Health Council of the Netherlands

Cytarabine

Evaluation of the effects on reproduction, recommendation for classification

2015/18

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Cytarabine*
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Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van cytarabine 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 Commissie 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 minister van Volksgezondheid, Welzijn en Sport en, eveneens ter kennisname, aan de staatssecretaris van Infrastructuur en Milieu.

Met vriendelijke groet,

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

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Cytarabine

Evaluation of the effects on reproduction,
recommendation for classification

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

to:

the Minister of Social Affairs and Employment

No. 2015/18, The Hague, July 23, 2015

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

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

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

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

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

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Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad cytarabine onder de loep genomen. Cytarabine is een pyrimidine-nucleoside-analoog. Het wordt gebruikt als cytostaticum, met name voor de behandeling van acute myeloïde leukemie. 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 voorplanting 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 cytarabine komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie cytarabine niet te classificeren wegens onvoldoende geschikte gegevens
- voor effecten op de ontwikkeling adviseert de commissie cytarabine 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 of via lactatie adviseert de commissie om cytarabine niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report, the Health Council of the Netherlands reviewed cytarabine. Cytarabine is a pyrimidine nucleoside analogue that inhibits the synthesis of DNA. It is used as an antineoplastic drug for the treatment of leukaemia, especially acute non-lymphoblastic leukaemia. 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 exposed occupationally. 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 the 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 cytarabine, these recommendations are:

- for effects on fertility, the Committee recommends not classifying cytarabine due to a lack of appropriate data
- for effects on development, the Committee recommends classifying cytarabine 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 cytarabine 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 cytarabine 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 2014, 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 comments received, and the replies by the Committee, can be found on the website of the Health Council.

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 Labelling for lactation

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

and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely independent of dosage), the labelling for effects during 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 on-line databases PubMed, Toxline and DART up to July 2012, and by searches on the Internet; an update was performed in TOXNET in September 2014. Literature was primarily selected on the basis of the text of the abstracts. Publications cited in the selected articles, but not retrieved during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted as well as several websites regarding (publications on) toxicology and health. References are divided in literature cited and literature consulted, but not cited.

The Committee describes both 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 cytarabine, 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 precludes assessment of the compound for reproductive toxicity
- sufficient data show that no classification for toxic to reproduction is indicated.

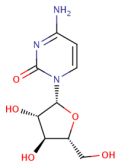
1.6 Final remark

The classification of compounds is based on hazard evaluation (Niesink et al., 1995) only, which is one of a series of elements guiding the risk evaluation process.[21] 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

Cytarabine

2.1 Introduction

name	:	cytarabine
IUPAC name	:	4-amino-1-[(2R,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl] pyrimidin-2-one
CAS name	:	2(1H)-pyrimidinone, 4-amino-1-β-D-arabinofuranosyl-
CAS registry number	:	147-94-4
synonyms	:	(arabinofuranosyl)cytosine; 1-(arabinofuranosyl)cytosine; 1-(β-D-arabinofuranosyl)- cytosine; 1-β-arabinofuranosylcytosine; 1-β-D-arabinofuranosylcytosine; 1-β-D-arabinosylcytosine; 4-amino-1-arabinofuranosyl-2-oxo-1,2-dihydropyrimidine; 4-amino-1-β-D-arabinofuranosyl-2(1H)-pyrimidinone; cytosine arabinoside; arabinocytidine; arabinosylcytosine; cytarabinoside
molecular formula	:	$C_9H_{13}N_3O_5$
colour and physical state	:	Odourless, white to off-white crystalline powder
structural formula	:	
molecular weight	:	243.2
melting point	:	212-213 °C
vapour pressure	:	1.1 x 10^{-9} Pa (estimated; at 25 °C)
solubility	:	soluble in water
Log $P_{octanol\text{-}water}$	:	-2.46 (estimated)

use	:	cytarabine is an antineoplastic drug for the treatment of leukaemia, especially acute non-lymphoblastic leukaemia. It is used in the Netherlands and internationationally for induction therapy as well as for consolidation therapy after the patient has achieved a complete remission. Route of administration is by injection or infusion (intravenous, subcutaneous, intramuscular or intrathecal). A widely accepted form of induction therapy includes standard-dose cytarabine 100-200 mg/m^2 (i.e. 2.7-5.3 mg/kg body weight, assuming a surface area of 1.6 m^2 for a 60-kg human), administered by continuous infusion for seven days, combined with three days of intravenous administration of anthracyclines (e.g. daunorubicin) or anthracenodianes, with or without addition of other agents (e.g. etoposide, fludarabine or cladribine). Consolidation therapy comprises treatment with additional courses of intensive chemotherapy, usually with higher doses of cytarabine (2-3 g/m^2; i.e. 53-80 mg/kg body weight).[28]
general toxicity	:	Acute toxicity is low (reported LD_{50}-values are: oral rat: >3,200 mg/kg, >5,000 mg/kg; oral mouse: 826 mg/kg, 3,150 mg/kg; intravenous rat: >5,000 mg/kg; intravenous mouse >7,000 mg/kg; intraperitoneal rat: 1,000 mg/kg, >5,000 mg/kg; intraperitoneal mouse: 1,000 mg/kg, 3,379 mg/kg). Cytarabine is mutagenic in vitro and clastogenic in vitro and in vivo. The carcinogenic potential of cytarabine has not been fully evaluated. In clinical use, adverse effects have included severe nausea and vomiting, bone marrow depression, low white blood cell, red blood cell and platelet counts, rash and hair loss, pain and redness of the palms and feet, respiratory distress, and neurological effects such as ataxia, dysphasia and nystagmus.
mechanism	:	Cytarabine, a pyrimidine nucleoside analogue, is an antimetabolite antineoplastic agent that inhibits the synthesis of DNA. Cytarabine competes with deoxycytidine triphosphate for incorporation into DNA during synthesis. Its actions are specific for the S-phase of the cell cycle.
kinetics	:	Following administration, cytarabine distributes into intracellular compartments with a volume of distribution approximately equal to body water. Its volume of distribution should therefore be substantially increased in the pregnant patient. Cytarabine is rapidly cleared from plasma, but the active metabolite (arabinofuranosyl-cytosine triphosphate; Ara-CTP) is retained intracellularly (13-42% of Ara-CTP 4 h after cytarabine administration). Accumulation of cytarabine in non-pregnant patients does not appear to occur even after successive doses. Approximately 80% of a cytarabine dose is eliminated 36 h after administration with the majority of the dose being excreted as uracil arabinoside. Cytarabine is approximately 13% protein bound and readily penetrates the blood brain barrier (Wiebe & Sipila).[34] Foetal plasma concentrations of cytarabine were 56.7% (± 22.6%; n=6) of the maternal concentration, ninety minutes after an intravenous injection (100 mg/kg) to mice on gestational day 18.5 (Van Calsteren et al.[3]).

Data from HSDB[19], unless otherwise noted.

2.2 Human studies

2.2.1 Fertility studies

Data on the effects of cytarabine on human fertility are difficult to interpret for several reasons, including use of regimens involving multiple agents, case series with small sample sizes, lack of pre-chemotherapy assessment, and inadequate long-term follow up.

Male fertility

Lenz and Valley reviewed the risks of infertility after chemotherapy using a MEDLINE search of articles from 1966-1996.[12] They concluded that male germ depletion probably occurs to some degree as a result of cytarabine therapy in acute leukaemia. No clear conclusions can be drawn, especially because all reported cases concerned combination therapy with other agents.

Lendon et al. investigated testicular biopsies in 44 boys (27 prepubertal and 17 pubertal) treated with various combination therapies for acute lymphoblastic leukaemia.[11] Cytarabine, when exceeding 1 g/m^2 (≈ 27 mg/kg body weight*), was associated with a statistically significant depression of the tubular fertility index in a multivariable model also including cyclophosphamide and the length of time between cessation of therapy and biopsy. The control group consisted of 16 boys who died within three weeks of diagnosis.

Some cases demonstrated that a man may be fertile and produce a normal child during chemotherapy for acute leukaemia with cytarabine in combination with other agents. These favourable reports remain anecdotal. Therefore they were not evaluated.

Female fertility

No relevant data are available.

* For a human adult, assuming a surface area of 1.6 m^2 for a 60 kg human.

2.2.2 *Developmental toxicity studies*

Case-control or cohort studies

Prospective or retrospective epidemiological studies examining the adverse effects of cytarabine during pregnancy are not available.

Case studies

Wiebe & Sipila reviewed 36 cases in which cytarabine was used in combination chemotherapy for the treatment of leukaemia in pregnancy.[34] The authors concluded that cytarabine can be used safely in the second and third trimester, but its use resulted in several cases of fetal anomalies when used during the first eight weeks of gestation. However, in almost all cases cytarabine was given in combination with other drugs. In only one case cytarabine was administered alone (Wagner et al.[33]). The woman delivered an infant with obvious extremity and ear deformities.

Caligiuri & Mayer reviewed 32 reported cases in which cytarabine was administered to pregnant women alone or in combination with other drugs.[2] The 32 pregnancies resulted in 18 normal infants (four of whom had first trimester exposure), two infants with congenital malformations (both with first trimester exposure) and several instances of neonatal distress resulting in one death and five therapeutic abortions. However, in almost all cases cytarabine was given in combination with other drugs. In only one case cytarabine was administered alone (Juárez et al.[8]). The woman was treated during the third trimester and gave birth at term. At the age of one year the infant born was normal.

In a letter to the editor, Morgenstern briefly referred to a number of extra cases of administration of cytarabine to pregnant women.[18] The original case reports were consulted.[13,16,17,20,29,32] Six pregnancies were described. In all cases the pregnant women received cytarabine as part of combination chemotherapy. One woman elected to have her pregnancy aborted. The other pregnancies resulted in normal, healthy infants. The time point at which this was determined varies from birth till the age of four years.

2.2.3 *Lactation*

No human studies on effects of cytarabine during lactation are available.

It is not known whether cytarabine is excreted in human milk.

2.3 Animal studies

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

2.3.1 *Fertility studies*

Male fertility

Cytarabine was administered at single intraperitoneal dose levels of 0, 100, 150 and 200 mg/kg body weight to groups of six male Swiss albino mice (Palo et al.[23]). Spermatocytic analysis was conducted 24 h and 4 wk post-treatment, and sperm morphology assays were conducted 8 wk post-treatment. Statistically significant and dose-dependent increases in the percentage of aberrant spermatogonial metaphases and chromosomal aberrations at 24 h post-treatment, and in the percentages of aberrant primary spermatocytes at wk 4 post-treatment were noted at all three dose levels. The percentage of abnormal sperm at wk 8 post-treatment was, however, not statistically significantly affected.

Female fertility

No data are available.

2.3.2 *Developmental toxicity studies*

No animal studies with oral administration of cytarabine are available.

Structural defects

Mice, intraperitoneal administration

Cytarabine was administered intraperitoneally at doses of 0, 0.5, 2 or 8 mg/kg body weight/day to pregnant Swiss mice (13-15/group) on gestational days 6-15

(Ortega et al.[22]). All dams survived to their scheduled termination on gestational day 18, and there were no abortions or early deliveries. Maternal body weight gain and feed intake during the treatment period were statistically significantly reduced at 2 and 8 mg/kg body weight/day. Maternal body weight and gravid uterine weight was statistically significantly reduced at 8 mg/kg body weight/day. The number of early and late resorptions per litter was increased ($p<0.001$ and $p<0.05$, respectively) and the number of life foetuses decreased ($p<0.001$) at 8 mg/kg body weight/day. Only 4 out of 13 dams in this group contained one or more life foetuses on gestational day 18. No effects on implantations, resorptions or viability were noted at 0.5 and 2 mg/kg body weight/day.

A dose-related and statistically significant decrease in foetal body weights occurred in all treatment groups. The number of stunted foetuses was increased ($p<0.05$) at 8 mg/kg body weight/day. External examination revealed phocomelia and short or absent tail in all foetuses of the 8 mg/kg body weight/day group.

The total incidence of soft tissue defects was statistically significantly increased at 2 and 8 mg/kg body weight/day; cleft palate and dilatation of cerebral ventricles were the main findings.

At 8 mg/kg body weight/day, skeletal malformations consisted of severe general retardation, incomplete ossification of the skull bones, fused and fragmented ribs, split vertebral arches, bifid vertebral centra and partial or complete absence of limb bones. At 0.5 and 2 mg/kg body weight, skeletal maturation was statistically significantly reduced (especially decreased numbers of ossified sacrococcygeal vertebrae as well as decreased percentage of foetuses with ossified calcaneus). Based on growth alterations, the NOAEL for maternal toxicity was placed at 0.5 mg/kg body weight/day. The developmental NOAEL was <0.5 mg/kg body weight/day.

Cytarabine was administered intraperitoneally at single doses of 0, 2, 10, 25, 50, 100 or 200 mg/kg body weight to 265 pregnant 1CR mice on gestational days 10, 10.5, 11, 11.5, 12, 12.5, 13, 14 or 16 (Kochhar et al.[10]). Injection of the mother with levels up to 200 mg/kg body weight at any time between gestational days 10.5 and 12 did not cause ‘any physical distress’. Embryo lethality depended on the dose level and stage of injection; e.g. a dose of 100 or 200 mg/kg body weight was completely embryo-lethal during gestational days 10.5-12, whereas 2 or 10 mg/kg body weight produced hardly any resorption at any stage.

Limb defects were induced during gestational days 10.5-12.5, not by treatment before or after this period. The effective dose was ≥10 mg/kg body weight. The dose of 2 mg/kg body weight was completely without effect. The

limb defects encountered were micromelia, phocomelia, hemimelia, ectrodactyly, polydactyly and adactyly.

Depending on the dose and time of injection, other defects included stunted growth, cleft palate, fusions of the vertebral bodies and of ribs, shortened abnormal tails, and micrognathia (both maxilla and mandible).

Cytarabine was administered intraperitoneally at single doses of 0, 10, 20 or 40 mg/kg body weight to pregnant CD-1 mice at various times from gestational day 10 till gestational day 12 (Manson et al.[14]). The dams were killed on gestational day 18. The number of litters examined were 18 in controls and 2-4 in the various groups at the various stages. No data on maternal toxicity were provided. There was a dose-related increase in both forelimb and hindlimb malformations (no statistics presented). Limbs were maximally sensitive to cytarabine between gestational day 10 (9 p.m.) and gestational day 11 (9 a.m.). Malformations (adactylous limbs with distally located blisters) ranged from 13%-100%, depending on the dose and time of exposure.

Cytarabine was injected intraperitoneally at single doses of 0 or 30 mg/kg body weight to groups of 4-12 pregnant CD-1 mice on gestational days 14 and 15 (Gray et al.[7]). No data on maternal toxicity were provided. Mortality in cytarabine-exposed offspring was considerably increased due to a failure of the lower incisors to develop. Many cytarabine-treated mice had narrow flattened skulls and some mice had thin cerebral hermispheres (no further details were given).

In a second experiment, cytarabine was injected intraperitoneally at a single dose of 0 or 30 mg/kg body weight to groups of 11-30 pregnant CD-1 mice on gestational days 8-9, 10-11, 14-15 or 17-18.[7] No data on maternal toxicity were provided.

No live pups were born to dams in the gestational days 8-9 and 10-11 treatment groups. All foetuses were resorbed in the gestational days 8-9 group, while a few malformed pups were born in the gestational days 10-11 group ($p<0.01$).

Growth was retarded by 25% (not statistically significant) in offspring in the gestational days 14-15 group and unaffected in the gestational days 17-18 group. Cytarabine-exposed offspring showed increased locomotor activity and were more aggressive (see ‘Cognitive effects’ below).

Cytarabine was injected intraperitoneally at a single dose of 0 or 5 mg/kg body weight to groups of 8 or 12 pregnant mice on gestational day 10.5 (Rahman et

al.[25]). Skeletal changes in offspring were evaluated on post-natal day 15 or 24. No data on maternal toxicity were provided. The body weights of newborns were similar in the treatment and control groups. In the treatment group, the incidence of external digit abnormalities (oligodactyly or polydactyly) of forelimbs and hindlimbs was about 50% and 20%, respectively, versus 0% in controls. The incidence of various abnormalities of carpal or tarsal bones (fusion, absence or deformation) was increased (no statistics presented) in the offspring of treated mice.

Cytarabine was injected intraperitoneally at single doses of 0, 0.5, 1 or 2 mg/kg body weight to groups of 6 to 10 pregnant mice on gestational day 10.5 (Rahman et al.[26]). Skeletal changes in offspring were evaluated on post-natal day 15. No data on maternal toxicity were provided. The number of foetuses per dam and the body weights of newborns were similar in the treatment groups and controls. The relatively low doses induced carpal and tarsal bone abnormalities (mostly fusions, ranging from 19% in the low-dose group ($p<0.01$) to 88% in the high-dose group ($p<0.01$)), without producing any other external or skeletal abnormalities.

Cytarabine was injected intraperitoneally at a single dose of 0 or 10 mg/kg body weight to pregnant Swiss Webster mice (22 or 24 animals per group) on gestational day 9 (Chiang et al.[4]). Dams were killed on gestational day 18. No data on maternal toxicity were reported. The incidence of resorptions and dead foetuses was statistically significantly increased to 20% in the treatment group (versus 5% in controls). The incidence of cleft palate and/or cleft lip was statistically significantly increased to 26% in the treatment group (versus 2.6% in controls). The length of the foetuses was statistically significantly decreased, and the incidence of minor skeletal variations (reduction of skeletal calcification) was increased (without statistical significance) in the treatment group.

Cytarabine was injected intraperitoneally at single doses of 0, 5 or 7.5 mg/kg body weight to groups of 8-19 pregnant Jcl:ICR mice on gestational days 8, 9.5 or 11 (Chiba et al.[5]). The offspring was killed on post-natal day 24. No data on maternal toxicity were provided. A 30% incidence of hip joint anomalies was observed only in the group exposed to 7.5 mg/kg body weight on gestational day 9.5, though the increase was not statistically significant. The types observed were femoral shaft dysplasia, pseudo arthrosis of the femur, femoral head dysplasia, acetabular dysplasia, fusion between the femoral head and acetabulum and

pseudo arthrosis of the coxal bone. Of the newborns in this group, 23% showed oligodactyly.

Rats, intraperitoneal administration

Cytarabine was injected intraperitoneally at single doses of 0, 15, 50, 100 or 200 mg/kg body weight to pregnant Wistar rats on gestational day 12 (Ritter et al.[27]). Pregnancy was terminated on gestational day 20.

The number of foetuses examined was 74-101 in the treatment groups and 477 in controls.

No data on maternal toxicity were provided. The percentage of malformations was 2% in controls, 16% in low-, 67% in mid- and 85% in high-dose groups (no *p*-values reported). The principal external malformations were ectrodactyly, brachydactyly, syndactyly, cleft palate, clubfoot and kinky tail. Internal abnormalities (hydrocephalus, hydronephrosis, diaphragmatic hernia and genital defects) occurred occasionally, particularly at high dosage.

CNS structural defects

Mice, intraperitoneal administration

Cytarabine was injected at 30 mg/kg body weight to pregnant Swiss ICR-JCL mice as a single dose, on gestational day 13 (8 mice), or twice, on gestational days 13 and 14 (16 mice) (Kasubuchi et al.[9]). Foetuses were examined within 24 h after the last injection. In addition, offspring of 6 mice were examined. No data on maternal toxicity were provided.

Within six hours after the first injection, pyknotic nuclei and nuclear debris were found at the matrix layer surrounding the lateral ventricles. Most of the matrix cells were killed by the treatment and had disappeared 24 h after the second injection.

Offspring examined after birth showed marked dilatation of the lateral ventricles, especially in the parieto-occipital region. From post-natal day 15, all offspring showed gradual increase of the cranial vault and subsequently died by 35 days of age.

Cytarabine was injected at a dose of 30 mg/kg body weight/day to pregnant Swiss ICR-JCL mice on gestational days 13.5 and 14.5 (Shimada et al.[30]). Foetuses of 6 mice were examined on gestational days 15, 16, 17 and 18. Five to

seven offspring of 15 mice were killed on post-natal days 1, 3, 7, 10, 20, 30, 60 and 120. No data on maternal toxicity were provided.

Severe damage in the matrix layer occurred in embryonic brains examined on gestational day 15, but regenerated partly on gestational day 17. Offspring examined from post-natal day 20 showed pronounced microcephaly. In offspring examined on post-natal day 1, 3 or 5, abnormal clusters of young neurons were found on the surface of the developing cerebral cortex. After post-natal day 20, the clusters gradually became indistinct but some vestigial groups of neurons were observed even at post-natal day 120. The hippocampus of young mice showed severe cytoarchitectural abnormalities.

Takano et al. examined the pathogenesis of grey matter heterotopia and microcephaly produced by cytarabine.[31] A total of 12 pregnant ICR-strain mice were injected with 30 mg/kg body weight on gestational days 13.5 and 14.5 and the offspring were examined. Four other (saline-injected) pregnant mice served as controls. No data on maternal toxicity were provided. Cytarabine disturbed the DNA-replication and migration of neuroepithelial cells in the ventriculate zone (BrdU-labelling, $p<0.001$) on post-natal day 15.5. Nestin-immunoreactive radial glial fibres and calretinin-positive subplate fibres were disrupted. TUNEL-reaction (detection of cells containing fragmented DNA) was remarkable throughout the cerebral hemisphere ($p<0.01$). Subcortical heterotopia in the cingulate cortex and subependymal nodular heterotopia in the dorsolateral part of the lateral ventricles became detectable on post-natal day 1. On post-natal day 32, microcephaly was apparent and subcortical heterotopia was observed to have increased in size. The authors concluded that cytarabine induces neuronal apoptosis throughout the cerebral hemisphere.

Rats, intraperitoneal administration

Cytarabine was injected at a single dose of 0 or 50 mg/kg body weight to pregnant rats of the Lister black and white hooded strain on gestational day 14 (Adlard et al.[1]). At birth, litters were reduced to eight pups for follow-up. Effects of cytarabine were evaluated in offspring at birth, on post-natal day 25 and in adult (15 wk old) offspring. Depending on the effect investigated, group sizes varied between 11 and 39 offspring rats per group.

No data on maternal toxicity were provided. The treatment resulted in statistically significant reductions in birth weight (-14%, $p<0.001$), brain weight at birth (-17%, $p<0.001$) and brain/body weight ratio (-9%, $p<0.001$). The brain

weight deficit at birth reflected a deficit in number of brain cells as assessed by total DNA.

At post-natal day 25, a 22% deficit in brain weight ($p<0.001$) with no significant effect on body weight was noted. Adult, 15 wk old, male offspring of treated mothers also showed microcephaly, in that brain weight was reduced by 15% ($p<0.001$) while body weight was normal.

Adult offspring of treated mothers showed an impairment in discrimination learning, when tested in a water T-maze (see 'Cognitive effects' below).

Cytarabine was injected at a dose of 0 or 280 mg/kg body weight/day to pregnant Wistar-Imamichi rats on gestational day 15 and 5-8 male offspring were examined on post-natal day 60 (Matsutani et al.[15]). No data on maternal toxicity were provided. Terminal body weight was statistically significantly (15%) reduced in the offspring of treated rats. The weight of the cerebral hemisphere, brain stem and cerebellum statistically significantly decreased to 60%, 75% and 89% of controls, respectively. DNA content (mg/region) in the brain stem and cerebellum decreased by 20% and 10%, respectively. Levels of norepinephrine, dopamine and serotonin in the cerebral hemisphere showed a statistically significant rise (almost two times the control value).

Mice and rats, subcutaneous administration

Cytarabine was injected at doses of 12.5, 25 or 50 mg/kg body weight/day to pregnant ICR Swiss mice on gestational days 16, 17 and 18 and to pregnant Sprague Dawley rats on gestational days 18, 19 and 20 (Percy[24]). Surviving offspring (17-57 per group) were killed on post-natal day 10 or 20. No data on maternal toxicity were provided. No data in concurrent controls were reported. Mortality of offspring rats was high at 25 (24%) and 50 mg/kg body weight/day (70%). Dose-related segmental cerebellar hyperplasia was noted in mice at 25 and 50 mg/kg body weight/day and in rats of all dose groups. Focal microcystic renal cortical dysplasia was noted in mice at 25 and 50 mg/kg body weight/day and in rats of all dose groups. Retinal dysplasia occurred in rats at 50 mg/kg body weight/day.

Cognitive effects

Mice, intraperitoneal administration

Cytarabine was injected at single doses of 0 or 30 mg/kg body weight to groups of 4-12 pregnant CD-1 mice on gestational days 14 and 15 (Gray et al.[7]). No data on maternal toxicity were provided. Cytarabine-exposed offspring (at least one animal per sex and per litter was tested) showed statistically significantly increased locomotor activity in a Figure-eight maze (hyperactivity) on post-natal day 22 (100% increase) and post-natal day 58 (43% increase). Cytarabine-exposed mice (85 days of age) were statistically significantly more aggressive (in a 'latency to attack the intruder test').

In a second experiment[7], cytarabine was injected intraperitoneally at a single dose of 0 or 30 mg/kg body weight to groups of 11-30 pregnant CD-1 mice on gestational days 8-9, 10-11, 14-15 or 17-18. No data on maternal toxicity were provided. No live pups were born to dams in the gestational days 8-9 and 10-11 treatment groups. Offspring (n=12) in the gestational days 14-15 group showed statistically significantly increased locomotor activity in the Figure-eight maze test on post-natal day 22. Behaviour in offspring in the gestational day 17-18 group was unaffected.

Rats, intraperitoneal administration

Cytarabine was injected at a single dose of 0 or 50 mg/kg body weight to pregnant rats of the Lister black and white hooded strain on gestational day 14 (Adlard et al.[1]).

No data on maternal toxicity were provided. Adult offspring of treated mothers (11 or 12 per group) showed an impairment in discrimination learning when tested in a water T-maze ($p<0.01$).

Rats, subcutaneous administration

Cytarabine was injected at a dose of 0 or 30 mg/kg body weight/day to pregnant Sprague Dawley rats on gestational days 19.5 and 20.5 (Elmer et al.[6]). This dosing regimen was chosen deliberately to investigate behavioural changes in the absence of severe anatomical lesions. Groups of 14-26 offspring were subjected to sensorimotor assessment on post-natal days 35 and 56. No data on maternal toxicity were provided. Disruption of the pyramidal cell layer in the

hippocampus was noted in cytarabine-exposed offspring. On post-natal day 35 no statistically significant neurocognitive changes were observed. On post-natal day 56, however, cytarabine-exposed offspring had lower acoustic startle amplitudes (acoustic startle test) (p<0.002) and diminished sensorimotor gating (prepulse inhibition of the acoustic startle response) (p<0.025).

2.3.3 Lactation

No animal studies on effects of cytarabine during lactation were available.

2.4 Conclusions

2.4.1 Fertility

The Committee proposes not classifying cytarabine for effects on fertility due to a lack of appropriate human and animal data.

2.4.2 Developmental toxicity

Prospective or retrospective epidemiological studies examining the adverse effects of cytarabine during pregnancy are not available. Several case reports on cytarabine treatment for leukaemia in pregnancy indicated both successful and unsuccessful maternal and foetal outcomes.[8,13,16,17,20,29,32,33] The data do not allow definite conclusions with respect to adverse perinatal outcomes or congenital malformations in humans. There are no animal studies available with oral administration of cytarabine. The rat and mouse studies with intraperitoneal or subcutaneous administration of cytarabine to pregnant rats or mice show a range of effects on development.[1,4-7,9,10,14,15,22,24-27,30,31] Most studies did not report on maternal toxicity. However, the developmental effects observed in two mouse studies, that of Kochhar et al. and that of Ortega et al., are not considered to be a non-specific consequence of maternal toxicity.[10,22] For effects on development, the Committee therefore recommends to classify cytarabine in category 1B (presumed human reproductive toxicant) and to label it H360D (may damage the unborn child).

2.4.3 Lactation

No human studies or animal studies on effects of cytarabine during lactation were available. The Committee therefore proposes not classifying cytarabine for effects on or via lactation.

Proposed classification for fertility

Lack of appropriate data precludes the assessment of cytarabine for effects on fertility.

Proposed classification for developmental toxicity

Category 1B, H360D.

Proposed labelling for effects during lactation

Lack of appropriate data precludes the assessment of cytarabine 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
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- J.G. Theuns-van Vliet
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  Reproductive Toxicologist, Zeist
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  Toxicologist, Weterings Consultancy BV, Rosmalen
- P.W. van Vliet, *Scientific Secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present document was prepared by Dr. B.A.R. Lina (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 *Cytarabine*
Your reference : DGV/BMO/U-932542
Our reference : U-791480/EvV/fs/543-K15
Enclosure(s) : 1
Date : July 23, 2015

Dear Minister,

I hereby submit the advisory report on the effects of cytarabine 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 Committee 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)
Professor W.A. van Gool,
President

Annex C

Comments on the public draft

A draft of the present report was released in 2014 for public review. The following organisation and persons have commented on the draft document:

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

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

Annex **D**

Regulation (EC) 1272/2008 of the European Community

3.7 Reproductive toxicity

3.7.1 Definitions and general considerations

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

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

(a) adverse effects on sexual function and fertility;

(b) adverse effects on development of the offspring.

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

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

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

3.7.1.3 Adverse effects on sexual function and fertility

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

3.7.1.4 Adverse effects on development of the offspring

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

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

3.7.2 Classification criteria for substances

3.7.2.1 Hazard categories

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

Table 3.7.1(a) Hazard categories for reproductive toxicants.

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

Table 3.7.1(b) Hazard category for lactation effects.

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

3.7.2.2 Basis of classification

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

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

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

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

3.7.2.3 Weight of evidence

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

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

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

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

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

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

3.7.2.4 Maternal toxicity

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

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

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

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

Maternal mortality:

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

Mating index

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

Fertility index

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

Gestation length

(if allowed to deliver)

Body weight and body weight change:

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

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

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

Food and water consumption (if relevant):

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

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

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

Post-mortem data:

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

3.7.2.5 Animal and experimental data

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

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

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

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

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

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

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

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

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

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

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

3.7.3 Classification criteria for mixtures

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

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

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

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

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

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

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

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

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

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

3.7.4 Hazard Communication

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

Table 3.7.3 Label elements for reproductive toxicity.

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

Annex **E**

Additional considerations to Regulation (EC) 1272/2008

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

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

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

* Organisation for Economic Cooperation and Development.

Annex **F**

Fertility and developmental toxicity studies

Table 1 Fertility studies in animals with cytarabine.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproductive organs/ effects on reproduction
Male fertility					
Palo *et al*., 2009	Swiss albino mice, 8-10 wk old	- Cytarabine was given once to groups of six male mice. - Spermatocytic analysis at 24 h and 4 wk post-treatment. - Sperm morphology assay at 8 wk post-treatment.	Single i.p. injection, 0, 100, 150 and 200 mg/kg bw.	No data on general toxicity.	- Statistically significant and dose-dependent increases in percentage of aberrant spermatogonial metaphases and chromosomal aberrations at 24 h post-treatment. - Statistically significant and dose-dependent increases in percentages of aberrant primary spermatocytes at wk 4 post-treatment at all three dose levels. - The percentage of abnormal sperm at wk 8 post-treatment was, however, not statistically significantly affected.
Female fertility					
No data available					

Table 2 Developmental studies in animals with cytarabine.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Developmental toxicity
Structural defects					
Ritter et al., 1971	Wistar rats Albino Farms stock	Cytarabine was administered to pregnant Wistar rats on gd 12. Pregnancy was terminated on gd 20. Embryos examined were 74-101 in each treatment group and 477 in controls.	Single i.p. injection, 0, 15, 50, 100 or 200 mg/kg bw	No data on maternal toxicity.	- The percentage of malformations was 2% in controls, 16% in low-, 67% in mid- and 85% in high-dose group (no p-values reported). - The principal external malformations were ectrodactyly, brachydactyly, syndactyly, cleft palate, clubfoot and kinky tail. - Internal abnormalities (hydrocephalus, hydronephrosis, diaphragmatic hernia and genital defects) occurred occasionally, particularly at high dosage.
Manson et al., 1977	Swiss-Cox albino mice	Cytarabine was administered to pregnant mice at various times on gd 10 - gd 12. The dams were killed on gd 18. The number of litters examined was 18 in controls and 2-4 in the various groups at the various stages.	Single i.p. injection of 0, 10, 20 or 40 mg/kg bw.	No data on maternal toxicity.	- There was a dose related increase in both forelimb and hindlimb malformations. - Limbs were maximally sensitive to cytarabine between gd 10 (9 p.m.) and gd 11 (9 a.m.). - Malformations (adactylous limbs with distally located blisters) ranged from 13%-100%, depending on the dose and time of exposure.
Kochhar et al., 1978	1CR mice	Cytarabine was administered to 265 pregnant mice on gd 10, 10.5, 11, 11.5, 12, 12.5, 13, 14 or 16. Dams were killed on gd15 or gd 18.	Single i.p. injection of 0, 2, 10, 25, 50, 100 or 200 mg/kg bw.	Injection of the mother with levels up to 200 mg/kg bw at any time between gd10.5-12 did not cause 'any physical distress'.	- Embryo lethality depended on the dose level and stage of injection; e.g. a dose of 100 or 200 mg/kg bw was completely embryo lethal during gd 10.5-12, whereas 2 or 10 mg/kg bw produced almost no resorptions at any stage. - Limb defects were induced during gd 10.5-12.5, not by treatment before or after this period. The effective dose was ≥10 mg/kg bw. The dose of 2 mg/kg bw was completely without effect. The limb defects were micromelia, phocomelia, hemimelia, ectrodactyly, polydactyly and adactyly. - Depending on the dose and time of injection, other defects included stunted growth, cleft palate, fusions of the vertebral bodies and of ribs, shortened abnormal tails, and micrognathia (both maxilla and mandible).

Gray et al., 1996	CD-1 mice	Cytarabine was administered to groups of 4-12 pregnant CD-1 mice on gd 14 and gd 15. At least one/sex/ litter was tested in a figure-eight maze.	Repeated i.p. injection 0 or 30 mg/ kg bw/d.	No data on maternal toxicity.	- Mortality in cytarabine-exposed offspring was considerably increased due to a failure of the lower incisors to develop. Many cytarabine-treated mice had narrow flattened skulls and some mice had thin cerebral hermispheres (no further details were given).
	CD-1 mice	In a second experiment, cytarabine was administered to groups of 11-30 pregnant CD-1 mice on gd 8-9, gd 10-11, gd 14-15 and gd 17-18.	Repeated i.p. injection 0 or 30 mg/ kg bw/d.	No data on maternal toxicity.	- No live pups were born to dams in the gd 8-9 and 10-11 treatment groups. All pups were resorbed in the gd 8-9 group, while a few malformed pups were born in the gd 10-11 group ($p<0.01$). - Growth was retarded by 25% (not statistically significant) in offspring in the gd 14-15 group and unaffected in the gd 17-18 group.
Ortega et al., 1991	Swiss mice	Cytarabine was administered to pregnant Swiss mice (13-15/group) on days 6-15 of gestation. Dams were killed on gd 18.	Repeated i.p injection of 0, 0.5, 2 or 8 mg/kg bw/d on gd 6-gd 15.	- All dams survived to their scheduled termination on gd 18. - There were no abortions or early deliveries. - Maternal body weight gain and feed intake during the treatment period was reduced at 2 and 8 mg/kg bw/d. - On gd 18, maternal body weight and gravid uterine weight was reduced at 8 mg/kg bw/d.	- NOAEL for maternal toxicity: 0.5 mg/kg bw/d. - Developmental NOAEL: <0.5 mg/kg bw/d. - Increased number of early and late resorptions/ litter ($p<0.001$ and $p<0.05$, respectively) and decreased number of life foetuses at 8 mg/kg bw/d ($p<0.001$). No effects on implantations, resorptions or viability at 0.5 and 2 mg/kg bw/d. - statistically significant dose-related decrease of foetal body weights in all treatment groups. - Increased number of stunted foetuses at 8 mg/kg bw/d ($p<0.05$). - External examination revealed phocomelia and short or absent tail in all foetuses of the 8 mg/kg bw/d group ($p<0.001$ in both cases). - Increased total incidence of soft tissue defects at 2 and 8 mg/kg bw/d; cleft palate and dilatation of cerebral ventricles were the main findings. - At 8 mg/kg bw/d, skeletal malformations consisted of severe general retardation, incomplete ossification of the skull bones, fused and fragmented ribs, split vertebral arches, bifid vertebral centra and partial or complete absence of limb bones. - At 0.5 and 2 mg/kg bw skeletal maturation was reduced (especially decreased numbers of ossified sacrococcygeal vertebrae ($p<0.05$ at both doses) as well as decreased percentage of foetuses with ossified calcaneus ($p<0.05$ and $p<0.001$, respectively).
Rahman et al., 1994	Jcl:ICR mice	Cytarabine was administered to groups of 8 or 12 pregnant mice on gd 10.5. Skeletal changes in offspring were evaluated on pnd 15 or pnd 24.	Single i.p. injection, 0 or 5 mg/kg bw.	No data on maternal toxicity.	- The body weights of newborns were similar in the treatment and control group. - The incidence of external digit abnormalities (oligodactyly or polydactyly) of forelimbs and hindlimbs was about 50% and 20%, respectively, versus 0 in controls. - Increased incidence of abnormalities of carpal or tarsal bones (fusion, absence or deformation) in the offspring of treated mice.

Chiang et al., 1995	Swiss Webster mice	Cytarabine was administered to groups of 22 or 24 pregnant mice on gd 9. Dams were killed on gd 18.	Single i.p. injection, 0 or 10 mg/kg bw.	No data on maternal toxicity.	- Increased incidence of resorptions and dead foetuses (20% versus 5% in controls). - Increased incidence of cleft palate and/or cleft lip (26% versus 2.6% in controls). - Decreased length of the foetuses in the treatment group, - Increased incidence of minor skeletal variations (reduction of skeletal calcification) in the treatment group.
Rahman et al., 1996	Jcl:ICR mice	Cytarabine was administered to groups of 6 to 10 pregnant mice on gd 10.5 Skeletal changes in offspring were evaluated on pnd 15.	Single i.p. injection, 0, 0.5, 1 or 2 mg/kg bw.	No data on maternal toxicity.	- The number of foetuses per dam and body weights of newborns was similar in the treatment groups and controls. - The relatively low doses induced carpal and tarsal bone abnormalities (mostly fusions, ranging from 19% in the low-dose group ($p<0.01$) to 88% in the high-dose group($p<0.01$)), without producing any other external or skeletal abnormalities.
Chiba et al., 1996	Jcl:ICR mice	Cytarabine was to pregnant mice on gd 8, 9.5 or 11. The offspring was killed on pnd 24.	Single i.p. injection, 0, 5 or 7.5 mg/kg bw.	No data on maternal toxicity.	- A 30% incidence of hip joint anomalies was observed only in the group exposed to 7.5 mg/kg bw on gd 9.5 (increase not statistically significant). The types observed were: femoral shaft dysplasia, pseudo arthrosis of the femur, femoral head dysplasia, acetabular dysplasia, fusion between the femoral head and acetabulum and pseudo arthrosis of the coxal bone. - 23% of the newborn in this group showed oligodactyly.
CNS structural defects					
Adlard et al., 1975	Rats of the Lister black and white hooded strain	Cytarabine was administered to pregnant rats on gd 14. At birth, litters were reduced to 8 pups for follow-up. Effects of cytarabine were evaluated in offspring at birth, on pnd 25 and in adult (15 wk old) offspring. Depending on the effect investigated, group sizes varied between 11-39 offspring rats/group.	Single i.p. injection, 0 or 50 mg/kg bw.	No data on maternal toxicity.	- Statistically significant reductions in birth weight (-14%, $p<0.001$)), brain weight at birth (-17%, $p<0.001$)) and brain/body weight ratio (-9%, $p<0.001$)). - The brain weight deficit at birth reflected a deficit in number of brain cells as assessed by total DNA. - At pnd 25, a 22% deficit in brain weight ($p<0.001$) with no significant effect on body weight was noted. Adult, 15 wk old, male offspring of treated mothers also showed microcephaly, in that brain weight was reduced by 15% ($p<0.001$) while body weight was normal. - Adult offspring of treated mothers showed impairment in discrimination learning when tested in a water T-maze ($p<0.01$) (see below).

Kasubuchi et al., 1977	Swiss ICR-JCL mice	Cytarabine was administered to pregnant mice as a single dose on gd 13 (8 mice), or twice on gd 13 and gd 14 (16 mice). Embryos were examined within 24 h after last injection. In addition, offspring of 6 mice were examined.	Single or repeated i.p. injection, 30 mg/kg bw(/d).	No data on maternal toxicity.	- Within six hours after the first injection, pyknotic nuclei and nuclear debris were found at the matrix layer surrounding the lateral ventricles. Most of the matrix cells were killed by the treatment. - 24 h after the second injection, most of the matrix cells had disappeared. - Offspring examined after birth showed marked dilatation of the lateral ventricles, especially in the parieto-occipital region. - From pnd15, all offspring showed gradual increase of the cranial vault and subsequently died by 35 days of age.
Shimada et al., 1982	Swiss ICR-JCL mice	Cytarabine was administered to. pregnant mice on gd 13.5 and gd 14.5. Embryos of 6 mice were examined on gd15, 16, 17 and 18. 5-7 offspring of 15 mice were killed on pnd 1, 3, 7, 10, 20, 30, 60 and 120.	Repeated i.p. injection with 30 mg/ kg bw/d.	No data on maternal toxicity.	- Severe damage in the matrix layer in embryonic brains examined on gd15 (regenerated partly on gd17). - Pronounced microcephaly in offspring examined from pnd 20. - Abnormal clusters of young neurons on the surface of the developing cerebral cortex in offspring examined on pnd 1, 3 or 5 (After pnd20 the clusters gradually became indistinct). - Severe cytoarchitectural abnormalities in the hippocampus of young mice.
Matsutani et al., 1983.	Wistar-Imamichi rats	Cytarabine was administered to pregnant rats on gd 15. Male offspring (5-8 per group) were examined on pnd 60.	Single i.p. injection, 0 or 280 mg/kg bw.	No data on maternal toxicity.	- Significantly (15%) reduced terminal body weight in offspring of treated rats. - Significantly decreased weight of the cerebral hemisphere, brain stem and cerebellum (60%, 75% and 89% of controls, respectively). - Decreased DNA content (mg/region) in the brain stem and cerebellum (20% and 10%, respectively). - Significant rise in norepinephrine, dopamine and serotonin levels (almost two times the control value).
Percy, 1999	ICR Swiss mice Sprague Dawley rats	Cytarabine was administered to pregnant mice on gd 16, 17 and 18 and to pregnant rats on gd 18, 19 and 20 - Surviving offspring (17- 57 per group) was killed on pnd 10 or pnd 20. - No data in concurrent controls were reported.	Repeated s.c. injection, 12.5, 25 or 50 mg/kg bw/d.	No data on maternal toxicity.	- High mortality of offspring rats at 25 (24%) and 50 mg/kg bw/d (70%). - Dose-related segmental cerebellar hyperplasia in mice at 25 and 50 mg/kg bw/d and in rats of all dose groups. - Focal microcystic renal cortical dysplasia in mice at 25 and 50 mg/kg bw/d and in rats of all dose groups. - Retinal dysplasia in rats at 50 mg/kg bw/d.

Takano et al., 2006	ICR strain mice	Cytarabine was administered to pregnant mice (n=12 treated and 4 controls) on gd 13.5 and gd 14.5. The pathogenesis of grey matter heterotopia and microcephaly was examined in offspring	Repeated i.p. injection with 0 or 30 mg/kg bw/d.	No data on maternal toxicity.	- Cytarabine disturbed the DNA replication and migration of neuroepithelial cells in the ventriculate zone (BrdU-labelling, $p<0.001$) on pnd 15.5. - Nestin-immunoreactive radial glial fibres and calretinin-positive subplate fibres were disrupted. - TUNEL-reaction (detection of cells containing fragmented DNA) throughout the cerebral hemisphere ($p<0.01$). - Subcortical heterotopia in the cingulate cortex and subependymal nodular heterotopia in the dorsolateral part of the lateral ventricles was detectable on pnd 1. - On pnd 32, microcephaly was apparent and subcortical heterotopia was observed to have increased in size. - The authors concluded that cytarabine induces neuronal apoptosis throughout the cerebral hemisphere.
Cognitive defects					
Adlard et al., 1975	Rats of the Lister black and white hooded strain	Cytarabine was administered to pregnant rats on gd 14. Adult offspring were tested (11 or 12 males/ group).	Single i.p. injection, 0 or 50 mg/ kg bw.	No data on maternal toxicity.	- Adult offspring of treated mothers showed an impairment in discrimination learning when tested in a water T-maze ($p<0.01$).
Gray et al., 1986	CD-1 mice	Cytarabine was administered to groups of 4-12 pregnant CD-1 mice on gd 14 and gd 15. At least one/sex/ litter was tested in a figure-eight maze.	Repeated i.p. injection 0 or 30 mg/ kg bw/d.	No data on maternal toxicity.	- Cytarabine exposed offspring showed significantly increased locomotor activity (hyperactivity) on pnd 22 (100% increase, $p<0.05$) and pnd 58 (43% increase, $p<0.05$). - Cytarabine-exposed mice (85 days of age) were significantly more aggressive (in a 'latency to attack the intruder test').
	CD-1 mice	In a second experiment, cytarabine was administered to groups of 11-30 pregnant CD-1 mice on gd 8-9, gd 10-11, gd 14-15 and gd 17-18.	Repeated i.p. injection 0 or 30 mg/ kg bw/d.	No data on maternal toxicity.	- No live pups were born to dams in the gd 8-9 and gd 10-11 treatment groups. - Offspring (n=12) in the gd 14-15 group showed significantly increased locomotor activity on pnd 22 ($p<0.001$). - Locomotor activity in offspring in the gd 17-18 group (n=10) was unaffected.

Elmer et al., 2004	Sprague Dawley rats	Cytarabine was administered to pregnant rats on gd 19.5 and gd 20.5. Groups of 14-26 offspring were subjected to sensorimotor assessment on pnd 35 or pnd 56	Repeated i.p. injection with 0 or 30 mg/kg bw/d.	No data on maternal toxicity.	- Disruption of the pyramidal cell layer in the hippocampus was noted in cytarabine-exposed offspring. - On pnd 35 no statistically significant neurocognitive changes were observed. - On pnd 56, cytarabine-exposed offspring had significantly lower acoustic startle amplitudes (acoustic startle test) (p=0.002, p=0.039) and significantly diminished sensorimotor gating (prepulse inhibition of the acoustic startle response) (p<0.025).

bw=body weight(s); BrdU=5-bromodeoxyuridine; CNS=central nervous system; d=day(s); gd=gestational day; h=hour(s); i.p.=intraperitoneal; n=number; NOAEL=no observed adverse effect level; pnd=post-natal day; s.c.=subcutaneaous; wk=week(s)

Health Council of the Netherlands

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