Health Council of the Netherlands

Hydroxyurea

Evaluation of the effects on reproduction, recommendation for classification

2014/10

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Hydroxyurea*
Uw kenmerk : DGV/MBO/U-932542
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Geachte minister,

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

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

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

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

Met vriendelijke groet,

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

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Hydroxyurea

Evaluation of the effects on reproduction,
recommendation for classification

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

to:

the Minister of Social Affairs and Employment

No. 2014/10, The Hague, April 3, 2014

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

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

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Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad hydroxyureum onder de loep genomen. Hydroxyureum is een geneesmiddel dat wordt gebruikt voor de behandeling van patiënten met chronische myeloïde leukemie, met essentiële thrombocytemie en polycythaemia vera of met sikkelcelanemie. Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Subcommissie Classificatie reproductietoxische stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (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 hydroxyureum komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie om hydroxyureum te classificeren in categorie 1B (*stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting*) en te kenmerken met H360F (*kan de vruchtbaarheid schaden*)

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

Executive summary

In the present report, the Health Council of the Netherlands reviewed hydroxyurea. Hydroxyurea is a drug used in the treatment of patients with chronic myeloid leukaemia, with essential thrombocytosis and polycytaemia vera or with sickle-cell anaemia 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. 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 hydroxyurea, these recommendations are:

- for effects on fertility, the Committee recommends classifying hydroxyurea in category 1B (*presumed human reproductive toxicant*) and labelling with H360F (*may damage fertility*)
- for effects on development, the Committee recommends classifying hydroxyurea 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 hydroxyurea 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 1B and 2) or compound with effects on or via lactation.

1.2 Committee and procedure

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

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

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

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

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

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

1.3 Effects on or via lactation

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

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

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

1.4 Data

Literature searches were conducted in the online databases Current Contents and Medline, starting from 1966 up to September 2012 and by searches on the Internet; an update was performed in TOXNET in June 2013. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted as well as several websites regarding (publications on) toxicology and health. References are divided into literature cited and literature consulted, but not cited.

The Committee describes both 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 is considered.

In the assessment of the potential reproduction toxic effects of hydroxyurea, 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.

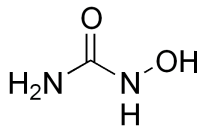
1.6 Final remark

The classification of compounds is based on hazard evaluation only (Niesink et al., 1995)[23], 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

Hydroxyurea

2.1 Introduction

name	:	hydroxyurea
CAS registry number	:	127-07-1
CAS name	:	urea, hydroxy-
synonyms	:	hydroxycarbamide; N-(aminocarbonyl)hydroxylamine; carbamohydroxamic acid; carbamohydroximic acid; carbamoyl oxime; hydroxycarbamine; hydroxylurea
colour and physical state	:	white, crystalline powder
molecular weight	:	76.06
molecular formula	:	$CH_4N_2O_2$
structural formula	:	
melting point	:	141 °C
boiling point	:	decomposes
vapour pressure	:	0.3 Pa (at 25 °C; estimated)
Log $P_{octanol/water}$	:	-1.80
solubility	:	very soluble in water; soluble in hot alcohol; insoluble in ethanol, benzene
use	:	In the Netherlands, hydroxyurea is registered for treatment of patients with chronic myeloid leukaemia, with essential thrombocytosis and polycytaemia vera, and with sickle-cell anaemia[9]; the starting doses: for sickle-cell anaemia 15 mg/kg bw/day usually followed by maintenance doses of 15-30 mg/kg bw/day; for chronic myeloid leukaemia 40 mg/kg; for essential thrombocytosis 15 mg/kg bw/day; for polycytaemia vera 15-20 mg/kg bw/day; in the latter three cases, maintenance doses are adjusted based on haematologic values.[10]

general toxicity	:	In humans, the major treatment-limiting and dose-related adverse effect of hydroxyurea is suppression of the bone marrow, resulting in neutropenia, myelosuppression, thrombocytopenia and anaemia. Hydroxyurea is cytotoxic.[17]
mechanism	:	Hydroxyurea is cytostatic by inhibition of ribonucleotide reductase, an enzyme important in creating deoxynucleosides for DNA replication in proliferating cells, which results in S-phase cytotoxicity. An increased sensitivity to radiation therapy is thought to be due to the arrest of malignant cells in G_1 phase. In the treatment of sickle cell disease, hydroxyurea induces the production of foetal haemoglobin, which results in prevention of the formation of sickle-shaped red blood cells. Additionally, it can reduce the frequency of painful crises by improving the movement of the sickle-shaped red blood cells through the blood vessels and the need for blood transfusions.[17]
kinetics	:	In humans, hydroxyurea is well absorbed after oral dosing and peak plasma levels are detected after 1-4 hours. Hydroxyurea is distributed in a volume that is similar to total body water, is concentrated in blood cells, such as erythrocytes and leukocytes, and it enters the cerebrospinal fluid and breast milk. Hydroxyurea is thought to be excreted via the hepatic metabolism and via renal excretion (unchanged hydroxyurea). In animals, hydroxyurea is well absorbed throughout the body after oral or intraperitoneal dosing. Hydroxyurea or its metabolites are distributed to the embryo in pregnant animals. The main metabolite of hydroxyurea is urea, which is present in the urine. The main route of elimination is by urinary excretion and occurs rapidly, with a half-life of <0.5 hours in rats and mice.[17]

Data from HSDB[22] unless otherwise noted.

2.2 Human studies

2.2.1 Fertility studies

Male fertility

In a retrospective multicentre study, Berthaut et al. (2008) studied the potential effects of hydroxyurea treatment on sperm parameters of patients with sickle cell disease. Semen samples were collected and analysed according to WHO criteria; parameters assessed included ejaculate volume, sperm concentration, total sperm count, motility, vitality and morphology. In 76 samples obtained from 34 patients before treatment, the percentages of abnormal values were 26%, 37%, 40%, 84%, 64% and 43%, respectively. In only three patients, all parameters were normal. In six samples obtained from five patients during treatment, percentages of abnormal values were 50%, 100%, 100%, 80%, 67% and 50%, respectively. All patients had abnormal parameters but none had azoospermia. In 26 samples obtained from eight patients after treatment, percentages of abnormal values were 36%, 76%, 68%, 88%, 75% and 77%, respectively. Seven patients had abnormal parameters and one patient was azoospermic, four years after treatment.[6]

In a few case reports, effects of hydroxyurea treatment on sperm parameters of patients with sickle cell disease (n=7), polycythaemia rubra vera (n=1) or thrombocythaemia (n=1) were described based on analyses of semen samples obtained during and after treatment. Data from semen samples taken before treatment were not available. Parameters assessed in samples obtained during treatment were generally impaired and did not always improve after cessation of therapy.[13,18,19]

Female fertility

There are no data on the fertility of women after treatment with hydroxyurea.

2.2.2 *Developmental toxicity studies*

In a clinical trial on the effect of hydroxyurea on reduction of painful crises in 153 female and 146 male patients with sickle cell anaemia, several pregnancies occurred. These patients were taken off further treatment, but the pregnancy outcomes were followed for up to 17 years in a descriptive manner (no statistics were performed). Out of a total of 52 pregnancy outcomes reported for female participants, six had known hydroxyurea usage at conception and sometime during gestation resulting in three elective abortions for unknown reasons, one full-term live birth, one premature live birth and one miscarriage. Three pregnancies had probable hydroxyurea usage throughout the entire pregnancy and resulted in two elective abortions and one miscarriage. Out of a total of 42 pregnancy outcomes reported for partners of male participants, ten had known hydroxyurea usage during conception resulting in two elective abortions, four full-term live births, one live birth at gestational age >37 weeks, one premature live birth and two miscarriages.[4]

Thauvin-Robinet et al. (2001) evaluated data of pregnancy outcome among 31 women treated with hydroxyurea (dose ranged from 0.5-6 g/day) for either essential thrombocythaemia (n=22), chronic myeloid leukaemia (n=6), chronic myeloid splenomegaly (n=2) or sickle cell disease (n=1), of which three received hydroxyurea throughout pregnancy, 22 during the first trimester, two during the first and second trimester and two during the third trimester (of the remaining two, the exposure time was not known). The 31 pregnancies resulted in 24 live-born infants (one twin), five induced abortions, one miscarriage and two in utero foetal deaths. Intrauterine growth retardation was found in 2/31 cases by ultrasound. Among the 24 live-born infants, nine were premature and three had

abnormalities including hip dysplasia, unilateral renal dilatation and pilonidal sinus. Five had neonatal respiratory distress considered to be the result of prematurity rather than pulmonary malformation. No malformations were seen in the two in utero foetal deaths. Pre- or postnatal chromosomal analysis was normal in 6/7 cases studied; the remaining case showed inherited inversion of chromosome 9.[30]

In a comprehensive report on the reproductive and developmental toxicity of hydroxyurea by the Center for the Evaluation of Risks to Human Reproduction (CERHR) of the (US) National Toxicological Program (NTP), case reports are presented and discussed concerning an additional 26 pregnancies in women to whom hydroxyurea was prescribed for the treatment of haematological malignancies, essential thrombocythaemia and sickle cell disease. Of the seven outcomes that were not normal, two were stillbirths, two were preterm deliveries and two were cases of intrauterine growth retardation, while one outcome was unknown.[17]

For further details, the Committee refers to the NTP-CERHR review[17].

The Committee is of the opinion that no conclusions concerning the potential developmental effects of hydroxyurea can be drawn from the studies presented above because of methodological deficiencies and the unknown influence of the underlying maternal illnesses.

2.2.3 Lactation

Sylvester et al. (1987) reported one case of excretion of hydroxyurea into breast milk. A patient with chronic myeloid leukaemia was treated orally during lactation with 500 mg hydroxyurea three times a day. Milk samples were collected at the start of the treatment and during seven days of lactation (two hours after the last dose of hydroxyurea each day). Due to methodological difficulties, hydroxyurea could be detected in only a few samples. The three reliable hydroxyurea milk concentrations were: day 1; 6.1 mg/L, day 3; 3.8 mg/L and day 4; 8.4 mg/L (mean 6.1±2.3 mg/L).[29]

2.3 Animal studies

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

2.3.1 *Fertility studies*

Male reproductive system

Mecklenburg et al. (1975) administered hydroxyurea at amounts of 3 mg/mL drinking water (equivalent to 300 mg hydroxyurea/kg bw/day, assuming a water intake of 100 mL/kg bw/day) to sexually mature rats (Holtzmann; n=90; controls: n=18) for 70 days, followed by a 30-day recovery period. Body weight at the end of the treatment was reduced in the treatment group. Germinal cell depletion was noted from 14 days after the start of the treatment and the severity increased with the duration of the treatment. This effect was ascribed to the inhibition of DNA synthesis by hydroxyurea. After cessation of the treatment, the germinal epithelium was re-established in most of the seminiferous tubules.[20]

Rich and De Kretser (1977) exposed rats (Sprague-Dawley; 60 days old; n=10/group) to amounts of hydroxyurea of 3 mg/mL drinking water (equivalent to 300 mg/kg bw/day) for three months. In treated rats, absolute testis weights were statistically significantly reduced (by 40%), destruction of the seminiferous epithelium occurred, and serum LH and FSH levels were significantly elevated.[24]

Jones et al. (2009) treated adult transgenic sickle cell mice (n=6/group/stage) by gavage with 0 or 25 mg hydroxyurea/kg bw/day for 28 or 56 days. Monthly body weights were similar between treated mice and controls. Hydroxyurea treatment statistically significantly decreased absolute testis weight on day 28 and 56. Concomitant with a 52% shrinkage of testis dimensions on day 56, testes from treated mice exhibited atrophic degeneration in the seminiferous tubules. Epididymides from treated mice showed a 25% shrinkage, along with 69% reduction in stored sperm density and 95% reduction in sperm motility on day 56.[15]

Wiger et al. (1995) injected mice (B6C3/F1/BOM M; six to eight weeks old; n=5/group) intraperitoneally with 0 or 200 mg hydroxyurea/kg bw/day for five days. Testes were examined at various stages after treatment. Atrophy of seminiferous tubules was seen in the treated mice five and ten days after the last exposure. The absolute and relative testis weights were reduced (40-45% lower than controls on days 27 and 33 after treatment). In addition, reduction in the proportion of the various spermatid stages and an alteration in sperm chromatin

structure were noted. Wiger et al. discussed that the primary cause of these findings was inhibition of DNA synthesis in the testes.[31]

Evenson and Jost (1993) treated mice (C57B/6JxC3H/HeJ F_1; 13 to 15 weeks old; n≥6/group) with intraperitoneal doses of hydroxyurea of 0, 25, 50, 100, 200, 400 or 500 mg/kg bw/day for five days. Whole testis, minced testicular cell suspensions and caudal epidydimal sperm cells were obtained eight or 29 days after treatment. Treatment did not affect body weights. At day 8, absolute testis weights were statistically significantly decreased at 400 and 500 mg/kg bw/day and testicular cell population ratios were altered at doses ≥100 mg/kg bw. At day 29, these testis changes were seen at doses ≥50 mg/kg bw/day. Evenson and Jost concluded that hydroxyurea inhibited DNA synthesis, causing maturation depletion of pachytene spermatocytes and, subsequently, depletion of meiotic daughter cells and differentiated cell types leading to mature sperm.[11]

Shin et al. (1999) administered single intraperitoneal doses of hydroxyurea of 0, 100, 200 or 400 mg/kg bw to mice (ICR; six to seven weeks old; n=3/group). Testes were examined at 0, 4, 8, 12, 24 and 48 hours after treatment. Both the number of apoptotic cells and the level of DNA fragmentation increased depending on the dose. The number of apoptotic cells increased continuously, peaked at 12 hours and reached control levels by 48 hours. Shin and Shiota discussed that apoptosis of damaged testicular cells is apparently a common response to toxicants, therefore protecting the next generation of germ cells from the damaged cell population.[27]

Ficsor and Ginsberg (1980) treated mice (CF1; 12 to 16 weeks old; n=3-4/group) intraperitoneally with doses of 0, 125, 250, 500 or 1,000 mg/kg bw/day for five days. Examinations were conducted 35 days after the last treatment. Terminal body weights were decreased at 1,000 mg/kg bw and absolute testis weights were statistically significantly decreased at 500 and 1,000 mg/kg bw. In all dose groups, the number of sperm extracted from the cauda was decreased and sperm motility was dose-dependently decreased.[12]

Singh and Taylor (1981) treated hamsters (inbred PD4 strain; ten to 12 weeks old; n=6-9/ group) with intraperitoneal doses of 0, 10, 50 or 250 mg/kg bw/day for five days. One, four and 10 weeks after treatment, two or three hamsters of each dose were examined. After an initial increase, body weight gradually declined with increasing levels of hydroxyurea. A progressive decline in sperm number with exposure to increasing dose levels of hydroxyurea occurred, which

was already evident at 10 mg/kg bw. No sperm abnormalities were induced at doses as high as 250 mg/kg bw.[28]

Female reproductive system

Sampson et al. (2010) investigated the effects of hydroxyurea on ovulation rate and embryo development in groups of 20 C57BL/6J female mice. Animals were treated with oral doses (gavage) of hydroxyurea of 30 mg/kg bw/day for up to 28 days; controls received saline (vehicle). Five days prior to cessation of treatment, mice were subjected to folliculogenesis induction with pregnant mare serum gonadotropin. Forty-eight hours after this induction, five mice/group were anaesthetized to collect blood for oestradiol-17ß (E_2) measurement; in the remaining mice, ovulation was induced with human chorionic gonadotropin (hCG) after which they were immediately caged with males for mating. Five plugged females/group were sacrificed for ovulation rate determination (about 15 hours post hCG); the remaining mice were sacrificed about 27 hours post hCG, ovaries excised and weighed and embryos harvested. Compared to controls, treated mice had decreased ovary weights, ovulation rates and circulating E_2 levels ($p<0.05$) and fewer embryos developing to the blastocyst stage (32% vs 60% in controls; $p<0.05$).[26]

2.3.2 Developmental toxicity studies

Since the original reports by Murphy and Chaube (1964)[21] and Chaube and Murphy (1966)[8], who showed that single intraperitoneal doses of hydroxyurea of 250 mg/kg bw or more given to Wistar rats on one of gestational days 9-12 produced a high proportion of foetuses with malformations, numerous studies on the developmental toxicity of hydroxyurea in a variety of animal species have been published. In many of these studies, single, often relatively high doses of hydroxyurea were administered at single gestational days and in some studies even as a positive control.

The Committee presents here only multi-dose studies; for a complete overview, the Committee refers to the comprehensive report of CERHR[17].

Aliverti et al. (1980) administered oral doses of hydroxyurea of 0, 50, 150, 300 or 450 mg/kg bw/day to female Sprague-Dawley rats (n=8-10/group; 2% Arabic gum in water-treated controls: n=27) during gestational days 6-15. The rats were killed on gestational day 21 and foetuses were subjected to external, visceral and skeletal examinations. There was no information on maternal toxicity. Foetal

body weights were reduced at levels ≥150 mg/kg bw. At levels ≥300 mg/kg bw, hydroxyurea induced postimplantation loss and developmental effects (most commonly: craniofacial abnormalities, abdominal wall defects, limb malrotation, hydrocephalus and ocular defects).[1]

Roll and Bär (1969) exposed female mice by gavage to doses of hydroxyurea of 0, 5, 10, 15 or 20 mg/animal (according to Roll and Bär ca. 0, 200, 400, 600 or 800 mg /kg bw/day) from gestational days 6-17. These doses were stated to be 'relatively non-toxic for the maternal animals' but no data were provided.

Twenty-one, 19 and 16 dams treated with 0, 400 and 800 mg/kg bw/day, respectively, underwent Caesarean section on gestational day 18, and implantation sites were examined and foetuses were assessed for skeletal abnormalities. Treatment caused statistically significant increases in the total number of resorptions (10, 23, 95%, respectively), particularly early (9, 12, 36%, respectively) and mid-term (1, 8, 57%, respectively) resorptions. At 400 mg/kg bw, foetal body weight was statistically significantly decreased. Skeletal evaluation showed an increased number of malformations including sternum defects (17% vs. 1% in controls), encephalocele (13% vs. 0.5%), thoracic vertebral defects (8% vs. 0%), cervical vertebrae fusion (5.9% vs 1%) and costal fusion (5.3% vs.1%). In the few surviving foetuses of the 800 mg/kg bw group, no malformations were observed but development was severely retarded.

Some dams (n=18, 29, 9 at 0, 200, 400 mg/kg bw, respectively; unspecified at higher levels) were allowed to deliver and pups were examined for external malformations, viability at birth and body weights until the end of the lactation period. At 600 and 800 mg/kg bw, complete resorption or abortion occurred. At 200 and 400 mg/kg bw, the number of stillbirths and pup mortality during the lactation period were increased and pup body weights were slightly decreased. External malformations were cleft palate (1.2%) and kinked tails (0.8%) at 200 mg/kg bw and cleft palate (1.5%) and encephalocele (3%) at 400 mg/kg bw (no malformation rates provided for the control group).

Roll and Bär also described treatment of dams during specific stages of pregnancy at similar and higher dose levels. Effects on resorptions and foetal weight were consistent with those observed after exposure during gestational days 6-17. Malformations commonly observed after hydroxyurea treatment on gestational days 6 or 7 included cleft palates, sternum defects, encephaloceles and vertebral defects. In addition to these effects, limb and tail defects occurred with exposures on gestational days 10 or 11.[25]

Khera (1979) gave hydroxyurea by capsules to female cats (n=17/group) at daily doses of 0, 50 or 100 mg/kg bw/day during gestational days 10-22. The cats were necropsied on gestational day 43, and foetuses were examined for external, visceral and skeletal malformations. At 100 mg/kg bw, maternal body weight gain was statistically significantly decreased. Only one cat of the 100 mg/kg bw group survived until necropsy. No maternal effects were noted at 50 mg/kg bw.

At 100 mg/kg bw/day, hydroxyurea induced a high number of non-pregnancy (ten not pregnant vs. five in controls) and resorptions with, consequently, few live foetuses. The one cat surviving until necropsy had two stunted live foetuses, one had no apparent anomaly, the other cyclopia. At 50 mg/kg bw, the number of litters with malformations and the overall number of malformed foetuses was higher than in controls (which was, according to Khera, of 'borderline statistical significance'). The malformations in this group were of various types but cleft palate and microphthalmia were most frequent.[16]

Asano and Okaniwa (1987) administered intraperitoneal doses of hydroxyurea of 0, 100 or 200 mg/kg bw/day to Sprague-Dawley and Wistar rats during gestational day 9-12. Information on maternal toxicity was not provided.

Groups of 15 to 16 Sprague-Dawley and five Wistar rats were sacrificed on gestational day 21 and examined for implantations, resorptions and live foetuses and the foetuses for sex, body weight and malformations. Compared to controls, there were no differences in the number of implantation sites, resorptions or life foetuses. In both strains, the weight of live foetuses was statistically significantly decreased at 200 mg/kg bw. At 200 mg/kg bw, the percentages of visceral malformations were statistically significantly increased (Sprague-Dawley: 44-51% vs. 1% controls; Wistar: 87-89% vs. 10% in controls). Malformations observed most commonly in both strains were dilatation of lateral ventricle, anophthalmia, microphthalmia and ventricular septal defect. In Wistar rats, also exencephaly, cleft palate and micrognathia were seen. At 100 mg/kg bw, no adverse effects were produced. Asano and Okaniwa noted that morphological effects of hydroxyurea were less severe in Sprague-Dawley rat foetuses than in Wistar rat foetuses.

Groups of 12 to 22 Sprague-Dawley rats were allowed to deliver spontaneously. Pups were reared by their biological mothers and observed up to postnatal day 21. At 200 mg/kg bw, there were statistically significant decreases in mean weights of male and female pups at birth and at postnatal day 21 and in viability index at postnatal day 4 and statistically significant increases in the number of male and female pups with malformations (53 and 43%, respectively; none in controls). Malformations most commonly observed were anophthalmia

(31% in males; 29% in females), hydrocephaly (39 and 11%, respectively) and microphthalmia (13 and 25%, respectively).[3]

Asano et al. (1983) treated Wistar rats with doses of hydroxyurea of 0, 25, 50 and 100 mg/kg bw/day (n=10-12/group) ('first study') or 0, 100 and 200 mg/kg bw/day (n=8-10/group) ('second study') during gestational days 9-12. Dams were allowed to deliver spontaneously. Litters were reared by biological mothers and observed for up to about eight weeks. No information on maternal toxicity was provided.

There were no differences in delivery index, number of stillbirth, body weight, postnatal growth and viability index at levels up to 100 mg/kg bw. At 200 mg/kg bw (second study), the frequency of stillbirth was increased ($p<0.05$) and the male body weight at birth was decreased ($p<0.05$). In addition, the frequency of malformations (eye defects, dilation of ventricles, cleft lip/palate) at birth and postnatal days 4, 14, 21 and 56 was statistically significantly ($p<0.01$) increased (to 20%, 69%, 88%, 63% and 69% of examined pups, respectively). At 100 mg/kg bw/day, eye defects, dilated ventricles and cranial enlargement were noted; the proportion of abnormalities in this group was: at postnatal day 4: 18% (first study) or 5.3% (second study); at postnatal day 14: 5.9% (second study); at postnatal day 21: 17% (first study) or 0% (second study); and at postnatal day 56: 7.5% (second study). At 25 and 50 mg/kg bw/day (first study), only a few cases of eye defects and dilated ventricles were observed versus none in controls, namely at postnatal day 4: dilated ventricle 4.4% and 7.7%, and at postnatal day 21: microphthalmia 1.3% and 1.2% of examined pups (n= 35-93 pups per sex) at 25 and 50 mg/kg bw/day, respectively. Delayed development of the female righting reflex was noted at two days of age, but statistical significance was obtained only at 25 mg/kg bw/day. The male free fall reflex was delayed between postnatal days 15-25; statistical significance was obtained at 100 and 200 mg/kg bw/day. The number of rearing in the open field test was increased ($p<0.05$) in females at 100 mg/kg bw/day. Rotorod performance and the acquisition rate of conditioned avoidance response were not affected.[2]

Chahoud and Paumgartten (2009) injected doses of 0, 250, 300, 350, 400, 450, 500 or 550 mg/kg bw intraperitoneally into Wistar rats (n=13-34 litters/group; controls: n=53 litters) on gestational day 11. Caesarean sections were performed on gestational day 21 and the foetuses were subjected to skeletal examinations. No information on maternal toxicity was given but Chahoud and Paumgartten stated that the single treatment in mid-gestation was an attempt to attenuating maternal toxicity and to avoiding marked embryo lethality.

Dose-related variations were observed at doses ≥250 mg/kg bw ($p<0.05$); at ≥250 mg/kg bw: increased percentages of dumbbell-shaped and bipartite ossification centres in thoracic and lumbar vertebrae (14% up to 87% at high-dose); at ≥300 mg/kg bw: increased occurrence of zygomatic bone fused to os maxilla (19% up to 84% at high-dose). Dose-related malformations occurred at doses ≥300 mg/kg bw ($p< 0.05$); at ≥300 mg/kg bw/d: absent tympanic bone (4.2% up to 91% at high-dose); at ≥400 mg/kg bw/d: cleft palate (4.8% up to 34% at high-dose), absent tibia (3.5% up to 44% at high-dose); at ≥450 mg/kg bw/d: bent ribs (0.8% up to 1.9 % at high-dose), bent clavicle (5.6% up to 20% at high-dose).[7]

Barr and Beaudoin (1981) administered intraperitoneal doses of hydoxyurea of 200-375 mg/kg bw to two stocks of Wistar rats (n=8-10 litters/group) at one or several six-hour intervals on gestational days 9-10.75. Caesarean sections were performed on gestational day 21. No information on maternal toxicity was given. Foetal and placental weights were decreased and malformations were increased in the treated rats. Statistically significant ($p<0.05$) increases in malformations observed most commonly included anopthalmia/microphtalmia (1.7-94%)*, hydrocephaly (1.9-35%), exencephaly (0-32%), maxillary hypoplasia (0-41%), cleft lip/palate (0-11.5%), protruding tongue (0-27%), hydronephrosis 7-58%), tail displasia (0-30%) and anal atresia (0-20%).[5]

Gupta and Jaffe (1982) injected Sprague-Dawley rats (n=5/group) subcutaneously with 0 or 160 mg hydroxyurea/kg bw/day on gestational days 17-20. Randomly selected female offspring were followed to their reproductive development. Treatment did not affect appearance or body weight of the dams. Offspring of hydroxyurea-treated rats did not show effects with respect to age of vaginal opening and first appearance of oestrus (n=20) or significant effects on oestrus cycle (n=6). Fertility of female offspring (n=9), determined by mating with untreated males, was not significantly affected.[14]

2.3.3 *Lactation*

No relevant animal studies on effects of hydroxyurea during lactation were available.

* Figures in brackets are ranges of percentages affected pups, depending on stock of rats and exposure stage.

2.4 Conclusions

Fertility

One multicentre study[6] and three case reports of men with sickle cell disease[13,18,19] suggest that hydroxyurea therapy reduces sperm counts and impairs sperm motility and morphology.

There were no data on the functional fertility of laboratory animals following treatment with hydroxyurea. Oral or intraperitoneal administration of hydroxyurea caused decreased testis weights and histological seminiferous tubular abnormalities in rats[13,20,24] and mice[11,12,15,27,31], decreased sperm counts in mice[12] and hamsters[28] and affected sperm morphology or motility in mice[12,15,31]. Oral administration to mice resulted in decreased ovary weights, ovulation rates and circulating E_2 levels and fewer embryos developing to blastocyst stage.[26]

Overall, the Committee concludes that the human data are not sufficient for classification. Based on the effects observed in laboratory animals, the Committee proposes to classify hydroxyurea for effects on fertility in category 1B (presumed human reproductive toxicant).

Developmental toxicity

No adequately designed human studies on developmental toxicity effects of hydroxyurea were available.

In various animal species, repeated oral or intraperitoneal administration induced increased numbers of resorptions, stillbirths and postnatal deaths, reduced pup weights and external, visceral or skeletal malformations.[1-3,16,25] For most studies, no or only limited information on maternal toxicity was available. However, the Committee considers that the nature and severity of the effects observed indicates that they occurred independently from maternal toxicity.

Therefore, based on the data from laboratory animal studies, the Committee proposes to classify hydroxyurea for effects on fertility in category 1B (presumed human reproductive toxicant).

Lactation

Hydroxyurea was excreted in human breast milk in an amount of 6.1±2.3 mg/L.[29] This value is based on a few observations in one subject only. Since there is no

information about a safe/acceptable daily intake of hydroxyurea either, it was not possible to calculate a safe level for hydroxyurea in human breast milk.

The Committee proposes not labelling hydroxyurea for effects on or via lactation due to a lack of appropriate human and animal data.

Proposed classification for fertility

Category 1B; H360F.

Proposed classification for developmental toxicity

Category 1B; H360D.

Proposed labelling for effects during lactation

Lack of appropriate human and animal data precludes assessment of hydroxyurea for effects on or via 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

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

The first draft of the present document was prepared by Dr. B.A.R. Lina and Dr. M.J.W. van den Hoven from TNO Triskelion BV, 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, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

The submission letter (in English)

Subject : Submission of the advisory report *Hydroxyurea*
Your reference : DGV/MBO/U-932542
Our reference : U-8076/HS/cn/543-J14
Enclosed : 1
Date : April 3, 2014

Dear Minister,

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

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

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

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

Annex C

Comments on the public draft

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

- T.J. Lentz, K. Krajnak, D. Murray, S. Rengasamy. National Institute for Occupational Safety and Health (NIOSH), Cincinnati OH, USA.

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

Annex **D**

Regulation (EC) 1272/2008 of the European Community

3.7 Reproductive toxicity

3.7.1 Definitions and general considerations

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

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

(a) adverse effects on sexual function and fertility;

(b) adverse effects on development of the offspring.

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

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

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

3.7.1.3 Adverse effects on sexual function and fertility

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

3.7.1.4 Adverse effects on development of the offspring

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

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

3.7.2 Classification criteria for substances

3.7.2.1 Hazard categories

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

Table 3.7.1(a) Hazard categories for reproductive toxicants.

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

Table 3.7.1(b) Hazard category for lactation effects.

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

3.7.2.2 Basis of classification

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

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

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

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

3.7.2.3 Weight of evidence

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

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

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

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

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

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

3.7.2.4 Maternal toxicity

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

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

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

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

Maternal mortality:

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

Mating index

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

Fertility index

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

Gestation length

(if allowed to deliver)

Body weight and body weight change:

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

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

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

Food and water consumption (if relevant):

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

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

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

Post-mortem data:

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

3.7.2.5 Animal and experimental data

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

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

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

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

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

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

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

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

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

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

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

3.7.3 Classification criteria for mixtures

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

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

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

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

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

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

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

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

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

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

3.7.4 Hazard Communication

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

Table 3.7.3 Label elements for reproductive toxicity.

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

Annex **E**

Additional considerations to Regulation (EC) 1272/2008

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

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

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

* Organisation for Economic Cooperation and Development.

Annex **F**

Fertility and developmental toxicity studies

Table 1 Fertility studies with hydroxyurea in animals.

authors	species	experimental period/ design	dose/route	general toxicity	effects on reproductive organs/ effects on reproduction
Male fertility					
Mecklenburg et al. (1975)	Holtzmann rats (n=90; controls: n=18; mature)	70 d, followed by a 30-d recovery period	0, 3 mg/mL of drinking water (ca. 300 mg/kg bw/d, assuming a water intake of 100 mL/kg bw/d)	decreased bw at the end of the treatment	germinal cell depletion from 14 days after the start of the treatment, severity increasing with the treatment duration; effect ascribed to the arrest of DNA synthesis by hydroxyurea. after cessation of the treatment, the germinal epithelium re-established in most of the seminiferous tubules
Rich/De Kretser (1977)	Sprague Dawley rats (n=10/ group; 60 d old)	3 mo	0, 3 mg/mL of drinking water (ca. 300 mg/kg bw/d, assuming a water intake of 100 mL/kg bw/d)	no data presented	absolute testis wt (g): 1.65±0.3 (controls), 0.66±0.3** caput epididymal wt (mg): 178±10, 88±7** serum LH levels (ng/mL): 1.3±0.1, 2.1±0.1** serum FSH levels (ng/mL): 378±27, 751±28** destruction of the seminiferous epithelium

Ficsor/ Ginsberg (1980)	CF1 mice (n=3-4/ group; 12-16 wk old)	5 d sacrifice: 35 d after treatment	0, 125, 250, 500, 1000 mg/kg bw/d; ip	decreased terminal bw at 1000 mg/kg bw/d	mean absolute testis wt (g): 277, 223, 242, 163*, 129*, resp. number of sperm (x10^6/mL): 124.8, 77.6*, 65.6*, 49.6*, 26.4* sperm motility (%): 46.6, 34.8, 38.7, 30.7*, 18.9*
Evenson/Jost (1993)	(C57B/6J x C3H/HeJ F1) mice (n≥6/ group; 13-15 wk old)	5 d sacrifice: 8, 29 d after treatment	0, 25, 50, 100, 200, 400, 500 mg/kg bw/d; ip	no effect on bw	at 8 d: statistically significantly decreased absolute testis wt at doses ≥400 mg/kg bw/d; altered testicular cell population ratios at doses ≥100 mg/kg bw/d at 29 d: statistically significantly decreased testis wt, altered testicular cell population ratios at doses ≥50 mg/kg bw/d Evenson/Jost concluded that hydroxyurea inhibits DNA synthesis, causing maturation depletion of pachytene spermatocytes and, subsequently, depletion of meiotic daughter cells and differentiated cell types leading to mature sperm
Wiger et al. (1995)	(B6C3/F1/ BOM M) mice (n=5/group; 6-8 wk old)	5 d sacrifice: 0, 5, 10, 27, 33, 45 d after treatment	0, 200 mg/kg bw/d; ip	bw: no effect during treatment; decreased bw gain during post-treatment d 0-5 and at d 45; during treatment period, animals showed signs of weakness	atrophy of seminiferous tubules on post-treatment d 5 and 10 decreased absolute and relative testis wt (40-45% lower than controls) on post-treatment d 27 and 33 reduced proportion of the various spermatid stages and altered sperm chromatin structure Wiger et al. discussed that inhibition of DNA synthesis in the testis was the primary cause of these findings
Shin et al. (1999)	ICR mice (n=3/ group; 6-7 wk old)	1 d sacrifice: 0, 4, 8, 12, 24, 48 h after treatment	0, 100, 200, 400 mg/kg bw; ip	no effect on bw and testis wt	dose-dependent increases in numbers of apoptotic cells and in levels of DNA fragmentation continuous increases in numbers of apoptotic cells, peaking at 12 h and reaching control levels by 48 h
Jones et al. (2009)	transgenic sickle cell mice (n=6/group/ stage; adult)	28, 56 d	0, 25 mg/kg bw/d; gavage	no effect on bw	statistically significantly decreased absolute testis wt on d 28 and 56 on d 56: 52% shrinkage of testis dimensions; atrophic degeneration in the seminiferous tubules; 25% shrinkage of epididymides; 69% decrease in stored sperm density; 95% decrease in sperm motility

Singh/Taylor (1981)	inbred PD4 strain hamsters (n=6-9/group; 10-12 wk old)	5 d sacrifice: 1, 4 and 10 wk after treatment	0, 10, 50, 250 mg/kg bw/d; ip	bw: initial increase to roughly 126, 114, 123% of controls, resp., at post-treatment wk 1, followed by gradual decrease to roughly 90, 86, 92% of controls, resp., at wk 12	progressively decreased sperm number with exposure to increasing dose levels of hydroxyurea occurred, which was already evident at 10 mg/kg bw/d. no sperm abnormalities
Female fertility					
Sampson et al. (2010)	C57BL/6J mice (n=20/group)	28 d sacrifice: at treatment d 25, 26, 28 d 23: ip injection of PSMG to induce folliculogenesis d 25: measurement of E_2 levels (n=5/group); ip injection of hCG and subsequent mating (n=15/group) about 15 h post hCG: determination ovulation rate (n=5/group) about 27 h post hCG: examination ovaries/embryos	0, 30 mg/kg bw/d; gavage	no data presented	decreased ovary wt*, ovulation rates*, circulating E_2 levels* number of embryos developing to the blastocyst stage: 32% (controls), 60%*

bw=body weight; d=day(s); E_2=oestradiol-17ß; h=hour(s); hCG=human chorionic gonadotropin; ip=intraperitoneal; mo=month(s); PSMG=pregnant mare serum; wk=week(s); wt=weight(s); *: $p<0.05$; **: $p<0.001$.

Table 2 Developmental toxicity studies with hydroxyurea in female animals.

authors	species	experimental period/design	dose/route	general toxicity	developmental toxicity
Aliverti et al. (1980)	Sprague-Dawley rats (n=8-10/ treatment group; n=27 controls)	gd 6-15 sacrifice: gd 21; foetuses examined for external, visceral, skeletal abnormalities	0, 50, 150, 300, 450 mg/ kg bw/d; oral	no data on maternal toxicity presented	mean foetal bw (g): 5.34±0.31, 5.31±0.41, 5.08±0.63, 3.85±0.50, 3.28±0.76, resp. number of resorptions+dead foetuses: 21, 9, 7, 52, 79 post-implantation loss (%): 5.7, 6.9, 5.5, 50.1, 69.6 number of viable foetuses: 375, 123, 121, 51, 30 number of foetuses with external abnormalities: 0/ 375, 0/123, 0/121, 4/51, 12/30; with visceral abnormalities: 0/196, 0/63, 0/63, 6/30, 15/15; with skeletal abnormalities: 0/179, 0/60, 0/58, 3/21, 11/14 most commonly observed abnormalities at 300 and 450 mg/kg bw/d external: cranial 0/51, 3/30, resp.; facial 1/51, 4/30; craniofacial dysgenia 3/51, 2/30; absent pinnae 0/51, 3/30; amelia/ phocomelia 0/51, 4/30; limb malrotation 0/51, 5/30 visceral: hydrocephalus 5/30, 7/16; eye 4/30, 13/16 skeletal: markedly reduced orbital bones 0/21, 5/14; reduced/absent/misshapen mandula 2/21, 6/14; vertebrae/sternebrae/ ribs dysgenesia 1/21, 10/14
Roll/Bär (1969)	NMRI mice (number exposed: see 'dose')	gd 6-17 dams allowed to litter; pups examined for external malformations, viability at birth, bw until weaning	0, 200, 400, 600, 800 mg/ kg bw/d (n=18, 29, 9, unspecified, unspecified, resp.); gavage	no data on maternal toxicity presented; stated to be 'relatively non-toxic for the maternal animals'	600 and 800 mg/kg bw/d: complete resorption or abortion number of pups: 154, 260, 66 at 0, 200, 400 mg/kg bw/d, resp. number of pups/dam: 8.6, 9.0, 7.3, resp. % of stillbirths: 3.3, 8.1, 12.1 pup mortality until pnd 21 (%): 9.7, 20.1 (p=0.0003), 24.3 (p=0.0001) mean pup bw at birth: 1.48±0.01, 1.39±0.01 (p=0.0002), 1.30±0.02 (p=0.0002) mean pup wt at weaning: 9.60±0.18, 9.39±0.13, 9.37±0.22
		gd 6-17 sacrifice: gd 18; dams examined for number of implantation sites; foetuses for skeletal abnormalities	0, 400, 800 mg/kg bw (n=21, 19, 16, resp.); gavage		number of implantations: 217, 200, 150, resp. total number of resorptions (%): 10.1, 33.5 p=0.0007), 94.7; of early resorptions (%): 8.7, 11.5, 36.0; of mid-term resorptions (%): 0.9, 7.5, 57.4; of late resorptions: 0.5, 4.5, 1.3 mean foetal bw (g): 1.17±0.01, 0.85±0.02 (p<0.0002), no data abnormalities observed at 0 and 400 mg/kg bw (%): sternebrae defects: 1.1, 17.1, resp.; encepalocele: 0.5, 12.5; missing/shortened tail: 0, 2.0; costal fusion: 1.1, 5.3; cervical vertebrae fusion: 1.1, 5.9; thoracic vertebrae defects: 0, 7.9; lumbar vertebrae defects: 0, 1.3 800 mg/kg bw: in the few surviving foetuses, no malformations but severe retardation of development

gd 6-7 sacrifice: gd 18 dams examined for number of implantation sites; foetuses for skeletal abnormalities	0, 600, 1200 mg/kg bw/d (n=21, 18, 12, resp.)	number of implantations: 217, 188, 139 total number of resorptions (%): 10.1, 56.4, 72.7; of early resorptions (%): 8.7, 48.4, 38.8; of mid-term resorptions (%): 0.9, 7.5, 29.4; of late resorptions: 0.5, 0.5, 4.5 mean foetal bw (g): 1.17±0.01, 1.00±0.02, 0.88±0.02 abnormalities observed (%): cleft palate: 0.5, 3.0, 23.7; sternebrae defects: 1.1, 16.7, 47.4; encephalocele: 0.5, 0, 15.8; missing/ shortened tail: 0, 0, 2.6; costal fusion: 1.1, 1.5, 2.6; cervical vertebrae fusion: 1.1, 6.1, 7.9; thoracic vertebrae defects: 0, 0, 15.8; lumbar vertebrae defects: 0, 0, 10.5
gd 10-11 sacrifice: gd 18 dams examined for number of implantation sites; foetuses for skeletal abnormalities	0, 600, 1200 mg/kg bw/d (n=21, 31, 23, resp.)	number of implantations: 217, 321, 222 total number of resorptions (%): 10.1, 8.1, 45.1; of early resorptions (%): 8.7, 10.9, 6.8; of mid-term resorptions (%): 0.9, 6.9, 36.5; of late resorptions: 0.5, 0.3, 1.8 mean foetal bw (g): 1.17±0.01, 1.08±0.01, 1.00±0.01 abnormalities observed (%): cleft palate: 0.5, 8.0, 28.7; sternebrae defects: 1.1, 4.2, 25.4; encephalocele: 0.5, 0.4, 9.8; missing/ shortened tail: 0, 8.4, 23.8; costal fusion: 1.1, 1.2, 4.1; cervical vertebrae fusion: 1.1, 0, 4.9; thoracic vertebrae defects: 0, 13.3, 55.5; lumbar vertebrae defects: 0, 5.3, 27.0; sacral vertebrae defects: 0, 0, 13.2; hexadactyly hind limb: 0, 0, 2.4; syndactyly forelimb: 0, 0, 3.2; syndactyly hind limb: 0, 0, 2.4; tibia aplasia: 0, 0, 9.8; shortened tibia: 0, 0, 4.9
gd 10 sacrifice: gd 18 dams examined for number of implantation sites; foetuses for skeletal abnormalities	0, 600, 900, 1200 mg/kg bw (n=21, 32, 13, 21, resp.)	number of implantations: 217, 333, 117, 182 total number of resorptions (%): 10.1, 12.9, 13.7, 25.3; of early resorptions (%): 8.7, 8.7, 8.6, 3.3; of mid-term resorptions (%): 0.9, 3.9, 3.4, 22.0; of late resorptions: 0.5, 0.3, 1.7, 0 mean foetal bw (g): 1.17±0.01, 1.15±0.01, 1.09±0.01, 1.03±0.02 abnormalities observed (%): cleft palate: 0.5, 1.7, 6.0, 19.3; sternebrae defects: 1.1, 4.7, 13.9, 25.9; encephalocele: 0.5, 0.4, 0, 0; missing/shortened tail: 0, 0, 13.9, 23.0; costal fusion: 1.1, 0.4, 2.0, 2.2; cervical vertebrae fusion: 1.1, 0.4, 0, 0; thoracic vertebrae defects: 0, 2.1, 26.7, 45.1; lumbar vertebrae defects: 0, 0, 6.0, 32.5; sacral vertebrae defects: 0, 0, 0, 25.1; hexadactyly hind limb: 0, 0, 2.4; syndactyly hind limb: 0, 0, 0, 6.7; tibia aplasia: 0, 0, 0, 7.4; shortened tibia: 0, 0, 4.9; ulna aplasia: 0, 0, 0, 1.5

		gd 11 sacrifice: gd 18 dams examined for number of implantation sites; foetuses for skeletal abnormalities	0, 600, 900, 1200 mg/kg bw (n=21, 17, 23, 30, resp.)		number of implantations: 217, 170, 264, 208 total number of resorptions (%): 10.1, 7.1, 12.5, 13.9; of early resorptions (%): 8.7, 4.7, 9.5, 7.7; of mid-term resorptions (%): 0.9, 1.8, 2.3, 4.8; of late resorptions: 0.5, 0.6, 0.7, 1.4 mean foetal bw (g): 1.17±0.01, 1.11±0.01, 1.09±0.01, 0.98±0.01 abnormalities observed (%): cleft palate: 0.5, 0, 0.9, 16.1; sternebrae defects: 1.1, 5.0, 3.9, 12.8; encephalocele: 0.5, 1.5, 0, 0.6; missing/shortened tail: 0, 0, 0, 5.6; thoracic vertebrae defects: 0, 0, 0, 17.3; lumbar vertebrae defects: 0, 0.7, 0, 5.3; hexadactyly hind limb: 0, 0, 3.9, 5.6; syndactyly forelimb: 0, 0, 9.5, 20.1; syndactyly hind limb: 0, 0, 0.9, 10.6; shortened tibia: 0, 0, 0, 1.2
Khera (1979)	cats (n=17/ group)	gd 10-22 sacrifice: gd 43; foetuses examined for external, visceral, skeletal abnormalities	0, 50, 100 mg/kg bw/d; oral (capsules)	no maternal toxicity at 50 mg/kg bw/d 100 mg/kg bw/d: decreased bw gain; only one cat survived until necropsy	number of cats aborted: 2/17, 1/17, 1/17, resp. number cats killed: 0/17, 0/17, 2/17 number of cats not pregnant: 5/17, 4/17, 10/17 number of cast having resorptions and no live foetuses: 3/17, 3/117, 3/17 number of cats with live foetuses: 7/17, 8/17, 1/17 total number of live foetuses: 40, 38, 2; of dead foetuses: 3, 0, 0; of resorptions: 20, 16, 13* mean foetal wt (g): 11.8±0.3, 11.3±0.4, 9.7±0.9* number of litters with abnormalities/number examined: 2/7, 5/8, 1/1; of foetuses with abnormalities/number examined: 4/40, 11/38, 1/2; of foetuses with visceral abnormalities/number examined: 1/19, 6/17, 1/1; of foetuses with skeletal abnormalities/number examined: 3/21, 5/21, 0/1 abnormalities observed (number of foetuses affected: controls: forked tongue and buccal cavity occupied by undifferentiated mass (1), fused ribs (1), sternebrae: distorded form (1), sternebrae: delayed ossification (1); 50 mg/kg bw: cleft palate (3), cleft palate, exencephaly, microcephaly, split eye lids, microphthalmia (1), cleft palate and lip, rudimentary kidneys, ectrodactyly, hind limb micromelia, taillessness (1), cleft lip and nose (1), bilateral microphthalmia (2), generalized oedema (1), fused ribs/ vertebrae (1), delayed ossification of calvarium (1), delayed ossification of digits/ sternum 91); 100 mg/kg bw: cyclopia (single medially located orbit containing globe, rudimentary nose and mandible (1)

Barr/ Beaudoin (1981)	two stocks of Wistar rats (n=8-10 litters/group); 'A' stock: colony maintained by one of the authors derived from Wistar stock originally from Albino Farms (Red Bank NJ, USA) 'B' stock: CFN Wistar purchased from Carworth (New York NY, USA)	at one of 8 6-intervals from gd 9.0 to 10.75 sacrifice: gd 21	0, 200 (at gd 9.0), 225 at gd 9.25), 250 (at gd 9.5), 275 at gd 9.75), 300 (at gd 10.0), 325 (at gd 10.25), 350 (at gd 10.5), 375 (at gd 10.75) mg/kg bw; ip	no data on maternal toxicity presented	'A' stock: resorptions (%): 10.6, 11.5, 9.4, 4.2, 7.3, 7.7, 11.1, 8.8, at 200, 225, 250, 275, 300, 325, 350, 375 mg/kg bw, resp. (no controls included , because 'purpose of the study was not to establish the teratogenicity of hydroxyurea but only to determine if there was a relation between the time of dosage and fetal outcome.') mean foetal wt (g): 4.91±0.06, 5.09±0.04, 5.26±0.05, 5.51±0.05, 5.39±0.04, 5.15±0.04, 5.35±0.05, 5.16±0.05 mean placental wt (g): 438±6, 497±8, 460±8, 465±7, 458±5, 432±8, 481±11, 431±6 malformations (%): 3.2, 71.8, 95.0, 71.3, 64.8, 67.6, 62.0, 62.5, 41.2 % of most commonly (i.e >10% in either 'A' or 'B' stock (see below) observed abnormalities: anophthalmia/microphthalmia: 55.5, 87.0, 51.3, 53.8, 35.3, 5.6, 1.7, 0; hydrocephaly: 13.6, 30.0, 18.3, 6.6, 1.0, 1.9, 3.3, 0; encephalocele: 3.6, 7.0, 3.5, 0, 0, 0, 0, 0; exencephaly: 8.2, 4.0, 2.6, 0, 0, 0, 0, 0; ear dysplasia: 6.4, 3.0, 1.7, 0, 0, 0.9, 0, 0; maxillary hypoplasia: 11.8, 12.0, 3.5, 0, 0, 0, 0, 0; facial asymmetry: 5.5, 2.0, 1.7, 0, 0, 0, 0, 0; pointed mandible: 3.6, 4.0, 7.0, 0, 0, 0, 0, 0; protruding tongue: 0.9, 5.0, 2.6, 0, 0, 0, 0, 0; cleft lip: 5.5, 3.0, 0.9, 0, 0, 0, 0, 0; hydronephrosis: 17.3, 42.0, 37.4, 28.6, 34.3, 49.1, 58.3, 34.2; left umbical artery: 8.2, 14.0, 11.3, 4.4, 11.8, 13.0, 9.2, 7.0; tail dysplasia: 4.5, 1.0, 0, 0, 0, 2.8, 1.7, 0; anal atresia: 0.9, 0, 0, 0, 0, 0, 0.8, 0 'B' stock: resorptions (%): 6.1, 12.9, 15.9, 9.4, 11.8, 9.9, 12.9, 15.8, 10.2, at 0, 200, 225, 250, 275, 300, 325, 350, 375 mg/kg bw, resp. mean foetal wt (g): 4.89±0.02, 4.26±0.05, 4.10±0.06, 4.29±0.06, 4.45±0.05, 4.41±0.05, 4.44±0.04, 4.35±0.04, 4.29±0.04 mean placental wt (g): 405±3, 397±5, 373±5, 399±6, 392±6, 370±6, 352±6, 355±5, 339±5 (decrease dose dependent: $p<0.01$) malformations (%): 3.2, 78.1, 91.8, 97.4, 93.7, 86.0, 77.9, 62.5, 57.7 % of most commonly (i.e. >10% in either 'A' (see above) or 'B' stock) observed abnormalities: anophthalmia/microphthalmia: 66.4, 91.0, 94.0, 90.6, 82.0, 47.5, 18.7, 6.2, at 200, 225, 250, 275, 300, 325, 350, 375 mg/kg bw, resp.; hydrocephaly: 25.0, 23.0, 34.5, 32.3, 14.0, 6.6, 4.7, 3.1; encephalocele: 10.2, 13.1, 9.5, 4.7, 0, 0, 0, 0; exencephaly: 8.6, 32.0, 25.0, 4.7, 0, 0, 0, 0; ear dysplasia: 8.6, 16.4, 29.3, 12.6, 4.0, 4.1, 1.6, 0; maxillary hypoplasia: 8.6, 41.0, 27.6,

					11.0, 2.0, 0, 0, 0; facial asymmetry: 3.1, 11.5, 5.2, 4.7, 1.0, 0, 0, 0; pointed mandible: 3.9, 10.7, 18.1, 6.3, 1.0, 0.8, 0.8, 0; protruding tongue: 7.8, 27.0, 20.7, 5.5, 1.0, 0, 0, 0; cleft lip: 3.9, 11.5, 3.4, 1.6, 0, 0, 0, 0; hydronephrosis: 5.5, 7.4, 12.9, 11.8, 7.0, 21.3, 21.1, 23.7; left umbical artery: 17.2, 20.5, 16.4, 11.0, 9.0, 13.9, 13.3, 14.4; tail dysplasia: 2.3, 5.7, 4.3, 1.6, 4.0, 27.9, 29.7, 27.8; anal atresia: 0, 0.8, 1.7, 0, 2.0, 14.8, 19.5, 2.1
Asano et al. (1983)	Wistar rats (n=10-12/ group)	gd 9-12 dams allowed to litter; sacrifice: pnd 21 litters reared by their biological mothers; at pnd 4, culled to 4 male and 4 female pups; offspring observed for morpholo-gical and behavioural development up to pnd 21; final sacrifice: pnd 21	0, 25, 50, 100 mg/kg bw/d; ip	no data on maternal toxicity provided	number of implantations: 186, 151, 159, 173, resp. delivery index (% of implantations): 91.4, 88.7, 91.2, 83.8 stillbirths (%): 0, 0, 2.8, 1.4 pnd 0: mean pup bw (g): males: 5.78±0.44, 5.94±0.66, 5.49±0.57, 5.64±0.57; females: 5.45±0.48, 5.57±0.57, 5.36±0.43, 5.22±0.70 no external malformations pnd 4: viability index (% of pups survived at birth): 98.8, 93.3, 95.7, 95.1 number of abnormal pups: 0, 2/45 (4.4%), 4/52 (7.7%), 9/50 (18%) malformations observed: dilated ventricular cavity (0, 2, 4, 1); anophthalmia (0, 0, 0, 9) pnd 21: mean pup bw (g): males: 54.5±4.1, 53.3±4.9, 50.9±4.7, 51.7±3.6; females: 52.7±3.5, 53.0±3.9, 49.7±4.8, 49.6±5.9 viability index (% of survived pups except for pups culled at pnd 4): 100, 97.5, 98.8, 100 number of pups with external malformations: 0, 1/78 (1.3%), 1/83 (1.2%), 14/86 (16.5%) malformations observed: microphthalmia: 0, 1,1,0; anophthalmia: 0, 0, 0, 13; enlarged cranical vault: 0, 0, 0, 10 behavioural effects: statistically significantly delayed development of the female righting reflex at pnd 2 at 25 mg/kg bw/d; statistically significantly delayed free fall reflex between pnd 15 and 25 in males at 100 mg/kg bw; statistically significantly increased numbers of rearing in open field test in postnatal wk 8 in females at 100 mg/kg bw no effects on ‘squares crossed’ number of ‘faecal boluses in open field test, on rotarod performance (in postnatal wk 8), on acquisisation rate of conditioned avoided response

	Wistar rats (n=8-10/ group)	gd 9-12 dams allowed to litter; offspring observed for morpholo-gical and behavioural development up to pnd 56	0, 100, 200 mg/kg bw/d; ip		number of implantations: 125, 160, 160, resp. delivery index (% of implantations): 90.4, 83.8, 70.6 stillbirths (%): 1.8, 4.5, 25.7* pnd 0: mean pup bw (g): males: 6.22±0.45, 5.77±0.61, 5.75±0.54*; females: 5.72±0.51, 5.48±0.67, 5.28±0.51 number of pups with external malformations: 0, 0, 17/84 (20.2%)* malformations observed at 200 mg/kg bw: head (exencephaly, meningocele, dilated ventricular cavity, enlarged cranial vault): 12; anotia: 1; cleft lip: 5; cleft palate: 2; micrognatia: 1; tail (kinky, brachyury): 2 pnd 4: viability index (% of survived pups at birth): 92.8, 91.4, 64.1 number of pups with malformations: 0/39, 2/38 (5.3%), 9/13 (69.2%)* malformations observed: head: 0, 2, 4; eye (anophthalmia, microphthalmia, pannus, corneal opacity, anterior synechia): 0, 1, 8 pnd 14: viability index (% of survived pups except for culled at pnd 4): 100, 94.9, 97.6 number of pups with malformations: 0/16, 1/17 (5.9%), 7/8 (87.5%)* malformations observed: eye: 0, 1, 6; head: 0, 0, 6 pnd 21: mean pup bw (g): males: 55.2±4.9, 53.8±6.4, 49.5±10.4; females: 53.6±4.8, 52.3±4.7, 46.9±14.4 viability index (% of survived pups for sacrificed pups at pnd 14 or 21): 100, 100, 100 number of pups with malformations: 0/19, 0/18, 5/8 (62.5%)* malformations observed at 200 mg kg/bw: head: 3; eye: 6 pnd 56: viability index (% of survived pups for sacrificed pups at pnd 14 or 21): 100, 100, 66.7* number of pups with malformations: 0/29, 3/40 (7.5%), 11/16 (68.8%)* malformations observed: head: 0, 1, 9; eyes; 0, 3, 6 behavioural effects: statistically significantly delayed free fall reflex in males at 100 and 200 mg/kg bw
Asano/ Okaniwa (1987)	Sprague-Dawley rats (n=15-16/ group)	gd 9-12 sacrifice: gd 21 dams examined for implanta-tions, resorptions, number of life foetuses; foetuses for malforma-tions.	0, 100, 200 mg/kg bw/d; ip	no data on maternal toxicity presented	number of implantations: 207, 212, 219, resp. number of resorptions: 15, 15, 19 mean number of live foetuses: 12.8±2.1, 13.1±1.7, 12.5±2.9 mean foetal wt (g): 4.82±0.43, 4.88±0.33, 4.48±0.47* % of foetuses with skeletal abnormalities: males: 0, 0, 51.1**; females: 1.1, 1.1, 43.8** abnormalities most commonly (i.e, >10%) observed at 200 mg/kg bw (in males and females, resp.): dilatation of lateral ventricle: 23/88 (26.1%), 15/112 (13.4%); anophthalmia: 16/88 (18.2%), 16/112 (14.3%); microphthalmia: 20/88 (22.7%), 19/112

				(17.0%); ventricular septal defect: 23/88 (26.1%), 17/112 (15.2%) [one control female: ventricular septal defect; one low-dose female: dilation of lateral ventricle]
Wistar rats (n=5/group)			no data on maternal toxicity presented	number of implantations: 63, 76, 78, resp. number of resorptions: 9, 4, 5 mean number of live foetuses: 10.8±3.3, 14.4±2.3, 14.6±2.1 mean foetal wt (g): 5.58±0.52, 5.14±0.10, 4.49±0.33** % of foetuses with skeletal abnormalities: males: 0, 0, 86.8**; females: 10.0, 6.7, 88.6** abnormalities most commonly (i.e >10%) observed at 200 mg/kg bw in males and females, resp.: exencephaly: 6/38 (15.8%), 0/35 ; dilatation of lateral ventricle: 21/38 (55.3%), 18/35 (51.4%); anophthalmia: 24/38 (63.2%), 17/35 (48.6%); microphthalmia: 8/38 (21.1%), 15/35 (42.9%) [one control female: double aortic arch; one low-dose female: ventricular septal defect]
Sprague-Dawley rats (n=12-22/ group)	gd 9-12 dams allowed to litter and sacrificed at pnd 21; litters reared by their biological mothers; at pnd 4, culled to 4 male and 4 female pups; pups examined for wt, viability and abnormalities; final sacrifice: pnd 21	0, 100, 200 mg/kg bw/d; ip	no data on maternal toxicity presented	number of implantations: 169, 168, 310, resp. delivery index (% of implantations): 89.9, 95.2, 79.7 stillbirths (%): 0.7, 0, 5.3 pnd 0: mean pup bw (g): males: 5.82±0.49, 5.55±0.45, 5.36±0.66*; females: 5.54±0.48, 5.31±0.49, 4.99±0.63** pnd 4: viability index (% of pups survived at birth): 100, 98.8, 87.6** number of pups malformed: males: 0/25, 0/27, 1/19 (5.3%); females: 0/30, 0/35, 5/34 (14.7%) abnormalities observed: at 200 mg/kg bw in males and females, resp.: dilation of lateral ventricle: 0/19, 1/31 (2.9%); anophthalmia: 0/19, 3/31 (8.8%); microphthalmia: 0/19, 2/13 (5.9%); ventricular septal defect: 1/19 (5.3%), 1/31 (2.9%) pnd 21: mean pup bw (g): males: 42.4±3.5, 41.7±2.9, 39.2±5.4*; females: 41.4±3.4, 40.3±3.2, 37.5±5.0* weaning index (% of pups survived after culling at pnd 4): 100, 100, 98.7 number of pups malformed: males: 0/50, 1/46 (2.2%), 37/70 (52.9%)**; females: 0/50, 1/50 (2%), 34/80 (42.5)** abnormalities observed at 200 mg/kg bw in males and females, resp.: hydrocephaly: 27/70 (38.6%), 9/80 (11.3%); anophthalmia: 22/70 (31.4%), 23/80 (28.8%); microphthalmia: 9/70 (12.9%), 10/80 (25%) [one low-dose male: microphthamia; one low-dose female: anophthalmia]

Chahoud/ Paumgartten (2009)	Wistar rats (n=13-34 litters/group; controls n=53 litters)	gd 11 sacrifice: gd 21 foetuses examined for skeletal abnormalities	0, 250, 300, 350, 400, 450, 500, 550 mg/kg bw/d; ip	no data on maternal toxicity presented	number of litters: 53, 18, 17, 21, 34, 17, 15, 13, resp. number of foetuses: 559, 154, 188, 213, 315, 125, 101, 70 resorptions (%, implantation): 4.1, 12.7, 6.0, 3.6, 13.0, 26.4, 35.2, 51.0 mean foetal bw (g): 4.40±0.37, 4.02±0.37*, 4.15±0.30*, 4.00±0.40*, 3.76±0.53*, 3.71±0.54*, 3.39±0.61*, 3.07±0.39* variations (%, foetuses): fused zygomatic bone 8.8, 10.3, 18.6*, 20.1*, 38.4*, 43.2*, 45.5*, 84.2*; misaligned sternebra sternum 4.3, 2.6, 4.2, 8*, 9.5*, 24*, 17.8*, 27.1*; wavy ribs 8.8, 1.3*, 0.5*, 0.5*, 2.5*, 2.4*, 3*, 0*; dumbbell-shaped ossification centre in lumbar vertebrae 0, 1.3*, 0.5, 6.1*, 15.2*, 25.6*, 10.8*, 12.8*; bipartite ossification centre in lumbar vertebrae 0, 0, 2.6*, 2.3*, 8.8*, 15.2*, 10.8*, 15.7*; dumbbell-shaped ossification centre in thoracic vertebrae 0.9, 13.6*, 38.8*, 46.4*, 64.7*, 69.6*, 63.3*, 61.4*; bipartite ossification centre in thoracic vertebrae 0.5, 3.9*, 4.8*, 12.2*, 25.3*, 26.4*, 36.6*, 61.4* malformations (%, foetuses): cleft palate 0, 0, 0, 0, 3.8*, 4.8*, 12.8*, 34.2*; absent tympanic bone 0, 0, 4.2*, 5.2*, 35.8*, 52.8*, 64.3*, 91.4*; absent tibia 0, 0, 0, 0, 3.5*, 17.6*, 27.7*, 44.2*; bent ribs 0, 0, 0, 0, 0, 0.8*, 1.9*, 1.4*; bent clavicle 0, 0, 0, 0, 0, 5.6*, 4.0*, 20.0*
Gupta/Jaffe (1982)	Sprague-Dawley rats (n=5/ group)	gd 17-20 examination of reproductive development of randomly selected female offspring	0, 160 mg/kg bw/d; sc	no gross abnormalities in offspring	no effect on age of vaginal opening and first appearance of oestrus (n=20) or on oestrus cycle (n=6) of female offspring no effect on fertility of female offspring (n=9), after mating with untreated males

bw=body weight; d=day(s); gd=gestational day(s); hr=hour(s); ip=intraperitoneal; n=number(s); pnd=postnatal day(s); sc=subcutaneous; wk=week(s); wt=weight(s);*: $p<0.05$; **: $p<0.01$.

Health Council of the Netherlands

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

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

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