Health Council of the Netherlands

3-Methylcholanthrene

Evaluation of the effects on reproduction, recommendation for classification

2014/09

Health Council of the Netherlands

3-Methylcholanthrene

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *3-Methylcholanthrene*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-8081/HS/cn/543-L14
Bijlagen : 1
Datum : 3 april 2014

Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van 3-methylcholantreen op de vruchtbaarheid en het nageslacht; het betreft ook effecten op de lactatie en via de moedermelk op de zuigeling.

Dit advies maakt deel uit van een uitgebreide reeks waarin voor de voortplanting giftige stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld.

Dit advies is opgesteld door een vaste commissie van de Gezondheidsraad, de Subcommissie Classificatie reproductietoxische stoffen. Het is vervolgens getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

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

Met vriendelijke groet,

prof. dr. W.A. van Gool,
voorzitter

Bezoekadres
Rijnstraat 50
2515 XP Den Haag
E-mail: h.stouten@gr.nl
Telefoon (070) 340 70 04

Postadres
Postbus 16052
2500 BB Den Haag
www.gr.nl

3-Methylcholanthrene

Evaluation of the effects on reproduction,
recommendation for classification

Subcommittee on the Classification of Reproduction Toxic Substances
A Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2014/09, The Hague, April 3, 2014

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

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

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

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

This report can be downloaded from www.healthcouncil.nl.

Preferred citation:
Health Council of the Netherlands. 3-Methylcholanthrene - Evaluation of the effects on reproduction, recommendation for classification. The Hague: Health Council of the Netherlands, 2014; publication no. 2014/09.

ISBN: 978-90-5549-993-9

Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad 3-methylcholantreen onder de loep genomen. 3-Methylcholantreen wordt gebruikt in biochemisch onderzoek om iso-enzymen van cytochroom P450 te induceren. Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de Minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Subcommissie Classificatie reproductietoxische stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen van de raad, hierna aangeduid als de commissie, kijkt zowel naar effecten op de vruchtbaarheid van mannen en vrouwen als naar effecten op de ontwikkeling van het nageslacht. Daarnaast worden effecten op de lactatie en via de moedermelk op de zuigeling beoordeeld.

Op basis van Verordening (EG) 1272/2008 van de Europese Unie doet de commissie een voorstel voor classificatie. De commissie komt tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie om 3-methylcholantreen te classificeren in categorie 2 (*stoffen die ervan verdacht worden dat zij toxisch zijn voor de menselijke voortplanting*) en te kenmerken met H361f (*wordt ervan verdacht de vruchtbaarheid te schaden*)
- voor effecten op de ontwikkeling adviseert de commissie 3-methylcholantreen te classificeren in categorie 1B (*stoffen waarvan verondersteld wordt*

dat zij toxisch zijn voor de menselijke voortplanting) en te kenmerken met H360D (*kan het ongeboren kind te schaden*)

- voor effecten op of via lactatie, adviseert de commissie om 3-methylcholantreen te kenmerken met H362 (*kan schadelijk zijn via de borstvoeding*).

Executive summary

In the present report the Health Council of the Netherlands reviewed 3-methylcholanthrene. 3-Methylcholanthrene is used in biochemical research to induce iso-forms of cytochrome P450. This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be occupationally exposed. The Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee, evaluates the effects on male and female fertility and on the development of the progeny. Moreover, the Committee considers the effects of a substance on lactation and on the progeny via lactation.

The Committee recommends classification according to Regulation (EC) 1272/2008 of the European Union. For 3-methylcholantrene, these recommendations are:

- for effects on fertility, the Committee recommends classifying 3-methylcholanthrene in category 2 (*suspected human reproductive toxicant*) and labelling with H361f (*suspected of damaging fertility*)
- for effects on development, the Committee recommends classifying 3-methylcholanthrene in category 1B (*presumed human reproductive toxicant*) and labelling with H360D (*suspected of damaging the unborn child*)

- for effects on or via lactation, the Committee recommends labelling 3-methylcholanthrene with H362 (*may cause harm to breast-fed children*).

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. This classification is performed by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety (DECOS). The classification is performed according to European Union Regulation (EC) 1272/ 2008 on classification, labelling and packaging (CLP) of substances and mixtures. The CLP guideline is based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The Subcommittee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as reproductive toxicant (category 1A and B and 2) or compound with effects on or via lactation.

1.2 Committee and procedure

This document contains the classification of 3-methylcholanthrene by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances, hereafter called the Committee. The members of the Committee are

listed in Annex A. The submission letter (in English) to the State Secretary can be found in Annex B.

In 2013, the President of the Health Council released a draft of the report for public review. The individuals and organizations that commented on the draft report are listed in Annex C. The Committee has taken these comments into account in deciding on the final version of the report.

The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development as well as lactation of the above mentioned compound.

Classification for reproduction (fertility (F) and development (D)):	
Category 1	Known or presumed human reproductive toxicant (H360(F/D))
Category 1A	Known human reproductive toxicant
Category 1B	Presumed human reproductive toxicant
Category 2	Suspected human reproductive toxicant (H361(f/d))
No classification for effects on fertility or development	
Classification for lactation:	
	Effects on or via lactation (H362)
	No labelling for lactation

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC) 1272/2008) presented in Annex D. The classification of compounds is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the Committee has agreed upon a number of additional considerations (see Annex E).

1.3 Effects on or via lactation

The recommendation for classifying substances for effects on or via lactation is also based on Regulation (EC) 1272/2008. The guideline defines that substances which are absorbed by women and have been shown to interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely

independent of dosage), the labelling for effects on or via lactation is based on risk characterization and therefore, it also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects on or via lactation when it is likely that the substance would be present in breast milk at potentially toxic levels. The Committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration leads to exceeding the exposure limit for the general population, e.g. the acceptable daily intake (ADI).

1.4 Data

Literature searches were conducted in the on-line databases XTOXLINE, MEDLINE and CAPLUS, up to February 2010 without a starting date; the final update was performed in TOXNET in October 2013. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided into literature cited and literature consulted but not cited.

The Committee describes both the human and animal studies in the text. The animal data are described in more detail in Annex F as well. Of each study, the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

1.5 Presentation of conclusions

The classification is given with key effects, species and references specified. In case a substance is not classified as toxic to reproduction, one of two reasons is given:

- lack of appropriate data precludes assessment of the compound for reproductive toxicity
- sufficient data show that no classification for toxic to reproduction is indicated.

1.6 Final remark

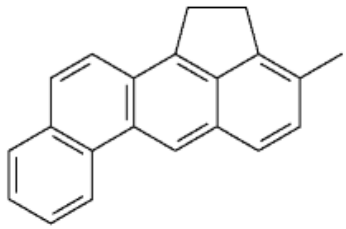
The classification of compounds is based on hazard evaluation only (Niesink et al., 1995)[16], which is one of a series of elements guiding the risk evaluation

process. The Committee emphasizes that for derivation of health-based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organizations.

Chapter 2

3-Methylcholanthrene

2.1 Introduction

name	:	3-methylcholanthrene
IUPAC name (old)	:	3-methylcholanthrene
CAS name	:	benz[j]aceanthrylene, 1,2-dihydro-3-methyl-
CAS registry number	:	56-49-5
EC/EINECS number	:	200-276-4
synonyms	:	20-methylcholanthrene; 1,2-dihydro-3-methyl-benz[j]aceanthrylene
colour and physical state	:	pale yellow, slender prisms
molecular weight	:	268.4
molecular formula	:	$C_{21}H_{16}$
structure	:	
melting point	:	179-180 °C
boiling point	:	280 °C at 10.7 kPa
relative density	:	1.28 (at 20 °C)
vapour pressure	:	$6x10^{-6}$ Pa (at 25 °C)
Log $P_{octanol\text{-}water}$	:	6.42 (experimental value)

solubility	:	insoluble in water; soluble in benzene, xylene, toluene; slightly soluble in amyl alcohol
use	:	experimentally in cancer research; in research to induce specific forms of cytochrome P450
general toxicity	:	3-Methylcholanthrene was found carcinogenic in mice and rats and mutagenic in bacteria, clastogenic in bone marrow *in vitro*, and induced DNA adducts in animals[14]; however, it is not classified as a carcinogen by IARC and it is not included in Annex VI of EC Regulation 1272/2008 nor is there any classification worldwide known to the Committee.
mechanism	:	3-Methylcholanthrene is an inert molecule by itself but metabolism of 3-methylcholanthrene by CYP enzymes leads to formation of chemically reactive intermediates that are capable of binding covalently to DNA. 3-Methylcholanthrene induces CYP1A1/1A2 by interacting with the arylhydrocarbon (Ah) receptor in the cytosol. The inducer-receptor complex is translocated into the nucleus where it binds to the Ah receptor nuclear translocator and this complex in turn interacts with specific regulatory elements, termed Ah receptor elements, on CYP1A1/1A2 and phase II enzyme genes, resulting in enhanced expression of the CYP1A1, CYP1A2 and phase II enzymes encoded by the *Ah* locus.[11-13] The *Ah* locus controls inducibility by 3-methylcholanthrene and numerous other polycyclic aromatic hydrocarbons, of at least 20 monooxygenase activities, including aryl hydrocarbon hydroxylase activity. There are genetic differences at this *Ah* locus, the inducible, responsive Ah^b genotype and the non-inducible, non-responsive Ah^d genotype, that play a role in the expression of e.g. reproduction toxic effects, including transplacental carcinogenesis. Polycyclic aromatic hydrocarbons such as 3-methylcholanthrene may be rapidly metabolized by inducible animals to reactive intermediates at the site of application, such that tissues distant from the site of application receive relatively low levels of exposure, so called presystemic drug elimination. If these hydrocarbons are administered systemically, however, non-inducible animals show increased susceptibility in tissues distant from the site of application.[4,25,26] The Ah receptor is ubiquitously expressed but the levels of Ah receptor mRNA and protein vary between tissues. Highest mRNA expression is observed in lung, liver, kidney, placenta and thymus, while lower expression is observed in the heart.[12]
kinetics	:	No data were found on the amount of oral or dermal absorption of 3-methylcholanthrene; 3-methylcholanthrene is metabolized in the liver, secreted in the bile and excreted in the faeces and reabsorbed from the intestine; up to 0.3% of 3-methylcholanthrene is transferred to the foetus after intravenous injection into maternal mice[21]; Shay et al. reported an amount of 0.1 µg/g 3-methylcholanthrene in the liver of pregnant Wistar rats given 200 mg/kg bw by gavage on gestational day 21 and killed 3 hours thereafter; the placenta contained 0.009 µg/g and the foetus 0.0013 µg/g.[18]

Data from[14,17], unless otherwise noted

2.2 Human studies

Fertility studies

No data were available regarding the effects of exposure to 3-methylcholanthrene on human fertility.

Developmental toxicity studies

No data were available regarding the effects of exposure to 3-methylcholanthrene on human developmental toxicity.

Lactation

No data were available regarding the excretion of 3-methylcholanthrene or the effects of exposure to 3-methylcholanthrene on human lactation or on infants during lactation.

2.3 Animal studies

Fertility and developmental toxicity studies with 3-methylcholanthrene in laboratory animals are summarized in Annex F.

Fertility studies

Male Sprague-Dawley rats were injected intraperitoneally with doses of 3-methylcholanthrene of 0 or 20 mg/kg bw/day in vegetable oil five days/week for four weeks. Treated rats had statistically significantly reduced seminal vesicle weights and body weights. Apart from peritonitis, gross pathological examination revealed no other abnormalities. The decrease was probably related to an impairment of testosterone formation in the gonads, since testosterone biosynthesis as well as the concentrations of cytochrome P450 and 17-α-hydroxylase activities in testicular microsomes were statistically significantly decreased. No histological changes were seen in testes.[20]

Adult and prepubertal (20-22 days old) Wistar rats were treated with 0 or 25 mg 3-methylcholanthrene/kg bw in olive oil intraperitoneally twice a week for one month. Effects on female and male reproductive status, offspring number and condition as well as sex steroid hormone and oestrogen receptor levels were examined.

No signs of general toxicity were noted. 3-Methylcholanthrene induced decreases in plasma testosterone concentrations ($p<0.05$) and testis weights ($p<0.005$) in adult but not in young male rats. Preputial glands were not affected. In adult females, the frequency of abnormal cycles was increased ($p<0.001$). Young female rats treated with 3-methylcholanthrene during their prepubertal period had more frequently abnormal cycles ($p<0.01$), a higher incidence of constant di-oestrus ($p<0.05$) and longer cycles of 6.8 days compared to 4.9 days for the controls ($p<0.01$). No effects were observed on plasma oestradiol and progesterone concentrations or uterine oestrogen receptor levels or on uterus and ovary weights.[10]

Borman et al. treated 28-days-old female Fischer 344 rats and B6C3F1 mice (n=6/species/group) with intraperitoneal injections of 3-methylcholanthrene in sesame oil (rats: 0.0015 to 60 mg/kg bw/day; mice: 0.0015 to 0.75 mg/kg bw/day) or with vehicle. After 15 days of treatment, ovaries were collected, histologically prepared and follicles were microscopically classified (primordial, primary or secondary) and counted.

In rats, body weights were decreased at doses ≥15 mg/kg bw, while no effects were noted on relative liver weights. No differences were observed in ovary size between treated and control rats. There was no effect on the numbers of primordial and primary follicles in the treated rats, but at doses ≥0.075 mg/kg bw the numbers of secondary follicles were approximately 50-60% of those in controls ($p<0.05$; no dose response).

In mice, treatment did not affect body or relative liver weights or ovary sizes. In treated mice, there was no effect on the numbers of secondary follicles but statistically significant decreases were observed in the numbers of primordial (by 60-75%) and primary follicles (by 40-50%) at doses ≥0.075 mg/kg bw.

Borman et al. referred to an earlier publication in which it was reported that a single intraperitoneal dose of 80 mg 3-methylcholanthrene/kg bw showed a 50% primordial follicle loss within two days in mice, suggesting that repeated exposure may result in a substantially higher toxicity to the ovary.[2]

Developmental toxicity studies

York et al. (1984) mated non-inducible females to inducible males and exposed the dams (n=15-34) to daily oral (gavage) doses of 3-methylcholanthrene of 0, 7, 21 or 63 mg/kg bw on gestational days 15, 16 and 17. Litter size was determined at postnatal days 1, 4, 7, 14 and 21. Twelve randomly selected offspring were killed at postnatal weeks 15 and 20 to determine the incidence of neoplastic lesions and the remaining offspring at 26 weeks of age for gross and histological evaluation and phenotype determination. The incidence of tumours and mean number per pup were analysed. Maternal weight gain was not affected.

There were 1, 1, 4 and 5 non-viable litters at 0, 7, 21 or 63 mg/kg bw/day, respectively. At postnatal day 4 and 21, the numbers of pups per litter were decreased being statistically significantly different from those in controls at 21 and 63 mg/kg bw/day. After weaning (postnatal day 21), survival rates did not change. Mean pup weight per litter was statistically significantly reduced at 63 mg/kg bw/day on postnatal day 7, 14 and 21. The number of inducible offspring was lower in the high-dose group and in the total 3-methylcholanthrene-treated group compared to the number of non-inducible offspring ($p<0.005$). When

analysed by *Ah* genotype, post-weaning pup weights were reduced across all treated inducible offspring groups ($p<0.005$); in the medium-dose group at 16 and 26 weeks and in the high-dose group at 13, 16, 20 and 26 weeks of age. No influence on sex ratio was noted. The pre-weaning toxicity may be due to transplacental and/or lactational exposure.[25,26]

Postmortem examinations at 26 weeks showed that the lung was the major site of neoplastic lesions. The percentages of nodule-bearing (i.e. hyperplasia and adenomas) and adenoma-bearing animals, the mean numbers of nodules per animal and the mean nodular sizes per animal were dose-relatedly statistically significantly increased across all treated groups, all responses being statistically significantly greater at the high dose than at the low dose. Of the animals killed ad interim, no data on neoplasms were presented.[26]

One group of 35 pregnant CF-1 mice was given three doses of 2.8 mg 3-methylcholanthrene within 24 hours by gavage and litters were left with the dams until weaning. Another group of 44 pregnant mice was treated similarly. Their litters were transferred immediately after birth to untreated dams and vice versa. An additional untreated control group consisting of non-pregnant females (n=44) and males (n=49) was included. Animals were observed up to 100 weeks. Dams showed a reduced survival rate and increased tumour incidence. The offspring from treated dams survived much shorter than the untreated control and untreated offspring foster-nursed by 3-methylcholanthrene-treated dams. All pregnant females treated with 3-methylcholanthrene and their offspring had one or more tumours. Tumours of the lung and malignant lymphomas were the most common in all groups.[22]

Pregnant female Wistar rats were given intraperitoneal injections of 3-methylcholanthrene (in corn oil) of 0 or 10 mg/kg bw/day for three days starting on gestational day 7. Rats were sacrificed on gestational day 20, foetuses were removed by Caesarean section and the number of sites of implantations and resorptions were recorded. Dams did not show signs of toxicity. The resorption rate was statistically significantly increased (60% vs. 6% in controls), and the mean percentage of dams with live foetuses (61% vs. 84%), the mean percentage of live foetuses (36% vs. 74%) and mean foetal weights (2.2±0.3 g vs. 2.8±0.6 g) were statistically significantly decreased. No malformations were noted.[9]

Shiverick et al. (1984) injected daily doses of 0 and 30 mg/kg bw 3-methylcholanthrene (in corn oil) intraperitoneally into pregnant Holzmann rats on gestational day 12-14 and animals were sacrificed on gestational day 15 or 20.

In the group killed on gestational day 15, no statistically significant effects were seen on maternal body weight gains, foetal resorption rates and foetal body weights. The group killed on gestational day 20 showed decreases in maternal weight gain, placental weight and foetal weight (all $p<0.05$).[19]

Offspring from Wistar rats treated with 25 mg/kg bw twice a week for one month in the fertility study of Konstandi did have a significantly lower oral body temperature at ten days of age ($p<0.05$; 30.33 vs. 30.83°C). Litter size and number of litters as well as pup weights and sex distribution were not affected. Konstandi et al. mentioned that no stillborn offspring or malformations were noted. Negative geotaxis, righting reflex and the cliff test showed no effect.[10]

In a study dealing with the effects of pretreatment with 3-methylcholanthrene on ethylenethiourea-induced developmental toxicity in female Swiss-Webster mice, groups receiving corn oil (n=9) and only 3-methylcholanthrene (n=7), respectively, were included. The mice were injected intraperitoneally with doses of 3-methylcholanthrene of 20 mg/kg bw/day on gestational day 9, 10 and 11. On gestational day 19, dams were killed for necropsy and foetal examination.

No data on maternal toxicity in the 3-methylcholanthrene-treated dams were presented. Uterine and foetal examinations did not reveal effects. No effects were observed on the percent resorptions, the number of dead foetuses and on the sex ratio. The mean number of live foetuses per dam (10.5 vs. 13.1 in corn-oil controls) and mean foetal weights (1.0 g vs. 1.3 g) were decreased. There were no increases in the incidences of cleft palates or paw deformities.[9]

Nebert et al. (1977) intraperitoneally injected inducible C57BL/6N mice and non-inducible AKR/N mice with doses of 3-methylcholanthrene of 0 or 70 mg/kg bw on gestational days 5, 7, 10 or 12. The number of litters amounted to 2-16 per group per gestational-day group. After injection on gestational day 5, 7, 10 and 12, the rates of prenatal mortality (i.e. the number of resorptions + stillborns/total number of implantations) were 13/13 (100%), 29/41 (71%), 5/32 (16%) and 9/32 (28%), respectively, in C57BL/6N mice (controls: 8/116 (7%)) and 22/104 (21%), 2/16 (13%), 4/49 (8%) and 12/49 (24%), respectively, in AKR/N mice (controls: 14/134 (10%)). Further, treatment at gestational day 7, 10 and 12 caused increases in the number of malformations (primarily club foot) in C57BL/6N mice while none were found in AKR/N mice and controls. No effects were seen on foetal weights or crown-rump lengths.[15]

Anderson et al. (1985) treated inducible pregnant (C57BL/6xDBA/2)F_1 and non-indu-cible pregnant DBA/2 mice with intraperitoneal doses of 3-methylcholanthrene of 5, 30, 60, 100, 135 and 175 mg/kg bw and 5 and 30 mg/kg bw, respectively, on gestational day 17. Control groups receiving vehicle (olive oil) alone were included. At about ten months of age, offspring were given 3-methylcholan-threne for phenotype determination, and sacrificed 48 hours later for complete necropsy. In (C57BL/6xDBA/2)F_1 progeny, no effects were observed at 5 mg/kg bw. At the other dose levels, the percentage of lung tumour-bearing animals ranged from 45 to 92% in inducible female progeny, 14-87% in non-inducible female progeny, 44-96% in inducible male progeny and 40-100% in non-inducible male progeny. In the control groups, 8% of the inducible female progeny had lung tumours and none of the animals in the other groups. In inducible female DBA/2 progeny, the percentages of lung tumour-bearing animals were 0 and 94%, respectively (controls: 11%), in non-inducible female progeny, 12 and 56%, respectively (controls: 4%), in inducible male progeny, 43 and 96%, respectively (controls: 0%) and in non-inducible male progeny, 21 and 75%, respectively (controls: 0%). Liver tumours were only found in male progeny. In the (C57BL/6xDBA/2)F_1 progeny, the percentages of liver tumour-bearing inducible animals ranged from 8 to 21% in the five higher dose groups while no tumours were seen at 5 mg/kg bw and in the control animals. In the non-inducible progeny, no liver tumours were observed except for the 100 mg/kg bw group (4%). In the DBA/2 progeny, the percentages of liver tumour-bearing inducible males were 7 and 42%, respectively (controls: 11%) and of liver tumour-bearing non-inducible males 0 and 33%, respectively (controls; 4%).[1]

Wessner et al. (1996) treated pregnant (DBA/2x(C57BL/6xDBA/2)) mice with intraperitoneal doses of 3-methylcholanthrene (in olive oil) of 0, 10 or 30 mg/kg bw on gestational day 17. Offspring born was immediately foster-nursed by untreated mothers and sacrificed after one year. Treatment caused increases in the percentages of lung tumour-bearing inducible male progeny of 0, 6 and 81%, respectively, of lung tumour- bearing non-inducible male progeny of 0, 0 and 35%, respectively, of lung tumour-bearing inducible female progeny of 0, 18 and 87%, respectively and of lung tumour-bearing non-inducible female progeny of 0, 0 and 50%, respectively (controls: 0%). In addition, there were increases in the numbers of liver tumour-bearing animals: 7, 0 and 63%, respectively, in inducible males; 8, 0 and 35%, respectively, in non-inducible males; 0, 0 and 7%, respectively, in inducible females; 0, 0 and 6%, respectively, in non-inducible females.[23]

Gressani et al. (1999) treated pregnant BALB/c mice (an 'intermediately' inducible strain) with single intraperitoneal injections of 0, 5, 15 or 45 mg/kg bw on gestational day 17. Mice were killed at six months of age. Lung tumours were enumerated and histologically classified. It was noted that no effect was seen on maternal weight gain, length of pregnancy, birth weight, foetal mortality or postnatal weight gain. The percentages of lung tumours were 0, 0, 20 and 100%, respectively; the number of tumours/mouse increased with dose.[5]

Jennings-Gee et al. (2006) examined the effects of in utero treatment with 3-methyl-cholanthrene (intraperitoneal doses of 45 mg/kw bw on gestational day 17) on the lung tumour incidence in the progeny of BALB/c and C57BL/6 mice and of two reciprocal crosses between these strains. Offspring was housed without further treatment for 12-18 months, depending on tumour latency. The numbers of animals with lung tumours (86, 100%, respectively) as well as tumour multiplicity (ca. 3 and 5-6 tumours/mouse, respectively) were high for the BALB/c and back crosses progeny compared to the C57BL/6 progeny (11% and <0.1, respectively).[6]

In a study of the effects of pretreatment with 3-methylcholanthrene on ethylenethiourea-induced developmental toxicity in groups of pregnant Syrian golden hamsters, receiving corn oil (n=7) or daily intraperitoneal doses of 3-methylcholanthrene of 20 mg/kg bw at gestational days 9, 10 and 11 (n=9/group), respectively, there were no effects on the numbers of resorptions, dead foetuses, live foetuses and runted foetuses, on the sex ratio and on mean foetal weights. No external or visceral anomalies were observed and for skeletal anomalies, the incidence of delayed ossification of the calvarium was comparable to that in controls (4/48 vs. 3/37). No data on maternal toxicity in the 3-methylcholanthrene-treated dams were presented.[7,8]

Naruse et al. (2002) investigated specifically the effect of 3-methylcholanthrene on the formation of foetal bone after subcutaneous injection of 1 mg/kg bw in mineral oil to pregnant BALB/c mice at gestational days 10, 12 and 14. Foetuses were removed on gestational days 15 and 17 and divided into two groups for skeletal and histological examination. Foetal weight was not affected. In the treated group, abnormalities in the first cervical and lumbar vertebrae and delays in ossification of the cervical and thoracic vertebrae and limbs were seen in 3/29 and 9/29 foetuses, respectively. Metacarpals of the treated group showed no subperiosteal bone matrix histologically. Maternal toxicity was not reported.[13]

Lactation

In a limited report of a preliminary study, two groups of female Wistar rats were given 2 mg 3-methylcholanthrene in olive oil by gavage six days/week for 28 days starting 18-24 hours after the litter was born and ending when the litter was weaned. In the first group, 2/22 males and 0/50 females developed reticulum-cell sarcoma in the mesenteric lymph nodes at 10 and 13 months of age and in the second group 1/21 males and 0/22 females developed lymphatic leukaemia at 86 days of age. 3-Methylcholanthrene was present in the milk in the stomach of pups.[18]

Because of deficiencies in the study design, the Committee cannot draw conclusions from this study.

Following single oral (gavage) administration of 10 mg 3-methylcholanthrene to lactating rats on postnatal day 7, amounts of 13 and 23 µg/g milk curd, respectively, were found in milk curd obtained 24 hours later from the stomach of two of their pups.[3]

West and Horton (1976) reported that after a single dose of 5 µCi (=235 µg) of 3-methylcholanthrene-6-^{14}C given orally in a pellet or dissolved in corn oil to lactating rats (n=2/group), 0.06 and 0.19% of total radioactivity, respectively, was calculated to be taken up by the total number in the litter through their mothers' milk within four hours. In rabbits given the same amount of ^{14}C-labelled compound in a pellet, 0.003% of the radioactivity administered was recovered from the milk within five days.[24]

2.4 Conclusion

Fertility

No human studies on fertility effects of 3-methylcholanthrene were available.

Repeated intraperitoneal injections of 3-methylcholanthrene to male rats caused impaired testosterone production and decreased seminal vesicle weights in the absence of histological testicular changes[19] or decreased plasma testosterone levels and testis weights[10]. Similar treatment affected oestrus cycle pattern[10] and decreased the number of secondary follicles in female rats[2] and decreased numbers of primordial and primary follicles in female mice[2].

Overall, studies in laboratory animals indicated that intraperitoneal injection of 3-methylcholanthrene may affect the testes in rats and folliculogenesis in rats

and mice. Animal studies on functional fertility or using routes more relevant to occupational exposure than intraperitoneal injection are lacking. Therefore, the Committee proposes to classify 3-methylcholanthrene for effects on fertility in category 2.

Developmental toxicity

No human studies on developmental toxicity effects of 3-methylcholanthrene were available.

In mice, repeated oral administration of 3-methylcholanthrene increased prenatal and pre-weaning mortality and numbers of progeny with neoplasms[25,26], while single administration resulted in decreased postnatal survival rates and increased numbers of tumour-bearing animals[22]. Following single or repeated intraperitoneal injections, increased prenatal mortality and decreased mean pup weights were seen in rats[10,19] and increased prenatal mortality, decreased foetal weights and increased tumour rates in progeny in mice[1,5,6,9,15,23].

Overall, oral studies in laboratory animals showed prenatal and postnatal effects, among which transplacental carcinogenicity in mice. In addition, similar findings were reported in intraperitoneal studies in rats and mice. Therefore, the Committee proposes to classify 3-methylcholanthrene for effects on development in category 1B.

Lactation

The Committee notes that 3-methylcholanthrene was found to have carcinogenic and genotoxic properties in laboratory animals (see Section 2.1).

Studies[3,18,24] suggested that oral administration of 3-methylcholanthrene to lactating rats may result in transfer into the breast milk of the treated rats. However, these studies showed deficiencies in design. Although the amounts excreted may have been relatively small, the Committee is of the opinion that the presence of genotoxic carcinogens, such as 3-methylcholanthrene, in breast milk should be avoided. Therefore, the Committee proposes to label 3-methylcholanthrene for effects on or via lactation.

Proposed classification for fertility

Category 2, H361f.

Proposed classification for developmental toxicity

Category 1B, H360D.

Proposed labelling for effects on or via lactation

H362.

References

1 Anderson LM, Jones AB, Riggs CW, Ohshima M. Fetal mouse susceptibility to transplacental lung and liver carcinogenesis by 3-methylcholanthrene: positive correlation with responsiveness to inducers of aromatic hydrocarbon metabolism. Carcinogenesis. 1985;6:1389-93.

2 Borman SM, Christian PJ, Sipes IG, Hoyer PB. Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. Toxicol Appl Pharmacol. 2000;167:191-8.

3 Dao TL, Bock FG, Crouch S. Level of 3-methylcholanthrene in mammary glands of rats after intragastric instillation of carcinogen. Proc Soc Exp Biol Med. 1959;102:635-8.

4 George JD, Manson JM. Strain-dependent differences in the metabolism of 3-methylcholanthrene by maternal, placental, and fetal tissues of C57BL/6J and DBA/2J mice. Cancer Res. 1986;46:5671-5.

5 Gressani KM, Leone-Kabler S, O'Sullivan MG, Case LD, Malkinson AM, Miller MS. Strain-dependent lung tumor formation in mice transplacentally exposed to 3-methylcholanthrene and post-natally exposed to butylated hydroxytoluene. Carcinogenesis. 1999;20:2159-65.

6 Jennings-Gee JE, Moore JE, Xu M, Dance ST, Kock ND, McCoy TP, et al. Strain-specific induction of murine lung tumors following in utero exposure to 3-methylcholanthrene. Mol Carcinog. 2006;45:676-84.

7 Khera KS. Ethylenethiourea-induced hindpaw deformities in mice and effects of metabolic modifiers on their occurrence. J Toxicol Environ Health. 1984;13:747-56.

8 Khera KS, Whalen C, Iverson F. Effects of pretreatment with SKF-525A, N-methyl-2-thioimidazole, sodium phenobarbital, or 3-methylcholanthrene on ethylenethiourea-induced teratogenicity in hamsters. J Toxicol Environ Health. 1983;11:287-300.

9 Khlood EB, Miyoshi H, Iwata H, Kazusaka A, Kon Y, Hadid AH, et al. Effects of concurrent exposure to 3-methylcholanthrene and vitamin A on fetal development in rats. Jpn J Vet Res. 1999;47:13-23.

10 Konstandi M, Pappas P, Johnson E, Lecklin A, Karageorgou M, Marselos M. Modification of reproductive function in the rat by 3-methylcholanthrene. Pharmacol Res. 1997;35:107-11.

11 Moorthy B. Persistent expression of 3-methylcholanthrene-inducible cytochromes P4501A in rat hepatic and extrahepatic tissues. J Pharmacol Exp Ther. 2000;294:313-22.

12 Mullen Grey AK, Riddick DS. Glucocorticoid and adrenalectomy effects on the rat aryl hydrocarbon receptor pathway depend on the dosing regimen and post-surgical time. Chem Biol Interact. 2009;182:148-58.

13 Naruse M, Ishihara Y, Miyagawa-Tomita S, Koyama A, Hagiwara H. 3-Methylcholanthrene, which binds to the arylhydrocarbon receptor, inhibits proliferation and differentiation of osteoblasts in vitro and ossification in vivo. Endocrinology. 2002;143:3575-81.

14 National Library of Medicine (NLM), editor. 3-Methylcholanthrene. In: Hazardous Substances Data Bank (HSDB) [Internet] [cited 2013 April]. Avaible from: http://toxnet.nlm.nih.gov.

15 Nebert DW, Levitt RC, Jensen NM, Lambert GH, Felton JS. Birth defects and aplastic anemia: differences in polycyclic hydrocarbon toxicity associated with the Ah locus. Arch Toxicol. 1977;39:109-32.

16 Niessink R, de Vries J, Hoolinger M. Toxicology principles and applications. Boca Raton, FL, USA: CRC Press; 1995.

17 O'Neil MJ, editor. 3-Methylcholanthrene. In: The Merck Index; an encyclopedia of chemicals, drugs, and biologicals. Whitehouse Station NJ, USA: Merck & Co., Inc.; 2006; p. 1043.

18 Shay H, Weinhouse S, Gruenstein M, Marx HE, Friedman B. Development of malignant lymphoma in some of the young rats suckled by mothers receiving methylcholanthrene by stomach tube only during the lactation period. Cancer. 1950;3:891-5.

19 Shiverick KT, Ortiz E, Fuhrman-Lane C, Crowley P. Effects of beta-naphthoflavone and 3-methylcholanthrene administered to pregnant rats on steroid metabolism in the isolated basal zone of the placenta. J Pharmacol Exp Ther. 1984;230:761-7.

20 Stripp B, Menard RH, Gillette JR. Effect of chronic treatment with phenobarbital or 3-methylcholanthrene on the male reproductive system in rats. Life Sci. 1974;14:2121-30.

21 Takahashi G, Yasuhira K. Macroautoradiographic and radiometric studies on the distribution of 3-methylcholanthrene in mice and their fetuses. Cancer Res. 1973;33:23-8.

22 Tomatis L, Turusov V, Guibbert D, Duperray B, Malaveille C, Pacheco H. Transplacental carcinogenic effect of 3-methylcholanthrene in mice and its quantitation in fetal tissues. J Natl Cancer Inst. 1971;47:645-51.

23 Wessner LL, Fan M, Schaeffer DO, McEntee MF, Miller MS. Mouse lung tumors exhibit specific Ki-ras mutations folliowng transplacental exposure to 3-methylcholanthrene. Carcinogenesis. 1996;17:1519-26.

24 West CE, Horton BJ. Transfer of polycyclic hydrocarbons from diet to milk in rats, rabbits and sheep. Life Sci. 1976;19:1543-51.

25 York RG, Manson JM. Neonatal toxicity in mice associated with the Ahb allele following transplacental exposure to 3-methylcholanthrene. Toxicol Appl Pharmacol. 1984;72:417-26.

26 York RG, Stemmer K, Manson JM. Lung tumorigenesis and hyperplasia in offspring associated with the Ahd allele following in utero exposure to 3-methylcholanthrene. Toxicol Appl Pharmacol. 1984;72:427-39.

Literature consulted but not cited

- Anderson LM, Jones AB, Riggs CW, Kovatch RM. Modification of transplacental tumorigenesis by 3-methylcholanthrene in mice by genotype at the Ah locus and pretreatment with beta-naphthoflavone. Cancer Res. 1989;49:1676-81.
- Anger DL, Crankshaw DJ, Foster WG. Spontaneous appearance of uterine tumours in vehicle and 3-methylcholanthrene-treated Wistar rats. Reprod Toxicol. 2006;22:760-4.
- Batra BK. A study of reproduction & ovarian histology in mice treated with a chemical carcinogen. Ind J Exp Biol. 1966;4:139-43.
- Donovan PJ, Smith GT, Nardone R. The mutagenic effects of 7,12-dimethylbenz[a]anthacene, 3-methylcholanthrene and benzo[a]pyrene to the developing Syrian hamster fetus measured by an in vivo/in vitro mutation assay. Mutat Res. 2004;554:111-20.
- Gressani KM, Rollins LA, Leone-Kabler S, Cline JM, Miller MS. Induction of mutations in Ki-ras and INK4a in liver tumors of mice exposed in utero to 3-methylcholanthrene. Carcinogenesis. 1998;19:1045-52.
- Guibert D, Duperray B, Pacheco H, Tomatis L, Turusov V. Etude biochimique du passage transplacentaire d'un agent cancérigène le 3-méthylcholantrêne, chez la souris. Therapie. 1971;26:1027-38.
- Jackson D, Robson JM. The effect of methylcholanthrene on growth and pregnancy. Br J Exp Pathol. 1958;39:133-8.
- Johri A, Yadav S, Dhawan A, Parmar D. Responsiveness of cerebral and hepatic cytochrome P450s in rat offspring prenatally exposed to lindane. Toxicol Appl Pharmacol. 2008;231:10-6.
- Juchau MR, Giachelli CM, Fantel AG, Greenaway JC, Shepard TH, Faustman-Watts EM. Effects of 3-methylcholanthrene and phenobarbital on the capacity of embryos to bioactivate teratogens during organogenesis. Toxicol Appl Pharmacol. 1985;80:137-46.
- Kazmin SD, Todor IN, Chekhun VE. Antitumor resistance formation in animals in early neonatal period. J Exp Clin Cancer Res. 2007;26:83-90.
- Miller MS, Jones AB, Chauhan DP, Park SS, Anderson LM. Induction of cytochrome P-450IA1 in fetal rat liver by a single dose of 3-methylcholanthrene. Biochem Biophys Res Commun. 1991;176:280-7.

- Miller MS, Leone-Kabler S, Rollins LA, Wessner LL, Fan M, Schaeffer DO, et al. Molecular pathogenesis of transplacentally induced mouse lung tumors. Exp Lung Res. 1998;24:557-77.
- Miller MS, Gressani KM, Leone-Kabler S, Townsend AJ, Malkinson AM, O'Sullivan MG. Differential sensitivity to lung tumorigenesis following transplacental exposure of mice to polycyclic hydrocarbons, heterocyclic amines, and lung tumor promoters. Exp Lung Res. 2000;26:709-30.
- Mizesko MC, Grewe C, Grabner A, Miller MS. Alterations at the Ink4a locus in transplacentally induced murine lung tumors. Cancer Lett. 2001;172:59-66.
- Nebert DW, Shum S, Boobis AR, Atlas SA. Developmental pharmacology and the Ah locus: regulatory and temporal genes affecting teratogenesis. Acta Biol Med Ger. 1979;38:519-29.
- Oato M, Schut H, Shiverick K. DNA modifications in rat placenta and maternal liver: effects of beta-naphthoflavone (BNF) and 3-methylcholanthrene (MC). FASEB J. 1990;4:A874.
- Rady P, Arany I, Uzvoelgyi E, Bojan F. Activity of pyruvate kinase and lactic acid dehydrogenase in mouse lung after transplacental exposure to carcinogenic and non-carcinogenic chemicals. Toxicol Lett. 1981;8:223-7.
- Savkur LD, Batra BK, Sridharan BN, Sanghvi LD. Effect of 20-methylcholanthrene on mouse embryos. J Reprod Fertil. 1962;3:422-4.
- Shay H, Gruenstein M, Weinberger M. Tumour incidence in F1 and F2 generations derived from female rats fed methylcholanthrene by stomach tube prior to conception. Cancer Res. 1952;12:296.
- Shendrikova IA, Aleksandrov VA. Comparative penetration of polycyclic hydrocarbons through the rat placenta into the fetus. Bull Exp Biol Med. 1974;77:169-71.
- Shiraiwa T, Kamiyama N, Kashiwayanagi M. Decreases in urinary pheromonal activities in male mice after exposure to 3-methylchoranthrene. Toxicol Lett. 2007;169:137-44.
- Shiverick KT, Ogilvie S, Medrano T. Polyaromatic compounds alter placental protein synthesis in pregnant rats. FASEB J. 1991;5:A1237.
- Strong LC, Hollander WF. Effects of methylcholanthrene in pregnant mice. J Natl Cancer Inst. 1947;8:79-82.
- Suchar LA, Chang RL, Thomas PE, Rosen RT, Lech J, Conney AH. Effects of phenobarbital, dexamethasone, and 3-methylcholanthrene administration on the metabolism of 17â-estradiol by liver microsomes from female rats. Endocrinology. 1996;137:663-76.
- Vore M, Soliven E. Hepatic estrone and estradiol glucuronyltransferase activity in pregnancy. Induction by pretreatment with 3-methylcholanthrene and phenobarbital. Drug Metab Dispos. 1979;7:247-51.
- Wang H, Huang M, Peng R, Le J. Influences of 3-methylcholanthrene, phenobarbital and dexamethasone on xenobiotic metabolizing-related cytochrome P450 enzymes and steroidogenesis in human fetal adrenal cortical cells. Acta Pharmacol Sin. 2006;27:1093-6.
- Wyrobek AJ, Bruce WR. Chemical induction of sperm abnormalities in mice. Proc Natl Acad Sci USA. 1975;72:4425-9.

- Xu M, Nelson GB, Moore JE, McCoy TP, Dai J, Manderville RA, et al. Induction of Cyp1a1 and Cyp1b1 and formation of DNA adducts in C57BL/6, Balb/c, and F1 mice following in utero exposure to 3-methylcholanthrene. Toxicol Appl Pharmacol. 2005;209:28-38.
- Xu M, Moore JE, Leone-Kabler S, McCoy TP, Swank A, Nelson GB, et al. Expression of glutathione S-transferases in fetal lung and liver tissue from parental strains and F1 crosses between C57BL/6 and BALB/c F1 mice following in utero exposure to 3-methylcholanthrene. Biochem Pharmacol

A	The Committee
B	The submission letter (in English)
C	Comments on the public draft
D	Regulation (EC) 1272/2008 of the European Community
E	Additional considerations to Regulation (EC) 1272/2008
F	Fertility and developmental toxicity studies

Annexes

Annex **A**

The Committee

- A.H. Piersma, *Chairman*
  Professor of Reproductive and Developmental Toxicology, Utrecht University, Utrecht and National Institute for Public Health and the Environment, Bilthoven
- D. Lindhout
  Professor of Medical Genetics, Paediatrician (not practising), Clinical Geneticist; University Medical Centre, Utrecht
- N. Roeleveld
  Reproductive Epidemiologist, Radboud university medical center, Nijmegen
- J.G. Theuns-van Vliet
  Reproductive Toxicologist, TNO Triskelion BV, Zeist
- D.H. Waalkens-Berendsen
  Reproductive Toxicologist, Zeist
- P.J.J.M. Weterings
  Toxicologist, Weterings Consultancy BV, Rosmalen
- A.S.A.M. van der Burght, *Scientific Secretary*
  Health Council of the Netherlands, Den Haag
- J.T.J. Stouten, *Scientific Secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present document was prepared by Dr. H.M. Barentsen, from the Regulatory Affairs Department of WIL Research Europe BV, Den Bosch, by contract with the Ministry of Social Affairs and Employment.

The Health Council and interests

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

Annex **B**

The submission letter (in English)

Subject : Submission of the advisory report *3-Methylcholanthrene*
Your reference : DGV/MBO/U-932542
Our reference : U-8081/HS/cn/543-L14
Enclosed : 1
Date : April 3, 2014

Dear Minister,

I hereby submit the advisory report on the effects of 3-methylcholanthrene on fertility and on the development of the progeny; it also concerns effects on lactation and on the progeny via lactation. This advisory report is part of an extensive series in which reproduction toxic substances are classified in accordance with European guidelines. This involves substances to which people may be exposed occupationally.

The advisory report was prepared by a permanent committee of the Health Council of the Netherlands, the Subcommittee on the Classification of Reproduction Toxic Substances. The advisory report was consequently reviewed by the Health Council's Standing Committee on Health and the Environment.

Today I sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their information.

Yours sincerely,
(signed)
Prof. dr. W.A. van Gool,
President

Annex C

Comments on the public draft

A draft of the present report was released in 2013 for public review. The following organisation and persons have commented on the draft document:

- T.J. Lentz, S. Reynolds, D. Murray, S. Rengasamy. National Institute for Occupational Safety and Health, Cincinnati OH, USA.

The received comments, and the reply by the Committee can be found on the website of the Health Council.

Annex **D**

Regulation (EC) 1272/2008 of the European Community

3.7 Reproductive toxicity

3.7.1 Definitions and general considerations

3.7.1.1 Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. The definitions presented below are adapted from those agreed as working definitions in IPCS/EHC Document No 225, Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals. For classification purposes, the known induction of genetically based heritable effects in the offspring is addressed in Germ Cell Mutagenicity (section 3.5), since in the present classification system it is considered more appropriate to address such effects under the separate hazard class of germ cell mutagenicity.

In this classification system, reproductive toxicity is subdivided under two main headings:

(a) adverse effects on sexual function and fertility;

(b) adverse effects on development of the offspring.

Some reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity. Nonetheless, substances with these effects, or mixtures containing them, shall be classified as reproductive toxicants.

3.7.1.2 For the purpose of classification the hazard class Reproductive Toxicity is differentiated into:

- adverse effects
  - on sexual function and fertility, or
  - on development;
- effects on or via lactation.

3.7.1.3 Adverse effects on sexual function and fertility

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

3.7.1.4 Adverse effects on development of the offspring

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

3.7.1.5 Adverse effects on or via lactation are also included in reproductive toxicity, but for classification purposes, such effects are treated separately (see Table 3.7.1 (b)). This is because it is desirable to be able to classify substances specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided for lactating mothers.

3.7.2 Classification criteria for substances

3.7.2.1 Hazard categories

3.7.2.1.1 For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Table 3.7.1(a) Hazard categories for reproductive toxicants.

Categories		Criteria
CATEGORY 1		Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
	Category 1A	Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.
	Category 1B	Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
CATEGORY 2		Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Table 3.7.1(b) Hazard category for lactation effects.

EFFECTS ON OR VIA LACTATION
Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

3.7.2.2 Basis of classification

3.7.2.2.1 Classification is made on the basis of the appropriate criteria, outlined above, and an assessment of the total weight of evidence (see 1.1.1). Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects.

The classification of a substance is derived from the hazard categories in the following order of precedence: Category 1A, Category 1B, Category 2 and the additional Category for effects on or via lactation. If a substance meets the criteria for classification into both of the main categories (for example Category 1B for effects on sexual function and fertility and also Category 2 for development) then both hazard differentiations shall be communicated by the respective hazard statements. Classification in the additional category for effects on or via lactation will be considered irrespective of a classification into Category 1A, Category 1B or Category 2.

3.7.2.2.2 In the evaluation of toxic effects on the developing offspring, it is important to consider the possible influence of maternal toxicity (see section 3.7.2.4).

3.7.2.2.3 For human evidence to provide the primary basis for a Category 1A classification there must be reliable evidence of an adverse effect on reproduction in humans. Evidence used for classification shall ideally be from well conducted epidemiological studies which include the use of appropriate controls, balanced assessment, and due consideration of bias or confounding factors. Less rigorous data from studies in humans shall be supplemented with adequate data from studies in experimental animals and classification in Category 1B shall be considered.

3.7.2.3 Weight of evidence

3.7.2.3.1 Classification as a reproductive toxicant is made on the basis of an assessment of the total weight of evidence, see section 1.1.1. This means that all available information that bears on the determination of reproductive toxicity is considered together, such as epidemiological studies and case reports in humans and specific reproduction studies along with sub-chronic, chronic and special study results in animals that provide relevant information regarding toxicity to reproductive and related endocrine organs. Evaluation of substances chemically related to the substance under study may also be included, particularly when information on the substance is scarce. The weight given to the available evidence will be influenced by factors such as the quality of the studies, consistency of results, nature and severity of effects, the presence of maternal toxicity in experimental animal studies, level of statistical significance for inter-group differences, number of endpoints affected, relevance of route of administration to humans and freedom from bias. Both positive and negative results are assembled together into a weight of evidence determination. A single, positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification (see also 3.7.2.2.3).

3.7.2.3.2 Toxicokinetic studies in animals and humans, site of action and mechanism or mode of action study results may provide relevant information which reduces or increases concerns about the hazard to human health. If it is conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals should not be classified.

3.7.2.3.3 If, in some reproductive toxicity studies in experimental animals the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome. These effects include small changes in semen parameters or in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments.

3.7.2.3.4 Data from animal studies ideally shall provide clear evidence of specific reproductive toxicity in the absence of other systemic toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects shall be assessed to the extent possible. The preferred approach is to consider adverse effects in the embryo/foetus first, and then evaluate maternal toxicity, along with any other factors which are likely to have influenced these effects, as part of the weight of evidence. In general, developmental effects that are observed at maternally toxic doses shall not be automatically discounted. Discounting devel-

opmental effects that are observed at maternally toxic doses can only be done on a case-by-case basis when a causal relationship is established or refuted.

3.7.2.3.5 If appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. Generally, the presence of maternal toxicity shall not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects. This is especially the case when the effects in the offspring are significant, e.g. irreversible effects such as structural malformations. In some situations it can be assumed that reproductive toxicity is due to a secondary consequence of maternal toxicity and discount the effects, if the substance is so toxic that dams fail to thrive and there is severe inanition, they are incapable of nursing pups; or they are prostrate or dying.

3.7.2.4 Maternal toxicity

3.7.2.4.1 Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms. In the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. This is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental outcome. Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity, along with any other factors which are likely to have influenced these effects, as weight of evidence, to help reach a conclusion about classification.

3.7.2.4.2 Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.

3.7.2.4.3 Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity.

3.7.2.4.4 Some of the end points used to assess maternal effects are provided below. Data on these end points, if available, need to be evaluated in light of their statistical or biological significance and dose response relationship.

Maternal mortality:

an increased incidence of mortality among the treated dams over the controls shall be considered evidence of maternal toxicity if the increase occurs in a dose-related manner and can be attributed to the systemic toxicity of the test material. Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation.

Mating index

(no. animals with seminal plugs or sperm/no. mated × 100) (*)

Fertility index

(no. animals with implants/no. of matings × 100)

Gestation length

(if allowed to deliver)

Body weight and body weight change:

Consideration of the maternal body weight change and/or adjusted (corrected) maternal body weight shall be included in the evaluation of maternal toxicity whenever such data are available. The calcula-

* () It is recognised that the Mating index and the Fertility index can also be affected by the male.

tion of an adjusted (corrected) mean maternal body weight change, which is the difference between the initial and terminal body weight minus the gravid uterine weight (or alternatively, the sum of the weights of the foetuses), may indicate whether the effect is maternal or intrauterine. In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.

Food and water consumption (if relevant):

The observation of a significant decrease in the average food or water consumption in treated dams compared to the control group is useful in evaluating maternal toxicity, particularly when the test material is administered in the diet or drinking water. Changes in food or water consumption need to be evaluated in conjunction with maternal body weights when determining if the effects noted are reflective of maternal toxicity or more simply, unpalatability of the test material in feed or water.

Clinical evaluations (including clinical signs, markers, haematology and clinical chemistry studies):

The observation of increased incidence of significant clinical signs of toxicity in treated dams relative to the control group is useful in evaluating maternal toxicity. If this is to be used as the basis for the assessment of maternal toxicity, the types, incidence, degree and duration of clinical signs shall be reported in the study. Clinical signs of maternal intoxication include: coma, prostration, hyperactivity, loss of righting reflex, ataxia, or laboured breathing.

Post-mortem data:

Increased incidence and/or severity of post-mortem findings may be indicative of maternal toxicity. This can include gross or microscopic pathological findings or organ weight data, including absolute organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio. When supported by findings of adverse histopathological effects in the affected organ(s), the observation of a significant change in the average weight of suspected target organ(s) of treated dams, compared to those in the control group, may be considered evidence of maternal toxicity.

3.7.2.5 Animal and experimental data

3.7.2.5.1 A number of internationally accepted test methods are available; these include methods for developmental toxicity testing (e.g. OECD Test Guideline 414), and methods for one or two-generation toxicity testing (e.g. OECD Test Guidelines 415, 416).

3.7.2.5.2 Results obtained from Screening Tests (e.g. OECD Guidelines 421 — Reproduction/ Developmental Toxicity Screening Test, and 422 — Combined Repeated Dose Toxicity Study with

Reproduction/Development Toxicity Screening Test) can also be used to justify classification, although it is recognised that the quality of this evidence is less reliable than that obtained through full studies.

3.7.2.5.3 Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads.

3.7.2.5.4 Evidence from in vitro assays, or non-mammalian tests, and from analogous substances using structure-activity relationship (SAR), can contribute to the procedure for classification. In all cases of this nature, expert judgement must be used to assess the adequacy of the data. Inadequate data shall not be used as a primary support for classification.

3.7.2.5.5 It is preferable that animal studies are conducted using appropriate routes of administration which relate to the potential route of human exposure. However, in practice, reproductive toxicity studies are commonly conducted using the oral route, and such studies will normally be suitable for evaluating the hazardous properties of the substance with respect to reproductive toxicity. However, if it can be conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals shall not be classified.

3.7.2.5.6 Studies involving routes of administration such as intravenous or intraperitoneal injection, which result in exposure of the reproductive organs to unrealistically high levels of the test substance, or elicit local damage to the reproductive organs, including irritation, must be interpreted with extreme caution and on their own are not normally the basis for classification.

3.7.2.5.7 There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding the inclusion within the criteria of a specific dose as a limit dose. However, some guidelines for test methods, specify a limit dose, others qualify the limit dose with a statement that higher doses may be necessary if anticipated human exposure is sufficiently high that an adequate margin of exposure is not achieved. Also, due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal model.

3.7.2.5.8 In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would

not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. Please also refer to the section on maternal toxicity (3.7.2.4) for further guidance in this area.

3.7.2.5.9 However, specification of the actual 'limit dose' will depend upon the test method that has been employed to provide the test results, e.g. in the OECD Test Guideline for repeated dose toxicity studies by the oral route, an upper dose of 1 000 mg/kg has been recommended as a limit dose, unless expected human response indicates the need for a higher dose level.

3.7.3 Classification criteria for mixtures

3.7.3.1 Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture

3.7.3.1.1 The mixture shall be classified as a reproductive toxicant when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 reproductive toxicant and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for Category 1A, Category 1B and Category 2 respectively.

3.7.3.1.2 The mixture shall be classified for effects on or via lactation when at least one ingredient has been classified for effects on or via lactation and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for the additional category for effects on or via lactation.

Table 3.7.2 Generic concentration limits of ingredients of a mixture classified as reproduction toxicants or foreffects on or via lactation that trigger classification of the mixture.

Ingredient classified as:	Generic concentration limits triggering classification of a mixture as:			
	Category 1A reproductive toxicant	Category 1B reproductive toxicant	Category 2 reproductive toxicant	Additional category for effects on or via l actation
Category 1A reproductive toxicant	≥ 0,3 % [Note 1]			
Category 1B reproductive toxicant		≥ 0,3 % [Note 1]		
Category 2 reproductive toxicant			≥ 3,0 % [Note 1]	
Additional category for effects on or via lactation				≥ 0,3 % [Note 1]

Note The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).
Note 1 If a Category 1 or Category 2 reproductive toxicant or a substance classified for effects on or via lactation is present in the mixture as an ingredient at a concentration above 0,1 %, a SDS shall be available for the mixture upon request.

3.7.3.2 Classification of mixtures when data are available for the complete mixture

3.7.3.2.1 Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients of the mixture. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual components. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of reproduction test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.7.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles

3.7.3.3.1 Subject to paragraph 3.7.3.2.1, where the mixture itself has not been tested to determine its reproductive toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

3.7.4 Hazard Communication

3.7.4.1 Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.7.3

Table 3.7.3 Label elements for reproductive toxicity.

Classification	Category 1A or Category 1B	Category 2	Additional category for effects on or via lactation
GHS Pictograms			No pictogram
Signal Word	Danger	Warning	No signal word
Hazard Statement	H360: May damage fertility or the unborn child (state specific effect if known)(state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H361: Suspected of damaging fertility or the unborn child (state specific effect if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H362: May cause harm to breast-fed children.
Precautionary Statement Prevention	P201 P202 P281	P201 P202 P281	P201 P260 P263 P264 P270
Precautionary Statement Response	P308 + P313	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405	
Precautionary Statement Disposal	P501	P501	

Annex **E**

Additional considerations to Regulation (EC) 1272/2008

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC)1272/2008) presented in Annex D. The classification of compounds is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the Committee has agreed upon a number of additional considerations:

- if there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the offspring, the compound will be classified in category 1A, irrespective of the general toxic effects (see Annex D, 3.7.2.2.1.)
- adverse effects in a reproductive study, occurring without reporting the parental or maternal toxicity, may lead to a classification other than category 1B, when the effects occur at dose levels which cause severe toxicity in *general* toxicity studies
- clear adverse reproductive effects will not be disregarded on the basis of reversibility per se

- the Committee dot not only use guideline studies (studies performed according to OECD* standard protocols) for the classification of compounds, but non-guideline studies are taken into consideration as well.

* Organisation for Economic Cooperation and Development.

Annex **F**

Fertility and developmental toxicity studies

Table 1 Fertility studies in laboratory animals with 3-metyhylcholanthrene: intraperitoneal.

authors	species	experimental period/design	dose/route	general toxicity	effects on reproductive organs/effects on reproduction
Stripp et al. (1974)	male Sprague-Dawley rats (n=10/group)	5 d/wk, 4 wk	0, 20 mg/kg bw/d (vehicle: vegetable oil)	decreased bw ($p<0.05$) (peritonitis); no gross abnormalities	reduced seminal vesicle weights ($p<0.01$); decreases in testosterone biosynthesis, cytochrome P450 concentrations, 17-α-hydroxylase activity (all $p<0.01$) in testicular microsomes ; no histopathological changes in testes
Konstandi et al. (1997)	adult and prepubertal male, female Wistar rats (n=4-11/ sex/ group	2x/wk, 1 mo; reproductive status, offspring number and condition, steroid hormone and oestrogen receptor levels	0, 25 mg/kg bw/d (vehicle: olive oil)	not reported	adult females: increased incidence of abnormal cycle ($p<0.001$), incl. pro-oestrous with leucocytes, oestrous with elevated number of nucleated cells, cycles with three di-oestrous, or cycles lasting 3, 6 or more d; increased incidence of constant di-oestrous ($p<0.0002$); increased length of oestrous cycle (5.9 d; controls: 4.64 d; $p<0.001$); prepubertal females: increased incidence of abnormal cycle ($p<0.01$), constant di-oestrous ($p<0.05$) and longer cycle (6.78 d; controls: 4.90 d; $p<0.01$); no effect on plasma oestradiol and progesterone or uterine oestrogen receptor levels, nor an effect on uterus and ovary weight in any female; males: decreases in testosterone levels, testis weights ($p<0.005$) in adults, but not in prepubertal; preputial glands not affected

Borman et al. (2000)	female Fischer 344 rats (n=6/group)	15 d; ovaries histologically prepared; follicles microscopically classified (primordial, primary, or secondary) and counted	0, 0.0015-60 mg/kg bw	decreased bw at 15 mg/kg bw and higher	no difference in ovary sizes; dose causing 50% loss of primordial follicles >60 mg/kg bw; number of primordial and primary follicles not affected; secondary follicles decreased
Borman et al. (2000)	female B6C3F_1 mice (n=6/group)	15 d; ovaries histologically prepared; follicles microscopically classified (primordial, primary, or secondary) and counted	0, 0.0015-0.75 mg/kg bw/d	no changes in body or liver weight	no difference in ovary sizes; dose causing 50% loss of primordial follicles = 0.045 mg/kg bw; number of primordial and primary follicles decreased to a similar degree; secondary follicles not affected; damage of primordial follicles first noted on d 12 [a single dose of 80 mg/kg bw caused a 50% primordial follicle loss within 2 d in B6 mice]

bw=body weight; d=day(s); mo=month(s); wk=week(s)

Table 2 Developmental toxicity studies in laboratory animals with 3-methylcholanthrene: oral (gavage).

authors	species	experimental period/design	dose/route	general toxicity	developmental toxicity
York et al. (1984a,b)	non-inducible female (DBA/2J) mice (n=15-34/group)	mated with inducible male mice (B6D2)F_1; treated on gd 15, 16,17; litter size determined at pnd 1, 4, 7, 14, 21; interim kills at postnatal wk 15 and 20 for determination of neoplastic lesions incidences;	0, 7, 21, 63 mg/kg bw/d (vehicle: corn oil)	bw not affected	1, 1, 4, 5 non-viable litters at 0, 7, 21, 63 mg/kg bw, resp.; at pnd 4 and 21, decreased number of pups at 21 and 63 mg/kg bw; survival not affected after weaning 63 mg/kg bw: reduced pup weight on pnd 7, 14 and 21; reduced number of inducible offspring; post-weaning pup weights reduced across all treated inducible offspring: at 21 mg/kg bw at 16 and 26 wk; at 63 mg/kg bw at 13, 16, 20, 26 wk; sex ratio not affected; no data on neoplastic lesions in animals killed at interim presented
		remaining offspring sacrificed wk 26 for gross and histological examinations and phenotype determination			lung major site of neoplastic lesions: incidence of: nodule-bearing offspring (total): 0/63 (0%), 10/25 (40%), 13/18 (72%), 28/34 (82%), resp.; nodule-bearing inducible offspring: 0/29 (0%), 2/10 (20%), 2/5 (40%), 4/7 (57%), resp.; nodule-bearing non-inducible offspring: 0/34 (0%), 8/15 (53%), 11/13 (85%), 24/27 (89%), resp. incidence of: adenoma-bearing offspring (total): 0/63 (0%), 6/25 (24%), 11/18 (61%), 25/34 (74%), resp.; adenoma-bearing inducible offspring: 0/29 (0%), 0/10 (0%), 2/5 (40%), 3/7 (43%), resp.; adenoma-bearing non-inducible offspring: 0/34 (0%), 6/15 (40%), 9/13 (69%), 22/27 (81%), resp.

					mean number of nodules in: offspring (total): 0.0, 1.80, 2.06, 6.47, resp.; in inducible offspring: 0.0, 1.10, 2.40, 7.71, resp.; in non-inducible offspring: 0.0, 2.27, 1.92, 6.15, resp. mean nodular cross-sectional area (mm) in: offspring (total): 0.0, 0.40, 1.21, 20.18, resp.; in inducible offspring: 0.0, 0.04, 0.25, 18.76; in non-inducible offspring: 0.0, 0.65, 1.58, 25.69, resp.
Tomatis et al. (1971)	female CF-1 mice (n=35 and 44/ group)	1-7 d before delivery; litters from treated animals were transferred to untreated dams and vice versa; additional untreated control group consisting of not-pregnant females and males included	0, 8.4 mg (vehicle: olive oil); given in 3 parts within 24 h	reduced survival rate; all treated females had one or more tumours	offspring from treated dams survived much shorter than the untreated control and untreated offspring foster-nursed by treated dams; offspring of treated dams had one or more tumours; offspring from untreated dams foster-nursed by treated dams showed a tumour incidence comparable to the untreated controls; tumours of the lung and malignant lymphomas were most common in all groups

bw=body weight; d=day(s); gd=gestational day(s); h=hour(s); n=number; pnd=postnatal day(s); wk=week(s)

Table 3 Developmental toxicity studies in laboratory animals with 3-methylcholanthrene: intraperitoneal.

authors	species	experimental period/design	dose/route	general toxicity	developmental toxicity
Khlood et al. (1999)	female Wistar rats (n: not specified)	gd 7-9 sacrifice: gd 20	0,10 mg/kg bw/d (vehicle: corn oil)	no signs of toxicity	increased resorption rate (0%, 60%, resp.); decreased mean percentages of dams with live foetuses (84%, 61%, resp.), mean percentages of live foetuses (74%, 36%, resp.), mean foetal weights (2.8±0.6 g, 2.2±0.3 g, resp.); no malformations
Shiverick et al. (1984)	female rats (strain not specified) (n=7-9/group)	gd 12-14 sacrifice: gd 15 or 20	0, 30 mg/kg bw/d (vehicle: corn oil)	gd 15: no effect on bw gain gd 20: decreased bw gain ($p<0.05$)	sacrifice gd 15: no effect on foetal resorption rate, foetal bw sacrifice gd 20: decreased placental weight (0.67±0.03 g, 0.48±0.01 g, resp.; $p<0.05$); foetal weight (3.94±0.02 g, 3.73±0.04 g, resp.; $p<0.05$)
Konstandi et al. (1997)	Wistar rats (n=5/sex/group	2x/wk, 1 mo; following treatment, animals mated for 1 wk; number of offspring, size, bw, malformations recorded on day of delivery; behaviouraltesting	0, 25 mg/kg bw/d (vehicle: olive oil)	not reported	offspring: litter size, number of litters, pup weight and sex distribution not affected; no stillborn offspring or malformations; negative geotaxis, righting reflex and cliff test not affected; at pnd 10: decreased oral body temperature (30.83±0.97 °C, 30.33±0.85 °C, resp.; $p<0.05$); no effect on vaginal opening (balanopreputial separation not examined)

		(negative geotaxis, righting reflex, cliff avoidance) , recording oral body temperature on pnd 8-10			
Khera et al. (1984)	female Swiss-Webster mice (n=7-9/group)	gd 10-12 sacrifice: gd19; foetal examination	0, 20 mg/kg bw/d (vehicle: corn oil)	not reported	decreased number of live foetuses/dam (13.1, 10.5, resp.), foetal weight (1.3 g, 1.0 g, resp.) no effect of treatment on percent resorptions, number of dead foetuses and sex ratio; no external, visceral or skeletal anomalies
Nebert et al. (1977)	female inducible C57BL/6N, non-inducible AKR/N mice (n=2-16/group	gd 5, 7, 10 or 12	0, 70 mg/kg bw (vehicle: corn oil)	not reported	number of resorptions+stillborns/total number of implantations: C57BL/6N mice: gd 5: 13/13 (100%); gd 7: 29/41 (71%); gd 10: 5/32 (16%); gd 12: 9/32 (28%); controls: 8/116 (7%); AKR/N mice: gd 5: 22/104 (21%); gd 7: 2/16 (13%); gd 10: 4/49 (8%); gd 12/49 (24%); controls: 14/134 (10%); malformations: C57BL/6N mice: gd 5: none; gd 7: 3 clubfoot; gd 10: 3 clubfoot; gd 12: 2 clubfoot, 1 unilateral anophthalmos; no malformations in AKR/N or control mice no effect on foetal weight or crown-rump length
Anderson et al. (1985)	female inducible (C57BL/6xDBA/2) F_1, non-inducible pregnant DBA/2 mice	gd 17 offspring sacrificed at about 10 mo of age for phenotype determination and complete necropsy	0, 30, 60, 100, 135, 175 mg/kg bw ((C57BL/6xDBA/2) F_1 mice); 0, 5, 30 mg/kg bw (DBA/2 mice)	not reported	lung tumour incidences in offspring of: (C57BL/6xDBA/2) F_1 mice: inducible males: 0/16, 11/25 (44%), 19/22 (86%), 24/25 (96%), 12/14 (86%), 7/8 (88%), resp.; inducible females: 2/25 (8%), 14/31 (45%), 17/28 (61%), 15/20 (75%), 5/7 (71%), 11/12 (92%), resp.; non-inducible males: 0/21 (0%), 13/30 (43%), 6/15 (40%), 18/23 (78%), 11/13 (85%), 12/12 (100%), resp.; non-inducible females: 0/22 (0%), 3/21 (14%), 4/17 (24%), 9/22 (41%), 13/15 (87%), 11/14 (79%); of DBA/2 mice: inducible males: 0/18 (0%), 13/30 (43%), 23/24 (96%), resp.; inducible females: 2/19 (11%), 0/11 (0%), 17/18 (94%), resp.; non-inducible males: 0/25 (0%), 5/24 (21%), 18/24 (75%), resp.; non-inducible females: 1/24 (4%), 3/26 (12%), 10/18 (56%), resp. average number of lung tumours/mouse in offspring of: (C57BL/6xDBA/2) F_1 mice: inducible males: 0, 2.0±1.3, 3.2±2.0, 7.1±7.5, 6.5±5.4, 5.6±4.2, resp.; inducible females: 1.0±0, 3.0±3.3, 1.9±1.3, 3.9±2.6, 3.6±3.7, 5.2±2.3, resp.; non-inducible males: 1.6±0.8, 1.5±0.5; 3.3±2.3, 3.1±2.1, 3.2±2.1, resp.; non-inducible females: 0, 1.0±0, 1.8±1.0, 2.7±1.9, 2.0±1.4, 2.1±1.1, resp. of DBA/2 mice: inducible males: 0, 1.2±0.4, 8.4±6.4, resp.; inducible females: 1.0±0, 0, 5.0±4.4;

					non-inducible males: 0, 1.2±0.4, 4.3±2.1, resp.; non-inducible females: 2.0±0, 2.3±1.5, 2.0±0.8 liver tumours found in male offspring only; incidences in offspring of: (C57BL/6xDBA/2) F_1 mice: inducible males: 0/16 (0%), 2/25 (8%), 3/22 (14%), 5/25 (20%), 3/14 (21%), 1/8 (12%), resp.; non-inducible males: 0/21 (0%), 0/30 (0%), 0/15 (0%), 1/23 (4%), 0/13 (0%), 0/12 (0%), resp.; of DBA/2 mice: inducible males: 2/18 (11%), 2/30 (7%), 10/24 (42%), resp.; non-inducible males: 1/25 (4%), 0/24 (0%), 8/24 (33%), resp. average number of liver tumours/mouse in offspring of (C57BL/6xDBA/2) F_1 mice: inducible males: 0, 1.0±0, 1.7±1.2, 2.0±1.4, 2.0±1.0, 1.0±0, resp.; non-inducible males: 0, 0, 0, 1.0±0, 0, 0, resp.; of DBA/2 mice: inducible males: 1.0±0, 1.0±0,, 2.4±1.3, resp.; non-inducible males: 1.0±0, 0, 1.6±1.1, resp.
Wessner et al. (1996)	female (DBA/2x(C57BL/6xDBA/2)) mice (number; unknown)	gd 17 offspring foster-nursed by untreated mothers and sacrificed after 1 year; lung and liver tumour formation examined in inducible and non-inducible offspring	0, 10, 30 mg/kg bw (vehicle: olive oil)	not reported	lung tumour incidence: inducible males: 0/14 (0%), 1/16 (6%), 13/16 (81%), resp.; inducible females: 0/12 (0%), 2/11 (18%), 13/15 (87%), resp.; non-inducible males: 0/13 (0%), 0/14 (0%), 7/20 (35%), resp.; non-inducible females: 0/14 (0%), 0/6 (0%), 9/18 (50%), resp. liver tumour incidences: inducible males: 1/14 (7%), 0/16 (0%), 10/16 (63%), resp.; inducible females: 0/12 (0%), 0/11 (0%), 1/15 (7%), resp.; non-inducible males: 1/13 (8%), 0/14 (0%), 7/20 (35%), resp.; non-inducible females: 0/14 (0%), 0/6 (0%), 1/18 (6%), resp.
Gressani et al. (1999)	female BALB/c mice (number: unknown)	gd 17; offspring foster-nursed by untreated mothers and sacrificed after 6 mo; lung tumours enumerated and classified	0, 5, 15, 45 mg/kg bw (vehicle: corn oil)	no effect on maternal bw gain and length of pregnancy	no effect on birth weight, foetal mortality or post-natal weight gain; lung tumour incidence: 0/11 (0%), 0/11 (0%), 3/15 (20%), 11/11 (100%), resp. number of tumours/mouse: 0, 0, 0.2±0.4, 2.6±1.3, resp.
Jennings-Gee et al. (2006)	female C57BL/6 (B6), BALB/c (C) mice (number: unknown)	mating within and between strains; gd 17; offspring housed for 12-18 mo, depending on tumour latency, examined for lung tumour	0, 45 mg/kg bw (vehicle: olive oil)	not reported	lung tumour incidence in offspring of: B6xB6: 0/16 90%), 5/46 (11%), resp.; CxB6: 8/36 (22%), 25/25 (100%), resp.; B6xC: 4/31 (13%), 19/19 (100%), resp.; CxC: 1/40 (3%), 30/35 (86%), resp. number of tumours/mouse in offspring of: B6xB6: 0, <0.1, resp.; CxB6: 0.15±0.38, 5.0±2.7, resp.; B6xC: 0.1±0.3, 5.8±3.2, resp.; CxC: <0.1, 3.3±3.2, resp.

Khera et al. (1983)	female Syrian golden hamsters (n=7-9/group)	gd 9-11 sacrifice: gd 15; foetal examination	0, 20 mg/kg bw/d (vehicle: corn oil)	not reported	no effect on number of resorptions, dead foetuses, live foetuses, runted foetuses and sex ratio; reduced foetal bw (by 6% compared to controls); no external or visceral anomalies observed; skeletal anomalies: incidence of delayed ossification of the calvarium comparable to that controls

bw=body weight; d=day(s); gd=gestational day(s); h=hour(s); mo=month(s); n=number; pnd=postnatal day(s); wk=week(s)

Table 4 Developmental toxicity studies in laboratory animals with 3-methylcholanthrene: subcutaneous.

authors	species	experimental period/ design	dose/route	general toxicity	developmental toxicity
Naruse et al. (2002)	female BALB/c mice (n=8-14/ group)	gd 10, 12, 14 sacrifice: gd 15,17; foetuses divided in 2 groups for skeletal and histological examination, respectively	1 mg/kg bw/ d (vehicle: mineral oil)	not reported	foetal weight not affected; abnormalities in first cervical and lumbar vertebrae in 3/29; delayed ossification of cervical and thoracic vertebrae and limbs in 9/29 (controls not affected); histologically, no subperiosteal bone matrix in metacarpals of treated group (n=29)

bw=body weight; d=day(s); gd=gestational day(s)

Health Council of the Netherlands

Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory reports that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.

www.healthcouncil.nl