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# Sundhedsmæssig vurdering af 5 tilsætningsstoffer til tobak

**Ministeriet for Sundhed og Forebyggelse**

**September 2009**

## *INDHOLDSFORTEGNELSE*



## *BILAG*

# 1 BAGGRUND

Ministeriet for Sundhed og Forebyggelse har bedt DHI om en nærmere vurdering af 5 tilsætningsstoffer til tobak.

Kræftens Bekæmpelse har udarbejdet en rapport vedr. tilsætningsstoffer i tobak, der belyser toksikologiske data for en række tilsætningsstoffer. DHI har i 2008 vurderet tilsætningsstoffer på det grundlag, som er anført i Kræftens Bekæmpelses rapport, og herudfra samt på baggrund af umiddelbart tilgængelige øvrige data kategoriseret de anvendte tilsætningsstoffer. Resultatet af DHI's kategorisering og umiddelbare vurdering foreligger i form af rapporten "Tilsætningsstoffer til tobak. Eksponering, risikovurdering og kategorisering" fra december 2008

Ministeriet for Sundhed og Forebyggelse har bedt DHI om en nærmere vurdering af 5 stoffer, hvor det skønnes relevant at søge og indhente data fra litteraturen.

Formålet er at kvalificere den sundhedsmæssige vurdering af 5 stoffer, der på baggrund af den foreløbige vurdering potentielt kan have særlige sundhedseffekter ved rygning, idet 4 af stofferne er anvendt som tilsætningsstoffer i tobaksvarer og ét stof dannes ved forbrænding af dels en række tilsætningsstoffer i tobak og dels fra selve tobakken.

DHI har indhentet eksisterende data i form af publicerede originalartikler og rapporter for på denne baggrund at kvalificere vurderingen. Der er søgt efter og indhentet publicerede data fra videnskabelige kilder, primært engelsksprogede. Ved datasøgning og vurdering af data til de toksikologiske profiler for enkeltstoffer er der søgt efter stoffernes forventede effekt ved rygning, herunder med særlig vægt på tilgængelig viden om evt. afhængighedsskabende effekt.

Der er udarbejdet toksikologiske profiler for følgende 5 stoffer:

- Menthol
- Acetaldehyd
- Acetophenon
- Cis-3-hexenol
- Benzylalkohol

De toksikologiske profiler er udarbejdet på engelsk og foreligger som bilag i denne rapport. For at lette tilgangen for ikke-fagfolk er der endvidere udarbejdet en tabel med nøgledata og hovedkonklusioner for de 5 stoffer. For hvert af de fem stoffer er der tillige udarbejdet mindre profiler på dansk, hvor data er trukket frem fra de toksikologiske profiler. Ved evt. tvivlsspørgsmål, er det i de egentlige toksikologiske profiler, der skal søges efter supplerende viden.

## 2 RESUMÈ

I forbindelse med kvalificering af den sundhedsmæssige vurdering af 5 tilsætningsstoffer til tobak, har DHI indhentet og vurderet data for 5 stoffer: Menthol, acetophenon, cis-3-hexenol, benzylalkohol og acetaldehyd. De første 4 stoffer anvendes alle som smagstilsætning (”flavour”) og sidstnævnte stof, acetaldehyd, er et af de stoffer, der dannes i højst mængde ved forbrænding af tobakken. Acetaldehyd dannes ud fra selve tobakken og fra andre stivelsesholdige og sukkerholdige kilder som f.eks. sukkerarter, kakao, lakrids, andre blade, frugt etc.

I forbindelse med redegørelsen for eksisterende data på sundhedspåvirkninger ved rygning, er der særligt set efter evt. data vedr. påvirkning af afhængighed. Det er – som nævnt i det tidligere arbejde - en diskussion i sig selv at afklare, hvornår et stof, der bidrager til en bestemt smag, kan siges at påvirke afhængigheden. Vi har i vort arbejde søgt at fokusere på, om der er fundet data, der kan underbygge en formodning om, at stoffet har yderligere effekt på afhængigheden. Dvs. om stoffet kan siges at have en særlig virkning på effekten/afhængigheden af nikotin, som er det stof, der medfører afhængighed af tobaksrygning.

Kun 1 – måske 2 - af de undersøgte 5 stoffer kan relateres til mulig påvirkning af rygemønstret:

Cis-3-hexenol er fundet beroligende/stressreducerede i test af dyr og øger tilsyneladende behagevirkningen ved rygning. Det kan derfor muligvis have en effekt på afhængigheden af tobaksrygning.

Menthol er ikke fundet at påvirke afhængigheden af nikotin, men ét studie viser, at menthol-cigaret-rygere i højere grad end andre rygere er ”wake-up-smokers”. Dvs. at menthol-cigaretrygere i højere grad end andre rygere ryger hurtigere efter de er vågnet om morgenen. Derfor kan menthol muligvis påvirke afhængigheden af tobak, om end det i så fald er mere indirekte end via nikotin-afhængigheden.

Ingen af disse to stoffer er i øvrigt fundet at bidrage til sundhedseffekter ved indtagelse/rygning.

For de 2 øvrige smagstilsætningsstoffer, acetophenon og benzylalkohol, er der tale om stoffer, der lugter og smager af bær/frugter. Begge stoffer indtages også via såvel kosten som via udeluft. Stofferne besidder lav sundhedsfare og der er ikke fundet studier, der underbygger en mistanke om påvirkning af afhængigheden af tobaksrygning.

Det sidste stof, acetaldehyd, er et stof, der bl.a. relateres til kræftrisikoen fra tobaksrygning. Stoffet kan endvidere virke toksisk i kroppen og give forgiftningssymptomer. Til yderligere oplysning er acetaldehyd også det stof, der kan give forgiftning i kroppen, hvis ikke man kan nedbryde alkohol (f.eks. ved overdosis, særlige asiatiske befolkningsgrupper eller antabusbehandling).

Acetaldehyd dannes ved pyrolyse (forbrænding) af glycerol, celluloseholdige stoffer/plantedele og nogle sukkerarter samt ikke mindst selve tobakken, der udgør langt den største kilde. Det er ved en rotteundersøgelse af acetaldehyd og nikotin fundet, at rotter med adgang til enten nikotin, acetaldehyd eller en blanding af disse foretrækker – og selvadministrerer oftere - blandingen med nikotin og acetaldehyd. Så det er muligt,

at acetaldehyd har en effekt på afhængigheden af nikotin. Da langt den største kilde til dannelsen af acetaldehyd som nævnt stammer fra selve tobakken, vil en fjernelse af de acetaldehyd-dannende tilsætningsstoffer fra tobakken dog blot medføre at rygetobak ville bestå af "mere" tobak i stedet og dermed vil en eliminering af tilsætningsstoffer ikke ændre væsentligt ved acetaldehydindtaget ved rygning.

Det samlede billede af problemstillingen omkring tilsætningsstoffer og tobak er særdeles komplekst og ingen stoffer vil formodentlig være undersøgt tilstrækkeligt til at man kan konkludere klart på rygeadfærd og dermed rygerrelaterede sygdomme. Det er muligt, at man ved undersøgelse af nogle af de øvrige tilsætningsstoffer vil kunne finde stoffer, der muligvis påvirker afhængigheden, således at man ville kunne samle en liste over flere uønskede tilsætningsstoffer til tobak.

## 3 SAMLET DATA OVERSIGT

| | Acetaldehyd | Acetophenon | Benzylalkohol | Cis-3-hexenol | Menthol |
|---|---|---|---|---|---|
| **Indtaget daglig mængde via rygning**[1] | Ca. 18-32 mg i dosis per dag fra tobak, heraf max. 2-4 mg fra tilsætningsstoffer.<br><br>Max. andel fra tilsætningsstoffer er 12% af estimeret indtag via rygning, sandsynligvis mindre. | Ca. 99 µg per kg bw per dag svarende til ca. 7 mg/dag fra rygning.<br><br>QNE 0,0006% | 0,05mg/kg bw daglig svarende til ca. 3,5 mg/dag for en ryger.<br><br>QNE 0,015% | Ca. 48 µg/dag<br><br>QNE: 0,00003% | Ca. 1,66 mg<br><br>QNE: 1,043% |
| **Stofkarakteristik** | Stoffet er et forbrændingsprodukt af sukkerarter, cellulose (plantestivelse) og glycerol.<br><br>Findes i planteekstrakter, tobaksrøg, inde- og udeluft, udstødningsgas og i vand. Årlig eksponering via udeluft er 1,0 µg/m3 (Sverige).<br><br>Der er målt fra 600- 1600 µg i røg fra én cigaret. | Aromatisk stof – dufter af bær/frugter (blomster /mandelagtig)<br><br>Eksponering via alm. udeluft, fødevarer, drikkevand, samt hudkontakt med forbrugerprodukter med indhold af stoffet.<br><br>Ca. 99% går videre uomdannet i røgen ved pyrolyse. | Findes naturligt i frugter, æteriske olier og tobak.<br><br>Bruges i kosmetik. | ”Grøn” smag.<br><br>Findes naturligt i planter og planteprodukter.<br><br>Dagligt indtag i Europa via kosten er estimeret til 4300 µg/dag per person. | Menthol anvendes i vid udstrækning i en lang række produkter bl.a. i fødevarer og kosmetik. |
| **Data** | Det diskuteres i litteraturen, om tilsætningsstofferne kan bidrage til den kræftfremkaldende effekt ved rygning set i forhold til, at de acetaldehyddannende tilsætningsstoffer udgør en | Er ikke undersøgt som enkeltstof i tobak.<br><br>Anvendes i fødevarer og er vurderet sikkert via indtagelse af WHO + JECFA[2]. | Lav toksicitet.<br>Ingen undersøgelser vedr. afhængighed.<br>Ref. Dosis beregnet af EPA til 0,3mg/kg/dag. | Lav toksicitet<br><br>Metaboliseres til carboxylsyrer i kroppen. Bliver til kuldioxid og vand i kroppen. | Menthol er ved indtagelse ikke fundet kræftfremkaldende.<br>Undersøgt af mange forskellige institutioner mht. effekter fra rygning. |

[1] Indtaget daglig mængde ved rygning af 20 cigaretter beregnet ud fra Quantum Not Exceeded (QNE), dvs. max. anvendt andel i tobakken af det pågældende tilsætningsstof i tobak. BW står for body weight, det vil sige at dosis måles i dosis per kg. legemsvægt.
[2] JECFA: Joint FAO/WHO Expert Committee on Food Additives (JECFA)

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| | forsvindende lille mængde af tobakkens bidrag. | Ét studie fra 1966 vedr. mulig påvirkning af elektriske impulser/hjerneaktiviteten. Grænseværdi herfor var 0.007 mg/m$^{3.}$ Ingen senere publikationer vedr. reaktioner på nervesystemet. | | | |
| **Sygdomsfremkaldende effekter** | Stoffet er fundet kræftfremkaldende i dyr. Internationalt anerkendt, at stoffet har kræftfremkaldende effekt.<br><br>Det er ved forbrænding og inhalation af tobak, der primært inhaleres acetaldehyd, der anses som hovedansvarlig for den kræftfremkaldende effekt ved rygning.<br><br>Acetaldehyd er i øvrigt det stof, der dannes ved nedbrydning af alkohol i kroppen – og kan give forgiftningssymptomer | Irriterende ved kontakt.<br><br>Lav akut toksicitet. | Lav toksicitet | Ingen fundne toksikologiske effekter ved inhalation. | Mentholcigaretrygere er ofte "voksne" rygere. I USA ryger flere sorte end hvide mentholcigaretter og selv om de ryger mindre, bliver de alligevel mere syge.<br><br>Diskussion af, om menthol kan øge den kræftfremkaldende effekt af cigaretter. Konklusioner i litteraturen fra mange forskellige kilder viser at dette ikke er tilfældet, ligesom menthol næppe har effekt på rygerelaterede sygdomme. Den evt. kræftfremkaldende effekt er derfor velundersøgt. |
| **Afhængighedsskabende?** | I et rotteforsøg er der fundet mulig sammenhæng mellem indholdet af acetaldehyd og lysten til at indtage nikotin (i drikkevand). Der kan derfor være tale om, at acetaldehyd kan have en synergistisk effekt med nikotin ved tobaksrygning og dermed muligvis kunne øge den afhængighedsskabende effekt. | Næppe. | Ingen fundne data. | Giver en frisk smag til prduktet. Kan tilsyneladende virke beroligende/reducerer stress i dyr.<br><br>Er patenteret i USA i 1970 pga. af dets behagelige effekt på rygeoplevelsen. Såfremt øgning af behagevirkning ved rygning anføres som effekt på afhængigheden af | Èt studie viser at mentholcigaretrygegre i højere grad er "Wake-up-smokers", det er derfor muligt at stoffet kan påvirke afhængigheden. Data, tyder ikke på, at menthol påvirker afhængigheden af nikotin. |

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| | | | | tobak, er dette mulig-vis tilfældet for cis-3-hexenol. | |
| **Regulering**[3] | B-værdi: 0,02 mg/m$^3$ | B-værdi: 0,01 mg/m$^3$ | B-værdi: 0,1 mg/m$^3$<br><br>JECFA ADI: 0-5 mg/kg af benzylforbindelser. | Ingen B-værdi | Ingen B-værdi |
| | Grænseværdier: Loftværdi i DK + USA: 25 ppm | Grænseværdier: I Danmark, Finland, USA, Belgien m.fl.: 5-10 ppm | Grænseværdi i arbejdsmiljøet: ingen i DK eller andre normalt sammenlignelige lande. I Polen: 240 mg/m3<br>USA: 10 ppm (44 mg/m3?) | Ingen grænseværdi. | Ingen grænseværdi |

[3] B-værdi er "Bidragsværdi", dvs. den værdi, som danske virksomheders udledning af et stof til udeluften maksimalt må være. Af hensyn til luftkvaliteten er der i Danmark fastsat B-værdier for en række stoffers maksimale bidrag ved udledning til udeluft.
JECFA ADI er den internationalt fastsatte acceptable daglige indtag af et stof via kosten.
Grænseværdier er arbejdsmiljøgrænseværdier. Det vil sige maksimale tilladte koncentrationer af et stof i arbejdsmiljøet.

# 4 STOFPROFILER

For alle 5 stoffer er der udarbejdet toksikologiske profiler med reference til originallitteraturen og med en mere indgående præsentation af fundne data. Profilerne er udarbejdet på engelsk. I det følgende præsenteres stofferne også i en kortfattet og lettere tilgængelig profil på dansk. Profilerne skal læses i sammenhæng med data fra tabellen ovenfor.

## 4.1 Acetophenon

### Forekomst og anvendelse

Acetophenon forekommer naturligt i bær, skaldyr, oksekød og nødder. Stoffet anvendes som smagsgiver i tobak.

Den almindelige befolkning kan blive eksponeret for acetophenon via indånding af den omgivende luft, ved indtagelse af fødevarer og drikkevand, og ved hudkontakt med forbrugerprodukter, der indeholder acetophenon. Det daglige indtag herfra er ca. 176 µg/dag/person. Rygeres eksponering fra cigaretter er for acetophenon ca. 99 µg/kg kropsvægt ved rygning af 20 cigaretter om dagen, svarende til ca. 7 mg per dag.

### Grænseværdier og fareklassificering

Der er ifølge vores datasøgninger ikke publiceret andre artikler omkring skadelige effekter på centralnervesystemet end ét russisk studie fra 1966. I dette blev det anbefalet at den gennemsnitlige, maksimale tilladte dosis blev sat til 0.003 mg/m$^3$ atmosfærisk luft for at undgå eventuelle effekter på centralnervesystemet.

Acetophenon er fareklassificeret som “Skadelig ved indtagelse” (Xn;R22) og ”Irriterende for øjne” (Xi;R36).

Acetophenon vurderes ikke til at være kræftfremkaldende for mennesker.

I arbejdsmiljøet er der et fastsat et 8 timers vægtet gennemsnit, der højst må være 10 ppm (svarende til 10 milligram per kubikmeter luft).

Acetophenon er ikke på Miljøministeriets Effektliste fra 2000 over stoffer, der udgør en alvorlig økotoksikologisk effekt på miljøet.

### Sundhedseffekter

Fra dyrestudier ved man at acetophenon bliver optaget, omsat og udskilt indenfor 24 timer. Stoffet udskilles primært i urinen og i noget mindre grad i afføringen.

Acetophenon er blevet testet af tobaksfirmaet Phillip Morris’ testlaboratorier i et studie hvor 333 ingredienser blev testet. Acetophenon blev ikke testet som enkeltstof, men i en gruppe af stoffer, og data tydede ikke på at nogen af de 333 testede ingredienser øgede den overordnede giftighed af tobak i nævneværdig grad.

WHO ved JECFA har vurderet acetophenon som smagsgivende ingrediens i fødevarer og undersøgelsen gav ikke anledning til bekymring omkring sikkerhed. Et amerikansk ekspertpanel fra aromastof- og ekstraktproducenters forening (FEMA) har vurderet acetophenon som værende "generally recognised as safe" (GRAS). Dette er dels baseret på, at stoffet hurtigt optages, omsættes og udskilles både i mennesker og dyr og dels på, at stoffet har en bred sikkerhedsmargin og et ringe genotoksisk og mutagent potentiale[4].

Ved bred sikkerhedsmargin forstås her, at forholdet mellem et konservativt estimat af indtaget og det no-observed-adverse-effekt[5]-niveau, der er bestemt på baggrund af subkroniske og kroniske studier er højt.

### *Konklusion*

Der findes begrænset litteratur omkring effekterne af at tilsætte acetophenon til tobak, og acetophenon som enkeltstof er ikke blevet undersøgt i studier af tobaksrygning.

Fra anvendelsen af acetophenon som smagsgivende ingrediens i fødevarer er der ikke rapporteret skadelige effekter og stoffet betragtes som sikkert at bruge. For acetophenon tilsat tobak tyder eksisterende data ikke på, at stoffet øger sundhedsrisikoen ved rygning, acetophenon er ikke undersøgt som enkeltstof i studier omkring rygning.

## *4.2 Acetaldehyd*

### *Forekomst og anvendelse*

Acetaldehyd forekommer som nedbrydningsprodukt af sukkerstoffer og alkohol i mennesker. Acetaldehyd er ikke et tilsætningsstof til tobak, men et pyrolyseprodukt (forbrændingsprodukt) af tilsatte og naturligt forekommende sukkerstoffer, cellulose og alkoholer. Acetaldehyd er således en af hovedkomponenterne i tobaksrøg.

Cellulose er den vigtigste bestanddel i cellevæggen i plaster, og den primære kilde til acetaldehyd er selve tobakken.

I et svensk studie er den gennemsnitlige koncentration af acetaldehyd i almindelig udluft angivet til ca. 1.0 µg/m$^3$. Mængden af acetaldehyd i tobaksrøg er rapporteret til være i størrelsesordenen 600-1500 µg per cigaret.

[4] Det genotoksiske og mutagene potentiale er bl.a. et udtryk for, om stoffet ændrer cellers arvelige egenskaber. Dette kan bl.a. give et praj om evt. forventede kræftfremkaldende effekter.

[5] No-observed-adverse-effect-level er det højeste niveau for dosis, hvor man ikke har fundet sundhedseffekter. Ved division af den beregnede mængde for indtag med denne dosis fås sikkerhedsmarginen. Bred – eller høj - sikkerhedsmargin udtrykker, at der er stor forskel mellem indtaget dosis og ned til den dosis, hvor vi ved der ikke er fundet sundhedseffekter.

### *Grænseværdier og fareklassificering*

I Danmark er bidragsværdien for acetaldehyd sat til 20 µg/m3. Bidragsværdien er en grænseværdi for en industrivirksomheds maksimalt tilladelige bidrag til tilstedeværelsen af et stof i luften.

I EU er acetaldehyd klassificeret som skadelig, og irriterende for øjne og respirationsorganer, med begrænset dokumentation for kræftfremkaldende egenskaber. Den Californiske miljøstyrelse har anført acetaldehyd som et stof kendt som kræftfremkaldende. Grænseværdien for arbejdsmiljøet er i USA 45 mg/$m^3$.

### *Sundhedseffekter*

Det tilgængelige data for acetaldehyds toksikologiske egenskaber tyder på at acetaldehyd er et af de vigtige toksiske stoffer i tobaksrøg, og at acetaldehyd muligvis er kræftfremkaldende.

Studier af tilsatte sukkerstoffer til tobak og tobaksrøgs sammensætning tyder på at der er sammenhæng mellem visse typer af sukkerstoffer og mængden af acetaldehyd i røgen. Tilsætningen af sukkerstoffer til tobak kan derfor forøge mængden af acetaldehyd i tobaksrøg, og dermed også påvirke tobaksrøgens toksicitet.

Den primære kilde er dog selve tobakken og en mindre mængde tilsætningsstof vil øge andelen af den rene tobak.

Det er i studier med rotter påvist at acetaldehyd kan medføre forøget selvadministreringen af nikotin, det er derfor muligt at acetaldehyd spiller en rolle i udviklingen af nikotinafhængighed.

### *Konklusion*

Acetaldehyd er muligvis et af de vigtigste toksiske stoffer i tobaksrøg og formentlig kræftfremkaldende. Eksperimentielle data viser, at der er sammenhæng mellem typen af sukker der tilsættes tobak og mængden af acetaldehyd i røgen, således at nogen sukkerarter tilsat tobakken giver forøget mængde acetaldehyd, mens andre sukkerstoffer ikke indvirker på mængden af acetaldehyd. Der foreligger eksperimentielle data, som indikerer at acetaldehyd kan spille en rolle i tobaksafhængighed.

## *4.3 Benzylalkohol*

### *Forekomst og anvendelse*

Benzylalkohol forekommer naturligt i frugter, samt æteriske olier fra eksempelvis jasmin, og i tobak. Benzylalkohol har en behagelig sød og frugtagtig duft. Benzylalkohol anvendes i kosmetik, og som smagsstof og konserveringsmiddel.

Benzylalkohol bliver endvidere anvendt som hjælpestof i den farmaceutiske industri.

Eksponeringen for benzylalkohol er ca 50 µg/kg kropsvægt svarende til ca. 3,5 mg for en person, der ryger 20 cigaretter om dagen.

### *Grænseværdier og fareklassificering*

Benzylalkohol er klassificeret som sundhedsskadelig (Xn; R20/22) i EU.

Det maksimale industrielle bidrag (B-værdi) for benzylalkohol er 0,1 mg $m^{-3}$ i Danmark og der findes flere anviste grænseværdier for benzylalkohol i industrier.

Den amerikanske grænse for arbejdsmiljøet er sat til 10 ppm, hvilket svarer til 44 mg $m^{-3}$. Der er ingen grænseværdier for arbejdsmiljøet i Danmark. I Polen er arbejdsmiljø-grænseværdien 240 mg $m^{-3}$.

### *Sundhedseffekter*

Antallet af studier omkring inhalation af benzylalkohol er meget begrænset. Effektkoncentrationerne i de få relevante studier er relativt høje. Hvis man forholder den meget lille mængde af benzylalkohol, der tilsættes til tobak, med den meget lave toksicitet, er det derfor vurderet at benzylalkohol ikke vil forøge toksiciteten af tobak af betydning.

Der er ikke fundet studier, der tyder på at benzylalkohol kan have effekt på tobakkens afhængighedsskabende egenskaber.

### *Konklusion*

Det er ikke sandsynligt at tilsætningen af benzylakohol til tobak forøger tobakkens toksicitet, og der er ingen indikationer på at benzylalkohol spiller en rolle i tobakkens afhængighedsskabende egenskaber.

## *4.4 Cis-3-hexenol*

### *Forekomst og anvendelse*

Cis-3-hexenol forekommer naturligt i mange planter og planteprodukter. Det daglige indtag i Europa af cis-3-hexenol er af WHO estimeret til 4300 µg/person/dag. Cis-3-hexenol har været brugt som additiv i cigaretter siden 1970.

Rygeres eksponering for Cis-3-hexenol er ca. 0,1 µg/kg kropsvægt om dagen ved rygning af 20 cigaretter.

### *Grænseværdier og fareklassificering*

Der er ikke identificeret nogen grænseværdier for cis-3-hexenol. I EU er cis-3-hexenol udelukkende klassificeret for dets brandfarlige egenskaber.

### *Sundhedseffekter*

Studier i dyr indikerer at den akutte toksicitet af Cis-3-hexenol er ganske lav, det er derfor usandsynligt at tilsætningen af stoffet til tobak, øger giftigheden af tobak.

Det skal dog bemærkes at Cis-3-hexenol er dokumenteret for at forøge behageligheden ved rygning, og det er vist eksperimentielt at cis-3-hexenol har angstdæmpende og beroligende egenskaber hos dyr, samt at stoffet kan reducere stress i mennesker. Det er således muligt at tilsætningen af cis-3-hexenol til tobak kan forøge tobakkens afhængighedsskabende egenskaber.

### *Konklusion*

Grundet den lave toksicitet af cis-3-hexenol er det usandsynligt at tilsætningen af stoffet til tobak vil kunne påvirke den samlede toksicitet af tobaksproduktet. Cis-3-hexenol er allerede i 1970èrne blevet påvist at kunne forøge den behagelige oplevelse ved at ryge tobak, og stoffet er eksperimentielt blevet påvist at kunne reducere stress hos mennesker. Det er derfor muligt at tilsætningen af cis-3-hexenol til tobak kan forøge de afhængighedskabende egenskaber af tobak.

## *4.5 Menthol*

### *Forekomst og anvendelse*

Menthol er en naturligt forekommende alkohol, der er ekstraheret fra planterne Mentha piperata og Mentha arvensis. Menthol kan også fremstilles syntetisk og det er det syntetiske produkt, der anvendes i mange kommercielle produkter.

Menthol anvendes mod kløe, som antiseptisk middel og som et kølende stof i kosmetiske produkter til huden, ligesom det anvendes som smagsgivende ingrediens i tandpasta og i andre produkter til mundhygiejne.

### *Grænseværdier og fareklassificering*

Den fælles ekspertkomité for tilsætningsstoffer til fødevarer, JECFA under AO/WHO, har fastsatte i 1968 et acceptabelt dagligt indtag (ADI) til 0-0,2 mg/kg kropsvægt. EU angiver ADI til at være 2 mg/kg kropsvægt. Forskellen i værdierne ligger sandsynligvis i brug af forskellige omregnings- og sikkerhedsfaktorer. I 1998 blev ADI ændret til 4 mg/kg kropsvægt af JECFA.

Menthol er ikke registreret på Miljøministeriet Effektliste fra 2000, der lister stoffer med særligt alvorlige økotoksikologiske effekter. Der er ikke fastsat nogen grænser for menthol i arbejdsmiljøet.

### *Sundhedseffekter*

Der findes enorme mængder af data omkring menthols sundhedseffekter i forbindelse med rygning. Mange studier af menthols indflydelse på udvikling af kræft og afhængighed af nikotin er amerikanske. Det skyldes det forhold at der er store afgrænsede grupper i den amerikanske befolkning, der henholdsvis ryger menthol og almindelige cigaretter, og at der findes ret forskellige sygdomsmønstre i disse to befolkningsgrupper.

Èt studie viser, at der ikke er forskel i blod- og urinprøver med hensyn til indhold af NNK (et kræftfremkaldende stof), nikotin og kulstofmonooxid efter rygning af henholdsvis almindelige og mentholcigaretter, mens et andet studie sætter spørgsmålstegn ved den prædiktive værdi af disse markører.

Med hensyn til om menthol har indflydelse på vejrtrækningen, inhalationsdybde, samt mængde af inhaleret tjære, tyder data ikke på, at det er tilfældet. Menthol har måske indflydelse på, hvordan man oplever det at ryge, men det giver sig ikke udslag i nogen objektivt målbare parametre.

Der er in vitro[6] data der tyder på at menthol kan forsinke omsætningen af nikotin i kroppen og nedsætte nyrenes evne til at udskille nikotin, men disse antagelser har ikke kunnet påvises i forsøg med mennesker.

Risikoen for udvikling af lungekræft og andre kræfttyper i luftvejene ser ud til at være forøget, især hos sorte mænd, der ryger mentholcigaretter, men en række undersøgelser tyder på, at det er noget andet end menthol, der er ansvarlig for denne forskel.

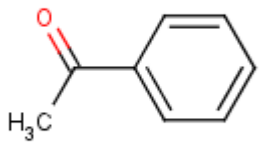
Der er enkelte studier, der tyder på, at sorte er mere afhængige af nikotin end hvide i og med at de har vanskeligere ved at holde op med at ryge, og de der ryger, ryger tidligere på dagen, end hvide rygere. Men det kan ikke konkluderes, at det er menthol, der er ansvarlig for denne forskel.

Der er en række antagelser omkring menthols mulige effekter på udvikling af luftvejskræft og afhængighed af nikotin, men der er ingen entydige resultater, der kan slå fast at det er menthol, der er den direkte årsag til at sorte rygere har højere forekomst af lungekræft og en øget afhængighed af nikotin.

[6] "In vitro", dvs. forsøg udført udenfor kroppen, typisk laboratorieeksperimenter udført på f.eks. celler i reagensglas.

# *B I L A G*

# Toxicological profile of Acetophenone




**SEPTEMBER 2009**

Ministeriet for Sundhed og Forebyggelse

## *CONTENTS*

# 1 INTRODUCTION

The purpose of the present report is to evaluate the impact of adding acetophenone to cigarette tobacco with regard to potentially increased health risk, with the primary focus on increased risk of cancer and increased dependence of nicotine. The evaluation is based on a literature search described below.

The search terms used were acetophenone, CAS 98-86-2, acetylbenzene, phenyl methyl ketone, CNS, neurotox, immunotox, smoke, smoking, inhalation, addiction or addictive.

The cited literature was collected from two different searches. The first search was made in the Toxnet cluster database, where data for the different toxicological end points were collected from HSDB, ChemIdPlus, Gene-tox, IRIS and CCRIS. The second search in the STN database (Toxcenter, Embase and Scisearch databases) did not give any relevant hits. Additional literature was provided by The Tobacco Manufacturers association of Denmark.

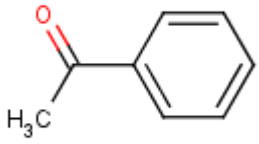
The literature on the effects of adding acetophenone to tobacco is very limited. The identified data either concern the classical toxicological aspects of acetophenone, not related to smoking, or concern the testing of a mixture of many additives, including acetophenone, that has been tested *in vitro* and *in vivo* for adding toxicological effects to tobacco.

The primary source of toxicological data is a safety evaluation from 2007 performed by the American Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) of a number of substances “Generally Regarded As Safe (GRAS)” (1) and a WHO/JECFA safety evaluation of certain food additives, including ketones, from 2001 (2).

# 2 IDENTIFICATION

| | |
|---|---|
| **Chemical Name** | Acetophenone |
| **Synonyms** | 1-Phenyl-1-ethanone<br>1-Phenylethanone<br>Acetofenon<br>Acetophenone<br>Acetylbenzene<br>Acetylbenzol<br>Benzene, acetyl-<br>Benzoyl methide<br>Benzoylmethide<br>Ethanone, 1-phenyl-<br>Hypnone<br>Ketone, methyl phenyl<br>Methyl phenyl ketone<br>Phenyl methyl ketone |
| **CAS no.** | 98-86-2 |

| | |
|---|---|
| **EINECS No.** | 202-708-7 |
| **Molecular formula** | C8-H8-O |
| **Structure** | |




## 3 USE AND OCCURRENCE

Acetophenone is the simplest aromatic ketone and is a natural component of berries, seafood, beef, and nuts (1). The substance is used as a flavorant in tobacco (3).

Acetophenone is used for production of resins, as a precursor for styrene, as raw material for the synthesis of some pharmaceuticals and as excipient for some drugs (4,5,6).

Monitoring data from NIOSH[1] indicate that the general population may be exposed to acetophenone via inhalation of ambient air, ingestion of food and drinking water and dermal contact with this compound and other consumer products containing acetophenone (3). The daily per capita intake of acetophenone is approximately 176 µg/day (1).

It can be calculated that smokers are exposed to 0.09856 mg acetophenone per kg bw per day when smoking 20 cigarettes per day.

## 4 PHYSICAL/CHEMICAL PROPERTIES

The substance is identified as given in the below table:

[1] The National Institute for Occupational Safety and Health is the United States federal agency responsible for conducting research and making recommendations for the prevention of work-related injury and illness.

| Property | Value |
|---|---|
| **Physical state** | Clear liquid or crystals(7) |
| **Molecular weight** | 120.15 (3) |
| **Melting point** | 20°C (8) |
| **Boiling point** | 202°C(8) |
| **Vapour pressure** | 0.397 mm Hg (25°C) (8) |
| **Density** | 1.033 (15°C) (3) |
| **Water solubility** | 6130 mg/l (25°C) (8) |
| **Log P octanol/water** | 1.58 (8) |
| **Other data** | |
| **Henry's law constant** | 1.04 x $10^{-5}$ atm-$m^3$/mole (25°C) (8) |
| **Atmospheric OH Rate Constant** | 2.74 x $10^{-12}$ $cm^3$/molecule-sec (25°C) (8) |

# 5 BIOTRANSFORMATON AND TOXICOKINETICS

In rabbits and dogs acetophenone is absorbed, metabolized and excreted as polar metabolites within 24 h (1). Acetophenone undergoes alpha-oxidation and subsequent oxidative decarboxylation to yield benzoic acid that is excreted mainly in the urine as hippuric acid (benzoylglycine) (1).

Acetophenone and structurally related aromatic ketones and alcohols have been shown to be absorbed rapidly from the gut, metabolized efficiently by the liver, and excreted primarily in the urine and to a very small extent in the faeces (2).

# 6 HEALTH EFFECTS

The effect of adding additives to tobacco has been evaluated in a test series by Phillip Morris test laboratories (9,10,11,12). A number of 333 ingredients added to typical commercial blended test cigarettes were evaluated in a bacterial mutagenicity screening (Ames test), a mammalian cell cytotoxicity assay (neutral red uptake) and a 90 days nose-only inhalation study in rats (9). The 333 ingredients were divided into three groups, and tested at a normal (low) level and a high level. No ingredients were evaluated on a single substance level. Acetophenone was included in the test series and was added to two of the three test groups. The conclusion from the studies was that addition of the ingredients to tobacco did not significantly add to the overall toxicity of cigarettes (9).

Acetophenone has also been evaluated as a flavour by WHO, JECFA[2], and it did not give rise to any safety concern (2). A group of 38 flavouring agents that included acetophenone and 36 structurally related aromatic secondary alcohols, ketones, and related esters by the Procedure for the Safety Evaluation of Flavouring Agents, that included

[2] The Joint FAO/WHO Expert Committee on Food Additives is an international scientific expert committee that is administered jointly by the Food and Agriculture Organization of the United Nations FAO and the World Health Organization WHO.

evaluation of estimated intake, metabolism and safety margin based on animal studies (2).

The group of aromatic substituted secondary alcohols, ketones, and related esters, including acetophenone, has been reaffirmed as GRAS (GRASr) by FEMA based, in part, on their rapid absorption, metabolic detoxication, and excretion in humans and other animals; their low level of flavor use; the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential (1).

## 6.1 Single exposure toxicity

### 6.1.1 Acute toxicity

The oral acute toxicity of aromatic substituted secondary alcohols, ketones, as acetophenone is low (1).

The listed values in the table apply to acetophenone.

| Route of exposure | Species | LD501) | Reference |
|---|---|---|---|
| Oral | Rats | 900-3200 mg/kg bw | (1) |
| Oral | Rats | 900 mg/kg bw | (13) |
| Oral | Mouse | 1780 mg/kg bw | (1) |
| Dermal | Guinea pigs | >20 mL/kg | (14) |

[1)] LD50 (mg/kg bw): Lethal dose to half of the animals.

### 6.1.2 Irritation/Corrosion

**Skin and Eyes**

Acetophenone is classified as irritating to skin or eyes in EINECS (15).

### 6.1.3 Allergy

No skin sensitisation was noted when 2% acetophenone in petrolatum was tested on humans (3).

## 6.2 Repeated exposure toxicity

### 6.2.1 General

| Route of exposure | Species | Time of exposure | NOAEL[1)] | Critical Effects | Reference |
|---|---|---|---|---|---|
| Oral | Rat | 28 days | 75 mg/kg bw (systemic toxicity 225 mg/kg bw (neurological effects)[1)] | Reduced forelimb grip strength and motor activity. Increased salivation. Reduced body weight gain. | (1) |
| Oral | Rats | 17 weeks | >1000 mg/kg bw | No reported effects | (1) |

1. NOAEL (mg/kg bw/day): No Observable Adverse Effect Level.

## 6.3 Genotoxicity

Acetophenone was tested for mutagenicity in Ames test, with and without metabolic activation, and the results were negative (1,16).

Likewise when tested for effects on bacterial DNA repair in *E. coli* polA the results were negative (17).

Acetophenone was tested for potential to induce chromosomal aberrations at doses of 800-1299 µg/ml in chinese hamster ovarian (CHO) cells, with a negative result without metabolic activation and with a positive result at 600-1000 µg/ml with metabolic activation (1).

## 6.4 Carcinogenicity

Acetophenone has been evaluated not to be a human carcinogen based on lack of human data and animal data (18).

## 6.5 Reproduction toxicity

In a developmental toxicology study where female rats were dosed via gavage for a minimum of 14 days until day three of lactation a NOAEL for reproductive effects were found to be 225 mg/kg bw. Above this dose level, at 750 mg/kg bw per day, the live birth index, pup survival during lactation and pup body weights were decreased (1).

# 7 REGULATORY INFORMATION

## 7.1 Environment

A hygienic evaluation of acetophenone as an atmospheric air pollutant is reported in a Russian paper from 1966 (19). The threshold of the reflex effect on the electrocortical brain activity of the most sensitive persons was 0.007 milligram per cubic meter. Under chronic inhalation conditions (24 hours/day for a total of 70 days) a 0.07 milligram per cubic meter concentration elicited in rats functional shifts, such as depressed cholinesterase activity, which were not elicited by 0.007 milligram per cubic meter of acetophenone. It was recommended that 0.003 milligram per cubic meter of acetophenone be officially adopted as the 24-hour average maximum allowable concentration for atmospheric air (19).

To our knowledge no other reports have been publiced since this publication from 1966 about adverse reactions to acetophenone in the central nervous system.

## 7.2 Classification and Health

EU: Acetophenone is classified as “Harmful if swallowed” (Xn;R22) and “Irritating to eyes” (Xi;R36) (20).

Acetophenone has been evaluated not to be a human carcinogen based on lack of human data and animal data (18).

Workplace Environmental Exposure Level (WEEL): The 8 hours Time-weighted Average is 10 ppm (3).

Acetophenone is not registered on the Effect List 2000 of the Danish Environmental Protection Agency as a substance causing particularly serious ecotoxicological effects (21).

# 8 EVALUATION

In 1993, the American Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) evaluated the group of secondary alcohols, ketones and related esters, including acetophenone as Generally Regarded As Safe (GRAS) as additives to food. This was based, in part, on their rapid absorption, metabolic detoxication, and excretion in humans and other animals; their low level of flavour use; the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential (1).

Evaluated as a flavouring agent by WHO/JECFA, acetophenone did not give rise to any safety concern (2).

# 9 CONCLUSION

The literature on the effects of adding acetophenone to tobacco is limited. The identified literature either concerns the classical toxicological aspects of acetophenone, which are not related to smoking, or it concerns the evaluation of a mixture of additives, including acetophenone. Acetophenone has not been evaluated as a single chemical compound for potential adverse effects in tobacco smoke.

However, it can be argued that it is the most realistic set-up to evaluate the mixture of additives actually used for the cigarette brands on the market, and not do an evaluation of single compounds. On the other hand it is difficult to evaluate the potential effect of at single compound if it has not been tested in an experimental set-up dedicated to investigate the effects of that single compound.

When acetophenone is used as a flavoring agent in foods no adverse effects have been reported and is regarded as safe. When added to tobacco data indicate that acetophenone does not add to the risk of smoking, but it must be noted that acetophenone has not been evaluated as a single compound.

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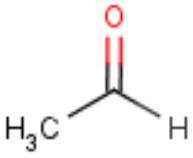
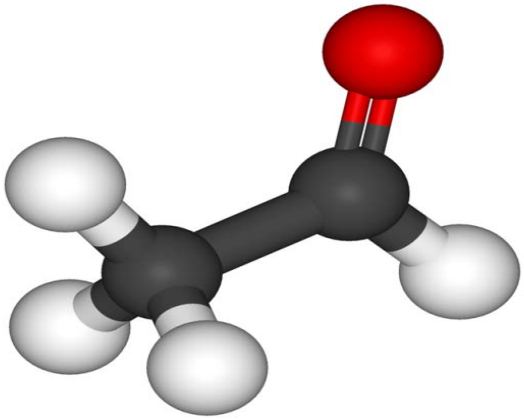
# Toxicological profile of Acetaldehyde



**SEPTEMBER 2009**

Ministeriet for Sundhed og Forebyggelse

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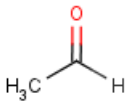
# 1 INTRODUCTION

The purpose of the present toxicological profile for acetaldehyde is to reveal the possible health effects on smokers by acetaldehyde in tobacco smoke, and to investigate if additives in tobacco lead to significant increase in acetaldehyde in tobacco smoke. Also possible effect of acetaldehyde in tobacco addiction was investigated. The search profile used was: acetaldehyde, CAS no 75-07-0, oncogen, tumor, neoplasm, carcinogen, addiction, and dependence.The profile has been compiled on searches in the following databases: ESIS, AtsDR, IRIS, IARC, InChem, DOSE, ChemID plus, HSDB, STN, and Toxline.

# 2 IDENTIFICATION

| | |
|---|---|
| **Chemical Name** | Acetaldehyde |
| **Synonyms** | Acetic ethanol, Ethylaldehyde |
| **CAS no.** | 75-07-0 |
| **EINECS/ELINCS No.** | 200-836-8 |
| **Molecular formula** | C2H4O |
| **Structure** | |




# 3 USE AND OCCURRENCE

Exposure to acetaldehyde may occur in its production and in the production of acetic acid and various other chemical agents. It is a metabolite of sugars and ethanol in humans and has been detected in plant extracts, tobacco smoke, engine exhaust, ambient and indoor air, and in water (15). The substance is a pyrolysis product of sugars, cellulose, and glycerol (16). In Sweden from December 1986 to August 1987, the mean yearly exposure to acetaldehyde from air pollution was 1.0 µg/m$^3$ (5). Acetaldehyde is a major component of tobacco smoke, being primarily produced by the combustion of (poly) saccharides (21). Stavanja et al. found that the acetaldehyde yield in tobacco smoke (800-880 µg/cigarette) was not significantly affected by the type of sugar used in the tobacco (24). Moir et al. measured the acetaldehyde yield in mainstream smoke between 872±101 and 1555±222 µg/cigarette, depending on the smoking condition used (20). Baker et al. found that addition of malic acid to tobacco gave rise to a significant increase in acetaldehyde yield from 626 µg per cigarette in control to 695 µg per cigarette from tobacco where 1700 ppm of malic acid was added (3). In another study Baker et al. found that the yield of acetaldehyde was strongly dependent on the

saccharide type added to tobacco (2). Carmines et al. reported significant yield of acetaldehyde from pyrolysis of licorice extract (7). Hecht reported 770-864 µg acetaldehyde per cigarette (13).

## 4 PHYSICAL/CHEMICAL PROPERTIES

The substance is identified as given in the below table:

| | |
|---|---|
| **Physical state** | Colourless liquid or gas (15) occur as vapour in tobacco smoke (23) |
| **Molecular weight** | 44.05 (15) |
| **Melting point** | -123 °C (9) (15) |
| **Boiling point** | 20.1 °C (9) 21 °C (15) |
| **Vapour pressure** | 902 mm Hg (45 °C) (9) |
| **Density** | 0.788 (16 °C) (15) |
| **Water solubility** | 1.00E+06 mg/l (25 °C) (9) |
| **Log P octanol/water** | Log Pow = -0.34 (9) |
| **Other data** | Pungent, fruity odor (15) Leafy green taste (15) |

## 5 BIOTRANSFORMATON AND TOXICOKINETICS

Available studies on toxicity indicate that acetaldehyde is absorbed through the lungs and gastrointestinal tract; however, no adequate quantitative studies have been identified (27). Absorption through the skin is probable (27). Following inhalation by rats, acetaldehyde is distributed to the blood, liver, kidney, spleen, heart, and other muscle tissues. Low levels were detected in embryos after maternal intraperitoneal injection of acetaldehyde (mouse) and following maternal exposure to ethanol (mouse and rat) (27). Acetaldehyde is a metabolic intermediate in humans. By far, the main source of exposure to acetaldehyde in the general population is through metabolism of ethanol. Several isoenzymic forms of acetaldehyde dehydrogenase (ALDH) have been identified in the human liver and other tissues. There is polymorphism for mitochondrial ALDH. Subjects that are homozygous or heterozygous for a point mutation in the mitochondrial ALDH corresponding gene have low activity of this enzyme, can only metabolize acetaldehyde slowly and are intolerant of ethanol. The liver is the most important metabolic site (15).

Acetaldehyde is apparently metabolized to N-nitroso-2-methylthiazolidine 4-carboxylic acid. This chemical was detected in the urine of human subjects during both oral and nasal breathing. A fraction of this may be formed as a two-step synthesis *in vivo* from acetaldehyde and L-cysteine to yield 2-methylthiazolidine 4-carboxylic acid, which is easily nitrosated (15).

# 6 HEALTH EFFECTS

## 6.1 Single exposure toxicity

Single dose LD50S in rats and mice and LC50S in rats and Syrian hamsters showed that the acute toxicity of acetaldehyde is low (27).

### 6.1.1 Acute toxicity

| Route of exposure | Species | LD50[1)] / LC50[2)] | Reference |
|---|---|---|---|
| Inhalation | Rat | 13,300 ppm / 24000 mg $m^{-3}$ (LC50 4h) | (1) |
| Inhalation | Rat | 37000 (LC50 0,5h) | (27) |
| Inhalation | Hamster | 17000 ppm ppm / 31000 mg $m^{-3}$ (LC50 4h) | (9) |
| Oral | Mouse | 900 (LD50) | (10) |
| Oral | Rat | 661 (LD50) | (10) |
| Intraperitoneal | Mouse | 500 (LD50) | (10) |

[1)] LD50 (mg/kg bw): Lethal dose to half of the animals.

[2)] LC50 (mg/$m^3$ air): Concentration in air lethal to half of the animals after 4 hours exposure

### 6.1.2 Irritation/Corrosion

**Skin**
Cutaneous erythema was observed in the patch testing of twelve human subjects of "oriental ancestry" (28).

**Eyes**
Acetaldehyde vapor irritation of the human eye is detectable at 50 ppm in air and becomes excessive for chronic industrial exposure above 200 ppm (27). Higher concentrations and extended exposure may injure the corneal epithelium, causing persistent lacrimation, photophobia and foreign body sensation. A splash of liquid acetaldehyde can be expected to cause painful but superficial injury of the cornea, with rapid healing; the liquid evaporates so rapidly at body temperature that contact is brief and self limited. (15) op cit (12).

**Inhalation**
Some effects of exposure are irritation and lung oedema (15). Kruysse et al. (17) reported from an inhalation study on Syrian Golden Hamster. The animals were exposed to 0-4560 ppm acetaldehyde over a 90 day period. The highest level induced growth retardation, ocular and nasal irritation, increased number of erythrocytes, increased weights of heart and kidney, and severe histopathological changes in the

respiratory tract, mainly necrosis, inflammatory changes, and hyper and metaplasia of the epithelium. At 1340 ppm treatment-related changes were increased kidney weights in males, and slight hyper and metaplastic changes of the tracheal epithelium. 390 ppm was considered no effect level (17). Woutersen et al. exposed male and female Wistar rats to acetaldehyde vapour at nominal concentrations of 0, 750, 1500 and 3000/1000 ppm during 6h/day, 5 days/week for up to 28 months. The highest concentration was gradually decreased due to severe growth retardation. Major compound-related effects included increased mortality, growth retardation, nasal tumours, and non-neoplastic nasal changes in all test groups. The nasal changes comprised of degeneration, hyperplasia, metaplasia and adenocarcinomas of the olfactory epithelium at all exposure levels. It was concluded that acetaldehyde was both cytotoxic and carcinogenic to the nasal mucosa in rats (29).

In humans, at low levels of exposure (concentrations up to 100 ppm (180 mg $m^{-3}$) in air), acetaldehyde is rapidly absorbed and metabolized. Inhalation at higher concentrations (greater than 100-200 ppm) can cause irritation to the mucous membranes and ciliastatic effects on the upper respiratory tract (15). Acetaldehyde may facilitate the uptake in the human body of other atmospheric contaminants by the bronchial epithelium because of its ciliotoxic and mucus coagulating effect (15).

**Effects on nervous system**
Acetaldehyde is reported to be less irritating but stronger central nervous depressant than formaldehyde (11).

Steinhagen and Barrows found a 50% decrease in respiratory rate in two strains of mice during inhalation of 5000 mg $m^{-3}$ acetaldehyde for 10 minutes (25).

### *6.1.3 Allergy*
Although a possible mechanism has been identified, available data are inadequate to assess the potential of acetaldehyde to induce sensitization (27).

## *6.2 Repeated exposure toxicity*

### *6.2.1 General*

| Route of exposure | Species | Time of exposure | LOAEL[1)]/ LOAEC[2)] | Critical effects | Reference |
|---|---|---|---|---|---|
| Inhalation | Wistar Rats | 13-52 weeks | 750 ppm (1350 mg m-3) | Growth retardation | (29) |
| Inhalation | Wistar Rats | 52 weeks | 750 ppm (1350 mg m-3) | Degenerative changes in the olfactory nasal epithelium | (29) |

| Route of exposure | Species | Time of exposure | LOAEL[1)]/ LOAEC[2)] | Critical effects | Reference |
|---|---|---|---|---|---|
| Inhala-tion | Wistar Rats | 28 months | 750 ppm (1350 mg m-3) | Incidence of nasal tumors | (29) |

1. LOAEL (mg/kg bw/day): Lowest Observable Adverse Effect Level
2. LOAEC (mg/m$^3$ air/day): Lowest Observable Adverse Effect Concentration

## 6.3 Cytotoxicity

Acetaldehyde has been demonstrated to induce single- and double-stranded DNA breaks in human lymphocytes (22).

## 6.4 Genotoxicity

Acetaldehyde induces chromosomal aberration and sister chromatid exchange in a variety of test systems. The mutagenic effect of acetaldehyde was studied at the hypoxanthine-guanine phosphoribosyl transferase locus in human lymphocytes in vitro by selection of mutant cell clones in medium containing thioguanine. Cells treated with 1.2-2.4 mM acetaldehyde for 24 hr or 0.2-0.6 mM acetaldehyde for 48 hr showed a dose-dependent decrease of cell survival and a 3- to 16-fold increase of the mutant frequency (15). In a study where provisional mutational spectra at the hypoxanthine phosphoribosyl transferase (HPRT) locus in vitro have been worked out for acetaldehyde in human (T)-lymphocytes, it was concluded that acetaldehyde predominantly caused large genomic deletions (18).

## 6.5 Carcinogenicity

There is inadequate evidence in humans for the carcinogenicity of acetaldehyde. There is sufficient evidence in experimental animals for the carcinogenicity of acetaldehyde. Overall evaluation from IARC is that acetaldehyde is possibly carcinogenic to humans (16). In a 28 months inhalation study on rats, exposed to 0, 750, 1500 and 3000/1000 ppm during 6 h/day, 5 days/week, It was concluded that acetaldehyde is both cytotoxic and carcinogenic to the nasal mucosa of rats (29). Homann et al. studied the effects of acetaldehyde on cell regeneration and differentiation of the upper gastrointestinal mucosa in rats. Administered orally to rats, acetaldehyde can cause hyperplastic and hyperproliferative changes in epithelia of the upper gastrointestinal tract (14).

## 6.6 Reproduction toxicity

Available data are inadequate for assessment of reprotoxicological effects.

## 6.7 Addiction

At concentrations comparable to those found in tobacco smoke (maximum effect at 4 µg/kg b.w. nicotine, 16 µg/kg b.w. acetaldehyde, intravenous), rats self-administered

around 5 times more acetaldehyde/nicotine mixture than nicotine or acetaldehyde alone (8). In another experiment on the self-administration of nicotine in adolescent rats, it was found that acetaldehyde in the concentrations similar to concentrations in tobacco smoke, interacted with nicotine to increase responding in a stringent self-administration acquisition test where nicotine alone is only weakly reinforcing (4). Harman and salsolinol condensation products of acetaldehyde and biogenic amines may be responsible for the observed reinforcing effect of acetaldehyde (26) (19).

# 7 REGULATORY INFORMATION

## 7.1 Environment

Acetaldehyde enters the environment during industrial production, as a product of incomplete combustion, and as a product of alcohol fermentation. Due to the high vapour pressure and low tendency for sorption onto soil, acetaldehyde is most likely to be present in air. Acetaldehyde is readily biodegradable with approximate half-lives 10-60 hours in air and 1.9 hour in water (27). No quantitative data on levels in ambient water were identified. Concentration in ambient air average about 5µg $m^{-3}$ (27).

## 7.2 Classification and Health

Acetaldehyde is classified harmful, irritation to eyes and respiratory system, limited evidence of a carcinogenic effect (Carc. Cat. 3; R40 - Xi; R36/37) by the European Commission.

The California Environmental Protection Agency has listed acetaldehyde as a substance known to cause cancer (6).

Threshold Limit Value (working environment) for acetaldehyde is 25 ppm (45 mg $m^{-3}$) in USA.

Max. allowable industrial contribution value for air (B-værdi) for acetaldehyde is 0.02 mg $m^{-3}$ in Denmark.

# 8 EVALUATION

The toxicological information on acetaldehyde indicates that acetaldehyde is possibly one of the important toxic compounds in tobacco smoke, and possibly also a carcinogen. Acetaldehyde is not added to tobacco, but appears as a pyrolysis product primarily from the tobacco itself and from many common additives, such as saccharides. Studies on sugars and tobacco smoke composition reveal that there is a strong relationship between the type of sugar added to tobacco and the yield of acetaldehyde. This means that addition of sugars to tobacco might have significant effect on the toxicity of cigarette smoke. In self-administering studies in rats it was shown that rats tend to administer more nicotine when acetaldehyde was present in drinking water than nicotine alone. It is possible that acetaldehyde plays a central role in tobacco addiction.

## 9 CONCLUSION

Acetaldehyde is possibly one of the most important toxic compounds in tobacco smoke. Experimental data suggest that there is a relationship between the type of sugars added and the acetaldehyde yield, meaning that some sugars added to tobacco leads to increased acetaldehyde yield, and others do not. There is experimental data suggesting that acetaldehyde might play a role in tobacco addiction.

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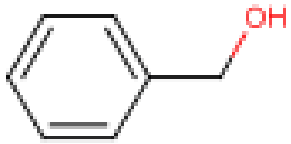
# Toxicological profile of Benzyl alcohol




**SEPTEMBER 200**

Ministeriet for Sundhed og Forebyggelse

## *CONTENTS*

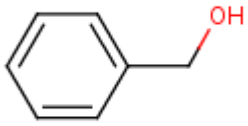
# 1 INTRODUCTION

The purpose of the present toxicological profile for benzyl alcohol is to reveal the possible health effects on smokers by the presence of benzyl alcohol in tobacco smoke. The search profile used was: benzyl alcohol, CAS no 100-51-6, oncogen, tumor, neoplasm, carcinogen, addiction, and dependence.The profile has been compiled on searches in the following databases: ESIS, AtsDR, IRIS, IARC, InChem, DOSE, ChemID plus, HSDB, STN, and Toxline.

# 2 IDENTIFICATION

| | |
|---|---|
| **Chemical Name** | Benzyl alcohol |
| **Synonyms** | Benzenemethanol, Phenyl-methanol, Phenylcarbinol, Phenyl-methyl Alcohol |
| **CAS No.** | 100-51-6 |
| **EINECS/ELINCS No.** | 202-859-9 |
| **Molecular formula** | C7-H8-O |

**Structure**




# 3 USE AND OCCURRENCE

Benzyl alcohol occurs naturally in fruits, essential oils, e.g. jasmine, and in tobacco (3). Benzyl alcohol has a pleasantly sweet and fruity odour (3). Benzyl alcohol is used in cosmetics, as flavour and preservative (24). Also, it is used in the manufacture of other benzyl compounds, and as a pharmaceutical aid (9). Quantity not exceed (QNE) in tobacco is reported to be 0,015% (21).

# 4 PHYSICAL/CHEMICAL PROPERTIES

The substance is identified as given in the following table:

| | |
|---|---|
| **Physical state** | Liquid (8) |
| **Molecular weight** | 108 g/mol (24) |
| **Melting point** | -15.3 $^{o}$C (7) |
| **Boiling point** | 205.3 $^{o}$C (6) |
| **Vapour pressure** | 0.094 mm Hg (25 $^{o}$C) (24) |
| **Density** | 1.0419 (16 $^{o}$C) (24) |
| **Water solubility** | 4.29E+04 mg/l (25 $^{o}$C) (5) |
| **Log P octanol/water** | Log Pow = 1.1(10) |
| **Other data** | Faint aromatic odour, sharp burning taste(24)<br>Benzyl alcohol will transfer 95% intact to smoke, during pyrolysis (2). |

# 5 BIOTRANSFORMATON AND TOXICOKINETICS

Benzyl alcohol is oxidised in the liver by the enzyme alcoholdehydrogenase (ADH) to benzaldehyde, which is oxidized to benzoic acid. Benzoic acid conjugates with the aminoacid glycine to form hippuric acid, which is excreted by the kidneys. Transformation and excretion is relatively fast. Within 6 hours from oral intake of benzyl alcohol, 75-100% is found in the urine as hippuric acid (24). It can be calculated that smokers are exposed to 0.05 mg benzyl alcohol per kg bw per day when smoking 20 cigarettes per day.

# 6 HEALTH EFFECTS

## 6.1 Single exposure toxicity

### 6.1.1 Acute toxicity

| Route of exposure | Species | LD50[1)] / LC50[2)] | Notes | Reference |
|---|---|---|---|---|
| Inhalation | Rats | >4178 (4h) (LC50) | (NOEC, No observed Effect Concentration) | (25) |
| Inhalation | Rats | 4417 (1000 ppm) (8h) (LCLo) | (LCLo, Lowest Concentration were death were observed) | (18) |
| Oral | Rats | 1230- 3200 (LD50) | (four studies referred in Nair, 2001) | (24) |
| Oral | Mouse | 1580 (LD50) | | (24) |
| Oral | Mouse | 1360 (LD50) | | (17) |

| Route of exposure | Species | LD50[1] / LC50[2] | Notes | Reference |
|---|---|---|---|---|
| Oral | Rabbit | 1040 (LD50) | | (16) |
| Dermal | Cat | 10000 (LDLo) | | (15) |
| Intravenous | Mouse | 324 (LD50) | | (14) |
| intraperitoneal | Mouse | 650 (LD50) | | (13) |
| Intraperitoneal | Rats | 400 (LD50) | - | (11) |

[1] LD50 (mg/kg bw): Lethal dose to half of the animals.

[2] LC50 ($mg/m^3$ air): Concentration in air lethal to half of the animals after 4 hours exposure

### *6.1.2 Irritation/Corrosion*

**Skin**

Undiluted benzyl alcohol was moderately irritating when applied to the depilated skin of guinea pigs for 24 hours (1). Benzyl alcohol has demonstrated a maximum incidence of sensitization of 1% of humans tested in patch testing (25).

**Eyes**

2% benzyl alcohol in saline water, 0.9% sodium chloride, applied to the eyes of rabbits, caused injury of endothelium, with severe bluish swelling of the cornea. Also, the irises became hyperemic and had poorly reactive pupils(20).

**Inhalation**

According to Lewis, Benzyl alcohol is moderately toxic by inhalation (23). In an inhalation study no mortality was seen at 4000 $mg/m^3$/4h (25).

**Effects on nervous system**

In human pain studies, benzyl alcohol was an effective local anaesthetic (24). It is used as a local anaesthetic and to reduce pain associated with lidocaine injection (4). In a study were cat was exposed to benzyl alcohol via the dermal route tremor and muscle weakness was observed at 10000 mg/Kg body weight (12).

### 6.1.3 Allergy

Benzyl alcohol gave both positive and negative results for sensitization in animals (25).

## *6.2 Repeated exposure toxicity*

### *6.2.1 General*

Benzyl alcohol exhibits relatively low repeated dose toxicity (25).

| Route of exposure | Species | Time of exposure | NOAEL[1]/ NOAEC[2] | Critical effects | Reference |
|---|---|---|---|---|---|
| Oral | Rat | 2 years | 400 NOAEL | Weigth gain Histopa-thologic le-sions | (25) |

| Route of exposure | Species | Time of exposure | NOAEL[1)]/ NOAEC[2)] | Critical effects | Reference |
|---|---|---|---|---|---|
| Oral | Mouse | 13 weeks | 200 NOAEL | Weigth gain Histopathologic lesions | (25) |

1. NOAEL (mg/kg bw/day): No Observable Adverse Effect Level.
2. NOAEC (mg/m$^3$ air/day): No Observable Adverse Effect Concentration.

## 6.3 Cytotoxicity

Ohmiya and Nakai (26) studied the uptake of benzyl alcohol by human erythrocytes, the binding of benzyl alcohol to celle membranes and hemolysis *in vitro*. The critical hemolytic level was estimated to 500 nmoles/mg protein.

## 6.4 Genotoxicity

Benzyl alcohol has shown no mutagenic activity in *in vitro* Ames tests, and has shown no genotoxic activity *in vivo* (25).

## 6.5 Carcinogenicity

No signs of carcinogenicity has been documented in long term carcinogenicity studies (25).

## 6.6 Reproduction toxicity

No data found

## 6.7 Addiction

No data found

# 7 REGULATORY INFORMATION

JECFA has established an ADI at 0-5 mg/kg for benzyl compounds like benzyl alcohol, benzyl acetate, benzaldehyde and benzoic acid (22).

Benzyl alcohol is included as a flavouring substance intended for use in or on foodstuffs, by the European Community in Commission Decision 2002/113/EC (19).

Reviewing a 2 year study, the EPA determined the human reference dose (RfD), for chronic oral exposure of 0.286 mg/kg/day, which was rounded to 0.3 mg/kg/day (24).

## 7.1 Environment

No data found.

## 7.2 Classification and Health

Benzyl alcohol is classified as harmful (Xn; R20/22) by the European Commision.

Max. allowable industrial contribution value (B-værdi) for benzyl alcohol is 0.1 mg $m^{-3}$ in Denmark,

Companies have provisionally advised exposure limits for benzyl alcohol. US Workplace Environmental Exposure Limit has set the limit value to 10 ppm, corresponding to 44 mg $m^{-3}$ (25).

No threshold limit value in Denmark. Threshold limit value in Poland is 240 mg $m^{-3}$.

# 8 EVALUATION

The number of studies concerning inhalation of benzyl alcohol is very limited. The effect concentrations in the few relevant studies are relatively high. The effect concentration from Ohmiya and Nakai (26) is very difficult to relate to expected effects on humans. Considering the small amount of benzyl alcohol added to tobacco, the relatively low toxicity of benzyl alcohol and the general toxicity of tobacco, it is therefore evaluated that benzyl alcohol will not increase the toxicity of tobacco significantly. There are no studies relating benzyl alcohol to tobacco addiction.

# 9 CONCLUSION

The addition of benzyl alcohol to tobacco is not likely to affect the overall toxicity of the product. There is no indication that benzyl alcohol plays a role in tobacco addiction.

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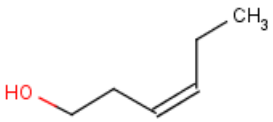
# Toxicological profile of Cis-3-hexenol




**SEPTEMBER 2009**

Ministeriet for Sundhed og Forebyggelse

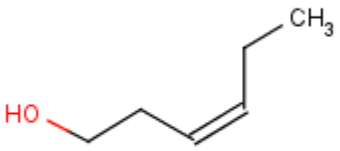
## *CONTENTS*

# 1 INTRODUCTION

The purpose of the present toxicological profile for cis-3-hexenol is to reveal the possible health effects on smokers by the presence of cis-3-hexenol in tobacco smoke. Also possible effect of cis-3-hexenol in tobacco addiction is reviewed. The search profile used was: cis-3-hexenol, CAS no 928-96-1, oncogen, tumor, neoplasm, carcinogen, addiction, and dependence. The profile has been compiled on searches in the following databases: ESIS, AtsDR, IRIS, IARC, InChem, DOSE, ChemID plus, HSDB, STN, Toxline.

# 2 IDENTIFICATION

| | |
|---|---|
| **Chemical Name** | Cis-3-hexenol |
| **Synonyms** | 3-Hexen-1-ol, 3-hexenol, Leaf alcohol |
| **CAS no.** | 928-96-1 |
| **EINECS/ELINCS No.** | 213-192-8 |
| **Molecular formula** | C6-H12-O |
| **Structure** | |




# 3 USE AND OCCURRENCE

Cis-3-hexenol occurs naturally in many plants and plant products (6). The daily intake in Europe per capita of cis-3-hexenol is estimated to 4300 µg/person/day (28). Cis-3-hexenol is a major component in green odor (20). Cis-3-hexenol has been in use as additive to cigarettes at least since 1970 (4). Quantity Not Exceeded (QNE) in tobacco is reported to be 0.00003% (14).

# 4 PHYSICAL/CHEMICAL PROPERTIES

The substance is identified as given in the following table:

| | |
|---|---|
| **Physical state** | Clear liquid, very faintly yellow (18) |
| **Molecular weight** | 100.18 (18) |
| **Melting point** | (N/A) |
| **Boiling point** | 156.5 $^{o}$C (9) |
| **Vapour pressure** | 0.86 mm Hg (27) |
| **Density** | 0.85 (16 $^{o}$C) (18) |
| **Water solubility** | 10000-16000 mg/l (25 $^{o}$C) (26) (19) |
| **Log P octanol/water** | Log Pow = 1.61(9) |
| **Other data** | Pine needle, grassy odor (25) Leafy green taste (10)<br>Odour threshold 19 ppb, in argon (8)<br>Flashpoint 44 $^{o}$C (18) |

# 5 BIOTRANSFORMATON AND TOXICOKINETICS

The metabolism of alcohols such as cis-3-hexenol in the mammalian body is well-understood. The mammalian body would effectively metabolize the alcohol to the corresponding aldehyde, which would then be metabolized to the corresponding carboxylic acid. The mammalian body has well-understood pathways for metabolism of carboxylic acids to carbon dioxide and water (24).

# 6 HEALTH EFFECTS

Cis-3-hexenol was shown inactive in a cytotoxicity test (29).

## 6.1 Single exposure toxicity

### 6.1.1 Acute toxicity

| Route of exposure | Species | LD50 (mg/kg bw) | Notes | Reference |
|---|---|---|---|---|
| Oral | Rat | 4700 | - | (18) |

| Route of exposure | Species | LD50 (mg/kg bw) | Notes | Reference |
|---|---|---|---|---|
| Intraperitoneal | Rat | 600 | - | (13) |
| Oral | Mouse | 7000 | | (18) |
| Dermal | Rabbit | >5000 | | (18) |
| Intraperitoneal | Mouse | 400 | | (18) |

### *6.1.2 Irritation/Corrosion*

**Skin**
No irritation was associated with a 24-hour dermal application of neat (undiluted) cis-3-hexenol with an occlusive dressing to either intact or abraded rabbit skin (23). Human subjects exhibited no signs or symptoms of irritation following a 48-hour dermal exposure to 4% cis-3-hexenol (in petrolatum) under an occlusive patch (22).

**Eyes**
No data found

**Inhalation**
No studies have been identified on the toxicological effects of cis-3-hexenol via inhalation. However Alford and Johnson (3) describe a study where cigarettes with added cis-3-hexenol were unanimously preferred over the control cigarettes. Also it was mentioned that the effect of cis-3-hexenol was a dramatic increase in smoke freshness and acceptability.

**Effects on nervous system**
A study on rats indicate that green odor (cis-3-hexenol and trans-2-hexenal) have an calming effect on autonomic stress response to novel environments (1). Cis-3-hexenol has been shown to potentiate GABA receptors in a similar way than by benzodiazepine, barbiturate, steroids and anesthetics, suggesting that Cis-3-hexenol have anxiolytic, anticonvulsant and sedative effects on the human mind (5). Cis-3-hexenol has been demonstrated to reduce stress in humans (16).

### *6.1.3 Allergy*

No data found

## *6.2 Repeated exposure toxicity*

### *6.2.1 General*

| Route of exposure | Species | Time of exposure | NOAEL 1) | LOAEL 2) | Critical effects | Reference |
|---|---|---|---|---|---|---|
| oral | Rat | 98 days | 120 | | Food intake | (12) |

| Route of exposure | Species | Time of exposure | NOAEL 1) | LOAEL 2) | Critical effects | Reference |
|---|---|---|---|---|---|---|
| | males | | | | Body weight gain | |
| oral | Rat males | 98 days | | 410 | Increased relative kidney weight Increased adrenal weight | (11) |

1. NOAEL (mg/kg bw/day): No Observable Adverse Effect Level.
2. LOAEL (mg/kg bw/day): Lowest Observable Adverse Effect Level

## 6.3 Genotoxicity

WHO has evaluated 42 flavouring substances that include linear and branched-chain aliphatic unsaturated and unconjugated alcohols, aldehydes, acids and related esthers for mutagenicity and genotoxicity. The negative results indicate that the substances including cis-3-hexenol are neither mutagenic nor genotoxic (28). The US EPA has performed a SAR (Structure-activity relation) analysis of cis-3-hexenol and came to the conclusion that cis-3-hexenol is not related to any known mutagens, carcinogens or developmental/reproductive toxicants (21).

## 6.4 Carcinogenicity

See above.

## 6.5 Reproduction toxicity

See above.

## 6.6 Addiction

Green odor, a mixture of equal amounts of trans-2 hexenal and cis-3-hexenol has been showed to attenuate immediate and long term stress in rats, by reducing the plasma concentration of adrenocorticotropic hormone (ACTH) (15). The relieving effect by Cis-3-hexenol has also been shown in humans. Cis-3-hexenol has been shown to potentiate GABA receptors in a similar way than by benzodiazepine, barbiturate, steroids and anesthetics, suggesting that Cis-3-hexenol has anxiolytic, anticonvulsant and sedative effects on the human mind (7). Cis-3-hexenol has been demonstrated to reduce stress in humans (17). The addition of cis-3-hexenol to tobacco has been patented in 1970 in US, due to its pleasant effect on the smoking experience (2,4).

# 7 REGULATORY INFORMATION

## 7.1 Environment

No data found

## 7.2 Classification and Health

EU Classification; R 10. Risk statement: Flammable. S 16. Safety statement keep away from sources of ignition – no smoking.

No Contribution value (B-værdi) for air pollution in Denmark for cis-3-hexenol

No threshold Limit values for cis-3-hexenol identified.

# 8 EVALUATION

Animal data indicate that the acute toxicity of Cis-3-hexenol is relatively low, it is therefore evaluated that the addition of the substance to tobacco will increase the overall toxicity of tobacco. However, Cis-3-hexenol is known to increase the pleasantness of tobacco when smoking, and is also experimentally shown in animals to have anxiolytic, anticonvulsant and sedative effects and to reduce stress in humans. It is therefore evaluated that the addition of the cis-3-hexenol to tobacco can assist to the addictive properties of tobacco.

# 9 CONCLUSION

Due to the low toxicity of cis-3-hexenol it is unlikely that the addition of the substance to tobacco will affect the overall toxicity of the product. Cis-3-hexenol has been shown to improve the smoking experience, and as cis-3-hexenol has been shown to reduce stress in humans, it is possible that the addition of the cis-3-hexenol to tobacco can assist to the addictive properties of tobacco.

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# Toxicological profile of Menthol

PLATE VII.—*Mentha piperita.* The source of Oleum Menthae Piperitae (peppermint oil). (From Jackson: *Experimental Pharmacology and Materia Medica.*)

**SEPTEMBER 2009**

Ministeriet for Sundhed og Forebyggelse

## *CONTENTS*

# 1 INTRODUCTION

The purpose of the present report is to elucidate the impact of adding menthol to cigarettes regarding potentially increased health risk, primarily increased risk of cancer and increased dependence of nicotine. The evaluation is based on a literature search described below. The literature on the potential effects of menthol in cigarettes is comprehensive. This report aims at answering the following questions: does menthol increase dependence on nicotine; does menthol increase the risk of smoking related illnesses such as lung cancer and heart disease.

The search terms used were: menthol, CAS no 89-78-1, oncogen, tumor, neoplasm, carcinogen, black, men, addiction, dependence, tobacco, and additives.

The used literature has been compiled from four different searches. The first search was made in the Toxnet cluster database, where data for the different toxicological end points were collected from HSDB, ChemIdPlus and CCRIS. An additional search was performed in the commercial cluster database STN, where data from Toxline, Embase and SciSearch were included. A third search was performed in PubMed and finally literature was also compiled from reference lists in the retrieved references.

A number of references were cited by many authors and these references were also evaluated by DHI. Also the most recent literature on menthol was evaluated. A lot of hypotheses have been put forward and we aimed at an evaluation of actual measurements and clinical prospective intervention studies, eventually supplied with *in vitro* data.

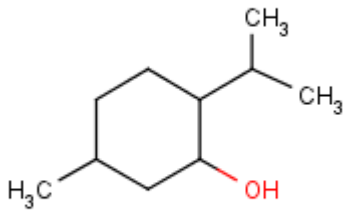
It is discussed in the literature if addition of menthol to tobacco increases the dependence on tobacco and whether it has additional adverse effects in addition to those induced by the content of tar and nicotine in the tobacco itself.

Overall, it is difficult to evaluate the adverse effects of additives in tobacco as the pyrolysis products of the additives may have effects on their own, and these effects are difficult to control (35).

The reasons for smoking mentholated cigarettes are reported to be due to a reduction in the irritating effects, a soothing effect on the lungs, and are said to be less harsh and smoother to smoke (3), but according to the Tobacco Manufacturers Association (TMA) of Denmark the typical menthol smoker is between 31 and 55 years of age, and they interpret the data in that way that it may take some time to get used to smoking menthol cigarettes, indicating that the opinion of the TMA in Denmark is that menthol cigarettes are not milder to smoke than non-menthol cigarettes.

## 2 IDENTIFICATION

| | |
|---|---|
| **Chemical Name** | Menthol |
| **Synonyms** | (+-)-Menthol<br>(1R,2S,5R)-Menthol<br>5-Methyl-2-(1-methylethyl)cyclohexanol,<br>(1alpha,2beta,5alpha)-<br>Hexahydrothymol<br>Menthacamphor<br>Peppermint camphor<br>Racementhol<br>p-Menthan-3-ol<br>rac-Menthol |
| **CAS no.** | 89-78-1 |
| **EINECS No.** | EINECS 201-939-0<br>EINECS 239-388-3 |
| **Molecular formula** | C10-H20-O |
| **Structure** | |




Menthol also exists with two other CAS numbers, 1490-04-6 and 2216-51-5, and they are different on some of the physical/chemical (d and l versions). Based on the report from The Danish Cancer Society, this report focuses on CAS no 89-78-1, which relates to the the racemic mixture.

## 3 USE AND OCCURRENCE

Menthol is a naturally occurring alcohol extracted from the Mentha piperata and M. arvensis plants (49). Synthetic processes are also used to produce d,l-menthol (the racemate) found in many commercial products (49). Most of the menthol used is reported to be synthetic (42). Menthol is used pharmaceutically as an antipruritic[1], antiseptic and cooling compound, and is also used as a flavouring in toothpaste and oral hygiene products (20).

Menthol may also be derived from cornmint oil where menthol is removed from the crude oil by freezing. Menthol for flavouring of tobacco may either come from the "dementholised" oil or from the extract (31).

[1] Against itching

# 4 PHYSICAL/CHEMICAL PROPERTIES

The substance is identified as given in the below table:

| | |
|---|---|
| **Physical state** | Crystals or granules (7) |
| **Molecular weight** | 156.27 (7) |
| **Melting point** | 41-43°C (7) |
| **Boiling point** | 212°C (7) |
| **Vapour pressure** | 0.0637 mm Hg (25°C) (11) |
| **Density** | 0.89 (7) |
| **Water solubility** | 456 mg/L (25°C) (11) |
| **Log P octanol/water** | 3.3 (11) |
| **Other data** | |
| **Odor** | Peppermint odor (7) |
| **Taste** | Peppermint taste (7) |
| **Henry's law constant** | $1.52x10^{-05}$ atm-$m^3$/mole (25°C, estimated value) (2) |
| **Atmospheric OH Rate Constant** | $2.4x10^{-11}$ $cm^3$/molecule-sec (25°C, estimated value) (1) |

# 5 BIOTRANSFORMATON AND TOXICOKINETICS

Metabolism of alcohol, as menthol, occur by conjugation with glucoronic acid which is then excreted as menthyl glucuronide (41). Even though the acute toxicity of menthol is said to be low and negative in a range of genotoxicity tests, some authors wonder why the complete details of the metabolism of menthol is lacking in humans (32).

The gluceronides of menthol are excreted either in urine or in faeces. Glucuronides of menthol are eliminated in urine by renal tubules as well as by glomerular filtration (5). In addition, glucuronides of menthol are excreted into bile (5). The distribution of the amounts that are excreted in urine and faeces, respectively, is species dependent (5), and has not been specified for humans.

# 6 HEALTH EFFECTS

Many studies on effects of smoking originates from US and are included in this report as they give some interesting data due to different smoking behaviours in black and white Americans. These differences may help answer the questions about dependence and disease risk by menthol cigarette smoking. Differences in the reported results may partly be explained by differences in representation of each gender and race group (Blacks and Whites) in the sample sizes.

Menthol is known or expected to influence the below mentioned smoking parameters and the potential adverse effects are discussed in the following paragraphs:

**Cooling effect**
In many papers, menthol is said to have a cooling and local anaesthetic effect that may permit greater and deeper inhalation of tobacco smoke (3). Green has tested the effects of menthol on cold and warmth perception and concludes that it is unclear, from a number of studies, whether a reduced heat perception was due to effects on warmth receptors, nociceptors or both (22,23,24). A receptor for menthol has recently been identified, the existence of which may explain the sensory effects of menthol. The receptor is called cold and menthol receptor 1 (CMR1) (12) or transient receptor potential melastatin 8 (TRPM8) (50). Topical application of menthol (40% menthol solution in ethanol) to the forearm of 10 human test persons resulted in increased sensation of pain at cold temperatures (cold hyperalgesia) and vasodilation at the site of application, but no vasodilation outside the area of application (axon reflex flare) (39).

These data indicate that menthol influence the sensation of pain at cold temperatures.

**Enhancement of drug absorption and increased exposure**
A study performed by Lorillard Tobacco Company revealed no difference in NNK (a class 1 carcinogen), nicotine, or carbonmonooxide in blood and urine between menthol and non-menthol heavy smokers, neither among white Americans nor black Americans (27).

The levels of urinary biomarkers following exposure to tobacco smoke have been shown not to be directly reflecting the exposure levels as the level of e.g. cotinine was lower in those smoking more than 20 cigarettes per day than in those smoking less than 20 cigarettes per day (34).

These data does not indicate increased exposure to nicotine, NNK or carbonmonooxide. Furthermore, they indicate that it may be misleading to evaluate exposure from plasma levels of the biomarkers.

**Respiration and inhalation, inhalation depth**
A study in humans showed that topical application of 1 or 5% menthol caused nasal congestion as measured by nasal resistance. In contrast to the measurements of nasal resistance, the test persons themselves felt that their nasal passages felt clear (19). This may document that the feeling of a nasal decongestant effect following inhalation of menthol actually occurs without objective decongestant action and perhaps even congestion.

A study with the purpose to investigate the effect of different tar yields from cigarettes on post-puff inhalation and exhalation depth and duration showed no significant relationship between tar yield and inhalation volume, inhalation tidal ratio or lung exposure time (17). No difference in inhalation tidal ratio was found between smokers of mentholated and unmentholated products (17).

These data indicate that menthol affects the sensation of different smoking parameters that do not give rise to objective measurements to support the sensations.

**Nicotine metabolism, menthol metabolism and metabolism of other compounds of the cigarette tobacco**

A study from 2004 showed that mentholated cigarette smoking did not increase the systemic levels of nicotine and carbon monoxide but did inhibit the metabolism of nicotine. Clearance of nicotine, especially renal clearance in African-Americans was lower following smoking of mentholated cigarettes, and this may be due to a decrease in CYP2A6 level and due to reduction in glucoronidation (metabolism of nicotine), as evidenced by a lower nicotine-glucorinide/nicotine ratio in the urine (13). Data from rats indicate that menthol may alter the hepatic drug-metabolizing enzyme levels (13), but it has not been tested whether this also applies to humans.

An in vitro study with human liver microsomal cells revealed that menthol inhibits the oxidation of nicotine as well as the P450 mediated 2A6 coumarin 7-hydoxylase (32). This may result in a less efficient metabolism of nicotine following smoking of mentholated cigarettes and a higher exposure to nicotine for a longer period of time compared to the levels after smoking cigarettes without menthol.

The potentially less efficient metabolism of nicotine and decreased renal clearance following smoking of mentholated cigarettes does not seem to be reflected in higher levels of the biomarkers systemically.

**Level of cotinine (a metabolite of nicotine)**

Cotinine is regarded as the best indicator of tobacco smoke exposure (42). Menthol cigarettes are typically higher in nicotine and tar (3), and Blacks have higher plasma cotinine concentrations and a higher ratio between cotinine and cigarettes per day (38).

No difference in cotinine level was found between smokers of menthol versus non-mentholated cigarettes in a Canadian/American study from 2007 (36). However, a paper from 1998 reports findings of higher concentrations of cotinine in male, black smokers compared to white smokers and Mexican American smokers (15). Unfortunately, no distinction was made in this study between smoking mentholated or non-mentholated cigarettes. The review by Richardson refers to several studies where black Americans have higher levels of cotinine in the blood (42).

**Smoking behaviour, including early initiation, time to first cigarette, dependency of nicotine and cessation**

Even though smoking prevalence is similar in white and black Americans (24.1% versus 23.2%), black Americans clearly prefer menthol cigarettes (76% versus 23% in white Americans). Most papers state that the prevalence of smokers is equal between black and white Americans but that black Americans smoke less cigarettes than white Americans (38,42). Furthermore, in one study only 37.3% of black “ever smokers” had quit smoking in contrast to 51% of Whites (3), suggesting that even though they smoke less they may be more addicted to smoking.

In a clinical trial of smoking cessation, where 5887 smokers were included, no difference in success of quitting smoking between smokers of plain versus menthol cigarettes was found as well as no difference in values of carbon monoxide and cotinine (36). Another study reports that black Americans show signs of being more addicted as a higher percentage of black Americans compared to white Americans are “wake-up” smokers that need to smoke within 10 minutes of awakening (42). In addition, in another study a difference was observed as significantly more Whites had been able to quit smoking

compared to Blacks, a difference that may be due to Blacks choosing mentholated cigarettes but no data support menthol to be responsible for this difference (46).

These data indicate that black Americans are more addicted to nicotine and that it cannot be related to smoking mentholated cigarettes.

**Risk of cancer**

In addition to the special smoking pattern in black Americans, also disease pattern differs. The incidence of lung cancer is approximately 50% higher in black Americans, and the risk of other smoking related illnesses as heart disease and stroke is also higher (42). It is discussed in the literature if the higher incidence of lung cancer observed in black, male Americans (43) may be due to smoking menthol cigarettes.

Carpenter et al report no increased risk for developing lung cancer for menthol cigarette smokers compared to plain cigarette smokers (16). Kabat and Hebert did not find any significant association between incidence of lung cancer and type of cigarette smoked (mentholated versus non-mentholated) in black and white Americans (29). In addition, a study from 2003 found no overall difference in lung cancer risk for Whites and Blacks with the same smoking habits (45).

Only one study reports an increased risk of lung cancer associated with mentholated cigarette use in male smokers, but not in female smokers (44). The relative risk of lung cancer associated with mentholated cigarette use was 1.45 in black men, corresponding to a 45% significantly increased risk of developing lung cancer (44). The study was a comprehensive prospective study including 11761 smokers (44).

The incidence and mortality of oesophageal cancer among Black Americans is over three times the rate for Whites (26). A study from 1989 in Black Americans showed no difference in the risk of developing oesophageal cancer when smoking mentholated cigarettes compared to smoking non-mentholated cigarettes (26).

The incidence of cancer in the oral cavity and pharynx is 50% higher in black Americans compared with white Americans (30). A case-control study revealed that menthol was not a risk factor for oropharyngeal cancer neither in Blacks nor in Whites (30).

One of the most recent studies reports no difference in lung cancer risk for smokers of menthol cigarettes, even though they cannot exclude a modest increase in risk in men due to methodological and sample size limitations (14).

Overall, the incidence of smoking related cancers are higher in black Americans, but the increased risk does not seem to be related to smoking mentholated cigarettes.

**Risk of cardiovascular effects**

The effect on heart rate (left and right ventricular systolic and diastolic function) in humans has been shown to be more adversely affected following smoking of mentholated cigarettes compared to regular cigarettes (18). Limitations to this study is sample size (18 smokers and 20 controls) and lack of measurements of e.g. cotinine and carbon monoxide that could better relate these results to results obtained in other studies (18).

**Conclusion**
Black Americans smoke more mentholated cigarettes than white Americans. They also have higher incidence of lung cancer even though they smoke less cigarettes. It has been a puzzle why black Americans become more ill from smoking than white Americans and one of the obvious differences is that they smoke more mentholated cigarettes. Many studies have investigated the impact of menthol but no clear conclusions can be made. This suggests that another factor than menthol may be responsible for the higher incidence of lung cancer in black Americans who smoke.

However, menthol does seem to affect dependence in the sense that more menthol smokers are "wake-up" smokers that need to smoke within 10 minutes after waking up.

Overall, menthol does not seem to be important for development of cancer and it is questionable which impact it has on development of dependence of nicotine as increased dependence has only been reported from one study.

## *6.1 Single exposure toxicity*

### *6.1.1 Acute toxicity*

| Route of exposure | Species | LD50[1)] | Reference |
|---|---|---|---|
| Intramuscular | Rat | 10 g/kg bw[1)] | (4) |
| Oral | Rat | 3180 mg/kg bw[1)] | (28) |

[1)] LD50 (mg/kg bw): Lethal dose to half of the animals.

### *6.1.2 Irritation/Corrosion*

Menthol is a moderate irritant to mucous membranes on inhalation (25).

### *6.1.3 Allergy*

Chronic urticaria with basophil leukopenia on challenge has been reported after contact with menthol in mentholated cigarettes (40).

Menthol may cause allergic reactions as contact dermatitis, flushing, and headache in certain individuals (31).

## 6.2 Repeated exposure toxicity

### 6.2.1 General

| Route of exposure | Species | Time of exposure | NOAEC[1)] | LOAEC[2)] | Critical effects | Reference |
|---|---|---|---|---|---|---|
| Inhalation of cigarette smoke (dose refers to particulate matter) | Fischer Rats | 1 h/day, 5 days/week for 13 weeks | < 200 mg/m$^3$ | 200 mg/m$^3$ | Carboxyhaemoglobin and serum cotinine lower in menthol exposed rats at all doses or 200 mg/kg bw, respectively. Epithelial changes. | (21) |

1. NOAEC (mg/m$^3$ air/day): No Observable Adverse Effect Concentration.
2. LOAEC (mg/m$^3$ air/day): Lowest Observable Adverse Effect Concentration

### 6.2.2 Genotoxicity

No data found.

### 6.2.3 Carcinogenicity

Menthol, 7500 ppm in diet, showed no carcinogenic effects in rats and mice (6).

Menthol may increase the risk of cancer, as the ratio of NNAL-Gluc/NNAL has been shown to be significantly lower in menthol versus non-menthol smokers, and this is interpreted as an indicator of lung cancer risk (37). NNAL-Gluc is the detoxified form of NNAL.

Combustion of menthol can give rise to 3,4-benzpyrenes which are carcinogenic (42) but menthol has not been shown to give rise to any measureable amounts of 3,4-benzpyrenes in smoke from mentholated cigarettes (33).

It is discussed in the literature whether smoking mentholated cigarettes increases the risk of lung cancer. From the literature there seem to be a tendency that smoking mentholated cigarettes increases the risk of lung cancer in black males, and may make no difference or even slightly decrease the risk in black females and white male and female (16,44,45). But the overall conclusion from the literature is that menthol does not increase the risk of lung cancer. The statement is based on results from case-control studies and does only address the relative risk. Limitations to the data are that they are based on retrospective analyses and that they rely on people's memory.

### 6.2.4 Reproduction toxicity

No data found.

## 6.3 Other

The psychotropic action (effect on the central nervous system) of menthol is reported to always be transient and unlikely to have any effects of this kind in man at the concentrations that occur in food or drinks (40).

### 6.3.1 Influence on dependence of nicotine

Menthol increases absorption of chemical combinations with menthol via the dermal route (49).

# 7 REGULATORY INFORMATION

The joint FAO/WHO expert committee on food additives (1968) has published a Monograph and specifications for menthol giving an unconditional acceptable daily intake (ADI) of 0-0.2 mg/kg bw (40). The European Council has listed menthol with an ADI of 2 mg/kg (40). The difference is assumed to result from use of different safety factors, and this affects the calculations and the ADI values. In 1998 this ADI value was changed to 4 mg/kg (8).

Menthol in cosmetics is not regulated.

## 7.1 Environment

The American Environmental Protection Agency (EPA) has listed menthol on List D, that contains “Pesticides of less concern” (48).

## 7.2 Classification and Health

Menthol is not registered on the Effect List 2000 of the Danish Environmental Protection Agency as a substance causing particularly serious ecotoxicological effects (9).

Menthol is not listed by the Danish Working Environment Authority (Arbejdstilsynet) (47), nor by the European Chemical Substances Information System (ESIS) (10).

No Contribution value (B-værdi) for air pollution exists in Denmark for Menthol.

# 8 EVALUATION AND CONCLUSION

As mentioned in the Introduction, this report aimed at answering the following questions: does menthol increase dependence on nicotine and does menthol increase the risk of smoking related illnesses such as lung cancer and heart disease.

The literature on the effects of adding menthol to tobacco is extensive. Conclusions are mainly based on differences among black and white Americans and the reason for this is

that black Americans smoke more mentholated cigarettes than white Americans which gives a unique opportunity to study in large groups the consequences of smoking either menthol or regular cigarettes. It is also a good basis for evaluation of the potential effects of menthol cigarette smoking on human health.

Many studies have investigated the impact of menthol but no clear conclusions have been made. This suggests that menthol as a single parameter is not responsible for the higher incidence of lung cancer observed in black Americans who smoke, and that this phenomenon must be ascribed to another factor than menthol. No data consistently supports the hypothesis that menthol increases the risk of developing smoking related diseases. The risk is high from smoking in general.

Menthol does seem to affect dependence in the sense that more menthol smokers are "wake-up" smokers that need to smoke within an hour after waking up but this is one of the only indicators of increased dependence of menthol cigarettes and data are inconclusive.

From the data presented in this report DHI evaluates that menthol does not significantly and consistently increase dependence on nicotine or the risk of developing smoking related diseases.

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# Sundhedsmæssig vurdering af 5 tilsætningsstoffer til tobak

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| Projekt | Projekt nr. |
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| Forfattere<br>Helle Westphal<br>Susanne Knoth Clausen<br>Frank Leck Fotel | Dato<br>September 2009 |
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| Nøgleord | Klassifikation |
|---|---|
| Toxicology of 5 additives for tobacco, acetaldehyde, acetophenone, benzylalcohol, cis-3-hexenol, menthol | ☐ Åben<br>☐ Intern<br>☒ Tilhører klienten |

| Distribution | Antal kopier |
|---|---|
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