Health Council of the Netherlands

Dexamethasone

Evaluation of the effects on reproduction, recommendation for classification

2016/15

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Dexamethasone*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-1024028/EvV/cn/543-R16
Bijlagen : 1
Datum : 18 oktober 2016

Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van dexamethason 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 Volksgezondheid van de Gezondheidsraad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van VWS en aan de staatssecretaris van IenM.

Met vriendelijke groet,

prof. dr. J.L. Severens
vicevoorzitter

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Dexamethasone

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. 2016/15, The Hague, October 18, 2016

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 and Sport, Infrastructure and the Environment, Social Affairs and Employment, and Economic Affairs. 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.

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Preferred citation:
Health Council of the Netherlands. Dexamethasone. Evaluation of the effects on reproduction, recommendation for classification. The Hague: Health Council of the Netherlands, 2016; publication no. 2016/15.

ISBN: 978-94-6281-086-0

Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad dexamethason onder de loep genomen. Dexamethason is een synthetisch steroïdhormoon (corticosteroïde). Het wordt gebruikt als geneesmiddel voor onderdrukking van ontstekingsreacties (aspecifiek anti-inflammatoir effect) en van (auto-)immuunprocessen. 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. Mensen die werkzaam zijn in de farmaceutische industrie, in apotheken of in ziekenhuizen kunnen tijdens hun werk in aanraking komen met dexamethason.

De Subcommissie Classificatie reproductietoxische stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) 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. Voor dexamethason komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie dexamethason niet te classificeren wegens onvoldoende geschikte gegevens

- voor effecten op de ontwikkeling adviseert de commissie dexamethason in categorie 1B (*stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting*) te classificeren en met H360D (*kan het ongeboren kind schaden*) te kenmerken
- voor effecten op of via lactatie adviseert de commissie om dexamethason niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report, the Health Council of the Netherlands reviewed dexamethasone. Dexamethasone is a corticosteroid. It is used as an anti-inflammatory or immunosuppressive agent. This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at the request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be occupationally exposed. Man can be occupationally exposed to dexamethasone in the pharmaceutical industry, in pharmacies or in hospitals.

The Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety (DECOS) 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 dexamethasone, these recommendations are:

- for effects on fertility, the Committee recommends not classifying dexamethasone due to a lack of appropriate data
- for effects on development, the Committee recommends classifying dexamethasone in category 1B (*presumed human reproductive toxicant*) and labelling with H360D (*may damage the unborn child*)

- for effects on or via lactation, the Committee recommends not labelling dexamethasone due to a lack of appropriate data.

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 present document contains the classification of dexamethasone by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances. The members of the Committee are listed in Annex A. The submission letter (in English) to the Minister can be found in Annex B.

In 2015 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 and 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
Hazard statement codes:	
H360F	May damage fertility.
H360D	May damage the unborn child.
H361f	Suspected of damaging fertility.
H361d	Suspected of damaging the unborn child.
H360FD	May damage fertility. May damage the unborn child.
H361fd	Suspected of damaging fertility. Suspected of damaging the unborn child.
H360Fd	May damage fertility. Suspected of damaging the unborn child.
H360Df	May damage the unborn child. Suspected of damaging fertility.
H362	May cause harm to breast-fed children.

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 Labelling for 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 a 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 exceeds 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 of Medline, starting from 1966 up to 2008, and by searches on the Internet; updates were performed in TOXNET, the latest one in July 2015. 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 as well as several websites regarding (publications on) toxicology and health. References are divided in 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.

In the assessment of the potential reproduction toxic effects of dexamethasone, the Committee also used data on adverse effects related to its application as a therapeutic agent.

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 preclude assessment of the compound for reproductive toxicity.
- Sufficient data show that no classification for toxic to reproduction is indicated.

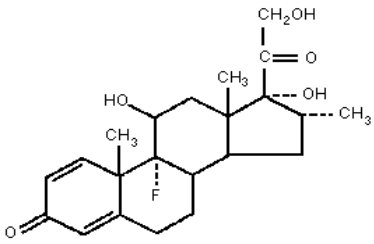
1.6 Final remark

The classification of compounds is based on hazard evaluation (Niesink et al., 1995[38]) only, 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

Dexamethasone

2.1 Introduction

name	:	dexamethasone
IUPAC name	:	(11β,16α)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione
CAS name	:	pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, 11β,16α)-
CAS registry number	:	50-02-2
EU/EINECS number	:	200-003-9
synonyms	:	16α-methyl-9α-fluoro-1,4-pregnadiene-11β,17α,21-triol-3,20-dione; 16α-methyl-9α-fluoro-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione; 16α-methyl-9α-fluoroprednisolone; 16α-methyl-9α-fluoro-Δ1-hydrocortisone; 1-dehydro-16α-methyl-9α-fluorohydrocortisone; 9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione; 9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione; 9α-fluoro-16α-methyl-1,4-pregnadiene-11β,17α,21-triol-3,20-dione; 9α-fluoro-16α-methyl-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione; 9α-fluoro-16α-methylprednisolone
colour/physical state	:	white to practically white crystalline powder
molecular weight	:	392.5
molecular formula	:	$C_{22}H_{29}FO_5$
formula	:	

melting point	:	262-264 ºC
vapour pressure	:	1.2 x10^{-11} Pa (at 25 ºC) (estimated)
Log $P_{octanol/water}$	:	1.83
solubility	:	10 mg/100 mL water (at 25 ºC); soluble in acetone, ethanol, chloroform
uses	:	as a synthetic glucocorticoid with anti-inflammatory and anti-rheumatic activity and immunosuppressant effects in a wide variety of disorders; antenatal administration to pregnant women, for instance to those at risk of delivering a child with congenital adrenal hyperplasia (off-label use in the Netherlands)[54]
occupational exposure		can occur in the pharmaceutical industry, in pharmacies or in hospitals
kinetics	:	toxicokinetic studies revealed rapid absorption after intramuscular administration to dogs and rats with peak plasma levels found after 30 minutes and six hours, respectively. Dexamethasone is rapidly excreted in urine and faeces. Dexamethasone esters are rapidly hydrolyzed in serum. Biotransformation in rats and humans is comparable and involves mainly hydroxylation to 6-hydroxy- and 20-dihydro-dexamethasone. However, there was additional evidence that at high (therapeutic) doses in people, dexamethasone is metabolized by an additional route involving epoxidation[28]
general toxicity	:	following repeated oral administration of dexamethasone to rats and dogs in short-term toxicity studies, the main target organs were the thymus and the adrenal gland. Corticosteroid concentrations in plasma and hepatic glycogen were reduced, whereas serum lipid levels were increased. In rats given oral doses of 0.0003-0.1 mg dexamethasone/kg bw/day for 90 days, the loss of thymus size, mass and cortical T cells, and morphological changes in the adrenal gland and a decrease in corticosterone and white blood cell counts were observed in male and female rats at doses above 0.01 mg/kg bw/day. Due to the decrease in white blood cell counts in female rats at 0.003 mg/kg bw/day, this dose was considered to be a marginal effect level. In a study with rats given oral doses of 0.0005-0.004 mg/kg bw/day dexamethasone for seven days, the corticosterone concentration was reduced in the highest-dose group and the activity of tyrosine aminotransferase in the liver was increased in a dose-related manner at 0.002 and 0.004 mg/kg bw/day. The No Observed Effect Level in this study was 0.0015 mg/kg bw/day[28]

Data from HSDB[57] unless otherwise noted

The adrenal cortex makes and secretes two different classes of hormones, the glucocorticoids and the mineralocorticoids. These hormones are involved in the regulation of metabolism and sodium and potassium balance, respectively. Dexamethasone is a synthetic derivative of the glucocorticoid hydroxycortisone. It is used for its anti-inflammatory and anti-rheumatic activity and its immunosuppressant effects. Since dexamethasone can pass the placental barrier and perinatal foetal serum concentrations are almost 100% of the maternal concentrations, maternal administration of dexamethasone to pregnant women is widely used therapeutically to promote lung maturation in human foetuses considered at risk of preterm delivery.

2.2 Human studies

2.2.1 Fertility studies

No studies were available regarding the effects of exposure to dexamethasone on human fertility.

2.2.2 Developmental toxicity studies

Carmichael and Shaw performed a population-based case-control study including 662 cases of orofacial clefts, 207 conotruncal heart defects, 265 neural tube defects, 165 limb reduction defects and 734 healthy controls, and collected information on medication use from one month before conception through the third month of pregnancy in maternal interviews a few years after delivery. The mother of one child with an orofacial cleft reported dexamethasone use, versus none of the control mothers.[9]

Dawes et al. determined the effect of administration of dexamethasone to pregnant patients on foetal heart rate and its variation in a retrospective analysis of computerized data derived from case studies. Dexamethasone (two doses of 12 mg intramuscularly, 12 hours apart, one to four occasions per patient) was given at weekly intervals to 28 pregnant women at weeks 27 to 32 of pregnancy. Foetal heart rate two days before and four days after dexamethasone treatment (n=28) and umbilical arterial flow velocity (n=19) were measured and analysed. The results showed an increase in foetal heart rate variation for up to one day (n=10, $p<0.01$). In case of foetal distress and reduced umbilical flow (n=18), only a small increase in foetal heart rate variation was observed.[11]

In a randomised controlled trial of pregnant women at increased risk of preterm delivery, Mulder et al. studied the effects of dexamethasone versus betamethasone (concentration and route of exposure not described) on foetal behaviour and foetal heart rate variation. The women (two groups of 30) were between weeks 26 and 33 of pregnancy and were studied daily over five successive days. For dexamethasone, an increase in foetal heart rate short term variation was found on day 1 ($p<0.05$). The observed change was transient, as the values returned to baseline on day 4.[35]

Senat et al. also studied the effects of dexamethasone and betamethasone on foetal heart rate of foetuses in a clinical trial. Pregnant women with preterm labour (n=40) received four intramuscular injections of 4 mg dexamethasone at weekly intervals starting between weeks 25 and 33 of pregnancy. No changes in foetal heart rate variability were found 1-7 days after dexamethasone treatment.[45]

Lee et al. investigated the neurodevelopmental outcome of extremely low birth weight infants exposed prenatally to dexamethasone. Dexamethasone was given as four 6 mg intramuscular doses administered with 12-h intervals during the admission for delivery. In this retrospective cohort study, infants weighing 401-1,000 grams at birth were included (n=408). Neonates with similar birth weights and receiving no steroids served as controls (n=153). Cerebral palsy, Barley scales of infant development, mental development, psychomotor development, blindness, hearing, neurodevelopmental impairment and unimpaired neurodevelopmental status at corrected gestational age were measured. There were no associations between prenatal dexamethasone exposure and any follow-up outcome, compared with no prenatal steroid exposure.[31]

Amador-Licona et al. investigated the effect of prenatal dexamethasone on the renal vascular resistance in preterm infants in a cross-sectional study. Pregnant women were treated with 6 mg dexamethasone intramuscularly, repeated every 12 hours for four doses, 48 hours or more before delivery between weeks 27 and 34 of pregnancy. The study included 37 infants treated with dexamethasone prenatally and 24 infants without treatment with prenatal steroids served as controls. Preterm infants who received prenatal dexamethasone and control infants showed no differences in birth weight or gestational age. The renal resistance measured between 12 and 72 hours after birth was decreased in treated infants (right renal artery p=0.001 and left renal artery p=0.01) without affecting renal volume and insulin levels.[1]

2.2.3 Lactation

No data are available regarding the excretion of dexamethasone in breast milk or the effects of exposure to dexamethasone on infants during the lactation period.

2.3 Animal studies

Fertility and developmental toxicity studies in laboratory animals are summarized in Annex F.

2.3.1 *Fertility studies*

Effects on female fertility

De Greef and Van der Schoot investigated the effects of dexamethasone on the ovarian activity in rats. Wistar rats were injected subcutaneously with daily doses of 0.1 mL dexamethasone sodium phosphate solution (0.25, 1 or 4 mg/mL saline). Several experiments were carried out. A control group was included (n=unknown). In the first experiment, rats were treated from the day of ovulation with 0.008 (n=6), 0.03 (n=7) or 0.1 (n=14) mg/kg body weight (bw) dexamethasone. The animals were killed on the first day following the observation of a cornified vaginal smear or eight to 23 days after the start of treatment. At autopsy, weights of the ovaries, adrenal glands and uterus were recorded. Ovaries were examined microscopically and fallopian tubes were examined for eggs. In the second experiment, daily treatment with 0.1 mg/kg bw dexamethasone was studied when treatment started two days before expected ovulation (n=17). Ten rats were killed two days later (to examine whether ovulation had occurred) and the remaining seven rats were killed after eight to 12 days of treatment. Uterine weight, ovarian weight and adrenal weight were recorded and ovaries were examined macroscopically and microscopically. In a third experiment (0.1 mg/kg bw dexamethasone from the day of ovulation, killed on day 9 of treatment; n=10) pseudopregnancy was investigated. Dexamethasone at the highest dose prolonged the ovarian cycle in the first experiment ($p \leq 0.05$). Histologically, the ovaries showed one generation of corpora lutea with signs of luteolysis and numerous ruptured follicles. All three treatment groups showed suppression of the weight of the adrenal glands at autopsy after 7-10 days. The seven rats killed after eight to 12 days of treatment in the second experiment showed a prolonged oestrous cycle. No differences were observed in the third experiment, except for an increased number of ovulation follicles after the start of treatment close to the expected time of ovulation and in pseudopregnancy ($p=0.05$). General toxicity was not described in the results.[12]

Maciel et al. investigated the effect of dexamethasone on ovarian follicular development and plasma hormone concentrations. Non-lactating Holstein cows were synchronized using prostaglandin F2α and one day after ovulation the animals (n=6) received a daily intramuscular injection of 0.044 mg dexamethasone/kg bw until the first dominant follicle stopped growing. Control animals (n=5) received vehicle injections (1 mL/100 kg bw/day of vehicle

containing ethanol, polyethylene glycol, and sterile-filtered Millipore water). Cows were weighed daily and blood samples were collected daily. There was no effect on body weight. Dexamethasone increased systemic glucose ($p<0.05$) and insulin concentrations ($p<0.05$), decreased plasma concentrations of insulin-like growth factor-I and II ($p<0.05$) without affecting insulin-like growth factor binding protein levels, did not affect the growth rate of dominant follicles, decreased plasma progesterone concentrations ($p<0.10$) without affecting gonadotropin levels and had no effect on plasma leptin concentrations.[32]

Effects on male fertility

Hatamoto et al. studied the effect of dexamethasone treatment on sperm parameters, seminal plasma antioxidant enzyme activity. Male adult Rottweiler dogs (n=18) were allocated to treatment groups (two groups were included to investigate whether oral supplementation with Vitamin E reduced adverse effects of dexamethasone). Dexamethasone was given intramuscularly for 7 days at a dose of 0.01 mg/kg bw/day. A control group was included. Body weight and food consumption were not affected. Semen collections were performed twice weekly for 14 weeks and blood samples to measure hormone levels were collected once a week. Dexamethasone treatment reduced ejaculate volume ($p=0.0002$) and increased thiobarbituric acid-reactive substances in the seminal plasma ($p<0.0001$). It also increased the number of sperm per ejaculate ($p=0.0122$), the percentage of abnormal sperm ($p=0.0002$) and the seminal plasma lipid peroxidation ($p=0.0264$). Furthermore, it elevated the activity of one of two antioxidant enzymes ($p=0.0264$).[20]

Gür et al. investigated the influence of dexamethasone on sperm characteristics and hyaluronidase activity of semen and serum. Akkaraman rams (n=7) were injected intramuscularly at a dose of 0.25 mg/kg bw for 2 days. A control group (n=7) was included. After the last administration, blood and semen samples were taken after 1, 2, 4, 24, 48, 72 and 96 hours. Semen evaluation and enzyme assays were performed. The results indicated that dexamethasone increases hyaluronidase activity in serum ($p<0.001$, except for the 1st hour) and semen ($p<0.001$, $p<0.01$, $p<0.05$), but decreases sperm concentration ($p<0.001$ except the 96th hour), semen volume ($p<0.05$) and sperm motility ($p<0.05$ except for the 72 and 96th hours) in rams. The rate of abnormal spermatozoa was not affected. Information on general toxicity was not provided.[18]

Barth et al. investigated the sequential appearance of sperm abnormalities after dexamethasone treatment. Bulls (n=8) of mixed Bos Taurus breeds with normal semen quality were treated with 20 mg dexamethasone intramuscularly daily for seven days. Untreated controls (n=4) were included. Beginning on the first day of treatment semen was collected in 4 bulls of each group three times a week for the first 25 days and then twice a week until day 42. Semen volume, concentration, motility, percent alive and differential counts of sperm abnormalities were recorded. Four animals treated for seven days were used to determine the effect on blood and testis tissue concentrations of testosterone. Control bulls were used for both semen and blood sampling. Dexamethasone induced lower basal, peak episodic and mean testosterone concentrations in blood ($p<0.05$). The concentrations in testis tissue were unchanged. There was a marked increase in sperm defects such as detached heads, midpiece defects and nuclear vacuoles (no statistics mentioned). However, all bulls, recovered to approximately pre-treatment levels of sperm defects by six weeks after initiation of treatment. General toxicity was not described in the results.[5]

2.3.2 *Developmental toxicity studies*

Teratogenic potential

No studies were available on the effects of oral administration of dexamethasone.

The relationship between glucocorticoids and teratogenic potential was studied by Jerome and Hendrickx by comparing the effect of triamcinolone acetonide and dexamethasone given to pregnant monkeys. Rhesus macaques (n=12) were treated intramuscularly with 1.0 or 10.0 mg/kg dexamethasone sodium phosphate at different days during pregnancy (between gestation days 23 and 49). A control group of two concurrent untreated animals and nine untreated control animals of a previous study was included. Pregnancies were terminated by hysterectomy on gestation day 100 ± 3. All foetuses were weighed, photographed, and examined for external and internal abnormalities. No effect on body weight was observed. The brain weight and the diameter of the cranial fossa were reduced in the highest dose group ($p<0.05$). The malformations observed appeared to be limited to the cranium, i.e. cranium bifidum* and aplasia

* a congenitally incomplete skull, often with an incomplete brain

cutis congenita* (2 out of 6 animals affected at 1.0 mg/kg, 4 out of 6 animals at 10.0 mg/kg). Presence or absence of maternal toxicity was not reported.[27]

Sauerbier studied the effect of prenatal dexamethasone as a function of timing of the dose. NMRI mice (n=12/group) were treated intramuscularly at doses of 10 or 50 mg/kg bw on gestation day 13 at four selected time points: 07:00, 13:00, 19:00 or 01:00 (according to 4 different breeding periods, i.e. 06:00-08:00; 12:00-14:00; 18:00-20:00 and 24:00-02:00). All mice were sacrificed at gestation day 18. Reproductive parameters were determined and foetuses were examined for external and skeletal abnormalities. Control groups (n=10), treated similarly with saline, were included. Cleft palate and resorptions were observed in all dexamethasone-treated groups. A maximum response, manifested by teratogenicity and embryolethality, was observed at 07:00 for both dose levels. A minimum response was seen at 19:00. The effects were dose-dependent. *P*-values were not mentioned. In this study it was possible to demonstrate a circadian rhythmicity in dexamethasone-induced cleft palate and embryolethality. Maternal toxicity was not described in the results.[44]

Ballard et al. treated pregnant CD-1 mice (n=unknown) in both eyes with 1 µl-drops of dexamethasone five times daily during gestation days 10-13. Four treatment groups were included: 1) control, saline; 2) low dose, approximately the human therapeutic concentration (not mentioned); 3) medium dose, one-half log unit above the low dose and 4) high dose, one log unit above the low dose. Mice were weighed throughout gestation and sacrificed at gestation day 18. Caesarian section was performed and all foetuses were processed for internal examination according to the Wilson method[58]. A dose-related increase in the incidence of cleft palate (all dose levels, $p<0.05$) and a dose-related increase in the incidence of sex organ abnormalities (dislocation, in the mid- and high-dose groups only, $p<0.05$) were observed. Maternal toxicity was not described in the results.[4]

Cardiovascular effects

No studies were available on cardiovascular effects of oral administration of dexamethasone.

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* the congenital absence or deficiency of a localized area of skin, usually on the scalp, with the base of the defect covered by a thin translucent membrane

In a mechanistic study of Torres et al. the maturation of the rat heart was investigated after prenatal dexamethasone treatment in rats. A subcutaneous, slow release, dexamethasone pellet (approximately 0.048 mg/day) was inserted to pregnant rats (n=8) beginning at gestation day 17. Control dams were unmanipulated (n=8). After 4-5 days of exposure, hearts were collected from neonatal rats within 24 h after birth. The organs were collected from 7-8 male and female offspring from treated and control mothers each. The effects on heart growth, cell number and DNA synthesis, extracellular matrix (ECM) and myosin heavy chain (MHC) mRNA expression were determined. Prenatal exposure to dexamethasone produced a higher heart/body weight ratio ($p<0.05$) and proliferative index (in females only, $p<0.05$) in association with a relative decrease in ECM content ($p<0.05$) and α-MHC mRNA (in males only, $p<0.05$), findings indicative for an immature heart as compared to the control group. Presence or absence of maternal toxicity was not reported. The average litter size and mortality was not different between the groups.[53]

Slotkin et al. investigated the effect of prenatal dexamethasone treatment on the development of the neonatal heart and kidney. Pregnant Sprague Dawley rats (n=unknown) were treated subcutaneously with 0.2 or 0.8 mg dexamethasone phosphate/kg bw/day on gestational days 17, 18 and 19. Controls received equivalent volumes of saline vehicle (1 ml/kg). Maternal weight gain was recorded and dams were allowed to litter. Pups were randomized and redistributed to nursing dams within the treatment group. At autopsy at weaning the body weight, heart weight and three biochemical markers of cell development in the heart were assessed: DNA content of the heart as an index of total cell numbers, DNA content per gram of tissue as an index of cell packing density and protein/DNA ratio as an index of relative cell size. Body and heart weights were lower ($p<0.01$). DNA content of the heart was diminished by dexamethasone at both doses ($p<0.01$). Cell packing density was decreased at first ($p<0.01$) and cells were enlarged ($p<0.01$). The conclusion from the results of this study was that foetal exposure to dexamethasone causes widespread inhibition of cell proliferation with disruption of parameters of cell differentiation and growth in the heart. Dexamethasone slowed maternal weight gain, but did not reduce the proportions of dams delivering pups or litter size at birth (data not shown).[47]

Dodic et al. investigated whether the dexamethasone-induced hypertension seen in a previous study[14] was associated with left ventricular hypertrophy and a reduced cardiac functional reserve (CO_{max-0}). Pregnant ewes (n=unknown) were

treated intravenously with 11.5 mg dexamethasone per day for 2 days at 27 days of gestation. Six female lambs served as a control group. Lambs were allowed to litter and surgery was performed at 50 days of age. Brief prenatal exposure led to the development of hypertension ($p<0.01$), left ventricular hypertrophy ($p<0.05$), and reduced cardiac functional reserve ($p<0.05$) in adult life. Maternal toxicity was not described in the results.[15]

Evidence for microvascular dysfunction after prenatal dexamethasone treatment in sheep was given by Molnar et al.. Dexamethasone was administered to pregnant ewes as three weekly courses of four intramuscular injections of 2 mg at 12h-intervals. Dexamethasone (n=7) or saline (n=7) was given at days 103, 110 and 117 of gestation. All ewes underwent Caesarean section and foetal femoral arteries were evaluated using wire myography. Foetal body weight was lower ($p<0.01$). Microvessel dysfunction, as expressed by enhanced endothelin-induced vasoconstriction ($p=0.003$ at the highest concentration), decreased endothelium-dependent relaxation ($p<0.05$) and normal endothelium-independent relaxation was observed, a combination which is associated with several forms of adult hypertension and thus adult cardiovascular health. Maternal toxicity was not described in the results.[33]

To determine the effects of a single course of maternally administered dexamethasone on foetal sheep in utero, Quaedackers et al. treated pregnant Romney/Suffolk ewes at gestation day 103 intramuscularly with two injections (24 hours apart) of 12 mg dexamethasone (n=8) or vehicle (n=7). Foetuses were continuously monitored for five days. In this study, a transient increase in blood pressure in the preterm sheep foetus was observed ($p<0.05$), with no sustained changes in vascular resistance. Maternal toxicity was not described in the results.[42]

The effect of maternal dexamethasone treatment on the development of foetal arteries was investigated by Hai and colleagues. Pregnant ewes were given a 6 mg intramuscular injection or placebo (0.9% saline) injection every 12h for 48h starting at gestation days 104, 105 or 106 (single dose group, n=unknown) and on gestation days 76, 84, 91, 98 and 105 of gestation in the repeated-dose group. The dose and treatment schedule were based on current treatment practice in pregnant women with premature labour. Foetuses were catheterized at 99-101 days of gestation and foetal arterial samples were collected on days 106-108. Thereafter, foetuses were removed and weighed and carotid arteries were dissected. Contraction and myosin light chain isoform expression were

investigated. Multiple dosing during pregnancy resulted in a reduced pup body weight ($p<0.05$). In response to 1 µM phenylephrine, arteries from foetuses of dexamethasone-treated ewes exhibited biphasic contractions ($p<0.0001$). The relaxation rate was higher in arteries from foetuses of dexamethasone-treated dams than control animals ($p<0.05$). Repeated maternal administration of dexamethasone induced an almost twofold increase in myosin $LC17_a$ isoform expression in the foetal arteries ($p<0.05$). Maternal toxicity was not described in the results.[19]

Neurodevelopmental effects

Oral administration

Hauser et al. studied the effect of prenatal dexamethasone treatment on maternal endocrinology (plasma cortisol and oestrogen titres) and postnatal physical growth, plasma and urinary adrenocorticotropic hormone and cortisol titres, and social and maintenance behaviour from birth to weaning in the common marmoset monkey. Pregnant marmosets received 5 mg dexamethasone/kg bw/day orally (tablets were crushed and suspended in fruit syrup) during early gestation (days 42-48) or late gestation (days 90-96). A control group received vehicle. The route of administration was chosen to reduce stress. Females were allowed to litter. There was no statistically significant effect of prenatal treatment on maternal body weight. The infants showed effects from postnatal day 56 on. Early administration of dexamethasone resulted in increased weight gain in the presence of normal skeletal growth ($p<0.05$), increased eating ($p<0.05$), and increased sympathetic autonomic nervous system arousal ($p<0.05$), increased time spent mobile ($p<0.05$); increased time spent eating ($p<0.05$), trends toward more solitary play ($p=0.053$) and tail hair piloerection ($p=0.085$). This phenotype shows similarity to that of the human metabolic syndrome. Late dexamethasone exposure (aiming at an equivalent developmental stage to human foetuses at risk of preterm delivery) was largely without effect on physical, endocrine, and behavioural measures across infancy.[21]

Based on the fact that prenatal stress is an important risk factor in schizophrenia, the effect of prenatal dexamethasone on the development of schizophrenia-like phenotypes was investigated by Hauser and colleagues. Wistar rats (n=14) were exposed to 0.1 mg/kg dexamethasone (dissolved in 0.01% ethanol) per day via drinking water between gestational days 15 and 21. A control group (n=12) was included receiving 0.01% ethanol. Maternal body weight was recorded during

gestation and rats were allowed to litter. During lactation females and pups were exposed to tap water only. A second study (n=10 rats/group) was performed according to the same procedure. A cross-fostering design (after culling to four males and four females) was used to allow dissociation of any direct prenatal effects on offspring from effects dependent on dexamethasone exposure from the rearing dam. Maternal behaviour was checked on postnatal day 1-14 and offspring was weaned on postnatal day 21. The offspring was tested for prepulse inhibition (PPI) and latent inhibition (LI), both known to be disrupted in schizophrenia patients. Pup birth weight was reduced by prenatal dexamethasone treatment ($p<0.05$) and a reduced body weight in adulthood was observed (in male pups only, $p<0.05$). The effect on maternal body weight was not reported. Dexamethasone-treated dams demonstrated an increased pup-directed behaviour ($p=0.029$). The study did not provide support for the hypothesis that prenatal dexamethasone exposure leads to schizophrenia-like deficits in PPI or LI.[22]

Dupouy et al. studied the effects of chronic dexamethasone exposure of mothers on different hormones of the hypothalamo-pituitary-adrenal axis (corticotrophin-releasing factor, adrenocorticotropic hormone, and corticosterone) in brain pieces (stalk, median eminence, and hypothalamus), pituitary gland, adrenals and plasma of 21-day-old rat foetuses. Pregnant rats (n=unknown) were treated with either plain tap water or water containing dexamethasone acetate (0.01 mg/ml) from gestation day 15 to 21. On day 21 the animals were sacrificed and the foetuses analysed. A marked reduction of body weight (-66% vs. controls, $p<0.001$), severe atrophy of the adrenals (-83%, $p<0.001$) and decreases in corticosterone concentrations in the adrenals (-74%, $p<0.001$) and in plasma (reduction to undetectable level, $p<0.001$) were observed after dexamethasone treatment. The adrenal changes correlated well with a drastic decrease in plasma adrenocorticotropic hormone (ACTH) concentrations (reduction to undetectable level, $p<0.001$) and pituitary ACTH-content (-93%, $p<0.001$). This low cortico-stimulating activity of the foetal pituitary glands was associated with a decreased corticotrophin-releasing factor (CRF) hypothalamic content (-57%, $p<0.001$) and concentration (-67%, $p<0.001$). These results suggest both pituitary and hypothalamic sites for the in vivo inhibiting action of dexamethasone on the rat hypothalamic-pituitary-adrenal axis in late gestation. Maternal toxicity was not described in the results.[17]

Brabham et al. examined the effects of prenatal dexamethasone on spatial learning and response to stress. Pregnant Sprague Dawley rats (n=20-24) received 0.0025 mg/ml dexamethasone in the evening water daily from gestation

day 15 until delivery. A control group receiving vehicle was included. In this study, a concentration of 0.01 mg/ml resulted in a high incidence of foetal abortion and a concentration of 0.005 mg/ml resulted in 50% maternal mortality on spontaneous vaginal delivery. All dexamethasone-treated animals received an equivalent amount of dexamethasone during 15-21 days of gestation, which averaged 0.27 ± 0.02 mg/kg/day (mean ± SD). After parturition pups were cross-fostered and reduced to eight or nine per dam. Spatial visual memory was evaluated in young adults (days 65-75 of age) with the Morris water maze. The cortisone response to restraint stress was examined at day 65 of age (naïve animals to behavioural testing) and expression of glucocorticoid and mineralocorticoid receptors mRNA was determined by in situ hybridization in brain tissue derived from 80-days old pups. Exposure to dexamethasone caused restlessness in mothers ($p<0.05$), low birth weights ($p<0.05$) and poor weight gain in the offspring ($p<0.05$). Males exposed to dexamethasone during pregnancy and cross-fostered to treated dams had impaired spatial learning ($p<0.05$), inability to rapidly terminate adrenocorticoid response to stress ($p<0.05$) and decreased hippocampal glucocorticoid receptor mRNA expression ($p<0.05$). Dexamethasone-exposed animals that were raised by vehicle-treated dams had adequate responses. The dexamethasone-treated dams showed a smaller increase of body weight at GD 15-18 ($p<0.001$), a larger one at two weeks after giving birth ($p=0.05$) and return to a normal one at three weeks after giving birth.[6]

Subcutaneous administration

Carlos et al. studied the mechanism of abnormalities to the nervous system structure and function after dexamethasone treatment. In this study dexamethasone was administered subcutaneously to pregnant rats during gestation days 17, 18 and 19. Dams (n=unknown) were given 0.2 mg/kg bw/day or 0.8 mg/kg bw/day. Control animals received equivalent volumes of saline (1 mg/kg). Maternal weight gain was recorded and pups were allowed to litter. At birth, pups were randomized within their respective treatment groups and redistributed to the nursing dams with litter size maintained at 9-11 pups. Animals were weaned at postnatal day 22. At autopsy (day not mentioned) the brain was investigated for weight, DNA content, protein content and DNA synthesis. The forebrain showed persistent elevations of DNA ($p<0.01$) and reduced protein/DNA ($p<0.01$), indicative of replacement of neurons with glia. Because the treatment period coincided with the timing of neuronal cell replication in the forebrain, but not in the other regions, these results suggest that

the critical period for lasting deficits of dexamethasone coincides with the peak of neuronal mitosis. Dexamethasone slowed maternal weight gain (data not shown).[8]

The effect of dexamethasone (0.050 mg/kg bw/day) injected subcutaneously to pregnant Sprague Dawley rats during gestation days 16-21 was investigated by Nagano and colleagues. A control group receiving vehicle was included; the number of dams in each group was not described. At postnatal day 2 the pups were randomized and redistributed to the dams within each treatment group and the litter size was maintained at six or seven male pups per dam. Behavioural tests, quantitative analysis of corticotropin-releasing factor (CRF), corticosterone levels and immunohistochemistry and image analysis were included. A reduced body weight in the offspring was observed in postnatal weeks 4, 7 and 10 ($p<0.01$ or $p<0.05$). Dexamethasone did not cause any apparent differences in pre-weaning maternal care between the dexamethasone-treated and control dams. Prenatal dexamethasone exposure decreased corticotropin-releasing factor mRNA in the hypothalamus ($p<0.01$) and disturbed the plasma corticosterone response to restraint stress in the offspring at postnatal week 4 (PNW4) ($p<0.01$). In contrast, it was not until PNW10 that increased anxiety-like behaviour emerged in the dexamethasone-exposed offspring ($p<0.05$). In association with the acquisition of increased anxiety-like behaviour at PNW10, glucocorticoid receptor expression was decreased in the amygdala in dexamethasone-exposed offspring at PNW7 ($p<0.01$) and PNW10 ($p<0.001$). Thus, prenatal exposure to dexamethasone hampered the neuroendocrinological development in the offspring during early life and it was suggested that this disturbance could result in the induction of increased anxiety-like behaviour in adulthood. Dexamethasone treatment had no apparent effect on maternal care during the pre-weaning period.[36]

The effect of prenatal and postnatal dexamethasone treatment on the serotonergic and dopaminergic systems was investigated by Slotkin and colleagues. Pregnant Sprague Dawley rats (n=unknown) were treated subcutaneously with doses of 0.05, 0.2 or 0.8 mg dexamethasone/kg bw/day on gestation days 17-19. Control rats received equivalent volumes (1 ml/kg) of saline vehicle. Females were allowed to deliver and pups were maintained at a litter size of 10. Randomization was repeated every 3-4 days to obviate any differences in maternal caretaking. Maternal toxicity was not described. To study the postnatal effects, pups were given 0, 0.05, 0.2 or 0.8 mg dexamethasone/kg bw/day on postnatal days 1-3 or 7-9 and the same randomization procedures were performed. On postnatal day 60

animals were decapitated. 5-hydroxytryptamine (5HT) receptor binding, 5HT concentration, 5-hydroxyindolacetic acid (5HIAA), dopamine (DA) concentration and turnover, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in the cerebral cortex and/or hippocampus. 5HT receptor binding and 5HT transporter binding were increased in the cerebral cortex during gestation and after birth ($p<0.0006$ and $p<0.0001$, respectively). 5HT concentration in the hippocampus and cerebral cortex were increased after birth ($p<0.002$), then decreased till levels below normal ($p<0.0006$). 5HT turnover was unchanged. DA concentration in the cerebral cortex was already increased during gestation ($p<0.03$), decreased till levels below normal at postnatal days 1-3 ($p<0.0001$) and returned to normal again by postnatal days 7-9. DA turnover in the cerebral cortex was unchanged. Results indicated that dexamethasone administration during phases of brain development, analogous to those in preterm infants, produces changes in indices of 5HT and DA synaptic function.[46]

Kreider et al. investigated the effect of prenatal dexamethasone at clinical doses on neurobehavioural development in adolescent and adult rats. Sprague Dawley rats (n=unknown) were treated subcutaneously with 0.2 mg dexamethasone/kg bw/day on gestation days 17, 18 and 19. A control group receiving equivalent volumes of saline (1 ml/kg) was included. Dams were allowed to litter and pups were randomized to nursing dams every 4 days until weaning. Behavioural tests were performed at postnatal days 31, 32, and 33 (figure 8 activity) and 45-68 (eight-arm radial maze). Dexamethasone had no statistically significant effect on maternal weight gain during pregnancy, gestation index, litter size, viability and sex ratio (data not shown). Dexamethasone treatment ablated the normal differences between sexes in locomotor activity by lowering the values in females to the levels of males ($p<0.0003$). Habituation of activity was similarly impaired in females to match the profile of males ($p<0.01$), while males in the dexamethasone group showed a partially feminized pattern of habituation ($p<0.004$). Dexamethasone delayed learning in males ($p<0.08$), while improving performance in females in the 8-arm radial maze ($p<0.04$). Values of hippocampal [3H]hemicholinium-3 binding, a biomarker for cholinergic synaptic activity, were increased in males to the levels of females after dexamethasone treatment ($p<0.007$). From this study it can be concluded that dexamethasone given at clinical doses has adverse effects on neurodevelopment, producing sex selective alterations in activity, learning and memory.[29]

Somm et al. examined the effect of dexamethasone treatment on growth and brain metabolism. Pregnant Sprague Dawley OFA rat dams were implanted subcutaneously with a minipump delivering either vehicle or dexamethasone at a dose of 0.1 mg/kg/day at gestational days 14-21. Litter size was smaller in the dexamethasone-treated group ($p<0.001$). For follow-up, litter size was brought to 10-12 pups. Perinatal body weight of pups was diminished by dexamethasone at postnatal days 1 and 21 ($p<0.001$). Pup hippocampal metabolism was reduced, as evidenced by lower concentrations of several brain metabolites and lower hippocampal gene expression at postnatal day 7 ($p<0.05$). Maternal body weight throughout gestation and weight gain during its third week were decreased ($p<0.001$ and $p<0.01$, respectively).[48]

Intramuscular injection

The effect of repeated low doses of maternally administered dexamethasone on growth in sheep during foetal life and the first 2 years of postnatal life was investigated by Kutzler and colleagues. Different cohorts were used. They all received four intramuscular injections of 29-33 µg/kg bw dexamethasone in saline or an equal volume of saline within 48 hours on gestation days 103-104, 110-111 and 117-118. In the first cohort twenty ewes were administered either dexamethasone (n=9) or saline (n=11) and autopsy was performed at gestation day 119. Foetal and placental measurements and immunocytochemistry were investigated. In cohort two, females were allowed to lamb. Sixteen female ewes were administered dexamethasone (n=8) or saline (n=8). Ewes were allowed to deliver and neonatal measurements (newborn body weight and organ weight within 12 hours after birth) were performed. In the last cohort, twenty-six ewes were administered dexamethasone (n=13) or saline (n=13). Ewes were allowed to lamb and postnatal measurements were performed biweekly for 8 weeks (body weight, biparietal diameter (BPD), crown-to-rump length (CRL), thoracic girth circumference (TGC), abdominal girth circumference (AGC), and radial bone length (RBL). The study showed that repeated maternal dexamethasone treatment at doses threefold lower than what women in preterm labour receive, results in decreased foetal body weight ($p<0.05$), prolonged gestation length ($p<0.05$), normal birth weight, decreased newborn brain weight ($p<0.05$) and biparietal diameter ($p<0.05$). Other postnatal growth measures were normal. Maternal toxicity was not described in the results.[30]

Neurotoxic effects induced by prenatal administration of dexamethasone to Rhesus monkeys during the early third trimester were investigated by Uno and

colleagues. Dexamethasone was given intramuscularly to pregnant monkeys on gestation day 132 (single injection with doses of 0.5, 5 or 10 mg/kg bw) or on days 132 and 133 (multiple injections at 12-h intervals with 0.125 x 4, 1.25 x 4 or 2.5 mg/kg x 4). A control group receiving vehicle was included. Maternal toxicity was not described in the results. Foetuses were delivered by Caesarean section on day 135 of gestation to study neurotoxic effects and at day 162 of gestation to investigate whether the effects seen at day 135 were persistent. Brain sections were investigated. A critical dose of dexamethasone given prenatally appeared to induce an irreversible reduction of the number of neurons in the hippocampus ($p<0.05$) and poorly differentiated hippocampal neurons.[56]

Miscellaneous routes of administration

The effect of prolonged low-dose dexamethasone treatment in early gestation was investigated in sheep by Moritz and colleagues. Pregnant merino ewes (n=18) were infused intravenously with approximately 0.020 mg dexamethasone/kg bw/day for 20 days. A control group (n=15) received saline. Ewes were killed on gestation day 45. Maternal and foetal organs were weighed and preserved. A second cohort of ewes (n=3 controls and n=4 dexamethasone-treated) were maintained until foetuses were at 130 day of gestation. At autopsy on gestational day 130 foetal organs were weighed and collected. Brain was further dissected. A last cohort was allowed to lamb and offspring was studied at 2 months of age. Immediate and permanent effects on the growth of the foetus and developing organs, and programming effects (gene expression levels of the angiotensin receptors, angiotensinogen, mineralocorticoid and glucocorticoid receptor in kidney and brain of foetuses at gestation day 130) were studied using the three cohorts. There were no persistent, long-term effects of prolonged low-dose dexamethasone treatment in normal ovine foetuses.[34]

Uno et al. also performed longitudinal studies of the juvenile monkeys with induced prenatal hippocampal deficiency. Rhesus monkeys (n=8) were treated with 5 mg dexamethasone/kg bw/day (route of administration not reported) at gestation days 132 and 133. A control group (n=3) receiving vehicle was included. After birth all infants lived with their mother for 1 year. Plasma cortisol levels were measured at 9 months of age and magnetic resonance images (MRI) of the brain were made at 20 months of age. Prenatal administration of dexamethasone induced an irreversible reduction in the size of the hippocampus. It also induced high plasma cortisol at the circadian baseline and post-stress

levels in the juvenile rhesus monkeys. Statistics were not reported. Maternal toxicity was not described in the results.[55]

Effects on the immune and endocrine systems

No studies were available on effects of oral administration of dexamethasone on the immune and endocrine systems.

Subcutaneous injection

Negić et al. investigated whether dexamethasone during pregnancy influenced the morphology of the pituitary, luteinising hormone (LH) and follicle stimulating hormone (FSH). Pregnant Wistar females were either treated with dexamethasone subcutaneously on gestation days 16-19 at doses of 1.0 or 0.5 mg/kg bw/day. The day when sperm was found was designed as gestation day 1. Control groups were injected with equal volumes of 0.9% saline. On gestation day 19 (n=8) and on gestation day 21 (n=8) rats were sacrificed. To compare the effects of pregnancy, a control group of virgin rats was included too (three daily injections and necropsy 24 h after the last treatment (n=8) or necropsy 72 h after the last treatment (n=8)). Histological analysis and morphometric analysis on pituitary glands was performed. The results demonstrated that pregnancy in rats led to a marked reduction of morphometric parameters (cell volume, volume density and number of cells) of LH and FSH compared to virgin control values ($p<0.05$). Moreover, daily dexamethasone treatment of pregnant females affected the size of LH and FSH cells on day 19 of pregnancy ($p<0.05$). The decrease in cell volume was reversible (normalization of LH and FSH cell function on day 21 of pregnancy). The results indicate that there are no prolonged effects of dexamethasone treatment during pregnancy on LH and FSH cells. Information on maternal toxicity was not provided.[37]

In a study performed by Stojanoski et al. the effect of prenatal dexamethasone treatment on the development of pituitary adrenocorticotrophic (ACTH) cells and adrenal glands in the offspring was investigated. Wistar rats (n=unknown) were injected subcutaneously with dexamethasone on gestation day 16 (1.0 mg/kg bw), 17 (0.5 mg/kg bw) and 18 (0.5 mg/kg bw). Control females received the same volume of saline vehicle. On day 19 of gestation autopsy was performed and foetuses were prepared for histological and morphometric measurements. Results showed maternal dexamethasone treatment in the period when foetal hypothalamo-pituitary-adrenal (HPA) axis begins to function, inhibited the axis.

Pituitary ACTH cells were fewer and had a smaller volume ($p<0.05$). In the adrenal organs cortical cells of the zona glomerulosa had reduced proliferative activity ($p<0.05$). Thus, reduced pituitary ACTH cell function and mitotic activity led to the suppression of adrenocortical cell multiplication in the zona glomerulosa. Information on maternal toxicity was not provided.[49]

Page et al. investigated whether prenatal exposure of rats alters Leydig cell steroidogenic capacity in immature and adult rats. Sprague Dawley rats were treated subcutaneously with 0.1 mg/kg bw per day dexamethasone (n=9) or saline and 0.4% ethanol (n=9) on gestation days 14-19 (day 0 is defined as the morning of appearance of the vaginal plug). Immature male offspring were weaned at postnatal day 21 and studied at day 35 days of age and adult males were killed and investigated at 90 days of age (n=26 dexamethasone-treated and n=27 control males for both immature and adult males). Maternal toxicity was not described. Immature male offspring showed decreased levels of testosterone in serum ($p<0.05$) and a reduced testosterone production in Leydig cells ($p<0.001$). Their serum ACTH and corticosterone concentrations were reduced ($p<0.001$). Mature male offspring demonstrated higher serum levels of these hormones ($p<0.001$). They also exhibited higher serum testosterone concentrations ($p<0.05$) and testosterone production in Leydig cells ($p<0.001$). The results demonstrate that a high level of maternal dexamethasone affects the steroid output from both the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes; Leydig cell testosterone production was affected in both pubertal and adult rats.[41]

The effects of prolonged dexamethasone administration to pregnant rats on the structure and function of the adrenal glands was investigated by Hristić and colleagues. Pregnant Wistar rats (n=10) were injected subcutaneously with 0.3 mg dexamethasone/kg bw/day during 5 days, starting from day 16 of pregnancy. A control group (n=10), that received the same volume of saline (0.3 ml/kg bw/day) was included. The first cohort of dams (number not mentioned) and foetuses were killed 24 h after the last injection. The second and third cohort were killed at postnatal days 3 and 14. The adrenal gland was investigated for morphometric parameters, metaphase index of cortical cells and histological analysis. The proliferative activity of adrenal cortical cells was inhibited and atrophic changes in the adrenal glands were seen. The main findings were a decreased volume of the foetal adrenal gland ($p<0.005$), its zona glomerulosa plus capsula ($p<0.005$) and its inner zone ($p<0.005$) as the consequence of atrophic changes in the gland and reduction of the volume and total number of

adrenocortical cells ($p<0.05$ and $p<0.005$, respectively). These morphometric changes were found in 3- and 14-day-old pups. Information on maternal toxicity was not provided.[26]

Holson et al. studied the effect of prenatal dexamethasone treatment (among other stressors) on the sexual behaviour of the male offspring. Pregnant Sprague Dawley derived CD rats (n=4/group) were treated subcutaneously with dexamethasone at 0 or 0.1 mg/kg bw/day on gestation days 14 to 21. At birth all litters were culled to 8 pups (4 ± 1 of each sex) and raised up to weaning. Litter size, number of stillbirths and sex ratio were not altered by dexamethasone, but a reduced pup birth weight (an effect still seen at postnatal day 85) was observed ($p<0.01$). Male adrenal weight at birth was reduced ($p<0.01$). Male sexual differentiation at birth, as measured by anogenital distance, was also decreased ($p<0.05$). Male sexual behaviour was assessed for three weeks, starting at postnatal day 90 by investigating the number of ejaculations per trial and the latency to first ejaculation after cohabitation with oestrous females, and the lordosis quotient after postgonadectomy exposure to stud males. Dexamethasone affected both sexual performance of adult males, as measured by the ejaculation parameters mentioned (both $p<0.05$), and the Lordosis quotient of castrated hormone-primed males after exposure to stud males ($p<0.05$). From the results of this study it can be concluded that prenatal dexamethasone exposure demasculinizes and feminizes male offspring. Maternal toxicity, demonstrated by diminished weight gain, was considerable ($p<0.0001$).[23]

Intraperitoneal injection

Bakker et al. examined the effects of prenatal dexamethasone treatment on the developing thymus, spleen and hypothalamo-pituitary adrenal axis. Female Wistar rats were given 400 µg of dexamethasone-21-phosphate (n=7) or saline (n=6) intraperitoneally on days 17 and 19 of pregnancy. A third group of pregnant rats (n=6) received no injection. After maternal dexamethasone treatment, the offspring showed reduced body weights compared to either control group, on the first day after birth. Thymus, spleen, hypothalamus and blood plasma of offspring were examined at several time intervals (1-20 days) after birth. Prenatal exposure to dexamethasone resulted in decreased T cell numbers in thymus and spleen on postnatal day 1 ($p<0.05$ or <0.005). T cell numbers in the spleen were reduced at all neonatal ages studied (postnatal days 1, 7 and 20; $p<0.05$ or <0.005). Regarding the hypothalamus, prenatal exposure to dexamethasone altered the pattern of neonatal changes in peptide expression in

corticotrophin-releasing hormone neurons. No effects were seen on adrenocorticotrophic hormone and corticosterone levels in plasma. Maternal toxicity was not described in the results.[2]

Bakker et al. also investigated whether maternal treatment with dexamethasone or saline alters the corticosterone response to a mild stressor in the offspring and whether maternal treatment results in long-term altered in vivo humoral and cellular immune responsiveness in the offspring. Pregnant Wistar rats (3 animals per group) were given saline or dexamethasone-21-phosphate at a dose of 1.2 mg/kg body weight intraperitoneally at days 17 and 19 of gestation. A third group of pregnant rats was left undisturbed. After maternal dexamethasone treatment, the offspring showed no altered corticosteroid response to a novel environment at 20 days of age, as compared to either control group. Furthermore, no effects of maternal dexamethasone exposure were seen on immuno-globulin-2a production after immunization with a conjugated pneumococcal polysaccharide at 6 weeks of age. Cellular immune responses, measured by an oxazolone-induced contact hypersensitivity response at 8 weeks of age, were lower in offspring of dexamethasone-treated dams as compared to saline-treated dams ($p<0.005$). The response was not different from that in offspring of untreated dams. Information on maternal toxicity was not provided.[3]

Yu et al. evaluated the immune programming influenced by prenatal dexamethasone. Pregnant Sprague-Dawley rats (6-8 animals per group) received saline or dexamethasone at 0.1 mg/kg/day at gestational days 14-20. Male offspring were killed at day 7 or day 120 after birth. Spleen were collected for immune analysis. Three out of five inflammation mediators were decreased at day 7, one of them, matrix metalloproteinase-9, still at day 120 ($p<0.05$). Upon concanavalin-A stimulation prenatal dexamethasone treatment reduced tumour necrosis factor-α production ($p<0.05$), but not interferon-γ production in spleen cells at day 120.[59]

Miscellaneous routes of administration

The effect of dexamethasone on the adrenal gland in foetal and neonatal rats was investigated by Hristić and colleagues. Wistar rats (n=5) were treated with a single dose (route unknown) of 1.5 mg dexamethasone /kg bw on day 16 of gestation. Group 2 included five females which received an equivalent volume of saline (1.5 ml) on the same day of gestation. Dams and foetuses were sacrificed at gestation day 21. Some neonatal animals were sacrificed on days 4 or 15 of

life. Adrenal glands of 20-day old foetuses, 3-day old neonatal rats and 14-day old neonatal rats were investigated with light and electron microscopy and stereological measurements were performed. Special attention was paid to necrotic changes and the presence of macrophages, multinuclear giant cells and lymphocytes. Findings were a decrease of both adrenal weight and volume, volume of the zona glomerulosa plus capsule, and volume of the inner zone, both in foetuses and 3-day-old rats (p-values are <0.05, <0.025 or <0.005). The number, but not the total volume of cells was decreased (p<0.05 or p<0.005). Morphological signs of necrosis were observed. In 14-day-old animals, the degree of atrophic changes in the adrenal cortex was reduced. The data demonstrated that even a single dose applied to pregnant rats during the period critical for the development of the hypothalamo-pituitary-adrenal system in foetuses leads to prominent changes in the structure of the adrenal cortex of the offspring that are partially maintained up to postnatal day 14. The changes suggest that cortical function was inhibited. Maternal toxicity was not described in the results.[25]

Effects on renal function

No studies were available on effects of oral administration of dexamethasone on renal function.

The effects of prolonged low-dose dexamethasone treatment in early gestation on blood pressure and renal function in adult sheep were investigated by Dodic and colleagues. Pregnant Mereno ewes were treated intravenously with dexamethasone (0.020 mg/kg bw/day) from gestation days 25 to 45 (n=11) or with saline (n=9). Ewes were allowed to lamb and blood pressure and renal function was studied in offspring at 2 years of age. From the results of this study there was no evidence of altered renal function or predisposition to adult hypertension.[16]

Ortiz et al. investigated whether prenatal exposure to dexamethasone adversely affects renal development and predisposes rats to develop renal disease and hypertension in later life. Pregnant Sprague Dawley rats (n=unknown) were given two daily intraperitoneal injections of 0.2 mg/kg bw dexamethasone on gestational days 11 and 12, 13 and 14, 15 and 16, 17 and 18, 19 and 20 or 20 and 21. A control group injected intraperitoneally with vehicle was included. Blood pressure, glomerular number and insulin clearance were measured in offspring at postnatal day 60 or 90. Length of gestation, litter size, body weight and kidney

weight at postnatal day 1 were not affected. In the offspring of dams treated at gestation days 15 and 16 the glomerular number was 30% reduced ($p<0.01$) and the systolic blood pressure was higher ($p<0.05$). After treatment at gestation days 17 and 18 a 20% reduction in the number of glomeruli was measured ($p<0.01$). No effects were seen in other treatment regimens. The results from this study showed that two daily doses of dexamethasone did not produce intrauterine growth retardation and that adult offspring of rats receiving dexamethasone at different times during pregnancy have a reduced number of nephrons and hypertension. Information on maternal toxicity was not provided.[39]

In a second study performed by Ortiz et al. the same experimental method was used. Pregnant rats (number and strain not reported) were treated intraperitoneally twice daily with 0 or 0.2 mg/kg bw dexamethasone on gestational days 11 and 12, 13 and 14, 15 and 16, 17 and 18, or 19 and 20. In this study the effect on neonates and the long-term effect at 6 to 9 months of age was measured. In the offspring of dams treated at gestation days 15 and 16 the glomerular number was 20% reduced at 6 to 9 months of age ($p<0.05$). This was similar to the reduction observed at 3 weeks of age. After treatment at gestation days 17 and 18 a 17% reduction in the number of glomeruli was measured at 6 months of age ($p<0.05$). After treatment at days 13 and 14, 15 and 16, or 17 and 18 of gestation blood pressure was elevated at 6 months of age ($p<0.05$). The day 13-14 group did not show a reduced glomerular number. Thus, prenatal treatment in rats at specific points during gestation resulted in a reduction in glomerular number, glomerulosclerosis, and hypertension. Hypertension was observed in animals that had a reduction in glomeruli as well as animals that did not have a reduction in glomerular number. Information on maternal toxicity was not provided.[40]

Slotkin et al. investigated the effect of prenatal dexamethasone treatment on the development of the neonatal heart (see earlier section) and kidney. Pregnant Sprague Dawley rats (n=unknown) were treated subcutaneously with 0.2 or 0.8 mg dexamethasone phosphate/kg bw/day on gestational days 17, 18 and 19. Controls received equivalent volumes of saline vehicle (1 ml/kg). Maternal weight gain was recorded and dams were allowed to litter. Pups were randomized and redistributed to nursing dams within the treatment group. At autopsy at weaning the body weight, kidney weight and three biochemical markers of cell development in the kidney were assessed: DNA content as an index of total cell numbers, DNA content per gram of tissue as an index of cell packing density and protein/DNA ratio as an index of relative cell size. Body and kidney weights

were lower (p<0.01). DNA content of the kidney was diminished (p<0.01). Cell packing density was increased (p<0.01 or p<0.05, depending on the dose of dexamethasone) and cells were enlarged, after an initial decrease in size (p<0.01). It was concluded from the results of this study that foetal exposure to dexamethasone causes widespread inhibition of cell proliferation with disruption of parameters of cell differentiation and growth in the kidney. Dexamethasone slowed maternal weight gain (data not shown).[47]

Tain et al. investigated the effect of dexamethasone on blood pressure and kidney. The purpose of the study was to examine whether maternal melatonin administration could attenuate the effects of dexamethasone. Sprague Dawley rats were injected subcutaneously with 0 or 0.1 mg/kg/day of dexamethasone on gestational days 16-22. Male pups (n=12/group) were examined and sacrificed at 16 weeks of age. Blood pressure, kidney histology and expression of a range of genes relevant to kidney development were investigated. Hypertension and reduced nephron numbers were observed (p<0.05). No changes were found in kidney expression of genes involved in apoptosis or nephrogenesis, except for a gene coding for one of the class I histone deacetylases. Some genes of the renin-angiotensin system playing a part in blood pressure control were upregulated (p<0.05). Maternal toxicity was not described in the results.[51]

Tain et al. investigated the effect of dexamethasone on blood pressure and kidney once more, to examine whether maternal citrulline administration could prevent the effects of dexamethasone. Sprague Dawley rats were injected subcutaneously with 0 or 0.2 mg/kg/day of dexamethasone on gestational days 15 and 16. Male pups (n=8-10/group) were examined and sacrificed at 16 weeks of age. Blood pressure and expression of a range of genes relevant to kidney development were investigated. Hypertension was observed (p<0.05). Various genes involved in apoptosis or nephrogenesis were upregulated (p<0.05). Maternal toxicity was not described in the results.[52]

Rogers et al. examined the effect of dexamethasone on blood pressure and kidney. Pregnant Sprague Dawley rats were injected subcutaneously with 0.6 mg/kg/day of dexamethasone on gestational days 16-20. Controls received vehicle. Litters were standardized to 10-12 pups per litter at birth. Both male and female offspring had reduced birth weights (p<0.05). They also had increased blood pressure at 7 weeks (males only), 37 weeks (females only, males not examined) and 65 weeks of age (p<0.05). Males had reduced nephron counts and increased glucocorticoid receptor gene expression in their kidneys (p<0.05);

female kidneys were not examined. Maternal weight gain during pregnancy (gestational days 19-21) was reduced ($p<0.05$).[43]

Effects on skeletal development

No studies were available on effects of oral administration of dexamethasone on skeletal development.

Pregnant Wistar rats (n=5) were treated intramuscularly with 0.1 mg/kg during gestation at days 9, 11 and 13 (i.e. the sensitive period of early foetal brain development) by Swolin-Eide and colleagues. Five control animals were injected intramuscularly with saline. Blood samples were collected 4 h after injection, to determine corticosterone concentrations. The dose level of 0.1 mg/kg was supposed to suppress corticosterone. Litters were weighed at birth and litters were adjusted to the same ratio of male/female pups. Six male and six female pups were killed at 6 weeks, the remaining males at 10 weeks of age and the remaining females at 12 weeks of age. Body weight, food intake, tissue weights, bone measurements and hormone analysis were performed. Dexamethasone did not induce maternal toxicity. Male pups showed transient increases in crown-rump length and tibia and femur lengths at 3-6 weeks of age ($p<0.05$ or <0.001). Cortical bone dimensions were altered in 12-week old females ($p<0.05$ or <0.001). Areal bone mineral densities of the long bones and the spine were unchanged in both male and female offspring.[50]

Effects on hearing

No studies were available on effects of oral administration of dexamethasone on hearing.

The effect of dexamethasone injected subcutaneously to pregnant Wistar rats (n=20-22) at a dose of 0.1 mg/kg/day during gestation days 14-21 was investigated by Hougaard and colleagues. Control animals received vehicle. The dose level and exposure period were based on reports of increased noise-induced hearing loss in offspring of rat dams treated during the last week of gestation with a different route of exposure (Canlon et al.[7]). Females were allowed to litter and in a subset of the male offspring, hearing was measured before, one day after, and one month after exposure to noise, to assess noise-induced hearing loss. The body weight gain during pregnancy of dams treated with dexamethasone was lower from gestation day 19 onwards ($p<0.001$). The number of live pups per

litter and the pup weight at postnatal day 3 (day 0 not reported) were lower than in the control group ($p<0.05$ and $p<0.001$, respectively). At weaning the body weight had returned to normal (data not shown). With respect to hearing loss, these data do not support the previous report of Canlon et al.[7], since the prenatal exposure to dexamethasone was not associated with enhanced noise-induced hearing loss compared to controls.[24]

Canlon et al. studied the effect of intraperitoneally injected dexamethasone (0.1 mg/kg bw) or vehicle once daily in rats from gestation day 14 until parturition. Auditory brain stem responses, the effects of moderate and intense noise exposure, the percentage of apoptotic nuclei after acoustic trauma and the effects of free radicals on acoustic exposure were measured. Prenatal treatment with dexamethasone increased the susceptibility of the inner ear to acoustic noise trauma in adult life. This acoustic trauma was reduced after treatment with a free radical scavenger. Information on maternal toxicity was not provided.[7]

Various effects measured in nonhuman primates

Oral administration

De Vries et al. studied the effect of prenatal dexamethasone exposure on the cardiometabolic and hypothalamic-pituitary-adrenal axis function. African vervet monkeys (*Chlorocebus aethiops*) were exposed to dexamethasone by diet at dosages of 0.050, 0.120 or 0.200 mg/kg bw/d from mid-gestation (gestational day not mentioned) up to birth (n=10/group). A control group was included and maternal parameters (weight, urine volume, blood pressure, plasma electrolytes) were measured. Monkeys were allowed to litter. Weight, head circumference, head length, biparietal diameter, crown-heel length, crown-rump length, tibia length, forearm length and hip width were measured at birth and at 2, 4, 6, 8, and 12 months of age. In addition motor function, ponderal index (weight/height^3), glucose tolerance test, dexamethasone suppression test, blood pressure measurements and 24-hour urine collections were performed and after necropsy organ weights, liver activity and pancreas histology were determined and investigated. Prenatal exposure to the tested, low doses of dexamethasone had no effect on maternal parameters and foetal birth weight. A disordered glucose-insulin homeostasis ($p<0.02$ or $p=0.051$, depending on dexamethasone dose), reduced pancreatic β cell mass ($p<0.0001$), and elevated blood pressure ($p<0.04$) and cortisol levels ($p<0.05$) in juvenile offspring were observed.[13]

Coe et al. summarized the developmental consequences of prenatal dexamethasone treatment in nonhuman primates. A review of literature in primates dating from 1977 to 2005 was undertaken. Most studies were performed with Rhesus monkeys (*Macaca mulatta*); in some studies *Macaca nemestrina* or *Papio* monkeys were used. Initiation of prenatal exposure varied from gestation days 120 to 143 with a duration of 2 to 42 days. Exposure typically involved intramuscular injections at different dose levels. Duration of treatment varied from 1 day to 6 weeks, with a typical exposure lasting 2 days. Across studies dosages varied from 0.1 to 15 mg/kg maternal bw/day. Endocrine physiology, foetal, placental and uterine physiology, physical growth, hepatic and pancreatic effects, lung function and brain alterations and immune consequences were investigated. The most prominent effects observed were:

- reduced cortisol levels in maternal blood (with a rapid recovery, returning to normal levels within 1-2 days after treatment)
- dexamethasone rapidly crosses the placenta (not bound to transporter proteins, not easily converted to cortisol by placental enzymes)
- transient increase in maternal and foetal blood pressure
- post-treatment effects on foetal adrenal glands (smaller) and cortisol levels in umbilical cord blood (reduced)
- reduced foetal body size and smaller head circumferences (with no effect on length of long limb bones)
- although airway functioning is usually improved by prenatal dexamethasone treatment, it appeared that very specific regimens are required (in this species 0.5 mg/kg bw for 4 times at 12 h intervals prior to delivery).
- reduced total absolute brain and cerebellum weights at preterm delivery and delivery at term
- morphological changes in the hippocampus.

Some physiological effects were found at the lowest concentration (suppression of adrenal activity), but more serious disturbances of growth and neural development occurred at dosages of > 2 mg/kg bw/day.[10]

2.4 Conclusions

2.4.1 Fertility

No human studies on fertility effects of dexamethasone were available.

Animal studies with respect to fertility were performed with small numbers of male or female animals from a variety of species.[5,18,20] The findings in males

were limited to sperm changes, such as a reduced sperm volume[18,20] and effects on sperm morphology[5,18]. Some of these effects diminished after a recovery period of 6 weeks. In female fertility studies[12,32] effects on hormone levels and ovulation were observed. Neither mating behaviour, nor pregnancy outcomes were investigated in animals exposed during adulthood. The Committee interprets the effects on sexual behaviour of male animals that had been exposed in utero to be related to developmental alterations rather than a direct effect on fertility.[23] Thus, functional fertility data are considered not to be available. Therefore, the Committee proposes not to classify dexamethasone for fertility due to a lack of appropriate data.

2.4.2 *Developmental toxicity*

Studies on the teratogenic potential of dexamethasone in humans included both prospective and retrospective ones.[1,9,11,31,35,45] Confounding by indication did not seem to play a role. Effects of limited significance, on foetal heart rate and renal resistance, were found in some of the studies.[1,11,35] It is uncertain whether these treatment-related effects are adverse. Together, the human studies do not allow classification of dexamethasone for developmental toxicity due to a lack of appropriate data.

A large number of animal studies with dexamethasone has been carried out. These experiments involve various animal species (nonhuman primates, rats, mice and sheep) and routes of administration (oral, intraperitoneal, subcutaneous, intravenous, intramuscular). They show a range of pre- and postnatal developmental effects, including cardiovascular and neurodevelopmental effects, as well as effects on the immune and endocrine systems, renal function, skeletal development and hearing.

The Committee considers the studies with oral administration as the most relevant ones: two studies in nonhuman primates and three in rats.[6,13,17,21,22] The primate studies demonstrate neurodevelopmental effects and effects on glucose-insulin and cardiovascular systems, without any evidence of maternal toxicity.[13,21] The rat studies also show neurodevelopmental effects.[6,17,22] In one of those studies maternal toxicity was detected[6], in the other ones no information on maternal toxicity was provided[17,22].

Some non-oral studies provide supporting evidence for developmental toxicity of dexamethasone. Among these are three studies in nonhuman primates with intramuscular administration.[27,55,56] The nature of the morphological effects in one of the studies[27], together with the neurodevelopmental effects seen in the

other ones[55,56], is suggestive for effects on the offspring, although a role of maternal toxicity cannot be ruled out. The other studies providing supporting evidence are three rat studies with subcutaneous or intramuscular administration in which maternal toxicity was absent.[29,36,50] They show neurodevelopmental and skeletal effects.

In some of the non-oral studies in which developmental toxicity was observed, that were carried out in rats, mice or sheep, maternal toxicity was reported to be present.[8,23,24,43,47,48] In the remaining studies no information on maternal toxicity was provided.[2-4,7,15,19,26,30,33,37,39-42,44,46,49,51-53,59]

Rat and mouse are common species for toxicity testing of substances. However, their relevance as predictive models for humans can be questioned in the case of dexamethasone, because rodents are typically viewed as “corticoid-sensitive” species, and their newborn pups are extremely immature at birth. The development of the rat kidney for example, is significantly different from that of the human kidney. Nephrogenesis ends by about 34 weeks of gestation in the human, but rats continue to form new nephrons until approximately 1 week after birth.[40] Although rodent studies contribute to the evidence on the developmental toxicity of dexamethasone, the crucial studies include two well-performed oral studies in primates. Moreover, similar types of adverse effect have been reported in primates and rodents. Therefore, the Committee takes both primate and rodent studies into account.

In conclusion, pre- and postnatal developmental effects have been observed in several studies in nonhuman primates and rodents in the absence of maternal toxicity. Consequently, the Committee proposes to classify dexamethasone in category 1B (presumed human reproductive toxicant) and to label it H360D (may damage the unborn child).

2.4.3 Lactation

No human or animal data were available regarding the excretion of dexamethasone in breast milk or the effects of exposure to dexamethasone on infants during the lactation period.

Proposed classification for fertility

A lack of appropriate data precludes assessment of dexamethasone for fertility.

Proposed classification for developmental toxicity

Category 1B, H360D.

Proposed labelling for effects on or via lactation

A lack of appropriate data precludes assessment of dexamethasone for lactation.

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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

- D. Lindhout, *chairman*
  Emeritus Professor of Medical Genetics, Paediatrician (not practising), Clinical Geneticist, University Medical Center, Utrecht
- N. Roeleveld
  Reproductive Epidemiologist, Radboud university medical center, Nijmegen
- J.G. Theuns-van Vliet
  Reproductive Toxicologist, Triskelion BV, Zeist
- T.G.M. Vrijkotte
  Epidemiologist, AMC, Amsterdam
- D.H. Waalkens-Berendsen
  Reproductive Toxicologist, Zeist
- P.J.J.M. Weterings
  Toxicologist, Weterings Consultancy BV, Rosmalen
- A.H. Piersma, *structurally consulted expert*
  Professor of Reproductive and Developmental Toxicology, Utrecht University, and National Institute of Public Health and the Environment, Bilthoven
- P.W. van Vliet, *scientific secretary*
  Health Council of the Netherlands, Den Haag

The first draft of this report was prepared by M.M. Tegelenbosch-Schouten MSc (TNO Quality of Life, Zeist, The Netherlands) 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, persons 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 Health Council to assess whether or not someone can become a member. An expert who has no financial but another clearly definable interest, can become a member under the restriction that he will not be involved in the debate on the subject to which his interest relates. If a person's interest is not clearly definable, he can sometimes be consulted as an expert. Experts working for a ministry or governmental organisation can be structurally consulted. 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 *Dexamethasone*
Your reference : DGV/BMO/U-932542
Our reference : U-1024028/EvV/cn/543-R16
Enclosure(s) : 1
Date : October 18, 2016

Dear Minister,

I hereby submit the advisory report on the effects of dexamethasone 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. It concerns 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 Public Health.

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

Yours sincerely,
(signed)
Professor J.L. Severens
Vice President

Annex C

Comments on the public draft

A draft of the present report was released in 2015 for public review. The following persons and organisation have commented on the draft document:

- T.J. Lentz, A.F. Hubbs. National Institute for Occupational Safety and Health (NIOSH), Cincinnati, OH, USA.

The comments received, 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 postnatal 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 does 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 animals.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Developmental toxicity	Remarks
De Greef (1987)	Wistar rats	Ovarian activity was measured with different methods	0 mg/rat (n=unknown), 0.008 mg/kg bw (n=6), 0.03 mg/kg bw (n=7), or 0.1 mg/kg bw (n=14) subcutaneously	Not described	Induction of prolactin-dependent luteal activity and large number of eggs after high doses	
Maciel et al. (2001)	Non-lactating Holstein cows (n=6) and controls (n=5)	Cows were treated from one day after ovulation until the first dominant follicle stopped growing.	0 or 0.044 mg/kg bw intramuscularly	No effect on bw. Increase systemic glucose and insulin (p<0.05). Decrease IGF-I and II (p<0.05)	Decrease plasma progesterone (p<0.10). No effect on the growth rate of dominant follicles	

Hatamoto et al. (2006)	Male adult Rottweiler dogs (n=18)	Dogs were treated for 7 days. Semen collection twice weekly for 14 weeks. Blood collection once a week	0 or 0.01 mg/kg bw/day intramuscularly	No effect on body weight or food intake (data not reported)	Reduced ejaculate volumes (p=0.0002). Increased thiobarbituric acid-reactive substances (p<0.0001). Increased number of sperm per ejaculate (p=0.0122), increased percentage of abnormal sperm (p=0.0002), increased seminal plasma lipid peroxidation (p=0.0264). Elevated activity of one of two antioxidant enzymes (p=0.0264)	
Gür et al. (2005)	Akkaraman rams (n=7/ group)	Rams were treated for 2 days. After last dose blood and semen samples taken after 1, 2, 4, 24, 48, 72 and 96 hrs	0 or 0.25 mg/kg bw intramuscularly	Not described	Increased hyaluronidase activity in serum (p<0.01, except after 1 hr) and sperm (p<0.001-0.05) Decreased sperm concentration (p<0.001, except at 96 hrs), semen volume (p<0.05) and sperm motility (p<0.05, except at 72 and 96 hrs). No effect on rate of abnormal spermatozoa	
Barth et al. (1994)	Mixed Bos Taurus Bulls (n=8) and controls (n=4)	Bulls were treated for 7 days. Sperm abnormalities were analysed	0 or 20 mg/bull intramuscularly	Not described	Lower basal, peak episodic and testosterone concentrations in blood (p<0.05). Concentration in testis tissue unchanged. Increase in sperm defects such as detached heads, midpiece defects and nuclear vacuoles (no statistics mentioned)	Recovery to pre-treatment levels of sperm defects after 6 wks

Abbreviations: bw=body weight; hr(s)=hour(s); n=number; wk(s)=week(s).

Table 2 Developmental toxicity studies in animals.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Developmental toxicity
Teratogenic potential					
Jerome and Hendrikx (1988)	Pregnant Rhesus monkeys (n=12). Sacrifice on GD100	Monkeys were treated at different days during GD 23-49	0, 1.0 or 10.0 mg/kg bw intramuscularly	Not described	No effect on body weight; brain weight reduced at 10.0 mg/kg (p<0.05); cranial fossa diameter reduced at 10.0 mg/kg (p<0.05); cranium malformations (2/6 animals affected at 1.0 mg/kg, 4/6 at 10.0 mg/kg)
Sauerbier et al. (1986)	NMRI Mice (n=12/group). and control (n=10) Sacrifice on GD 18	Mice were treated on GD13 at 07:00, 13:00, 19:00 or 01:00	10 or 50 mg/kg bw intramuscularly	Not described	Cleft palate and resorptions at all dose levels. Effect dose-dependent (p-values not mentioned)

Ballard et al. (1977)	Pregnant CD-1 mice (n=unknown). Sacrifice on GD 18	Mice were treated five times daily during GD10-13	Therapeutic concentration (not specified) or vehicle, ocular application	Not described	Increased incidence of cleft palate. Increased incidence of sex organ abnormalities
Cardiovascular effects					
Torres et al. (1997)	Pregnant rats (n=8/group)	Rats were treated from GD17 for 4-5 days. Foetal heart was investigated	0.0048 mg/day subcutaneously, (slow release pellet); controls unmanipulated	Not described	Higher heart/body weight ratio (p<0.05) and proliferative index (p<0.05). Decrease in ECM-content (p<0.05) and α-MHC mRNA (p<0.05)
Slotkin et al. (1991)	Pregnant Sprague Dawley rats (n=unknown)	Rats were treated on GD 17, 18 and 19. Foetal heart and kidney were investigated	0, 0.2 or 0.8 mg/kg bw/day subcutaneously	Maternal body weight gain slower, but specifics not reported	Lower body weight (p<0.01); lower heart weight, DNA-content, cell packing density (all p<0.01) and enlarged cells (p<0.01)
Dodic et al. (2001)	Pregnant treated ewes (n= unknown) and control (n=6)	Ewes were treated for 2 days at 27 days of gestation. Allowed to litter and surgery at PND 50	11.5 mg/day intraveneously	Not described	Hypertension (p<0.01), left ventricular hypertrophy (p<0.05), and reduced cardiac functional reserve (p<0.05) in adult life
Molnar et. al. (2002)	Pregnant ewes (n=7) and controls (n=7)	Ewes were administered as three weekly courses of four intramuscularly injections at 12 h-intervals	0 or 2 mg/ewe intramuscularly	Not described	Body weight lower (p<0.01). Microvessel dysfunction: enhanced endothelin-induced vasoconstriction (p=0.003 at highest concentration), decreased endothelium-dependent relaxation (p<0.05); normal endothelium-independent relaxation
Quaedackers et al. (2005)	Pregnant Romney/ Suffolk ewes (n=8) and control (n=7)	Ewes were treated with two injections (24 h apart) at GD 103. Allowed to litter and litter monitored for 5 days	2 injections of 0 or 12 mg (24 h apart) intramuscularly	Not described	Transient increase in blood pressure (p<0.05). No effect on vascular resistance
Hai et al. (2002)	Pregnant ewes (n=unknown)	Ewes were treated with single doses (starting GD 104, 105, or 106) or multiple doses (starting GD 76, 84, 91, 98 and 105). Foetuses catheterized at GD 99-101. Foetal arteries investigated	0 or 6 mg/day intramuscularly	Not described	Reduced pup body weight (p<0.05). Biphasic contractions in foetal arteries (p<0.05); Increased relaxation rate in foetal arteries (p<0.05); Increased myosin $LC17_a$ isoform expression in fetal arteries (p<0.05)

Neurodevelopmental effects					
Hauser et al. (2007)	Pregnant Marmoset monkey	Monkeys were treated during early (GD42-48) or late (GD90-96) gestation	0 or 5 mg/kg bw/day orally	No effect on maternal body weight	Early treatment: Increased weight gain in the presence of normal skeletal growth (p<0.05), increased eating (p<0.05), increased sympathetic autonomic nervous system arousal (p<0.05); increased time spent mobile (p<0.05); increased time spent eating (p<0.05), trends toward more solitary play (p=0.053) and tail hair piloerection (p=0.085). Late treatment: no effect
Hauser et al. (2006)	Wister rats (n=14) and controls (n=12)	Rats were treated between GD15-21. Offspring tested for prepulse inhibition (PPI) and latent inhibition (LI)	0 or 0.1 mg/kg/day in drinking water	Effect on maternal body weight not reported. Increased pup-directed behaviour (p=0.029)	Reduced pup birth weight (p<0.05)and adult body weight (p<0.05, male pups only). No effect on PPI and LI
Dupouy et al. (1987)	Wistar rats (n=unknown)	Rats were treated from GD 15-21. Effect on hormones of the hypothalamo-pituitary-adrenal axis measured in hypothalamus, pituitary gland, adrenals and plasma in the offspring	0 or 0.01 mg/ml in drinking water	Not described	Reduction of body weight (p<0.001), severe atrophy of the adrenals (p<0.001), and decreases in adrenal and plasma corticosterone concentrations (p<0.001). Decrease in plasma adrenocorticotrophic hormone (ACTH) concentration and pituitary ACTH content (p<0.001). Decreased corticotrophin-releasing factor hypothalamic content and concentration (p<0.001)
Brabham et al. (2000)	Pregnant Sprague Dawley rats (n=20-24)	Rats were treated daily from GD15 until delivery	0 or 0.0025 mg/ml in the evening water	Maternal body weight showed a smaller increase at GD 15-18 (p<0.001), a larger one at 2 wks after giving birth (p=0.05) and returned to normal levels at 3 wks after giving birth	Restlessness in mothers (p<0.05). Low birth weights and poor weight gain in the offspring (p<0.05). Impaired spatial learning (p<0.05), inability to rapidly terminate adrenocorticoid response to stress (p<0.05) and decreased hippocampal glucocorticoid receptor mRNA expression (p<0.05). Dexamethasone exposed animals reared to vehicle dams had adequate reponses
Carlos et al. (1992)	Pregnant rats (n=unknown)	Rats were treated during GD17, 18 and 19. Pups after weaning investigated for brain defects	0, 0.2 or 0.8 mg/kg bw/day subcutaneously	Maternal weight gain slowed (data not reported)	The forebrain showed persistent elevations of DNA (p<0.01) and reduced protein/DNA (p<0.01), indicative of replacement of neurons with glia

Nagano et al. (2008)	Pregnant Sprague Dawley rats (n=unknown)	Rats were treated during GD16-21. Neuroendocrinological development pups investigated.	0 or 0.005 mg/kg bw/day subcutaneously	No effect on maternal care during preweaning	Reduced body weight in the offspring at PNW 4, 7 and 10 (p<0.01 or p<0.05). Decreased corticotropin-releasing factor mRNA in hypothalamus (p<0.01), disturbed plasma corticosterone response to restraint stress at PNW 4 (p<0.01), increased anxiety-like behaviour (p<0.05), decreased glucocorticoid receptor expression amygdale at PNW7 (p<0.01) and PW10 (p<0.001). Neuroendocrinological development altered in pups
Slotkin et al. (2006)	Pregnant Sprague Dawley rats (n=unknown)	Dams were treated during GD17-19. Pups were treated during PND1-3 or 7-9. Pups were sacrificed on PND60 and brain was investigated	Dams; 0, 0.05, 0.2 or 0.8 mg/kg bw/day subcutaneously. Pups; 0, 0.05, 0.2 or 0.8 mg/kg bw/day	Not described	5HT receptor binding and 5HT transporter binding increased in the cerebral cortex during gestation and after birth (p<0.0006 and p<0.0001, respectively). 5HT concentration in hippocampus and cerebral cortex increased after birth (p<0.002), then decreased till levels below normal (p<0.0006). 5HT turnover unchanged. DA concentration in cerebral cortex already increased during gestation (p<0.03), decreased till levels below normal at PND 1-3 (p<0.0001) and returned to normal by PND 7-9. DA turnover in cerebral cortex unchanged
Kreider et al. (2005)	Pregnant Sprague Dawley rats (n=unknown)	Rats were treated on GD17, 18 and 19. Behavioural tests at PND31, 32, and 33	0 or 0.2 mg/kg bw/day subcutaneously	No effect on maternal weight gain during pregnancy	No effect on gestation index, litter size, viability and sex ratio. Normal sex differences in locomotor activity ablated by lowering values in females to levels of males (p<0.0003). Habituation of activity similarly impaired in females to match profile of males (p<0.01), while males showed a partially feminized pattern of habituation (p<0.004). Delayed learning in males (p<0.08), while improving performance in females in the 8-arm radial maze (p<0.04). Values of hippocampal [3H]hemicholinium-3 binding increased in males to levels of females (p<0.007)

Somm et al. (2014)	Pregnant Sprague Dawley OFA rats	Rats were treated on GD14-21. Growth investigated at PND1 and -21, and brain metabolism at PND7	0 or 0.1 mg/kg bw/day subcutaneously	Maternal body weight throughout gestation and weight gain during the third week decreased (p<0.001 and p<0.01, respectively)	Litter size smaller (p<0.001). Perinatal body weight diminished at PND1 and 21 (p<0.001). Lower concentrations of several brain metabolites and lower hippocampal gene expression at PND7 (p<0.05)
Kutzler et al. (2004)	Pregnant ewes (n=9) and control (n=11) used for prenatal development (cohort 1) ---------- n= 8 (treated and control cohort 2) or n=13 (treated and control cohort 3) used for postnatal development	Ewes were treated on GD103-104, 110-111 and 117-118	0 or 8 mg per ewe intramuscularly (4 doses of 2 mg per ewe within 48 hrs; a dose corresponds to 29-33 µg/kg bw)	Not described	Decreased foetal body weight (p<0.05). Prolonged gestation length (p<0.05). Normal birth weight. Decreased newborn brain weight (p<0.05) and biparietal diameter (p<0.05). Various other postnatal growth measures normal
Uno et al. (1990)	Rhesus Monkeys (n=unknown)	Monkeys were treated, Single; GD132 or Multiple; GD132 and 133. Caesarean section GD135 for acute effects or GD162 for chronic effects. Brain investigated	Single; 0, 0.5, 5 or 10 mg/kg bw. Multiple; 0 (4x 0), 0.5 (4x 0.125), 5 (4x 1.25) or 10 (4x 2.5 mg/kg). Intramuscularly	Not described	Irreversible deficit and poorly differentiated hippocampal and cortal neurons
Moritz et al. (2002)	Pregnant merino ewes (n=18) and control (n=15)	Ewes were infused (route not mentioned) for 20 days. Ewes were killed on GD45. A second cohort was killed on GD130. In a third cohort the lambs were studied at 2 months of age	0 or 0.020 mg/kg bw/day	Not described	No effects

Uno et al. (1994)	Rhesus monkeys (n=8) and control (n=3)	Monkeys were treated on GD132 and 133. In infants cortisol levels and brain was investigated at 20 months of age	0 or 5 mg/kg bw/day (route unknown)	Not described	Irreversible reduction of hippocampal size and high plasma cortisol and post stress levels (statistics not reported)
Effects on immune and endocrine system					
Negić et al. (2007)	Pregnant Wistar females. Sacrifice on GD19 (n=8) and on GD21 (n=8) and two control groups (n=8/group)	Rats were treated on GD16-19. Autopsy at GD 19 or GD21. Foetal pituitary investigated	0, 1.0 or 0.5 mg/kg bw/day subcutaneously	Not described	Marked reduction of morphometric parameters of LH and FSH (p<0.05). Size of LH and FSH cells on day 19 of pregnancy affected. Reversible decrease in cell volume (normalization of LH and FSH cell function on day 21 of pregnancy). No prolonged effects of dexamethasone treatment during pregnancy on LH and FSH cells
Stojanoski et al. (2006)	Wistar rats (n=unknown)	Rats were treated on GD16, 17 and 18. Autopsy at GD19 and pituitary and adrenal glands investigated	1.0 (GD 16), 0.5 (GD 17 and 18) mg/kg bw/day subcutaneously; Controls received vehicle	Not described	Pituitary gland: smaller number and volume of ACTH cells (p<0.05). Adrenal organs: reduced proliferative activity of cortical cells of zona glomerulosa (p<0.05)
Page et al. (2001)	Spraque Dawley rats (n=9/group)	Rats were treated on GD14-19. Allowed to litter and immature and adult males investigated	0 or 0.1 mg/kg bw/day subcutaneously	Not described	Immature male offspring showed decreased serum testosterone (p<0.05) and testosterone production in Leydig cells (p<0.001). Serum ACTH and corticosterone were reduced (p<0.001). Mature male offspring demonstrated higher serum ACTH and corticosterone (p<0.001), and higher serum testosterone (p<0.05) and testosterone production in Leydig cells (p<0.001)
Hristić et al. (1997)	Pregnant Wistar rats (n=10/group)	Rats were treated during 5 days, started on GD16. First cohort killed after 24 h after the last injection. Second and third cohort killed on PND3 and 14. Structure and function of adrenal gland investigated	0 or 0.3 mg/kg bw/day subcutaneously	Not described	Decreased volume of foetal adrenal gland (p<0.005), its zona glomerulosa plus capsula (p<0.005) and its inner zone (p<0.005), atrophic changes in the gland, reduction of the volume and total number of adrenocortical cells (p<0.05 and p<0.005, respectively). These morphometric changes were found in 3- and 14-day-old pups

Holson et al. (1995)	Spraque Dawley derived CD rats (n=4/ group)	Rats were treated on GD14 to 21. Sexual behaviour offspring measured	0.1 mg/kg bw/day subcutaneously	Considerable reduction in maternal weight gain (p<0.0001)	Reduced pup birth weight (effect still seen at PND85) (p<0.01). Male adrenal weight at birth reduced (p<0.01); male anogenital distance reduced (p<0.05). Adult male sexual performance (number of ejaculations per trial and latency to first ejaculation after cohabitation with oestrous females (p<0.05)) affected, and lordosis quotient following exposure of castrated hormone-primed males to stud males affected (p<0.05)
Bakker et al. (1995)	Wistar rats (n=7) and two control groups (n=6)	Rats were treated on GD 17 and 19. Effects on thymus, spleen, hypothalamus and blood plasma of offspring were examined at PND 1, 7 and 20	0 or 400 µg of dexamethasone -21-phosphate intra-peritoneally, or no injection	Not described	Reduced pup birth weight compared to either control group, on PND 1. Decreased T cell numbers in thymus and spleen on PND 1 (p<0.05 or <0.005). T cell numbers in spleen were reduced at all neonatal ages studied (PND 1, 7 and 20; p<0.05 or <0.005). Altered pattern of neonatal changes in peptide expresion in corticotropin-releasing hormone neurons. No effects on adrenocorticotrophic hormone and corticosterone levels in plasma
Bakker et al. (1998)	Wistar rats (3 animals per group)	Rats were treated on GD 17 and 19. Effects on corticosterone response to a mild stressor and on humoral and cellular immune responsiveness were measured	0 or 1.2 mg/kg bw dexamethasone -21-phosphate intra-peritoneally, or no injection	Not described	No altered corticosteroid response to a novel environment at 20 days of age, as compared to either control group. No effects on immunoglobulin-2a production after immunization with conjugated pneumococcal polysaccharide at 6 wks of age. Oxazolone-induced contact hypersensitivity responses at 8 wks of age, was lower compared to saline controls (p<0.005), but not different from untreated controls
Yu et al. (2014)	Sprague-Dawley rats (6-8 animals per group)	Rat were treated at gestational days 14-20. Male offspring were killed at day 7 or day 120 after birth. Spleens were collected for immune analysis	0 or 0.1 mg/kg bw/day dexamethasone intra-peritoneally	Not described	Three out of five inflammation mediators decreased at day 7, one of them, matrix metalloproteinase-9, still at day 120 (p<0.05). Upon concanavalin-A stimulation prenatal dexamethasone treatment reduced tumour necrosis factor-α production (p<0.05), but not interferon-γ production in spleen cells at day 120

Hristić et al. (1995)	Wistar rats (n=5/group)	Rats were treated on GD16. Dams and foetuses sacrificed on GD21 en some neonatal animals sacrificed at PND 4 or 15. Adrenal glands investigated	Single dose of 0 or 1.5 mg/kg bw (route unknown)	Not described	Decreased adrenal weight and volume, volume of the zona glomerulosa plus capsule, and volume of the inner zone, both in foetuses and 3-day-old rats (p-values <0.05, <0.025 or <0.005). Decreased number, but not total volume of cells (p<0.05 or p<0.005). Morphological signs of necrosis. In 14-day-old animals, degree of atrophic changes in adrenal cortex reduced
Effects on renal function					
Dodic et al. (2003)	Pregnant Mereno ewes (n=11) and controls (n=9)	Ewes were treated from GD25 to 45. Ewes allowed to lamb and blood pressure and renal function in offspring investigated	0 or 0.002 mg/kg bw/day intravenously	Not described	No evidence of altered renal function or predisposition to adult hypertension
Ortiz et al. (2001)	Pregnant Sprague Dawley rats (n=unknown)	Rats were treated on GD11 and 12, 13 and 14, 15 and 16, 17 and 18, 19 and 20 or 20 and 21. Blood pressure, glomerular number and inulin clearance were measured in offspring at PND60 or 90	two daily intraperitoneal injections of 0 or 0.2 mg/kg bw	Not described	Length of gestation, litter size, body weight and kidney weight at PND1 not affected. In the offspring of dams treated at GD15 and 16 the glomerular number was 30% reduced (p<0.01) and the systolic pressure was higher (p<0.05). After maternal treatment at GD17 and 18 a 20% reduction of glomeruli was measured (p<0.01). No effects in other treatment regimens
Ortiz et al. (2003)	Pregnant rats (n=unknown)	Rats were treated on GD11 and 12, 13 and 14, 15 and 16, 17 and 18, or 19 and 20. Effects on neonates and longterm effects were investigated	Twice daily 0 or 0.2 mg/kg bw intra-peritoneally	Not described	In the offspring of dams treated at GD15 and 16 the glomerular number was 20% reduced at 6 to 9 months of age (p<0.05). This was similar to the reduction at 3 wks of age. After treatment at GD17 and 18 the glomerular number was 17% reduced at 6 months of age (p<0.05). After treatment at days 13 and 14, 15 and 16, or 17 and 18 of gestation blood pressure was elevated at 6 months of age (p<0.05). The GD13-14 group did not show a reduced glomerular number
Slotkin et al. (1991)	Pregnant Sprague Dawley rats (n=unknown)	Rats were treated on GD 17, 18 and 19. Foetal heart and kidney were investigated	0, 0.2 or 0.8 mg/kg bw/day subcutaneously	Maternal body weight gain slower, but specifics not reported	Body and kidney weights lower (p<0.01). DNA content of the kidney diminished (p<0.01). Cell packing density increased (p<0.01 or p<0.05, depending on the dose of dexamethasone) and cells enlarged, after initial decrease in size (p<0.01)

Tain et al. (2014)	Pregnant Sprague Dawley rats (n=unknown)	Rats were treated on GD 16-22. Offspring blood pressure and kidney were investigated at 16 wks of age (n=12/group)	0 or 0.1 mg/kg bw/day subcutaneously	Not described	Hypertension and reduced nephron numbers (p<0.05). No changes in kidney expression of genes involved in apoptosis or nephrogenesis, except for a gene coding for a class I histone deacetylase. Some genes of the renin-angiotensin system upregulated (p<0.05)
Tain et al. (2014a)	Pregnant Sprague Dawley rats (n=unknown)	Rats were treated on GD15-16. Offspring blood pressure and kidney were investigated at 16 wks of age (n=8-10/group)	0 or 0.2 mg/kg bw/day subcutaneously	Not described	Hypertension (p<0.05). Various genes involved in apoptosis or nephrogenesis upregulated (p<0.05)
Rogers et al. (2014)	Pregnant Sprague Dawley rats (n=unknown)	Rats were treated on GD16-20. Offspring blood pressure and kidney were investigated up until 65 wks of age (n=10-12/group)	0 or 0.6 mg/kg bw/day subcutaneously	Maternal weight gain during pregnancy (GD19-21) was reduced (p<0.05)	Both male and female offspring had reduced birth weights (p<0.05). Increased blood pressure at 7 wks (males only), 37 wks (females only, males not examined) and 65 wks of age (p<0.05). Males had reduced nephron counts and increased glucocorticoid receptor gene expression in kidneys (p<0.05); female kidneys not examined
Effects on skeletal function					
Swolin-Eide et al. (2002)	Pregnant Wistar rats (n=5/group)	Rats were treated during GD9, 11 and 13. Allowed to litter and 6 ♂ and 6 ♀ killed at 6 weeks. Remaining ♂♂ at 10 weeks, remaining ♀♀ at 12 weeks of age	0 or 0.1 mg/kg bw/day intramuscularly	Maternal toxicity was not observed	Male pups showed transient increases in crown-rump length and tibia and femur lengths at 3-6 wks of age (p<0.05 or p<0.001). Cortical bone dimensions altered in 12-week old females (p<0.05 or p<0.001). Areal bone mineral densities of long bones and spine unchanged in both male and female offspring
Effects on hearing					
Hougaard et al. (2007)	Pregnant Wistar rats (n=20-22)	Rats were treated during GD14-21, females allowed to litter and hearing loss investigated	0 or 0.1 mg/kg/day subcutaneously	The body weight gain during pregnancy of dams was lower from GD19 onwards (p<0.001)	The number of live pups and the pup weight at PND3 (day 0 not reported) were lower (p<0.05 and p<0.001, respectively). At weaning they had returned to normal (data not shown). No effect on hearing loss
Canlon et al. (2003)		Rats were treated from GD14 until parturition. Females allowed to litter and hearing loss investigated	0 or 0.1 mg/kg bw intra-peritoneally	Not described	Increased susceptibility of the inner ear to acoustic noise trauma in adult life. This acoustic trauma was significantly reduced after treatment with the free radical scavenger

Effects in nonhuman primates					
de Vries et al. (2007)	African vervet monkeys (n=10/group)	Monkeys were treated from mid-gestation (exact day unknown) up to birth	0, 0.05, 0.12 or 0.2 mg/kg bw/d by diet	No effect on maternal parameters	No effect on foetal birth weight. A disordered glucose-insulin homeostasis (p<0.02 or p=0.051, depending on dexamethasone dose), reduced pancreatic β cell mass (p<0.0001), and elevated blood pressure (p<0.04) and cortisol levels (p<0.05)
Coe et al. (2005)	Rhesus monkeys (*Macaca nemestrina* or *Papio* monkeys)	Exposure varied: initiation at GD120 to 143, duration 2 to 42 days	Intramuscular injections at different dose levels, varying from 0.1 mg/kg bw/day to 15 mg/kg bw/day	Reduced maternal cortisol levels and increased maternal blood pressure	Transient increase in foetal blood pressure, post-treatment effects on foetal adrenal glands (smaller) and cortisol levels in umbilical cord blood (reduced), reduced foetal body size and smaller head circumferences, increase in weight of foetal liver, higher glycogen content and elevated blood glucose increased insulin levels, reduced total brain and cerebellum weights at preterm delivery and delivery at term, morphological changes in the hippocampus, decreased thymus and spleen weights and reduced lymphocyte proliferative responses

Abbreviations: bw=body weight; GD=gestation day(s); hr(s)=hour(s); n=number; PND= postnatal day(s); PNW=postnatal week(s); wk(s)=week(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 opinions 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.

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