA in mice with daily intraperitoneal injections at doses of 0.2 to 0.6 mmol/kg/day on gestation days 8-10. They found malformations only at exposure levels of maternal toxicity (41).

No effects on reproduction were observed in CD-1 mice when TMA (550 000  $\mu\text{g}/\text{kg}$ ) was given orally on days 7 through 14 of gestation (72).

No treatment-related effects on foetal development were seen in rats treated orally with MA in corn oil at a dose of 140 000  $\mu\text{g}/\text{kg}/\text{day}$  from days 6-15 of gestation. Furthermore, no treatment-related effects on reproduction were observed with MA at doses up to 55 000  $\mu\text{g}/\text{kg}/\text{day}$  over 2 generations (170).

PA caused malformations at a high frequency when 200, 100, 50, or 25  $\mu\text{g}$  of PA per egg was injected into the air chamber of the egg of 3-day chicken embryos. A dose-response effect was found for early (105).

In conclusion, there is no evidence of teratogenic effects. However, only a few cyclic anhydrides have been tested.

## 11. Observations in man

Observations on human exposure are based on case reports of occupational sensitisations, industrial surveys, and some experimental human studies. In early reports the information on exposure levels is limited.

In humans, cyclic anhydrides cause irritation and sensitisation after direct contact with the skin and the mucous membranes or after exposure by inhalation (215).

The irritative symptoms (itching, lacrimation, sneezing, rhinorrhoea, cough, and dyspnoea) begin immediately at exposure to high concentrations of dust or vapours. The most common allergic diseases are rhinoconjunctivitis and asthma, both immediate-type IgE-mediated allergies. Also late-type respiratory symptoms with specific IgG antibodies have been described. A less frequent consequence is the severe disease called pulmonary disease-anaemia syndrome, and contact eczema, contact urticaria, allergic laryngitis, and allergic alveolitis. One case of haemolytic anaemia in an MA-exposed worker has also been reported.

### 11.1. Irritation and sensitisation

Conjunctival, nasal, and bronchial irritation is a common immediate feature following exposure to acid anhydrides (11, 126, 135, 136, 214). On mucous membranes and on sweating skin, they are hydrated to acids and can cause irritation, reddening, corneal damage, caustic dermatitis, and burns (122, 126).

The human nasal irritation threshold for PA has been reported to be  $30\,000\ \mu\text{g}/\text{m}^3$  and that for MA is  $5\,480\ \mu\text{g}/\text{m}^3$ . However, exposure duration, generation of particles and particle sizes were not given (162).

Nielsen *et al.* studied 60 workers exposed to mean levels of  $400\ \mu\text{g}/\text{m}^3$  PA with peak levels of  $6\,600\ \mu\text{g}/\text{m}^3$  during the loading of reactors. Of the 35 heavily exposed workers, 5 (14%) had asthma and 24 (69%) reported work-related symptoms of rhinitis and/or conjunctivitis. All the asthmatics were loaders. Four subjects had positive specific IgE, and 1 of the 4 suffered from asthma (136). The results indicate an irritative, non-allergic effect of PA on the mucous membrane. In a follow-up study, no significant effect on large airways, small airways, or alveolae was observed in the group of loaders when they were compared with the unexposed controls (130). When 20 HHPA-exposed subjects with work-related nasal symptoms were investigated, only 11 HHPA sensitised persons had positive reactions in the specific nasal challenge tests. The challenge test was negative in 9 subjects, all of them without HHPA sensitisation according to skin prick tests and determination of HHPA specific IgE antibodies but who nonetheless had irritant symptoms upon HHPA exposure (135).

Irritant haemorrhagic rhinitis has been reported as a result of MA exposure (18).

There is one report of anhydride-induced reactive airways dysfunction syndrome (RADS). RADS is characterised by damage of the bronchial epithelia followed by neurogenic inflammation and asthma (51). The person experienced acute mucosal symptoms immediately after an accidental 10-minute exposure to a

high concentration of PA. Symptoms of asthma developed, and 2 months later a nonspecific bronchial hyperreactivity was verified, which resolved after about 3.5 years (45).

## **11.2 Effects of repeated exposure on organ systems**

Allergic reactions of the skin and conjunctiva and allergic respiratory manifestations are well known effects of occupational exposure to cyclic anhydrides. Respiratory diseases include occupational allergic rhinoconjunctivitis and occupational asthma. Urticaria and allergic rhinoconjunctivitis often precede asthma. Cases of haemorrhagic alveolitis, haemorrhagic anaemia, allergic alveolitis, and allergic laryngitis have also been reported in association with exposure to anhydrides.

In industrial surveys the prevalence of occupational asthma due to different anhydrides has varied between 8-18% in those exposed to PA, 2-11% for TMA exposure, 15% for HHPA exposure, 11% for MHHPA exposure and 3-39% for TCPA exposure (203). According to the Finnish Register of Occupational Diseases, cyclic acid anhydrides caused the following numbers of diseases relative to the total numbers in 1997-1999: asthma 1/902, rhinitis 14/1 001 and contact urticaria 8/478. There were no reports of allergic alveolitis or allergic contact dermatitis (97-99).

There are no data showing an effect on other organ systems.

### *11.2.1 Allergic dermatitis*

DSA sensitised a laboratory technician who prepared tissues for electron microscopy. The delayed-type allergic reaction was verified with positive patch tests with 0.5-1% DSA in acetone. Tests on 15 controls were negative. The patient did not react to epoxy resin, accelerator or another hardener (57).

A worker who used a horizontal boring machine contracted allergic contact dermatitis when exposed to MHHPA. Patch testing with a dilution series of MHHPA in petrolatum gave the following positive results: 2%, 1% and 0.5% ++; 0.25% and 0.125% +. Immunohistochemical and electron microscopic observations indicated that the patch test reactions were conventional delayed allergic reactions. The patient also had an immediate-type contact dermatitis due to exposure to MHHPA, verified with positive skin prick tests and presence of specific IgE antibodies, and also erythematous skin symptoms in a chamber provocation test with MHHPA (96).

In a cross-sectional dermatological examination, 190 workers at 5 ceramics factories were investigated. The patch test series included MA (1% in ether) and PA (1% in petrolatum). Two workers had a positive patch test reaction to MA; no other data were available on these cases. The authors reported that, earlier, MA had been involved in 3 cases of skin sensitisation (129).

In 1955 urticarial reactions were reported among workers in PA production by Menschick (126) and Baader (11). There are, however, only a few case reports of immediate-type dermatitis due to acid anhydrides. MHHPA induced contact

urticaria in a worker from a factory where electronic components were filled with MHHPA-cured epoxy resin. The sensitisation was confirmed with the open test, which gave a positive reaction with the filling mixture and the hardener tested separately. Open tests with all the other components were negative, as were the epicutaneous tests with all the components. Open tests with the hardener were negative for 12 control persons (83). Tarvainen reported 2 cases of contact urticaria, one due to MHHPA, and the other due to MTHPA. Neither person had had skin contact with the anhydride; instead the exposure had only been airborne. Symptoms of urticaria began after 2 months of exposure. Later, conjunctivitis, rhinitis, and asthma symptoms also developed. An IgE-mediated allergy was diagnosed by means of skin prick tests and specific IgE antibodies (174). Another case of airborne contact urticaria due to MHHPA and HHPA has been reported for a winder in the electronics industry (95). CA has caused contact urticaria in a welder exposed to welding fumes of chlorinated polyester paint containing CA. An IgE-mediated allergy was verified with skin prick tests and specific IgE antibodies, and an open test with CA was also positive (102). MA has caused contact urticaria in an operator exposed to MA dust in a firm manufacturing polyester resin (94).

In conclusion, allergic dermatitis due to cyclic acid anhydrides is rare. There are only two case reports on delayed-type contact allergy, due to DSA and MHHPA. IgE mediated contact urticaria is more common, induced by contact or sometimes even airborne exposure to MA, MHHPA, MTHPA, HHPA and CA.

#### *11.2.2. Respiratory allergies*

Kern reported the first case of occupational asthma caused by an organic acid anhydride as early as 1939 (100). During the last 10 years a growing number of cases of asthma or rhinoconjunctivitis due to different acid cyclic anhydrides has been reported. The symptoms have been those of typical occupational asthma and rhinitis. After a symptom-free latency period the worker experiences symptoms when exposed to acid anhydrides at the workplace. The diagnosis has been based on the exposure, symptoms, and a cause-effect relationship proved with immunological tests or challenge tests. In industrial surveys the diagnosis has often been based on questionnaire and immunological studies.

Reports of occupational asthma are presented in Table 10 according to the exposure. The more rare sensitising anhydrides are PMDA (epoxy glue (125), production of PMDA (18)), HA (manufacture of synthetic flame-retardants, (160)) and CA (in fumes of polyester paints, (102)). There are no reports of THPA- or TBPA-induced cases of occupational allergy.

Data from the industrial surveys on the findings of antibody formation and symptoms are compiled in Table 11.

**Table 10.** Type of industry or occupation in reports of occupational rhinitis and asthma.

Acid anhydride	Exposure situation/occupation	Reference
PA	PA production	(128)
	Production of alkyd or unsaturated polyester resins	(13, 136, 196)
	Paint factory	(43, 100)
	Varnish production	(54)
	Plastic grinder	(188)
	Chemical foreman	(120)
	Tyre and rubber manufacture	(27)
	Meat wrapper/price label fume	(110)
TMA	TMA production	(216)
	Mixing of TMA powder to epoxy resin	(164)
	Epoxy powder painting	(43, 109)
	Manufacture of cushioned flooring	(13)
MA	Epoxy resin insulation	(64, 171)
	MA production	(50)
	Alkyd or polyester resin production	(13, 108)
HHPA	Epoxy resin moulding	(127)
	Isolating of components for the electronics industry	(195)
	Production of electrical capacitors	(195)
	Production of epoxy hardener	(26)
MHHPA	Filling of electric components	(163)
MTHPA	Epoxy resin coating	(133, 137, 199)
TCPA	Epoxy resin production	(168)
	Epoxy resin coating	(76)

**Table 11.** Effects of cyclic acid anhydrides in occupationally exposed.

Anhydride/ Exposure level ( $\mu\text{g}/\text{m}^3$ )	No of exposed	Exposure duration m=months, yr=years	Symptoms, effects	Specific IgE	Specific IgG	Skin prick tests	Reference
<b>PA</b> <300-13 000 (TWA)	118	2 m-40 yr	Rhinitis 28 (24%) Asthma 21 (18%)				(196)
6 600 (1 500-1 7400) (TWA)	35	0-43 yr	Conjunctivitis 16 (46%) Rhinitis 14 (40%) Rhinoconjunctivitis 6 (17%) Asthma 5 (14%)	0.9 (0.5-28)	2.5 (0.7-7.1)		(136)
<100 (TWA)	25	0.3-40 yr	Conjunctivitis 5 (20%) Rhinitis 5 (20%) Rhinoconjunctivitis 3 (12%) Asthma 0 (0%)	1.5 (0.4-2.7)	1.5 (0.6-3.1)		
0	22		No symptoms	1.0 (0.4-2.2)	1.1 (0.5-3.2)		
<b>TMA</b> 1 700-3 600 (TWA)	9	m-10 yr	Irritation 4/9 Asthma 3/9	1/9	4/9		(109)
10-2 100	18	8.6 yr	Rhinitis 1/18 Asthma 3/18	1/18, total TMA-HSA antibodies 4/18			(20)

**Table 11 . Cont.**

Anhydride/ Exposure level ( $\mu\text{g}/\text{m}^3$ )	No of exposed	Exposure duration m=months, yr=years	Symptoms, effects	Specific IgE	Specific IgG	Skin prick tests	Reference
<b>TMA</b>							
<1-100	11	2 yr	No symptoms	No antibody response			(124)
170 (GM)	8	Not given	Asthma/rhinitis 2/8	2.5%			(213)
87	39			0%			
<0.55	98		Asthma 1/98	0%			
<0.41	123			0%			
<0.53	42			0%			
full-shift							
<10	63	>1 m				TMA-HSA: 1/63 (OR 1.00)	(13)
10-40	36					5/36 (OR 10.00)	
>40	8					2/8 (OR 20.67)	
full-shift							
<10	44	>1 m	Risk of new work-related respiratory symptoms: 5/44 (OR 1.00)				(13)
10-40	13		6/13 (OR 5.94)				
>40	3		1/2 (OR 7.42)				

**Table 11. Cont.**

Anhydride/ Exposure level ( $\mu\text{g}/\text{m}^3$ )	No of exposed	Exposure duration m=months, yr=years	Symptoms, effects	Specific IgE	Specific IgG	Skin prick tests	Reference
<b>HHPA</b>							
3 800 (1 300-8 200)	11	Not given	Asthma 1/11 Rhinitis/conjunctivitis 9/11	4/11 (36%)	7/11 (64%)		(127)
1 900 (600-3 100)	16		Asthma 3/16 Rhinitis/conjunctivitis 13/16	7/16 (43%)	4/16 (25%)		
(HHPA and MHHPA)							
0	26	5 yr		0%			(195)
<10	37	6 yr		19%			
<10 (peak exposures)	19	10 yr		26%			
<10 (no peak exposures)	15	2.5 yr		0%			
10-<50	32	3.5 yr		34%			
$\geq 50$	26	5.5 yr		19%			
(HHPA and MHHPA)							
38.7 (1-189)	37	33 (1-85) m		27%		21.6%	(193)
(HHPA and MTHPA)							
not measured	110	9.1 (6.8-36) yr	Work-related respiratory symptoms 14/110	17/110 (15.4%)			(35, 36)
after improvements <0.5-36	6	<4 yr		0/6			



**Table 11. Cont.**

Anhydride/ Exposure level ( $\mu\text{g}/\text{m}^3$ )	No of exposed	Exposure duration m=months, yr=years	Symptoms, effects	Specific IgE	Specific IgG	Skin prick tests	Reference
<b>MTHPA</b>							
20-150	55	2.0 (0-6.0) yr	Eyes/upper airway 65%	2.2%	24%		(134, 192)
5-20 variable formerly 20-150, unexposed 1-5 yr unexposed referents	70 19 26 33		56% 42% 69% 9%	16% 16% 8% 0%	4% 5% 0% 0%		
10.1 (9-38) 5.9 (0-50)	81 45	26 (2-38) m 40 (1-105) m		8.6% 8.9%		4.9% 2.2%	(193)
Plant A 25.5- 63.9 (GM)	37	5.6 (0.2-13) yr	More symptoms of eyes, nose and pharynx in plant A ( $p<0.02$ ). No asthma in either plant.	64.9%			(202)
Plant B 4.93- 5.49 (GM)	58	12.4 (1.3-20) yr	More eye and nose symptoms in IgE-sensitised workers ( $p<0.05$ ).	65.5%			
<b>TCPA</b>							
1. Moulders, constant exposure 210-390 (GM)	7	2.5 (0.1-8.1) yr	Work-related asthma 35%, mainly in moulding				(118)
2. Intermittent exposure	6		(work-related wheezing 27%, shortness of breath 29%, chest tightness 39%).	43%	57%		
3. Coil assemblers	33			67%	67%		
4. Office workers	6			25%	34%		
				0%	0%		

GM: geometric mean.

### *Determinations of specific antibodies*

The proof of IgE mediation in immediate-type asthma or rhinitis due to acid anhydrides is convincing. When Kern reported the first case of asthma and rhinitis due to PA in 1939, he already had evidence of the immunological background. The scratch test with PA in crystalline form and diluted 1:1 000 in alcohol gave positive reactions. The tests with control patients were negative. The passive transfer test was also positive (100). Maccia and his colleagues were the first to find specific IgE in the serum of a patient with asthma due to PA in 1976. The patient experienced an immediate asthmatic reaction in the provocation test with PA dust (120).

Specific IgE has been found to PA, TMA, MA, TCPA, HHPA, HA, MHHPA, MTHPA, and CA (36, 76, 118, 120, 127, 134, 136, 160, 164, 176, 192, 193, 195, 201, 216). The induction time for positive specific IgE antibodies has been 8.8 (range 1-35) months when HHPA-, MHHPA-, and MTHPA-exposed workers were followed. The incidence of sensitisation was 4.1 cases/1 000 months at risk (193). The half time for specific IgE antibodies after cessation of the exposure was 1 year for TCPA and 0.9 year for MTHPA (186, 191). Commercial tests are available for measuring specific IgE antibodies for some anhydrides (123).

Inhibition studies and passive transfer studies have supported the specificity of IgE antibodies. However, cross-reactivity between some acid anhydrides has been reported (36, 119, 176, 190).

Immediate-type skin tests with acid anhydride-HSA conjugate have correlated well with the finding of specific IgE (17, 76, 143, 190, 192, 193, 216).

The location and specificity of the IgE antibody for the epitopes present on the acid anhydride (hapten)protein complex have been studied. It has been postulated that the reaction of acid anhydride with albumin alters the albumin to form a new antigenic determinant or that the hapten is altered at the antibody-combining site (19, 143, 210, 216). The formation of new antigenic determinants on the albumin site explains the cross-reactivity in the radioallergosorbent tests (RAST). There is evidence that in patients sensitised to TCPA and TMA, the antibody combines with the anhydride and the adjacent portion of the HSA molecule, whereas in patients sensitised to PA, the antibody is specific to the hapten (176). TMA is claimed to form unique antigenic determinants that do not bind significantly with antibodies formed by sensitisation to PA, HHPA and HA. This may explain why significant cross-reactivity with TMA has not been found in inhibition studies (19, 176, 216).

The workers exposed to either MTHPA or both HHPA and MHHPA have showed a marked cross-reactivity in skin prick tests, in the specific IgE determinations as well as in inhibition studies to MTHPA-HSA, HHPA-HSA, and MHHPA-HSA but not to PA-HSA and MA-HSA. For PA-exposed subjects the cross reactivity with other anhydrides was less pronounced (190).

The serum levels of specific IgE antibodies and interleukin-4 and interleukin-13 as Th<sub>2</sub> lymphocyte markers and interferon-gamma as a Th<sub>1</sub> lymphocyte marker were measured in 147 MTHPA-exposed workers. Work-related eye and nasal symptoms due to MTHPA were associated to the levels of interleukin-4 and also

interleukin-13. A shift in the balance between Th<sub>1</sub> and Th<sub>2</sub> cells was suspected due to the IgE mediated allergic response (204).

Specific IgG antibodies have been studied especially in connection with sensitisation to TMA. Specific IgG antibodies against TMA-HSA have been correlated with late-onset occupational asthma due to TMA. They have also been found in the pulmonary disease-anaemia syndrome due to TMA, as have IgG antibodies to erythrocyte conjugate (142, 144, 145, 164, 177).

Specific PA-IgG antibodies have been found in a worker exposed to PVC degradation products and phthalic acid esters. This finding indicates exposure to phthalic anhydride (131).

No cross-reactivity with PA, MA, HHPA, or TCPA was found when the specificity of IgG antibodies against TMA-HSA conjugate was investigated. In an equal test TMA-HSA was able to inhibit IgG bound to both PA-HSA and MA-HSA. Another antibody was suspected (53).

Nielsen and his co-workers have studied IgE, IgG, IgG<sub>4</sub>, and IgM antibodies specific for PA in workers from alkyd or unsaturated polyester resin production. The mean level of exposure was 6 600 (1 500-17 400)  $\mu\text{g}/\text{m}^3$  (time-weighted average, TWA) during loading and for full workday 400  $\mu\text{g}/\text{m}^3$ . Of the heavily exposed workers, 69% had rhinitis and/or conjunctivitis and 5 workers (14%) had PA-induced asthma. There was a significant ( $p=0.01$ ) difference between the heavy and low exposure groups only for specific IgG against PA. Only 1 worker with asthma had an increased specific IgE level. The subjects with asthma had significantly higher values for specific IgG than the asymptomatic subjects. Specific IgG<sub>4</sub> was found in 4 persons, 3 had asthma and 1 had rhinitis. The authors concluded that specific IgG is an index of PA exposure, and that specific IgG<sub>4</sub> may be a pathogenetic factor in asthma (136).

In TMA-exposed workers, the levels of IgG<sub>1-4</sub> subclasses against TMA-HSA did not differ between workers with and without a TMA-induced immunological lung disease (52).

In MTHPA-exposed workers no association between work-related symptoms and specific IgG<sub>4</sub> antibodies was found, but the intensity of the exposure was the major determinant of specific IgG<sub>4</sub> levels (201, 205).

#### *Reports of allergic occupational diseases due to cyclic anhydrides: PA*

Wernfors *et al.* studied 118 workers in four plants producing alkyd or unsaturated polyester resins. Forty-eight were current employees and 70 were former employees. The PA dust concentrations during the loading of the reactors and in the handling of bags were very high, up to 13 000  $\mu\text{g}/\text{m}^3$ . The fraction of respirable dust was about 40%. The authors found 28 (24%) persons with work-related rhinitis and 21 (18%) with asthma. Eleven per cent had symptoms of chronic bronchitis. In 10 of the 21 asthmatics, rhinitis preceded the asthmatic symptoms. The latency period before the onset of the respiratory symptoms ranged from 1 month-16 year. A positive skin-scratch test was found in 3 of the 11 asthmatics but in none of the non-asthmatics. A Prauznitz -Küstner test was

positive with serum of 2 asthmatics. The same patients were challenge tested. The bronchial provocation tests with PA powder  $6\ 000\ \mu\text{g}/\text{m}^3$  (5 minutes) caused a dual asthmatic reaction in the first patient, whereas the test with  $500\ \mu\text{g}/\text{m}^3$  was negative. The second patient experienced a dual reaction when challenged with the lower PA concentration (196).

When the exposure level of PA was measured (Table 11), the full day TWA for PA was approximately  $400\ \mu\text{g}/\text{m}^3$ , well below the Swedish exposure limit value  $6\ 000\ \mu\text{g}/\text{m}^3$  at that time. The authors then suggested a ceiling value below  $6\ 000\ \mu\text{g}/\text{m}^3$  for PA (136).

*Reports of allergic occupational diseases due to cyclic anhydrides: TMA*

Both immediate- and late-type respiratory allergies have been reported due to TMA. Both begin after a latency period. Specific IgE antibodies have been found in immediate-type rhinitis and asthma (144, 214). In the late-type respiratory syndrome the worker experiences coughing, wheezing and dyspnoea, starting 4-8 hours after the exposure. The respiratory symptoms have been accompanied by malaise, chills, fever, myalgia and arthralgia. No specific IgE antibodies are found, but IgG and IgA antibodies are present (144, 214). The disease has not been verified with challenge tests. An irritant reaction has been postulated (183).

Pulmonary disease-anaemia syndrome due to TMA is a disease first reported by Rice *et al.* in 1977. Herbert and Orford reported 7 more cases in 1979, and Ahmad *et al.* presented another 2 in 1979. All cases had been exposed to fumes from TMA-cured epoxy resin sprayed on hot pipes. The symptoms were cough, haemoptysis, dyspnoea, pulmonary infiltrates, restrictive respiratory defect, hypoxaemia, and anaemia. The symptoms ranged from mild to very severe. A possible fatal case was identified (2, 74, 142, 157). IgG antibodies have been found to both human serum conjugate and erythrocyte conjugate of TMA. Open lung biopsy has shown intact alveolar septae and extensive intra-alveolar haemorrhage with granular pneumocyte hyperplasia. Immunofluorescent studies were negative, suggesting that the antibodies were not involved in the tissue injury (2, 74, 144, 157, 214). Patterson *et al.* did not find any IgE antibody activity against trimellityl-HSA (TM-HSA) in workers with pulmonary disease-anaemia syndrome. IgG activity against TM-HSA did not differ from the level in other workers exposed to TMA fumes under similar work conditions. IgG, IgA and IgM antibodies were found against TMA-human erythrocytes (142). Later, antibodies to TMA-human erythrocytes were also found in workers with asthma due to TMA, but not in unexposed persons (177).

All 9 workers from a barrel manufacturing plant were investigated because of their exposure to TMA. The levels of TMA in the personal breathing zone were  $1\ 700$ - $3\ 600\ \mu\text{g}/\text{m}^3$ . One worker was asymptomatic. Four workers had TMA-induced irritant effects. Three had symptoms and IgG levels consistent with late-type respiratory syndrome. One of them also had specific IgE against TMA. One worker had bronchitis not related to TMA (109).

In a factory preparing epoxy resin coating material the exposure level for TMA was less than  $180 \mu\text{g}/\text{m}^3$  after improvements in work hygiene. During two years no TMA related diseases were found among 11 workers (124).

In the TMA manufacturing industry, a 12-year (1976-1987) clinical and immunological evaluation of 196 workers was carried out. The diagnosis was based on results of a questionnaire, an interview and immunological studies for total TMA antibodies and TMA-specific IgE. IgE-mediated immediate-type asthma or rhinitis was found in 21 workers and late-type asthma in 10 workers, and yet another worker had pulmonary disease-anaemia syndrome. A total of 113 workers had only irritant symptoms, and 46 were asymptomatic. A low level of total antibodies was found for TMA-HSA in 16% and 8%, respectively. No data were available on the exposure. There was, however, an annual decline in the number of sensitised workers due to improvements at the workplace (212).

In the same factory, in a cross-sectional study of 474 workers in 1988-1989 with a very similar setting, five exposure groups were assigned by an industrial hygienist on the basis of job history and the results of personal monitoring of some employees in each exposure class. Seven per cent had an immunological syndrome due to TMA exposure, and 32% had irritant symptoms with low specific total and IgE antibody levels. Sixty-two per cent had no work-related symptoms and very few or no antibodies. The mean total antibody levels and mean IgE antibody levels decreased according to the exposure level. TMA specific IgE antibodies were found only in the high exposure group ( $0.54\text{-}6\ 500 \mu\text{g}/\text{m}^3$ , geometric mean  $170 \mu\text{g}/\text{m}^3$ ) whereas the findings were negative in four other groups with exposure less than  $6\text{-}970 \mu\text{g}/\text{m}^3$  (geometric mean  $87 \mu\text{g}/\text{m}^3$ ) of TMA. The sensitisations and illnesses due to TMA were concentrated into the three upper exposure groups, and therefore efforts to reduce exposure were suggested. A group of new employees not evaluated earlier for an occupational disease were analysed for total TMA-HSA antibody levels by age, sex, date of hire and smoking. Only current or former smoking was associated with the total antibody levels ( $\chi^2=6.45$ ,  $p=0.01$ ). Atopic status was not assessed (213). One year after 29 sensitised workers had been moved to low-exposure jobs, their symptoms and pulmonary functions had improved and the specific antibody levels had decreased (60).

One case of allergic alveolitis has been reported in connection with exposure to both TMA and PA. The worker was exposed to the dust and fumes of polyester powder paint during a malfunction of the ventilation of the factory hall. The paint contained small amounts (<1%) of both TMA and PA. The diagnosis was based on the follow-up of the symptoms and on the findings in chest radiographs and BAL, as well as on the presence of fever and a slight reduction in the transfer factor after a short re-exposure at work (153).

*Reports of allergic occupational diseases due to cyclic anhydrides: MA*  
Durham reported in 1987 the results of the MA challenge tests of 2 MA-exposed workers (a storeman and a batch weigher). In both cases control tests were negative and dual asthma reactions due to MA were verified (37). In another

report a worker developed cough, rhinitis, breathlessness and wheezing after one month of exposure to PA and MA. The dust exposure during the emptying of bags was  $330 \mu\text{g}/\text{m}^3$  for PA and  $170 \mu\text{g}/\text{m}^3$  for MA. The worker was a smoker. An atopic tendency was found in the skin prick tests, and he had nonspecific bronchial hyperreactivity. In the bronchial challenge tests with MA crystals he experienced a dual asthmatic reaction, whereas the test with PA was negative. The dust concentrations were well below those measured from the workplace. No skin prick tests or antibody determinations were carried out (108).

A worker in MA production who had previously had MA-related asthmatic symptoms developed severe haemolytic anaemia. He relapsed two weeks after the return to work the next year. Afterwards he remained stable as long as he avoided MA exposure. He had high titre of MA-specific IgE but not IgG antibodies. The Coombs test was positive but no antibodies to MA red-cell conjugate were found. There were no symptoms of pulmonary haemorrhage in conjunction with pulmonary disease-anaemia syndrome due to TMA exposure (50, 81).

#### *Reports of allergic occupational diseases due to cyclic anhydrides: HHPA*

In 1985 Moller reported the first cases of occupational sensitisation to HHPA from exposure to epoxy resin moulding. Twenty-seven workers were evaluated on the basis of the results of a questionnaire, pulmonary function tests, and antibody determinations. The concentration of HHPA was very high when compared to the more recent studies (Table 11). All 4 cases with symptoms of occupational asthma had specific IgE to HHPA conjugates, as did 6 of 22 cases with rhinitis and/or conjunctivitis. In the specificity studies both HHPA-HSA and PA-HSA were effective inhibitors (127).

Erosions of the nasal mucosa and epistaxis have been described in connection with IgE-positive occupational rhinitis due to HHPA. All the patients also had high titres of specific IgG. The HHPA concentration was not determined, but it was not considered high enough to cause tissue destruction (61). The same group followed 28 employees with HHPA-induced asthma or rhinitis for least one year after removal from exposure. The symptoms and spirometry normalised in all but 1 worker. There was a decline in the HHPA-specific IgE in 25 workers (59).

Chee *et al.* diagnosed a case of asthma due to HHPA in the manufacture of epoxy resins. The hardener consisted mainly of HHPA but also of an amine as an additive. In the process the hardener was heated to  $130^\circ\text{C}$ . The long-term follow-up of peak expiratory flow showed deterioration during the workweek and improvement during days off. The authors also carried out challenge tests. A control test where an empty tin was heated was negative. When 200 ml of pure HHPA was heated to  $92^\circ\text{C}$ , the patient experienced a severe immediate and also a late long-lasting asthmatic reaction. No skin prick tests or antibody determinations were carried out (26).

Nielsen *et al.* investigated the allergic aetiology of work-related nasal symptoms of HHPA-exposed workers. Nasal challenge tests with HHPA-HSA conjugate were carried out. The results were positive in 11 subjects sensitised to HHPA (positive skin prick tests and specific IgE antibodies) and negative in 9

non-sensitised subjects, in spite of work related irritation symptoms in the non-sensitised individuals (135).

*Reports of allergic occupational diseases due to cyclic anhydrides: MHHPA*

MHHPA has caused sensitisation and respiratory symptoms (96). A rare case of specific MHHPA-induced laryngitis has been reported in an electrician. A skin prick test with MHHPA conjugate was positive, and specific IgE antibodies to the conjugate were found. The laryngeal reaction was verified by a challenge test with MHHPA (163).

*Reports of allergic occupational diseases due to cyclic anhydrides: MTHPA*

Nielsen *et al.* found a case of work-associated asthma and rhinitis in a subject exposed to MTHPA used as an epoxy-curing agent in the preparation of barrels for grenade firearms. MTHPA-HSA-specific IgE antibodies were found, but specific IgG antibodies were not present. A skin prick test was positive with the same conjugate but negative with PA-HSA (137). Later the work force of the factory was examined for symptoms, exposure, antibody response, lung functions and risk factors. In a group consisting of 140 currently and 26 formerly exposed workers, they found higher frequencies of work-related symptoms of the eyes (31 versus 0%), nose (53 versus 9%), pharynx (26 versus 6%) and asthma (11 versus 0%) than among 33 controls. The rate was 16 for positive skin prick tests with MTHPA-HSA conjugate, 18 for specific IgE antibodies and 12 for specific IgG antibodies, while for the controls all the tests were negative. There were statistically significant exposure-response relationships between the exposure and symptoms of the eyes and upper airways, dry cough, positive skin prick tests and specific IgE and IgG antibodies. The concentration of MTHPA was under  $150 \mu\text{g}/\text{m}^3$ . The median exposure time for specific IgE positivity was 5 (range 0.2-5) years. A close association between specific IgG<sub>4</sub> and total specific IgG was found. Up to 91-95% inhibition of the IgE-MTHPA-HSA binding was reached with MTHPA. There were no significant differences in lung functions between sensitised and non-sensitised subjects. No association between sensitisation and either atopy or smoking was found. They found that significantly more former workers than current ones had positive skin prick tests with common allergens; atopic workers tended to withdraw from the exposure early, perhaps even before clear work-related symptoms appeared (133, 134, 192).

The decline in serum antibodies to MTHPA was followed in 10 workers after the cessation of exposure an average of 54 months earlier. The mean individual half-time for specific IgE was 0.9 years, and for specific IgG it was 0.4 years. In addition, 4 workers who moved to jobs with significantly reduced exposure were followed, and corresponding decreases in antibodies were seen. No correlation was found between the half-times of specific IgE and total IgE, atopy, smoking or gender (191).

Yokota *et al.* studied the workers at two plants where condensers for electric appliances were manufactured and MTHPA was used as a hardener in the epoxy resin insulation. Some workers had had work-related nasal symptoms. At first the

authors studied 28 workers exposed to MTHPA levels between 1.09 and 22.4  $\mu\text{g}/\text{m}^3$ . Specific IgE antibodies were detected in 9 (32%) of the workers. Eight of them had nasal symptoms. The authors also found that the total IgE levels were significantly higher in the group with specific IgE, and suggested that total IgE can be used in the selection of workers (199).

A later study group consisted of 148 workers, of whom 66% had specific IgE antibodies. The authors further found that the relative risk for elevated MTHPA-specific IgE for smoking was 4.1. In the group with a high total IgE, the relative risk of producing IgE antibodies to MTHPA was 4.7 in a comparison with the group with a low total IgE (<80 IU/ml) (200).

In a study of the exposure-response relationship, the minimal level for an association between MTHPA exposure and work-related symptoms was 15-22  $\mu\text{g}/\text{m}^3$ . It was suggested that exposure above 15  $\mu\text{g}/\text{m}^3$  should be avoided to prevent the development of occupational allergic diseases among most workers (202).

In 1991, Drexler *et al.* investigated 110 workers exposed to HHPA and MTHPA and found that 20 of them were sensitised according to skin prick tests and specific IgE determination. In challenge tests occupational asthma or rhinitis was verified for 6 cases. The concentrations of acid anhydrides in air were not measured. In the end of 1991 the hygienic conditions at the plant were improved. In 1995 a new study was carried out that also included people who had left the plant since 1991. The relative risk of people sensitised in 1991 leaving the plant between 1991 and 1995 was 2.6 compared with people without any sign of sensitisation. The rate of sensitisation was 21%. None of the 6 persons employed after 1991 showed evidence of sensitisation. Five of the 6 persons with verified sensitisation in 1991 were still at the workplace. All of them reported fewer symptoms than before. The exposure level was measured in 1995, and it was <0.5-36  $\mu\text{g}/\text{m}^3$  (35, 36).

#### *Reports of allergic occupational diseases due to cyclic anhydrides: TPCA*

In 1987 five cases of occupational asthma due to TPCA in epoxy resin were reported. The diagnoses were confirmed in 3 workers by dual asthmatic reactions in inhalation challenge tests. TPCA-bovine serum albumin conjugate was used to detect specific IgE antibodies. No antibodies were found (168).

TPCA has been used as an epoxy-resin curing agent since 1979 in an epoxy-coating process in a factory making electronic components in the United Kingdom. Seven cases of occupational asthma were diagnosed in 1979-1980. Inhalation challenge tests with controlled TPCA concentrations from 1.3-961.1  $\mu\text{g}/\text{m}^3$  with both immediate and late reactions confirmed the diagnoses. Specific skin prick tests were positive, and specific IgE antibodies against TPCA-HSA conjugate were detected. The factory was closed in 1980 (76, 184). A 12-year follow-up showed that symptoms at first improved, but then remained unchanged. All the cases still had daily or weekly symptoms. The bronchial hyperresponsiveness had also persisted. Specific IgE antibodies fell slowly with a half-time of 1 year (12, 186).



In a Canadian cross-sectional study of 56 workers in a factory using TCPA-cured epoxy resin for about 2 years, the prevalence of work-related respiratory symptoms was high, 27-39%. According to a questionnaire, occupational asthma was present in 35% of the workers. Thirty-one per cent had specific IgE, and 39% had specific IgG against TCPA-HSA. The mean TCPA concentration was between 210-390  $\mu\text{g}/\text{m}^3$ . The prevalence of specific IgE was highest among those who had the jobs with the highest exposure. After the ventilation was improved and exposure was reduced to less than 110  $\mu\text{g}/\text{m}^3$ , the symptoms decreased, and no new cases of occupational asthma were found (118).

*Reports of allergic occupational diseases due to cyclic anhydrides: PMDA*

In 1980, Meadway reported 2 cases of asthma from exposure to PMDA-cured epoxy resin when glue was mixed, used and cured. The workers underwent challenge tests simulating work with the mixture, and the results were positive. Five other workers had negative test (125).

Next report on PMDA is from year 1995. A cross-sectional and follow-up study was carried out with 92 workers with exposure mainly to PDMA but also to PA, MA or TMA. Of those with less than one year of exposure, 56 workers had work-related symptoms. Eighteen had dyspnoea, and 8 had bronchial obstruction. Specific IgE antibodies were found only in 15 workers, of them 12 had symptoms. The authors also found 11 cases of haemorrhagic rhinitis that occurred after more than 15 years of exposure. Only one of the cases had specific IgE antibodies. After 10 months both the symptoms and the bronchial obstruction of the group no longer exposed had diminished, whereas the workers still exposed had comparable pathological findings in the re-examination. The symptoms were reversible in workers whose exposure stopped after a work period of about 0.7 years. No relevant specific IgG antibodies were detected in this group (18).

A case of acute haemorrhagic alveolitis associated with anaemia similar to pulmonary disease-anaemia syndrome due to TMA has been reported for PMDA exposure. PMDA in powder form was used as a cross-linking agent in epoxy resin production. High concentrations of PMDA-specific IgG antibodies were found and also TMA-specific IgG antibodies, but not PA- or MA-specific IgG antibodies. The patient had no specific IgE antibodies. The Coombs test was negative (29).

*Reports of allergic occupational diseases due to cyclic anhydrides: HA*

A worker exposed to HA powder in the manufacture of a synthetic flame retardant developed after one year rhinitis, hives, and wheezing at work. He and 2 of the 6 other symptomatic workers of all 20 tested had specific IgE antibodies for HA-HSA conjugate. In RAST inhibition studies HA-HSA showed the highest inhibition. A cross-reactivity was found with HHPA-HSA but not with PA and TMA conjugates (160).

### *Reports of allergic occupational diseases due to cyclic anhydrides: CA*

A welder developed work-related urticaria and asthmatic symptoms when exposed to the thermal degradation fumes from a polyester paint. The paint contained both alkyd resins and chlorinated polyester resin. A new sensitising anhydride, CA, was identified from the hardener. A skin prick test with CA-HSA conjugate was positive, as was an open test for urticaria. CA-HSA-specific IgE antibodies were also detected. Significant decreases in peak expiratory flow measurements were seen after short (10 seconds) welding procedures (102).

### *Concluding remarks*

The reports of occupational asthma and rhinitis due to PA are mainly dated to the years 1980-1990. The case reports and industrial surveys on TMA-induced work-related respiratory symptoms are likewise not very recent. During last decade MTHPA and HHPA have been the main topics of concern. The exposure levels have decreased with improved work hygienic control, but exposure peaks still occur.

The respiratory allergies have mainly been IgE mediated. Specific IgE antibody determinations and skin prick tests are relevant methods when investigating the aetiology of work-related symptoms. Specific IgE antibodies cannot, however, be used to assess exposure at the work place. The half-time of specific IgE antibodies after cessation of the exposure has been about 1 year.

The 12-year follow-up study of TCPA-induced asthma showed a poor prognosis for occupational asthma despite cessation of the exposure. Equally long follow-up studies are not available on occupational asthma induced by other cyclic acid anhydrides.

### *11.2.3 Predisposing factors for cyclic acid anhydride-related allergy*

Venables and co-workers studied the effect of smoking and atopy on antibody E production in 300 TCPA-exposed workers. Atopy was defined as at least one positive skin prick test to common allergens. Twenty-four of the workers had specific IgE to TCPA. Twenty of them (83.3%) were current smokers compared with 133 (48.2%) of 276 without antibodies ( $p < 0.01$ ). Atopy was also more common in those with specific IgE, but not significantly so. Smoking and atopy interacted, the prevalence of antibody being 16.1% for atopic smokers, 11.7% for nonatopic smokers, and 8.3% for atopic non-smokers ( $p > 0.025$ ). The authors concluded that smoking may interact with atopy in the production of specific-IgE antibodies to TCPA (185).

In a study of 55 workers highly exposed to MTHPA no significant differences were found between the subgroups for atopy and smoking habits according to IgE sensitisation. In the same study with a total of 144 exposed workers the group of former workers had positive skin prick tests to common allergens significantly more often than unexposed controls. The authors suggested that atopic workers tend to withdraw from exposure early because their mucous membranes are more reactive and therefore atopic persons do not seem to run a particular risk of sensitisation to small reactive organic molecules (134).

In a recent prospective study, 163 workers exposed to HHPA, MHHPA and MTHPA were followed for 32 (1-105) months. The mean combined exposure level was 15 (range <1-189)  $\mu\text{g}/\text{m}^3$ . Thirteen per cent of the workers had positive specific IgE. An association was found between exposure and atopy, respectively, and the induction of specific antibodies. The odds ratio for atopics to get IgE sensitised as compared with non-atopics was 6.0 (95%CI 2.2-16.6). Subjects with a mean exposure of  $>15 \mu\text{g}/\text{m}^3$  had a significantly higher risk for development of specific IgE (odds ratio 3.4; 95%CI 1.2-9.4) than subjects with a mean exposure of  $<10 \mu\text{g}/\text{m}^3$ . The risk for atopics was comparable with the risk for the subjects in the most exposed group. The effect of smoking was not significant (193).

In a Canadian study of 148 MTHPA-exposed workers a correlation was found between total IgE and specific IgE ( $p<0.0001$ ). Current smoking was significantly associated with specific IgE production only in the group with low total IgE,  $<100 \text{ kU}/\text{ml}$  (200).

Altogether 119 TMA-exposed workers were followed for five years to determine whether they would develop a respiratory disease. Sixteen had TMA-specific IgE antibodies and 3 of them had asthma in the beginning. Another 6 developed asthma during the 5-year follow-up period. One of the 102 workers with no specific IgE antibodies developed asthma. Specific IgG antibodies were detected in 44 subjects, 6 had a non-immediate respiratory disease in the beginning and 2 more were found after five years. None of the IgG-negative workers developed a respiratory disease (58). In a study of 57 HHPA-exposed workers, 7 had IgE and IgG-mediated respiratory disease whereas 9 had only IgE-mediated disease. An association was found between the development of respiratory disease and specific IgE and IgG antibodies, as well as an association with the level of exposure but not with smoking (62). Skin prick test reactivity to common allergens in 33 employees with respiratory symptoms due to HHPA showed that atopy had only a marginal clinical significance as a risk factor for disease (63).

In a case-control study of 16 persons with TMA asthma and 44 similarly exposed controls, determinations of specific IgE against four common environmental allergens were carried out. Fifty-six per cent of the cases and only 29% of the controls were found to be atopic (172).

Risk factors for sensitisation and respiratory symptoms were evaluated in a historical cohort study consisting of 506 workers from four factories. The cohort was defined as all workers with exposure to anhydrides for more than one month since the beginning in 1960. Three factories manufacturing resins used principally PA, but also MA and TMA were used. One factory produced cushioned flooring and used only TMA. The exposure was assessed retrospectively, by job. The current full-shift and task-specific exposure measurements, the past exposure data and qualitative information were used and exposure estimates were calculated in the job-time-exposure matrices (181). The questionnaire, comprising employment history, respiratory symptoms and smoking habits, was completed by 401 workers (79%). Skin prick tests with common inhalant allergens and anhydride conjugates were carried out. Thirty-four persons (8.8%) had respiratory symptoms related to

anhydride exposure, and 12 (3.2%) were sensitised according to the skin prick tests with anhydride conjugates. Sensitisation was associated with work-related respiratory symptoms and with smoking, at the time of the exposure to acid anhydride. An exposure-response relationship was not found overall, but in the factory with exposure to TMA an increased prevalence of sensitisation and work-related symptoms with increasing full-shift exposure: <10, 10-40  $\mu\text{g}/\text{m}^3$  and >40  $\mu\text{g}/\text{m}^3$  of TMA was observed. The relation was apparent within the current occupational exposure standard of 40  $\mu\text{g}/\text{m}^3$  and it was not modified significantly by smoking or atopy. Exposure to PA or MA was found to be uncommon as a cause of sensitisation at the low exposure levels measured, 8.9-11.9  $\mu\text{g}/\text{m}^3$  and 1.8-2.8  $\mu\text{g}/\text{m}^3$ , respectively (13).

The association of human leukocyte antigen (HLA) allele frequency and specific IgE antibody to acid anhydride-HSA conjugates has been investigated to determine a possible genetic influence on sensitisation. Thirty workers with work-related respiratory symptoms with specific IgE antibodies had been exposed to PA, TMA, or TCPA. Thirty referents were exposed to PA or TMA. A similar proportion of both the cases and referents were atopic and smokers, the other risk factors for this sensitisation. A significant excess of HLA-DR3 loci were found in cases with specific IgE to acid anhydrides when compared with the controls (50% versus 14%). A relationship was found between HLA-DR3 and specific IgE antibodies for TMA and possibly for TCPA but not for PA. The difference in the epitope was suggested as the reason for the different findings (206).

Altogether 57 HLA antigens were analysed in MTHPA- or HHPA-exposed workers. Fifty-three were sensitised according to the specific IgE antibodies and positive skin prick test. Forty-seven non-sensitised exposed workers were controls, as was a group of unexposed subjects. Only for HLA-A25 and HLA-A32 statistically significant differences were found between the sensitised and non-sensitised workers, 0% versus 9% and 0% versus 13%, respectively. An HLA-antigen-associated immune protective factor was suspected to reduce the risk of sensitisation. An association HLA-DR3 was not found for MTHPA-sensitised workers, as in an earlier study on TMA-sensitised workers (132).

#### *Concluding remarks*

In TCPA-exposed workers smoking and atopy have been found to interact in the production of specific-IgE antibody to TCPA. In a prospective study on HHPA-, MHHPA- and MTHPA-exposed workers, a significant effect of atopy, as well as of exposure, on sensitisation was observed. The influence of smoking on sensitisation was not significant.

The influence of smoking and atopy on TMA sensitisation has been non-significant. Increasing full-shift exposure to TMA has been a predisposing factor for sensitisation.

There are also two studies on the genetic background of sensitised persons. A relationship was found between HLA-DR3 and specific IgE antibodies for TMA and possibly for TCPA but not for PA. When MTHPA- and HHPA- exposed

workers were investigated no such association was found but an HLA-A25 and HLA-A32 associated immunoprotective factor was suspected.

### **11.3 Genotoxic effects**

There are no data on genotoxic effects of cyclic acid anhydrides in humans.

### **11.4 Carcinogenic effects**

In a case-control study mortality from lung cancer was investigated in a plant producing acetylene and PA. After control for age and smoking, the odds ratio for lung cancer mortality among the 43 subjects exposed in the factory was 5.6 (95%CI 1.9-16.2). The corresponding odds ratio for 99 referents from other work environments in the region was 1.7 (95%CI 0.9-3.5). There was, however, exposure also to phthalates and soot (156).

### **11.5. Reproductive and developmental effects**

There are no data on the developmental effects of cyclic acid anhydrides on human reproduction.

## **12. Dose-effect and dose-response relationships**

### **12.1. Single and short-term exposures**

The acute effects of cyclic anhydrides on animals have generally been found to be irritation of mucous membranes after direct exposure (Table 7).

The human nasal irritation threshold for PA has been reported to be 30 000  $\mu\text{g}/\text{m}^3$  and that for MA to be 5 480  $\mu\text{g}/\text{m}^3$  (162). However, exposure type and duration, generation of anhydrides, and particle sizes were not given. In early reports, irritation of the mucous membranes was a common immediate response in workers highly exposed to acid anhydrides in powder form (11, 126, 136). In workers exposed to high levels of TMA, rhinorrhoea, cough, and dyspnoea have been reported already after the first exposure (214). In more recent studies irritant symptoms of eyes and upper airways have been detected after relatively low exposure (150  $\mu\text{g}/\text{m}^3$  TWA) of MTHPA (134).

Immunisation of animal species verified with specific IgE and IgG antibodies, followed after cutaneous administration or single intradermal injection with free or conjugated PA, TMA, MA, HHPA, MHHPA, MTHPA, THPA and SA (Table 7 and 8). Repeated short-term exposures (subcutaneous or inhalation) with PA or the corresponding conjugate induced specific IgG antibody formation in a dose-response manner in guinea pigs. In challenges after sensitisation, more positive tests were seen when a higher dose was used (165) (Table 8).

In inhalation challenge tests in rats, concentrations of 30-300  $\mu\text{g}/\text{m}^3$  of TMA dust induced haemorrhagic lung lesion and antibodies, whereas 10  $\mu\text{g}/\text{m}^3$  had no effects (211).

## 12.2 Long-term exposure

Long-term dose-response studies (animals) are few. Rats, hamsters and monkeys were exposed to 1 100-9 800  $\mu\text{g}/\text{m}^3$  concentrations of MA 6 hours/day 5 days/week for 6 months. Nasal and ocular irritation and metaplasia of nasal mucosa were found at all the exposure levels only in rodents being obligate nose breathers (171).

Human data derived from industrial surveys have been compiled in Tables 10 and 11.

In a survey with a total exposure time of about 40 years, 46% in the high exposure group (1 500-17 400  $\mu\text{g}/\text{m}^3$  of PA) had conjunctivitis, 40% had rhinitis, and 14% had asthma. In the low exposure group (<100  $\mu\text{g}/\text{m}^3$ ) the corresponding figures were 20% for conjunctivitis and rhinitis, and 0% for asthma. There was no association between specific IgE antibodies and PA exposure (136).

In a historical cohort study of PA- and MA-exposed workers sensitisation was found to be uncommon at the low exposure levels measured, 8.9-11.9  $\mu\text{g}/\text{m}^3$  and 1.8-2.8  $\mu\text{g}/\text{m}^3$ , respectively (13).

When TCPA had been in use for two years, 35% of the 56 exposed workers had occupational asthma according to a questionnaire, and 31% had specific IgE antibodies. The exposure levels to TCPA in preforming and moulding had been between 140-590  $\mu\text{g}/\text{m}^3$ . The next year, after the ventilation was improved, the TCPA exposure fell to 30  $\mu\text{g}/\text{m}^3$  in preforming and to <10-110  $\mu\text{g}/\text{m}^3$  in moulding. After that, work-related symptoms decreased and no new cases of occupational asthma have been reported (118).

In a historical cohort study of TMA-exposed workers, the prevalence of sensitisation and work-related symptoms increased with increasing exposure. In workers exposed to 10-40  $\mu\text{g}/\text{m}^3$  or >40  $\mu\text{g}/\text{m}^3$  of TMA compared to the workers exposed to <10  $\mu\text{g}/\text{m}^3$ , the odds ratio for positivity was for TMA skin prick tests 10.0 and 20.7 and for the risk of new work-related respiratory symptoms 5.9 and 7.4, respectively (13).

In workers exposed to HHPA and MHPA at levels of <10, 10-50 and >50  $\mu\text{g}/\text{m}^3$  specific IgE antibodies were found. There were no differences in levels of the specific IgE antibodies between the exposure groups. The workers in the low exposure group had, however, occasionally had higher exposure peaks (195). Among MTHPA-exposed workers, even at low levels of exposure (5-20  $\mu\text{g}/\text{m}^3$ ) 56% had symptoms of the eyes and upper airways, 9% had asthma, and 16% had MTHPA specific IgE antibodies. The corresponding numbers were 65%, 11%, and 22% in the more heavily (20-150  $\mu\text{g}/\text{m}^3$ ) exposed groups (134, 192).

When HHPA- and MHPA-exposed workers were followed for a mean period of 33 months (range 1-85), 27% had been sensitised and had specific IgE

antibodies. Altogether 21.6% had positive reactions in skin prick tests with corresponding HSA-conjugates. The exposure level was 38.7 (range 1-187)  $\mu\text{g}/\text{m}^3$ . In workers exposed to MTHPA at levels 10.1 (range 9-38) and 5.9 (range 0-50)  $\mu\text{g}/\text{m}^3$  the percentage of positivity for specific IgE antibodies were 8.6% and 8.9% and for skin prick tests 4.9% and 2.2%, respectively. The observation times were 26 (range 2-38) and 40 (range 1-105) months. There was no statistically significant difference between peak exposure levels in sensitised and nonsensitised workers in these studies (193).

#### *Concluding remarks*

Immunisation has been induced by a single intradermal dose of various cyclic anhydrides in animals. Exposure to high as well as low levels has without any latency period caused irritant symptoms in workers. Metaplasia of nasal mucosa has been seen in animals after long-term exposure to high levels of MA. The data on dose-response of sensitisation, and work-related symptoms are too limited for determining any lowest observable adverse effect level, but in industrial surveys TCPA and PA have induced sensitisation and asthma in workers exposed to the concentrations 140 and 1 500  $\mu\text{g}/\text{m}^3$ , respectively. TMA, HHPA, MHPA, and MTHPA have caused both sensitisation and work-related symptoms at exposure levels as low as 5-50  $\mu\text{g}/\text{m}^3$ .

### 13. Previous evaluations by national and international bodies

The documentation of the American Conference of Governmental Industrial Hygienists (ACGIH) was revised for PA in 2000, for TMA in 1993 and for MA in 2000. PA was defined as an irritant and sensitiser of the skin and respiratory tract and potent ocular irritant. The potency of irritancy was considered lower than that of MA. TMA was defined as irritant to the respiratory tract, the eyes, and skin and also as sensitiser in repeated exposure. To prevent sensitisation, a ceiling value was recommended in 1993. MA was defined as an irritant of the skin, eyes and upper respiratory tract, and as a sensitiser of the respiratory tract. According to ACGIH, PA and MA are not classifiable as human carcinogens (1).

The Health and Safety Executive (HSE) in the United Kingdom published a document on three acid anhydrides in 1996 (PA, TMA, and MA). The principal adverse effects of exposure in humans were stated to be asthma and respiratory tract irritancy and, for TMA, also other respiratory effects, which may be accompanied by systemic toxicity. Asthma was regarded as a serious health effect, and therefore the assignment of maximum exposure limits was indicated for each substance. A special notation of the respiratory sensitisation properties of each substance was added (158).

SA has been evaluated by the International Agency for Research on Cancer (IARC); limited evidence of a carcinogenic effect was reported for animals, but SA was not classifiable as carcinogen in humans (group 3) (78).

Chlorendic acid, the hydrolysis product of CA, has been evaluated by IARC. The evidence was sufficient for the carcinogenicity of chlorendic acid in experimental animals. There are no data on its carcinogenicity in humans, but, overall, it was concluded that chlorendic acid is possibly carcinogenic to humans (Group 2B) (79). In the evaluation of the International Programme on Chemical Safety (IPCS), separate carcinogenicity studies with CA were not considered necessary, since the compound is metabolically transformed to chlorendic acid (80).

In the European Union commission directives on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, PA, TMA, MA, HHPA, MHHPA, MTHPA, THPA, TCPA, HA and PMDA have been classified as sensitisers by inhalation and skin contact (39, 40). All of them have also been classified as irritants. In addition, PA and MA have been classified as harmful when swallowed because of the acute toxicity in animal studies. No occupational exposure limits for cyclic acid anhydrides have been set by the European Union.

## 14. Evaluation of human health risk

### 14.1 Groups at extra risk

When TCPA-exposed workers were investigated, smoking was found to interact with atopy in the production of specific IgE antibodies (185). On the other hand in a historical cohort study of TMA-exposed workers, there was an increased prevalence of sensitisation and work-related symptoms with increasing exposure but not with smoking or atopy (13). In another study, smoking was associated with specific IgE production to MTHPA only when the total IgE was low (200). In a prospective study of HHPA-, MHHPA-, and MTHPA-exposed workers an association was found between atopy and specific IgE antibodies. The effect of smoking was not significant (193).

In conclusion, atopy seems to predispose for sensitisation against cyclic acid anhydrides whereas no conclusion can be drawn with respect to smoking.

### 14.2 Assessment of health risk

The most common symptom from exposure to cyclic acid anhydrides is irritation of the mucous membranes (Tables 7 and 8). In long-term animal studies with MA, metaplasia of nasal tissue has been developed. Irritant nasal and ocular symptoms have been usual findings in industrial surveys (18, 130, 135, 214).

PA, TMA, MA, HHPA, MHHPA, MTHPA, TCPA, PMDA, HA and CA are capable of inducing IgE-mediated sensitisation followed by allergic diseases, e.g. allergic rhinitis often associated with allergic conjunctivitis and bronchial asthma (Table 10). The number of reports on MA, MHHPA, PMDA, HA or CA induced sensitisation is small. The prevalence of occupational asthma due to other cyclic anhydrides has varied between 2-39% in industrial surveys (203).



The allergic skin diseases caused by acid anhydrides include rare cases of IgE-mediated contact urticaria due to HHPA, MHHPA, MTHPA, MA and CA (83, 94, 95, 102, 174), and even more rare cases of allergic contact dermatitis of type IV allergy (57, 96).

Sensitisation and work related symptoms have been induced already at low levels of exposure, at 10-40  $\mu\text{g}/\text{m}^3$  for TMA, at 10-50  $\mu\text{g}/\text{m}^3$  for HHPA and MHHPA (mixed exposure in industrial surveys), and at 5-20  $\mu\text{g}/\text{m}^3$  for MTHPA. For PA and TCPA the corresponding exposure levels have been higher, 1 500-17 400  $\mu\text{g}/\text{m}^3$  and 140-590  $\mu\text{g}/\text{m}^3$ , respectively (Table 12). The prevalence of sensitisation has increased with increasing exposure (Table 10).

When workers were exposed to a PA dust concentration of less than 100  $\mu\text{g}/\text{m}^3$ , no asthmatics were found, but half of the workers had conjunctivitis and/or rhinitis (136). In a historical cohort study the exposure to PA or MA was found to be an uncommon cause of sensitisation at the low exposure levels measured, 8.9-11.9  $\mu\text{g}/\text{m}^3$  and 1.8-2.8  $\mu\text{g}/\text{m}^3$ , respectively (13).

After reduction of TCPA exposure to less than 110  $\mu\text{g}/\text{m}^3$  no new cases of work-related asthma were reported (118). Lower concentrations, between 4.1-66.7  $\mu\text{g}/\text{m}^3$  of TCPA have, however, induced asthma reactions in challenge tests of sensitised workers with occupational asthma (184).

After the elimination or reduction of exposure, asthma has persisted although it has improved in some of the follow-up studies. Occupational rhinitis often precedes asthma, and therefore the early detection of rhinitis is important for the prevention of more severe bronchial asthma.

The severe pulmonary disease-anaemia syndrome due to TMA and PMDA with assumed high levels of exposure has been rare (29, 144, 212).

There are no reports on THPA induced work-related diseases.

SA injected subcutaneously has induced subcutaneous sarcomas in rats. IARC has stated that there is limited evidence for the animal carcinogenicity of SA and that it is not classifiable as carcinogen in humans (78).

### **14.3 Scientific basis for an occupational exposure limit**

The data for setting occupational exposure limits is based on studies with information on dose-response effects. The number of the studies is limited, and some of them are not very recent. All cyclic anhydrides are considered irritants of the mucous membranes and skin.

Data on the main critical effects, sensitisation (specific IgE antibodies), and work-related symptoms (rhinoconjunctivitis and asthma) of PA, TMA, HHPA, MHHPA, MTHPA, and TCPA are compiled in Table 12 in relation to exposure levels. Occupational exposure limits for PA, TMA, and MA are presented in the Appendix.

The critical effects are as follows:

*Methyl tetrahydrophthalic anhydride* (MTHPA): irritation of mucous membranes of the eyes and airways and sensitisation-induced work-related diseases. Sensitisation, work-related rhinoconjunctivitis, and asthma have been verified for workers exposed to MTHPA levels of 5-20  $\mu\text{g}/\text{m}^3$ .

*Hexahydrophthalic anhydride* (HHPA) and *Methyl hexahydrophthalic anhydride* (MHHPA): irritation of mucous membranes of the eyes and airways and sensitisation-induced work-related diseases. Sensitisation in workers exposed to HHPA levels of 10-50  $\mu\text{g}/\text{m}^3$  has been found.

*Trimellitic anhydride* (TMA): irritation of mucous membranes of the eyes and airways and sensitisation-induced work-related diseases. In a historical cohort study of 116 workers with TMA exposure at the level of 10-40  $\mu\text{g}/\text{m}^3$ , sensitisation was found according to specific IgE antibodies, as were work-related respiratory symptoms.

*Tetrachlorophthalic anhydride* (TCPA): irritation of mucous membranes of the eyes and airways and sensitisation-induced work-related respiratory diseases. Exposure to TCPA concentrations of 140-590  $\mu\text{g}/\text{m}^3$  has caused both sensitisation and work-related asthma. No new cases of work-related asthma have been reported after decreasing the exposure to less than 110  $\mu\text{g}/\text{m}^3$  (118). In challenge tests of a worker with TCPA asthma, a significant asthmatic response has been induced with concentrations between 4.1-66.7  $\mu\text{g}/\text{m}^3$  of TCPA.

*Phthalic anhydride* (PA): irritation of mucous membranes of the eyes and airways and sensitisation-induced work related diseases. Both work-related rhinoconjunctivitis and sensitisation, but no asthma, have been found in workers exposed to less than 100  $\mu\text{g}/\text{m}^3$  of PA.

*Maleic anhydride* (MA): irritation of mucous membranes of the eyes and airways and sensitisation-induced work-related diseases. Nasal metaplasia has been found in animal studies (1 100-9 800  $\mu\text{g}/\text{m}^3$  of MA). There is not enough data available for dose-response assessment in humans.

*Tetrahydrophthalic anhydride* (THPA): data insufficient for assessing the critical effect on humans.

**Table 12.** Critical effects (sensitisation and work-related symptoms) in man with corresponding exposure levels of cyclic acid anhydrides.

Acid anhydride	Exposure level ( $\mu\text{g}/\text{m}^3$ )	Critical effect	Reference
PA	1 500 –17 400	Sensitisation Asthma	(136)
TCPA	140-590	Sensitisation Work-related respiratory symptoms	(118)
TMA	10-40	Sensitisation Work-related respiratory symptoms	(13)
HHPA and MHPA	10-50	Sensitisation	(195)
MTHPA	5-20	Sensitisation Rhinoconjunctivitis Asthma	(134, 202)

## 15. Research needs

Dose-response studies of several sensitising cyclic anhydrides are few or even lacking. Such studies are needed for assessing occupational exposure limits, especially since the sensitising properties of the cyclic anhydrides are not similar.

Furthermore follow-up studies are needed to determine the prognosis of asthma due to cyclic anhydrides.

Smoking and atopy as risk factors for sensitisation are not fully understood and need to be further investigated.

Assessing exposure when products manufactured from cyclic anhydrides are used in work processes is difficult. More knowledge is needed on the tasks expected to involve anhydrides as thermal degradation products.

Long-term inhalation studies on animals are needed to evaluate the carcinogenicity of acid anhydrides.

## 16. Summary

Keskinen H. *The Nordic Expert Group for Criteria Documentation of Health Risk from Chemicals and the Dutch Expert Committee on Occupational Standards.*

136. Cyclic acid anhydrides. *Arbete och Hälsa* 2004;15:1-74.

Cyclic acid anhydrides are widely used in the manufacture of resins and a variety of chemicals. Since the introduction of phthalic anhydride (PA) several new derivatives of cyclic acid anhydrides have come into use. Exposure to anhydrides occurs either in powder form or as fumes when anhydrides are used at elevated temperatures or when they are released as thermal degradation products. The technical product of a cyclic acid anhydride may contain other related anhydrides as impurities.

The critical effects of cyclic acid anhydrides are sensitisation, rhinoconjunctivitis and asthma. Occupational rhinoconjunctivitis and asthma due to cyclic acid anhydrides are usually IgE-mediated. Irritant work-related rhinoconjunctivitis is often found without sensitisation to anhydrides.

Allergic contact dermatitis due to anhydrides is rare.

The sensitising potency of cyclic acid anhydrides varies. According to limited data, trimellitic (TMA), hexahydrophthalic (HHPA), and methyl tetrahydrophthalic anhydride (MTHPA) are more sensitising than PA and tetrachlorophthalic anhydride (TCPA). Based on immunological cross-reactivity, the sensitising potency of methyl hexahydrophthalic anhydride (MHHPA) might be near that of HHPA and MTHPA. The level of exposure needed to cause specific IgE antibody production and work-related symptoms in mucous membranes and respiratory organs may be less than  $10 \mu\text{g}/\text{m}^3$  for the more highly sensitising anhydrides.

*Keywords:* allergic contact dermatitis, allergy, asthma, conjunctivitis, contact urticaria, cyclic acid anhydrides, hexahydrophthalic anhydride, irritation, maleic anhydride, methyl hexahydrophthalic anhydride, methyl tetrahydrophthalic anhydride, occupational disease, occupational exposure limit, phthalic anhydride, pulmonary disease-anaemia syndrome, rhinitis, tetrachlorophthalic anhydride, tetrahydrophthalic anhydride, trimellitic anhydride.

## 17. Summary in Swedish

Keskinen H. *The Nordic Expert Group for Criteria Documentation of Health Risk from Chemicals and the Dutch Expert Committee on Occupational Standards.*

136. Cyclic acid anhydrides. *Arbete och Hälsa* 2004;15:1-74.

Cykliska syraanhydrider har en utbredd användning vid tillverkning av hartser och en mängd andra kemikalier. Sedan ftalsyraanhydrid (PA) började användas har flera nya derivat av syraanhydrider kommit i bruk. Exponering för anhydrider sker antingen i form av pulver eller som rök när anhydriderna upphettas eller frigörs som termiska nedbrytningsprodukter. Den tekniska produkten av en cyklisk syraanhydrid kan innehålla liknande syraanhydrider som förorening.

De kritiska effekterna för cykliska syraanhydrider är sensibilisering, rinokonjunktivit och astma. Yrkesrelaterad rinokonjunktivit och astma till följd av syraanhydrider är i regel IgE-medierade. Irritativ arbetsrelaterad rinokonjunktivit förekommer ofta utan föregående sensibilisering för syraanhydrider.

Allergiskt kontakteksem av syraanhydrider är sällsynt.

Sensibiliseringspotentialen hos de cykliska syraanhydriderna varierar. Enligt begränsade data är trimellitsyra- (TMA), hexahydroftalsyra- (HHPA) och metyltetrahydroftalsyraanhydrid (MTHPA) mer sensibiliserande än PA och tetraklorftalsyraanhydrid (TCPA). Baserat på immunologisk korsreaktivitet har metylhexahydroftalsyraanhydrid (MHHPA) likartad sensibiliseringspotential som HHPA och MTHPA. De exponeringsnivåer som erfordras för att ge specifikt IgE svar och arbetsrelaterade symtom på slemhinnor och andningsorgan kan vara lägre än  $10 \mu\text{g}/\text{m}^3$  för de mer kraftigt sensibiliserande syraanhydriderna.

*Nyckelord:* allergi, allergisk kontaktdermatit, astma, cykliska syraanhydrider, ftalsyraanhydrid, hygieniskt gränsvärde, hexahydroftalsyraanhydrid, irritation, konjunktivit, kontakturtikaria, maleinsyraanhydrid, metylhexahydroftalsyraanhydrid, metyltetrahydroftalsyraanhydrid, rinit, tetrahydroftalsyraanhydrid, tetrakloroftalsyraanhydrid, trimellitsyraanhydrid, trimellitsyraanhydrid-alveolit, yrkessjukdom.

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## 19. Databases used in the search for literature

In the literature search the following databases were used (June 2001):

NIOSH TIC

Medline

Toxline

Rtecs

Tomes

HSE-line

CISDOC

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## Appendix

### Occupational exposure limits for cyclic acid anhydrides in air.

#### Phthalic anhydride (TWA)

Country	ppm	$\mu\text{g}/\text{m}^3$		Comments	Year	Reference
Denmark		1 000			2002	(1)
Finland		200			2002	(2)
Germany		1 000	MAK	SEN	2003	(3)
Iceland	1	6 000		SEN	1999	(4)
The Netherlands		1 000			2002	(5)
		2 000	CL			
Norway		2 000		SEN	2003	(6)
Sweden		2 000		SEN	2000	(7)
		3 000	CL			
United States					2003	(8)
ACGIH	1	6 100		SEN		
NIOSH	1	6 000				
OSHA	2	12 000				
Great Britain		4 000	MEL	SEN	2002	(9)
		12 000	STEL			

Abbreviations: CL, ceiling; MAK, maximum workplace concentration; MEL, maximum exposure limit; SEN, sensitiser; STEL, short term exposure limit; TWA, time weighted average.

#### Trimellitic anhydride (TWA)

Country	ppm	$\mu\text{g}/\text{m}^3$		Comments	Year	Reference
Denmark		40			2002	(1)
Finland	0.005	40			2002	(2)
Germany		40	MAK	SEN	2003	(3)
Iceland	0.005	40	STEL		1999	(4)
The Netherlands		40	CL		2002	(5)
Norway	0.005	40		SEN	2003	(6)
Sweden		40		SEN	2000	(7)
		80	CL			
United States					2003	(8)
ACGIH	0.005	40	CL			
NIOSH	0.005	40				
OSHA	0.005					
Great Britain		40	MEL	SEN	2002	(9)
		120	STEL			

Abbreviations: CL, ceiling; MAK, maximum workplace concentration; MEL, maximum exposure limit; SEN, sensitiser; STEL, short term exposure limit; TWA, time weighted average.

Maleic anhydride (TWA).

Country	ppm	$\mu\text{g}/\text{m}^3$		Comments	Year	Reference
Denmark	0.1	400			2002	(1)
Finland		410			2002	(2)
		810	STEL			
Germany		410	MAK	SEN	2003	(3)
Iceland	0.1	400		SEN	1999	(4)
The Netherlands		400			2002	(5)
Norway	0.2	800		SEN	2003	(6)
Sweden	0.3	1 200		SEN	2000	(7)
United States						(8)
ACGIH	0.1	400		SEN	2003	
NIOSH	0.25	1 000				
OSHA	0.25					
Great Britain		1 000	MEL	SEN	2002	(9)
		3 000	STEL			

Abbreviations: CL, ceiling; MAK, maximum workplace concentration; MEL, maximum exposure limit; SEN, sensitiser; STEL, short term exposure limit; TWA, time weighted average.

In addition to the occupational exposure limits for phthalic anhydride, trimellitic anhydride and maleic anhydride presented in tables, the corresponding limits for two other cyclic acid anhydrides have been given in Finland in 2000: Methyltetrahydrophthalic anhydride  $170 \mu\text{g}/\text{m}^3$  and Tetrachlorophthalic anhydride  $200 \mu\text{g}/\text{m}^3$ , ceiling  $400 \mu\text{g}/\text{m}^3$  (4).

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