Health Council of the Netherlands

Uranium and its compounds

Evaluation of the effects on reproduction, recommendation for classification

2016/05

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Uranium and its compounds*
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Geachte minister,

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

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

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

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

Met vriendelijke groet,

prof. dr. J.L. Severens
vicevoorzitter

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Uranium and its compounds

Evaluation of the effects on reproduction,
recommendation for classification

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

to:

the Minister of Social Affairs and Employment

No. 2016/05, The Hague, May 09, 2016

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

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

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

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.

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Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad uranium en uraniumverbindingen onder de loep genomen. Uranium komt in variërende concentraties in de natuur voor; het bestaat uit drie isotopen, die alle radioactief zijn (^{234}U, ^{235}U en ^{238}U). Daarnaast bestaan er 19 uraniumisotopen als gevolg van menselijke activiteiten. Natuurlijk uranium bestaat uit 99,27% ^{238}U, 0,72% ^{235}U, en 0,01% ^{234}U per massa-eenheid. Verarmd uranium bestaat uit een lager percentage ^{234}U en ^{235}U, en een hoger percentage ^{238}U vergeleken met natuurlijk uranium, terwijl verrijkt uranium uit een hoger percentage ^{234}U en ^{235}U, en een lager percentage ^{238}U bestaat vergeleken met natuurlijk uranium. Voor een juiste evaluatie van de reproductietoxische effecten van uranium zou onderscheid gemaakt moeten worden tussen stralingseffecten en chemische effecten. Volgens het Amerikaanse Agency for Toxic Substances and Disease Registry (ATSDR) zijn de risico's van natuurlijk en verarmd uranium vooral het gevolg van de chemische kenmerken van uranium en niet zozeer het gevolg van radioactiviteit, in tegenstelling tot verrijkt uranium. Omdat verrijkt uranium en uraniumisotopen die ontstaan als gevolg van menselijke activiteiten uitgesloten zijn van de wetgeving voor classificatie en labeling, is het voorliggende advies gericht op natuurlijk en verarmd uranium.

Uranium wordt in de industrie gebruikt als laagverrijkte metalen of als keramische UO_2-brandstofkorrels. Verarmd uranium wordt gebruikt in traagheidsbesturingsapparaten en tolkompassen, als contragewicht in helikopters en vliegtuigen, als afschermingsmateriaal en in Röntgenapparatuur. Het kan ook

worden gebruikt voor de productie van pantserdoordringende munitie. Uraniumdioxide wordt gebruikt om de levensduur van gloeidraden in lampen gebruikt voor fotografie en filmprojectoren te verlengen. Uraniumverbindingen worden ook gebruikt voor kleurschakeringen in de leer- en houtindustrie en de fotografie, en als beits in de zijde- en houtindustrie. Ammoniumdiuranaat wordt gebruikt om gekleurd glazuur te maken voor keramiek. Uraniumcarbide is een goede katalysator voor de productie van synthetisch ammonia.

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 uranium en uraniumverbindingen komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de Commissie om uranium en uraniumverbindingen niet te classificeren wegens onvoldoende geschikte gegevens
- voor effecten op de ontwikkeling adviseert de Commissie uranium niet te classificeren wegens onvoldoende geschikte gegevens.
  De Commissie adviseert om uranium*verbindingen* te classificeren in categorie 2 (*stoffen die ervan verdacht worden dat zij toxisch zijn voor de menselijke voortplanting*) en te kenmerken met H361d (*wordt ervan verdacht het ongeboren kind te schaden*)
- voor effecten op of via lactatie adviseert de Commissie om uranium en uraniumverbindingen niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report the Health Council of the Netherlands reviewed uranium and uranium compounds. Natural uranium, which is found in varying concentrations in rocks and soil, consists of three isotopes, all of which are radioactive (^{234}U, ^{235}U en ^{238}U). In addition, 19 uranium isotopes originate from human activities. Natural uranium consists of 99.27% ^{238}U, 0.72% ^{235}U, and 0.01% ^{234}U by mass. Depleted uranium contains a decreased amount of ^{234}U and ^{235}U, and an increased amount of ^{238}U when compared to natural uranium, while enriched uranium contains an increased amount of ^{234}U and ^{235}U, and a decreased amount of ^{238}U when compared to natural uranium. For a proper evaluation of the effects of uranium on reproductive toxicity, a distinction should be made between radiation hazards and chemical hazards. According to the U.S. Agency for Toxic Substances and Disease Registry (ATSDR), natural and depleted uranium are more likely to be chemical hazards than radiation hazards, in contrast to enriched uranium. Since enriched uranium and uranium isotopes originating from human activities are excluded from the classification and labelling legislation, the current assessment of uranium focuses on natural and depleted uranium.

Uranium is used in the commercial nuclear power industry as low-enriched metal or ceramic UO_2 fuel pellets. Depleted uranium is used in inertial guidance devices and gyro compasses, as counterbalances for helicopter rotors, and aircraft control surfaces, as radiation shielding material, and as X-ray targets. It can also be used in the manufacture of armour-piercing ammunition for the

military. Uranium dioxide is used to extend the lives of filaments in large incandescent lamps used in photography and motion picture projectors. Uranium compounds are used in photography for toning, in the leather and wood industries for stains and dyes, and in the silk and wood industries as mordants. Ammonium diuranate is used to produce coloured glazes in ceramics. Uranium carbide is a good catalyst for the production of synthetic ammonia.

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, uranium and uranium compounds these recommendations are:

- for effects on fertility, the Committee recommends not classifying uranium and uranium compounds due to a lack of appropriate data
- for developmental toxicity, the Committee recommends not classifying uranium due to a lack of appropriate data.
  The Committee recommends classifying uranium *compounds* in category 2 (*suspected human reproductive toxicant*) and labelling with H361d (*suspected of damaging the unborn child*)
- for effects on or via lactation, the Committee recommends not labelling uranium and uranium compounds due to a lack of appropriate data.

Chapter 1

Scope

1.1 Background

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

1.2 Subcommittee and procedure

This document contains the classification of uranium and uranium compounds 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 2015, the President of the Health Council released a draft of the report for public review. The individuals and organizations that commented on the draft report are listed in Annex C. The Committee has taken these comments into account in deciding on the final version of the report. The received comments, 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 compounds.

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 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 on-line databases Toxline, Toxcenter and Medline starting from 1945 up to 2009; updates were performed in TOXNET/TOXLINE until June 2015. Literature was selected primarily on the basis of the text of the abstracts with a focus on natural and depleted uranium. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited. Data are described in the text and animal studies with respect to fertility and development are summarized in Annex F. Of each study, the quality of the study (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

1.5 Presentation of conclusions

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

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

1.6 Final remark

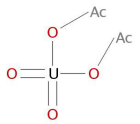
The classification of compounds is based on hazard evaluation (Niesink et al.[29]) only, which is one of a series of elements guiding the risk evaluation process. The Committee emphasizes that for derivation of health-based occupational exposure limits, these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organizations.

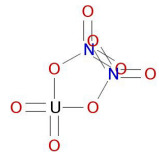
Chapter 2

Uranium and uranium compounds

2.1 Introduction

name	:	uranium
CAS registry number	:	7440-61-1
EINECS number	:	231-170-6
synonyms	:	uranium-234, uranium-235, uranium-238, ^{234}U, ^{235}U, and ^{238}U
colour and physical state	:	silver-white, lustrous, radioactive metal
formula	:	U
atomic weight	:	238.03
boiling point	:	4131 °C
melting point	:	1135 °C
solubility	:	insoluble in water, alkalis, alcohol; soluble in acids
stability and reactivity	:	ignites in oxygen at about 170 °C (1)
use	:	^{235}U is used in atomic and hydrogen bombs ^{234}U and ^{235}U are used as nuclear fuel on power reactors
EU classification	:	not classified in Annex I of Directive 67/548/EEC

name	:	uranyl acetate
CAS registry name	:	uranium, bis(acetato-κO)dioxo-, (T-4)-
CAS registry number	:	541-09-3
EINECS number	:	208-767-5
synonyms	:	uranyl diacetate; uranium diacetate dioxide; uranium oxyacetate; bis(acetato)dioxouranium; diacetatodioxouranium; bis(acetato-O)dioxouran
colour and physical state	:	yellow crystalline powder
chemical formula	:	$C_4H_6O_6U$
structural formula	:	
molecular weight	:	388.12
boiling point	:	275 °C
melting point	:	-
solubility	:	freely soluble in water acidified with acetic acid, slightly soluble in alcohol
Stability and reactivity	:	decomposes before reaching bp of 275 °C; loses $2H_2O$ at 110 °C, decomposes in hot water
use	:	reagent for precipitation of sodium; in dry copying inks and as activator in bacterial oxidation processes
EU Classification	:	not classified in Annex I of Directive 67/548/EEC

name	:	uranyl nitrate
CAS registry name	:	uranium, bis(nitrato- κO)dioxo-, (T-4)-
CAS registry number	:	10102-06-4
EINECS-number	:	233-266-3
synonyms	:	uranyl dinitrate; uranium oxynitrate; uranium nitrate oxide; uranium dinitrate dioxide; dinitratodioxyuranium; bis(nitrato-O)dioxouranium
colour and physical state	:	yellow, rhombic crystals
chemical formula	:	N_2O_8U
structural formula	:	
molecular weight	:	394.04
boiling point	:	decomposes at 118 °C
melting point	:	60 °C
solubility	:	soluble in water and oxygenated solvents, in ethanol, ether
use	:	source of uranium dioxide; extraction of uranium into non-aqueous solvents
EU Classification	:	not classified in Annex I of Directive 67/548/EEC

Data from ECHA, HSDB, IUCLID and Merck.[11,15,21,32]

Uranium exists in several isotopic forms, all of which are radioactive. The most toxicologically important of the 22 currently recognized uranium isotopes are ^{232}U and ^{233}U, originating from human activities, and ^{234}U, ^{235}U, and ^{238}U, occurring naturally. Natural uranium, including uranium ore, is comprised of 99.27% ^{238}U, 0.72% ^{235}U, and 0.01% ^{234}U by mass.[20] Enriched uranium contains an increased amount of ^{234}U and ^{235}U, and a decreased amount of ^{238}U when compared to natural uranium, while for depleted uranium the opposite is true.[2] For a proper evaluation of the effects of uranium on reproductive toxicity, a distinction should be made between radiation hazards and chemical hazards. According to the U.S. Agency for Toxic Substances and Disease Registry (ATSDR), natural and depleted uranium are more likely to be chemical hazards than radiation hazards, in contrast to enriched uranium.[2] Since enriched uranium and uranium isotopes originating from human activities are excluded from the classification and labelling legislation, the current assessment of uranium focuses on natural and depleted uranium. When a publication does not mention the use of depleted or enriched uranium specifically, it is assumed that natural uranium is used. Since the three uranium isotopes chemically behave the same, the chemical risks found for depleted and natural uranium will also account for enriched uranium. However, for enriched uranium, ionizing radiation may play an additional role in reproductive toxicity (see ATSDR-document regarding ionizing radiation[1]).

Uranium is extracted chemically from ores and converted into chemical forms usable in industry. It is used in the commercial nuclear power industry as low-enriched metal or ceramic UO_2 fuel pellets.[2] Depleted uranium is used in inertial guidance devices and gyro compasses, as counterbalances for helicopter rotors, and aircraft control surfaces, as radiation shielding material, and as X-ray targets. It can also be used in the manufacture of armour-piercing ammunition for the military. Uranium dioxide is used to extend the lives of filaments in large incandescent lamps used in photography and motion picture projectors. Various uranium compounds are used in photography for toning, in the leather and wood industries for stains and dyes, and in the silk and wood industries as mordants. Ammonium diuranate is used to produce coloured glazes in ceramics. Uranium carbide is a good catalyst for the production of synthetic ammonia. Consequently, all these compounds can cause occupational exposure. Most of them have not been investigated for reproductive toxicity. The only exceptions are uranyl

acetate and uranyl nitrate, of which the main chemical and physical characteristics are listed above. They have been investigated in animal studies. The epidemiological studies concern depleted (i.e. metallic) uranium, or uranium from natural sources (unspecified compounds, presumably mixtures).

Occupational exposure to uranium and uranium compounds occurs mainly through inhalation. The uptake of the inhaled uranium into the body predominantly depends on the size of the aerosol particles and the chemical form of the uranium. The ICRP distinguishes three classes of compounds on the basis of their solubility in biological fluids: fast, moderate and slow, reflecting the rate of elimination from the lungs.[17,18] Slowly soluble uranium compounds, such as (metallic) uranium and uranium oxides, do not dissolve easily and can consequently remain in the lungs for years. More soluble compounds, on the other hand, can enter the bloodstream in a matter of days or even hours. Uranyl acetate and uranyl nitrate belong to the most readily absorbed compounds.

Once in the blood, the uranium is distributed throughout the body and reaches the organs. It preferentially distributes to bone, liver, and kidney. Approximately 67% of the uranium in the blood is filtered out in the kidneys and leaves the body in urine within 24 hours.[2] Uranium can be transferred to the foetus during pregnancy. Concentrations in the foetus and the placenta are 10% at the most of those in maternal blood.[33]

The toxicity of uranium also depends on its chemical form. With increasing dose the kidneys are affected first. Chronic inhalation exposure to uranium compounds dissolving fast and to uranium compounds dissolving moderately fast mainly causes chemical damage to kidney tubular cells. Chronic inhalation exposure to barely soluble uranium compounds mainly leads to damage to the airways.[2] Consequently, the primary target organ of inhaled barely soluble compounds is the lung. The primary target organ of dissolved uranium compounds is the kidney. The no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) for renal toxicity following inhalation vary depending on the uranium compound, the animal species used for testing and the duration of the experiment.[2]

Inhaled uranium is associated with only a low cancer risk, with the main risk being associated with the co-inhalation of other toxic and/or carcinogenic agents,

such as the radioactive transformation products of radon gas and cigarette smoke.[2,16] The International Agency for Research on Cancer (IARC) has not published a classification report on uranium.

2.2 Human studies

2.2.1 Fertility

Zaire and colleagues examined Namibian uranium miners that were exposed long-term to a low dose of uranium, from natural sources (six-fold increase in uranium excretion in urine when compared to controls). The miners showed a 3-fold increase in chromosome aberrations in their peripheral blood lymphocytes ($p<0.0001$). Further changes included decreased neutrophil counts ($p<0.004$) and marginally increased lymphocyte counts ($p<0.03$). In addition, a decrease in serum LH and FSH levels was observed ($p<0.008$).[35]

McDiarmid et al. examined 50 male Gulf War veterans with retained depleted uranium (DU) fragments (exposed to DU by means of friendly fire) 10 years after their first exposure. Cohorts were stratified based on urine uranium concentrations. Observed health effects were related both to subtle but biologically plausible perturbations in central nervous system function and to a general measure of mutagen exposure. No statistically significant differences between high and low uranium exposure groups were observed for luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone, or thyroid measures. The incidences of subnormal sperm count and motility characteristics were not statistically significantly different between the high and low urine uranium groups. Total sperm count, total progressive sperm, and total rapid progressive sperm (as categorized by the World Health Organization) in the high urine uranium group were increased compared to the low urine uranium group ($p<0.05$).[27]

McDiarmid et al. examined 35 male Gulf War veterans with retained DU fragments (exposed to DU by means of friendly fire) 16 years after their first exposure. Cohorts were stratified based on urine uranium concentrations. The high-uranium group showed a trend toward higher concentrations of urine b2 microglobulin compared to the low-uranium group (81.7 vs. 69.0 mg/g creatinine; $p=0.11$) and retinol binding protein (48.1 vs. 31.0 mg/g creatinine; $p=0.07$). No statistically significant differences between urine uranium groups

were observed in any of the World Health Organization (WHO, 1987) criteria semen characteristics measured.[26]

2.2.2 Development

Müller et al. reported that male uranium miners were found to have more first-born female children than expected (statistically significant, p not reported). The authors suggested that uranium damaged the y-chromosomes of the miners.[28]

Sumanovic-Glamuzina et al. determined the prevalence of major congenital malformations in West Herzegovina, a part of Bosnia and Herzegovina, immediately and five years after 1991-1995 military activities, which allegedly included the use of weapons with DU. The study included all live-born and stillborn neonates and excluded all aborted foetuses in two one-year cohorts (1995 and 2000) of neonates in the Maternity Ward of the Mostar University Hospital. Malformations were recorded according to the recommendations of the EUROCAT protocol. Major malformations were found in 2.16% of neonates in 1995 (95% confidence interval (CI) 1.49-2.82%) and in 2.26% in 2000 (95% CI 1.50-3.01%). These rates did not differ from the average prevalence in EUROCAT centers.[31]

2.2.3 Lactation

Wappelhorst et al. measured the transfer of uranium (compounds not further specified) from food to milk in nursing mothers. The transfer factor (the portion of uranium intake passed on in the milk) was calculated as the uranium concentration in food (g/kg) divided by the uranium concentration in milk (g/L). Average uranium intake of the mothers was 1.9 ± 1.0 µg/day (0.03 ± 0.02 µg/kg bw/day). Mean uranium content of human milk was 0.03 µg/L. The transfer factor for uranium was calculated to be 21.3.[34]

The International Commission on Radiological Protection (ICRP) assessed the transfer of uranium from maternal milk to the infant. Maternal intake included acute and chronic intake by ingestion or inhalation during pregnancy or breastfeeding. The transfer of uranium from maternal blood to breast milk was calculated using biokinetic modelling. The maximum transfer factors from mother to child were expressed as a proportion of radioactivity in blood. The results indicate that a small fraction of the uranium ingested by mothers is transferred to the milk.[19]

2.3 Animal studies

2.3.1 Fertility

Oral studies

Maynard and co-workers exposed weanling rats (50/sex/group) in pairs for 195 days to a diet containing 0 or 2% uranium nitrate hexahydrate.[25] 2% in the feed is equivalent to 1,000-1,500 mg/kg bw.[12] After 195 days, all animals were fed control diet for another 170 days. In the first 30 days, 4 females and 5 males from the uranium group died. In the remainder of the year, 10% of controls died, compared to 17% of the uranium group. Uranium exposure reduced body weight gain in males and females (after 7 months males and females weighed 65 and 60 grams less than controls). Degeneration of the kidneys was observed in the first week. Between weeks 4 and 6 regeneration and between week 8 and 14 also tubular atrophy was observed. After the recovery period, no renal abnormality was observed. In addition, testicular lesions were observed in uranium exposed rats, including degeneration and atrophic changes (tubuli with loss of spermatozoa, absence of spermatids, loss of primary spermatocytes, presence of multinucleated cells). It was shown that 14/44 uranium exposed rats had irregular oestrus cycles, accompanied by irregular (n=1) or no (n=13) matings, whereas only 2/45 control rats had irregular oestrus cycles (accompanied by no matings). A reduction in females that had at least one litter was observed (44/45 in control group compared to 30/44 in the uranium group). Moreover, the number of litters per female was decreased. In the recovery period, the amount of litters in the uranium group returned to normal. Statistics were not mentioned.[25]

Other studies indicate that uranium does affect male fertility. Maynard and coworkers provided male rats with a chronic diet of uranyl nitrate hexahydrate for 1-2 years (331 mg U/kg bw/day) (Maynard and colleagues (1953), cited in [2,8]). This caused severe degeneration in the testes and depletion of germ cells. Female rats given oral doses of 664 mg U/kg bw/day as uranyl nitrate hexahydrate for 2 years had reduced litter sizes. Analysis of general toxicity was not reported and it is unclear whether controls were included.

In addition, in a study of Malenchenko et al. uranyl nitrate hexahydrate added to the drinking water of male Wistar rats for 4 months (0.1%, ~ 50 mg/kg bw) resulted in decreased testes weight ($p<0.05$), testicular lesions, and necrosis of

spermatocytes and spermatogonia. Spleen weight and spleen index were increased ($p<0.05$) and morphological changes in the thyroid gland were observed. Analysis of general toxicity was not reported.[24]

Llobet et al. provided male Swiss mice (24/group) with drinking water containing uranyl acetate dihydrate (doses of 0, 10, 20, 40, and 80 mg/kg/day) for 64 days. Animals were mated with untreated females (16/group) for 4 days. No clinical signs of toxicity were observed. A non-dose-related decrease in the pregnancy rate of the females was observed in all dose groups (25-35% in uranium treated animals vs. 81% in the control group ($p<0.01$ or $p<0.05$). Body weights were slightly reduced at 80 mg/kg/day (35.8 ± 2.04 g vs. 37.6 ± 2.53 g in the control group, $p<0.05$). No statistically significant differences in the numbers of total implantations, early and late resorptions, and live and dead foetuses were observed. Testicular function was not affected by uranium, as evidenced by normal testes and epididymis weights and normal spermatogenesis. Also histopathologic examination of the testes in mice killed after 64 days of treatment did not reveal any significant effects of uranium on tubule diameter, tubule alterations, and interstitial alterations (focal atrophy, binucleated cells), with the exception of an increase in Leydig cells vacuolization at 80 mg/kg/day ($p<0.05$). Based on effects on pregnancy rates in this study, the no observed adverse effect level (NOAEL) for reproductive toxicity of uranium is below 10 mg/kg/day.[23]

Gilman et al. exposed weanling Sprague-Dawley rats (10/sex/group) for 28 days to uranyl nitrate hexahydrate (UN) in drinking water (< 0.001, 0.96, 4.8, 24, 120 and 600 mg UN/L, corresponding to up to 35 mg/kg bw for males and 40 mg/kg bw for females). No general toxicity was observed. In addition, no changes in reproductive organ weights were found in the epididymis, testes, ovary, or uterus. Next, they exposed weanling Sprague-Dawley rats (15/sex/group) for 91 days to UN in drinking water (0, 0.96, 4.8, 24, 120, or 600 mg UN/L, corresponding to up to 37 mg/kg bw for males and 54 mg/kg bw for females). General toxicity consisted of histopathological lesions in the kidney and liver, in both males and females, in all groups including the lowest exposure group ($p<0.001$, $p<0.01$ or $p<0.05$). Renal lesions of tubules (apical nuclear displacement and vesiculation, cytoplasmic vacuolation, and dilation), glomeruli (capsular sclerosis), and interstitium (reticulin sclerosis and lymphoid cuffing) were already observed in the lowest exposure groups. A lowest observed adverse effect level (LOAEL) of 0.06 and 0.09 mg UN/kg body wt/day was reported for male and female rats,

respectively). No statistically significant changes in reproductive organ weights were found in the epididymis, testes, ovary, or uterus.[14]

Gilman et al. also exposed New Zealand rabbits to uranyl nitrate hexahydrate (UN) in the drinking water (males: 0, 0.96, 4.8, 24, 120, or 600 mg UN/L, corresponding to up to 28.70 mg UN/kg/day; females: 0, 4.8, 24, or 600 mg UN/L, corresponding to up to 43.02 mg UN/kg/day) for 91 days. The hematological and biochemical parameters were not affected in a statistically significant exposure-related manner. Dose-dependent differences consisted of histopathological changes limited primarily to kidney. Changes in renal tubules were characteristic of uranium toxicity (LOAEL for males is 0.05 mg UN/kg bw/day and LOAEL for females is 0.49 mg U/kg bw/day in this study). No statistically significant histopathological or organ weight changes in the epididymis, ovary, testes, or uterus were found.[13]

Albina et al. exposed male Sprague Dawley rats (8-12/group) to uranyl acetate dihydrate in the drinking water at doses of 0, 10, 20 and 40 mg/kg bw/day during 3 months. At the end of the experimental period, male rats were mated for 2 weeks with untreated females. On gestation day 14, one half of pregnant rats were euthanized; the remaining dams were allowed to deliver and wean their offspring. No deaths or clinical signs of toxicity, including body weight gain were observed in the dams at any dose. A decrease in the pregnancy rate was observed at 20 mg/kg bw/day only ($p<0.05$). The numbers of pregnancies were 9/12, 6/8, 4/8 and 6/8 in females mated to males exposed to 0, 10, 20 and 40 mg/kg bw/day, respectively. Thus, no dose-effect relationship was observed. No statistically significant differences in the number of total implants per litter and the number of viable and nonviable implants per litter were observed.[4]

The results reported by Albina et al. are also reported by Linares et al. However, Linares et al. also examined the effects of uranium on male reproduction parameters. The number of spermatids per testis was significantly decreased by uranium administration at 20 and 40 mg/kg bw/day ($p<0.05$). Histopathological examination of the testes in male rats killed after 3 months of treatment revealed few differences in the tubule and interstitial alterations (progressive but not statistically significant cellular loss, Sertoli cells or germinal cells, with cytoplasmic vacuolization) between control and uranium-exposed animals.[22]

Sánchez et al. treated adult female Sprague Dawley rats with uranyl acetate dihydrate in the drinking water at doses of 0, 40 and 80 mg/kg bw/day for

4 weeks before mating with untreated males, and continued this treatment during pregnancy and lactation. No statistically significant effects were found on maternal body weight gain or number of implants (total, viable and non viable)/ litter.[30]

Arnault and colleagues treated female C57Bl×CBA mice for 15 weeks with uranyl nitrate in drinking water. A first group (0, 12.5 or 100 mg/kg bw/day) was euthanized immediately after the end of the 15 weeks administration period; a second group (0, 1.25 or 12.5 mg/kg bw/day) was paired with untreated males after exposure. Dams were euthanized 3 months after the end of the exposure period. No general toxicity was observed on body weight, kidney weight and behavior. Mice from the first group had fewer large antral follicles (Ø >200 µm) than the untreated mice ($p<0.05$), but the findings were not dose-related. The animals showed a normal amount of secondary and early preantral follicles (Ø 70-110 µm). By contrast, dams in the second group had a normal amount of large antral follicles, but more secondary and early pre-antral follicles than untreated mice ($p<0.05$). The effects were not dose-related. Changes in follicle atresia were not observed.[5]

2.3.2 *Developmental toxicity*

Oral studies

Domingo et al. dosed pregnant Swiss mice (20/group) with uranyl acetate dihydrate (0, 5, 10, 25 or 50 mg/kg bw/day, top dose 1/5 of acute LD_{50}) by gavage on gestation day 6-15 and sacrificed on gestation day 18 to assess potential maternal and foetal toxicity. Maternal toxicity (reduced weight gain and food consumption, increased relative liver weight) was seen at all doses ($p<0.001$, $p<0.01$ or $p<0.05$). Dose-related foetotoxicity was observed, manifested as reduced foetal body weight and length, an increase in the incidence of stunted foetuses and external and skeletal malformations, and developmental variations ($p<0.001$, $p<0.01$ or $p<0.05$). External malformations included a dose-related increase in the incidence of cleft palate (statistically significant at 10 mg/kg bw/day and higher doses) and an increase in hematomas ($p<0.05$ at 5 and 50 mg/kg bw/day). Undeveloped renal papillae were seen in the 5 and 25 mg/kg bw/day groups. A dose-related increase in the incidence of skeletal abnormalities (bipartite sternebrae and reduced or delayed ossification of the hind limb, fore limb, skull, and tail) was seen in the 25 and 50 mg/kg bw/day

groups ($p<0.001$, $p<0.01$ or $p<0.05$). Embryolethality was not found at any of the dose levels tested.[10]

Domingo et al. also dosed pregnant Swiss mice (20/group) with uranyl acetate dihydrate (0, 0.05, 0.5, 5 or 50 mg/kg bw/day, top dose 1/5 of acute LD_{50}) by gavage from gestation day 13 through postnatal day 21. Two dams in the 5 mg/kg bw/day group and two dams in the 50 mg/kg bw/day group died during lactation. In contrast to the study described above, effects on body weight or food intake were not observed. Further maternal toxicity was not analysed. Number of litters or litter size was not affected on postnatal day 0. However, litter size was decreased at postnatal day 21 at 50 mg/kg bw/day (5.5 vs. 8.8 in controls, $p<0.05$). The viability index (number of pups viable at day 21/number of pups born) and the lactation index (number of pups viable at day 21/number of pups retained at day 4) were decreased in the 50 mg/kg bw/day group ($p<0.05$). A reduction in liver weight was observed in pups of all dose groups ($p<0.01$ or $p<0.05$). No statistically significant differences were observed in pup weight or body length at birth. No statistically significant differences were observed in developmental signs (pinnae unfolding, lower incisor eruption, eye opening), or in pup weight or body length.[9]

Sánchez et al. treated adult female Sprague Dawley rats with uranyl acetate dihydrate in the drinking water at doses of 0, 40 and 80 mg/kg bw/day for 4 weeks before mating with untreated males, and continued this treatment during pregnancy and lactation. No statistically significant effects were found on maternal body weight gain or number of implants (total, viable and non viable)/ litter. No statistically significant effects were found on the body weight of the pups at post-natal day 1. However, the body weight of the pups (male and female) was decreased on postnatal days 4 (at 40 mg/kg bw/day), 12 and 21 (at 40 and 80 mg/kg bw/day, $p<0.05$). No statistically significant effects of uranium were noted on physical development, neuromotor maturation, and behavior in the offspring.[30]

Arnault and colleagues treated female C57Bl×CBA mice for 15 weeks with uranyl nitrate in drinking water. A first group (0, 12.5 or 100 mg/kg bw/day) was euthanized immediately after the end of the 15 week administration period; a second group (0, 1.25 or 12.5 mg/kg bw/day) was paired with untreated males after exposure. Dams and their female pups were euthanized 3 months after the end of the exposure period. In the dams, no effect was observed on body weight, kidney weight and behavior. Female pups had fewer large antral follicles

(Ø >200 µm) than the untreated mice ($p<0.05$), but the effect was not dose-related. They had a normal amount of secondary and early preantral follicles (Ø 70-110 µm).[5]

Subcutaneous injection

The effects of daily maternal subcutaneous injections of uranyl acetate dihydrate (0, 0.5, 1, and 2 mg/kg bw/day, top dose ~ 1/10 of the acute subcutaneous LD_{50}) from day 6 to day 15 of gestation were evaluated by Bosque et al. in Swiss mice (25 plug-positive dams/dose group). External, internal soft-tissue and skeletal examinations of foetuses were performed on gestation day 18. Dose-related maternal toxicity occurred in all uranium-treated groups as evidenced primarily by deaths (0, 1, 2, and 7 deaths at 0, 0.5, 1 and 2 mg/kg bw/day, respectively) and decreased weight gain during gestation (at all dose levels, ($p<0.001$, $p<0.01$ or $p<0.05$) and decreased body weight at termination (1 and 2 mg/kg bw/day, $p<0.01$ and $p<0.001$, respectively). In addition, maternal liver weight (absolute and relative) and kidney weight (absolute) were decreased in the 1 and 2 mg/kg bw/day dose groups ($p<0.001$ or $p<0.05$). Embryo toxicity was also noted in all uranium-exposed groups (increased percentage of post-implantation loss, $p<0.01$). Foetotoxicity was indicated by a significant reduction in foetal weight at 1 and 2 mg/kg bw/day and by a dose-dependent increase in the number of total internal (renal hypoplasia) and total skeletal (decreased ossification) defects, statistically significant at all doses ($p<0.001$, $p<0.01$ or $p<0.05$). An increase in malformations (cleft palate and bipartite sternebrae) was only detected at 1 and 2 mg/kg bw/day ($p<0.001$, $p<0.01$ or $p<0.05$).[7]

Bosque and coworkers subcutaneously injected pregnant Swiss mice on gestation day 10 with a single dose of 4 mg/kg body weight uranyl acetate dihydrate (saline was used as control). Mice were killed on gestation day 18. Maternal toxicity included death (20%, compared to 0% in controls), lethargy and reduced body weight gain ($p<0.01$). Uranyl acetate administration resulted in a significantly increased percentage of resorptions and dead foetuses (together 36.3 vs 2.21%, $p<0.001$). In addition, decreases in foetal body weight ($p<0.001$) and increases in the percentage of skeletal anomalies (78.6 vs 10.7% foetuses affected in treated vs control group, $p<0.05$) were observed. Skeletal alterations included reduced ossifications, wavy ribs and dorsal hyperkyphosis.[6]

Albina et al. injected pregnant Sprague Dawley rats (11-12/group) subcutaneously with uranyl acetate dihydrate (0, 0.415 or 0.830 mg/kg bw/day)

on gestation days 6 to 15. Cesarean sections were performed on gestation day 20. Maternal toxicity was noted at 0.830 mg/kg bw/day (decreased body weight gain, decreased absolute and relative kidney weight ($p<0.05$)). Foetotoxicity was evidenced at 0.415 and 0.830 mg/kg/day by reductions in foetal body weight (dose-dependent, $p<0.05$) and increases in the percentage of skeletally affected foetuses (16, 55 and 88% in controls, at 0.415 and at 0.830 mg/kg/day, respectively ($p<0.003$)). The skeletal abnormalities observed were delayed ossifications.[3]

2.4 Conclusion

2.4.1 Fertility

The human data on the potential effect of uranium and uranium compounds on fertility originate from surveillance studies of uranium miners and Gulf war veterans. As both populations may have been in contact with other toxic substances, the reported effects on fertility are not sufficient to classify uranium or uranium compounds for having effects on fertility in humans.

The animal studies all concern uranium compounds. The studies in rats and mice with oral administration of these compounds do not show consistent outcomes. Arnault et al. found a reduced number of follicles in female mice, in the absence of general toxicity.[5] Malenchenko et al. demonstrated male sex organ toxicity, but also found signs of toxicity in other organs.[24] Lloblet et al.[23], and Albina et al. and Linares et al.[4,22] showed reduced pregnancy rates in mice and rats, respectively. They did not provide information as to whether general toxicity occurred. The effects on fertility were not dose-dependent in any of these studies. Another study, by Sánchez et al., showed unchanged pregnancy rates, so no effects on functional fertility, in the absence of general toxicity.[30]

In conclusion, based on the inconsistent results, and limited information on general toxicity, the committee recommends not to classify uranium and uranium compounds for fertility due to a lack of appropriate data.

2.4.2 Developmental toxicity

The available human data, restricted to uranium miners and a post-war population, are not sufficient to allow a conclusion regarding developmental effects of uranium or uranium compounds.

The animal data all concern uranium compounds and demonstrate prenatal as well as postnatal developmental effects of uranium compounds administered orally. Effects on offspring, including decreased litter size, litter viability and pup weight, were observed in rats and mice orally treated with uranium compounds.[5,9,30] These postnatal effects occurred without maternal toxicity. The prenatal effects seen in mice treated orally included reduced foetal body weight and length, and increased numbers of stunted foetuses and external and skeletal malformations.[5,9,10,30] The effects seen in one of the studies were dose-dependent.[10] They were, however, accompanied by maternal toxicity. Overall, the Committee considers that exposure to uranium compounds can cause prenatal and postnatal effects that may be independent of maternal toxicity.

The findings from the studies in rats and mice treated orally are supported by those from studies in rats and mice injected subcutaneously.[3,6,7]

Based on the prenatal and postnatal effects seen in animals, the Committee recommends to classify uranium *compounds* in category 2 (*suspected human reproductive toxicant*) and to label them with H361d (*suspected of damaging the unborn child*). The Committee recommends not to classify uranium due to a lack of data.

2.4.3 Lactation

Two publications are available regarding the transfer of uranium from food to milk in nursing mothers. In one of these the results of measurements in milk are reported, in the other transfer factors from maternal blood to milk based on biokinetic modelling. Together, the studies indicate that a small portion of the uranium ingested by mothers is transferred to milk. There are no further human or animal data regarding the secretion of uranium compounds in milk or regarding the effects on offspring. Therefore, the Committee is of the opinion that due to a lack of appropriate data uranium and uranium compounds should not be labelled for effects during lactation.

Proposed classification for fertility

Lack of appropriate data precludes the assessment of uranium and uranium compounds for effects on fertility.

Proposed classification for developmental toxicity

Lack of appropriate data precludes the assessment of uranium for developmental toxicity. Proposed classification of uranium *compounds*: category 2; H361d.

Proposed labelling for effect during lactation

Lack of appropriate data precludes the assessment of uranium and uranium compounds 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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The first draft of the present document was prepared by Dr. B. Tiesjema, from the National Institute of Public Health and the Environment (RIVM) in Bilthoven, by contract with the Ministry of Social Affairs and Employment.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, persons are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the Health Council to assess whether or not someone can become a member. An expert who has no financial but another clearly definable interest, can become a member under the restriction that he will not be involved in the debate on the subject to which his interest relates. If a person's interest is not clearly definable, he can sometimes be consulted as an expert. Experts working for a ministry or governmental organisation can be structurally consulted. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

The submission letter (in English)

Subject : Submission of the advisory report *Uranium and its compounds*
Your reference : DGV/BMO/U-932542
Our reference : U-962581/EvV/jh/543-F16
Enclosure(s) : 1
Date : May 18, 2016

Dear Minister,

I hereby submit the advisory report on the effects of uranium and its compounds on fertility and on the development of the progeny; it also concerns effects on lactation and on the progeny via lactation.

This advisory report is part of an extensive series in which reproduction toxic substances are classified in accordance with European guidelines. It concerns substances to which people may be exposed occupationally.

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

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

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

Annex C

Comments on the public draft

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

- T.P. Kuipers, on behalf of the Military Healthcare & Occupational Health Expertise Co-ordination Centre, Support Command, Ministry of Defence, Doorn, The Netherlands
- T.J. Lentz, J. Anderson, C. Johnson. 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 does not only use guideline studies (studies performed according to OECD* standard protocols) for the classification of compounds, but non-guideline studies are taken into consideration as well.

* Organisation for Economic Cooperation and Development.

Annex **F**

Fertility and developmental toxicity studies

Table 1 Fertility studies (oral) in animals.

authors	compound	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs and reproduction
Maynard et al., 1949	uranium nitrate hexahydrate	rats	195 days, followed by a recovery period of 170 days	oral administration in diet, at 0 or 2% $UO_2(NO_3)_2$* $6H_2O$ (200-300 mg/rat/day)	mortality: In the first 30 days, 4 females and 5 males from the uranium group; 10% of controls and 17% of the uranium group in the remainder of the year. Reduction of body weight gain in males and females (after 7 months males and females weighed 65 and 60 grams less than controls). Degeneration of the kidneys in the first week; between weeks 8 and 14 tubular atrophy; no renal abnormality after the recovery period.	testicular lesions, including degeneration and atrophic changes (tubuli with loss of spermatozoa, absence of spermatids, loss of primary spermatocytes, presence of multinucleated cells). Irregular oestrus cycles (14/44) compared to 2/45 in control rats; irregular (1/44 vs 0/45 in controls) or no (17/44 vs 4/45 in controls) matings; reduction in females that had at least one litter (44/45 in control group compared to 30/44 in the uranium group); reduced number of litters per female. In the recovery period, the amount of litters in the uranium group returned to normal. No statistics mentioned

Maynard et al., 1953 cited in ATSDR, 2013 and Domingo, 2001	uranyl nitrate hexahydrate	rats	12 or 24 months	oral administration in diet, at dose levels of 331 mg U/kg bw/day (males) and 664 mg U/kg bw/day (females)	not reported	severe degeneration in the testes, depletion of germ cells (males) reduced litter sizes (females) [no dose-response data available]
Malenchenko et al., 1978	uranyl nitrate hexahydrate	male Wistar rats	4 months	administration in drinking water at 0.1% $UO_2(NO_5)$* $6H_2O$ (~50 mg/kg bw)	Spleen weight and spleen index were increased (p<0.05) and morphological changes in the thyroid gland were observed (not further analyzed)	decreased testes weight (p<0.05), testicular lesions, and necrosis of spermatocytes and spermatogonia
Llobet et al., 1991	uranyl acetate dihydrate	male Swiss mice	64 days (before mating to untreated female mice)	oral administration in drinking water resulting in doses of 10, 20, 40, and 80 mg $UO_2(CH_3COO)_2$* $2H_2O$/kg bw/day	no clinical signs of toxicity were observed, except for a reduced body weight at the highest dose (p<0.05)	non-dose-related decrease in pregnancy rate (25-35% in uranium treated animals vs. 81% in the control group, p<0.01 or p<0.05). No statistically significant differences in total implantations, early and late resorptions, or number of live and dead foetuses. Normal testes and epididymis weights, normal spermatogenesis. Increase in Leydig cells vacuolization at 80 mg/kg/day (p<0.05)
Gilman et al., 1998a	uranyl nitrate hexahydrate	Sprague-Dawley rats (10/sex/group)	28 days	oral administration in drinking water at doses of < 0.001, 0.96, 4.8, 24, 120 and 600 mg $UO_2(NO_3)_2$* $6H_2O$ /L (up to 35.3 mg/kg bw/day (males) and 40.0 mg/kg bw/day (females)	no general toxicity observed	no statistically significant changes in reproductive organ weights in epididymis, testes, ovary or uterus

Gilman et al., 1998a	uranyl nitrate hexahydrate	Sprague-Dawley rats (15/sex/group)	91 days	oral administration in drinking water at doses of 0, 0.96, 4.8, 24, 120, or 600 mg $UO_2(NO_3)_2$* $6H_2O$ /L (up to 36.73 mg/kg bw/d (males) and 53.56 mg/kg bw/d (females))	in all dose groups, males and females: histopathological lesions in the kidney and liver, renal lesions of tubules (apical nuclear displacement and vesiculation, cytoplasmic vacuolation, and dilation), glomeruli (capsular sclerosis), and interstitium (reticulin sclerosis and lymphoid cuffing) (p<0.001, p<0.01 or p<0.05)	no statistically significant changes in reproductive organ weights in epididymis, testes, ovary or uterus
Gilman et al., 1998b	uranyl nitrate hexahydrate	New Zealand White rabbits	for 91 days	oral administration in drinking water at doses of 0, 0.96, 4.8, 24, 120, or 600 mg $UO_2(NO_3)_2$* $6H_2O$ /L (up to 28.70 mg/kg bw/day) in males and 0, 4.8, 24, or 600 mg UN/L (up to 43.02 mg/kg bw/day) in females	changes in renal tubules (LOAEL males 0.05 mg U/kg bw/day, females 0.49 mg U/kg bw/day)	no statistically significant histopathological or organ weight changes in epididymis, testes, ovary or uterus
Albina et al., 2005	uranyl acetate dihydrate	male Sprague Dawley rats	males were exposed for 3 months and then mated to untreated females	0, 10, 20 or 40 mg $UO_2(CH_3COO)_2$* $2H_2O$ /kg bw in drinking water	no effects on body weight gain of the dams, maternal toxicity not further analyzed	decrease in pregnancy rate at 20 mg/kg bw/day (p<0.05) (no dose-effect relationship); no effects on number of implants, number of viable implants and number of dead foetuses per litter

Linares et al., 2005	uranyl acetate dihydrate	male Sprague Dawley rats (8/group)	males were exposed for 3 months and then mated to untreated females	0, 10, 20 or 40 mg $UO_2(CH_3COO)_2$* $2H_2O$ /kg bw in drinking water	No deaths or clinical signs of toxicity, including body weight gain at any dose	at 20 mg/kg bw/day: decrease in pregnancy rate (p<0.05) (9/12, 6/8, 4/8 and 6/8 in females mated to males exposed to 0, 10, 20 and 40 mg/kg bw/day respectively); no statistically significant differences in number of total implants and number of viable and nonviable implants per litter; decreased spermatid number/testis at 20 and 40 mg/kg bw/day (p<0.05). Histopathological examination testes after 3 months revealed few differences in the tubule and interstitial alterations (progressive but not statistically significant cellular loss, Sertoli cells or germinal cells, with cytoplasmic vacuolization) between control and uranium-exposed animals
Sánchez et al., 2006	uranyl acetate dihydrate	adult female Sprague Dawley rats	for 4 weeks before mating with untreated males, as well as during pregnancy and lactation	at doses of 0, 40 and 80 mg $UO_2(CH_3COO)_2$* $2H_2O$ /kg bw/day in the drinking water	no relevant effects on maternal body weight gain	no effects on number of litters or number of implants (total, viable and non viable)/litter
Arneault et al., 2008	uranyl nitrate	female C57Bl×CBA mice	group 1: 15 weeks, directly followed by analysis. group 2: 15 weeks, just before gestation Analysis after 3 months	5, 50 or 400 mg/L $UO_2(NO_3)_2$* $6H_2O$ in drinking water (~ 1.25, 12.5 and 100 mg U/kg bw/day)	no effects on body weight, kidney weight and behavior	dams first group and female pups: fewer large antral follicles (Ø >200µm) than controls (p<0.05). Dams second group: more secondary and early pre-antral follicles (Ø 70-110µm) than controls (p<0.05)

bw=body weight(s); gd=gestational day(s); pnd=post-natal day(s); U=uranium; UN=uranyl nitrate hexahydrate.

Table 2 Developmental studies (oral) in animals.

authors	compound	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs and reproduction
Domingo et al. 1989a	uranyl acetate dihydrate	pregnant Swiss mice (20/group)	dosed on gd 6-15 and sacrificed on gd 18	0, 5, 10, 25 or 50 mg $UO_2(CH_3COO)_2$* $2H_2O$ /kg bw/day by gavage	at all doses: reduced weight gain and food consumption, increased relative liver weight (p<0.001, p<0.01 or p<0.05)	dose-related foetotoxicity, manifested as reduced foetal body weight and length, increased incidence of stunted foetuses and external and skeletal malformations, and developmental variations (p<0.001, p<0.01 or p<0.05). External malformations included an increase in the incidence of cleft palate (at 10 mg/kg bw/day and higher doses, (p<0.01) and hematomas (p<0.05 at 5 and 50 mg/kg bw/day). In the 5 and 25 mg/kg bw/day groups: undeveloped renal papillae. Increased incidence of skeletal abnormalities (bipartite sternebrae and reduced or delayed ossification of the hind limb, fore limb, skull, and tail) in the 25 and 50 mg/kg bw/day groups (p<0.001, p<0.01 or p<0.05) Embryol-ethality was not found
Domingo et al., 1989b	uranyl acetate dihydrate	pregnant Swiss mice (20/group)	from gd 13 through pnd 21	0, 0.05, 0.5, 5 or 50 mg $UO_2(CH_3COO)_2$ * $2H_2O$ /kg bw/day by gavage	effects on body weight or food intake were not observed. Two dams in the 5 mg/kg bw/day group and 2 dams in the 50 mg/kg bw/day group died during lactation	number of litters or litter size not affected on postnatal day 0, but decreased litter size at postnatal day 21 at 50 mg/kg bw/day (5.5 vs. 8.8 in controls) (p<0.05); decreased viability index (number of pups viable at day 21/number of pups born) and the lactation index (number of pups viable at day 21/number of pups retained at day 4) at 50 mg/kg bw/day

						(p<0.05); reduction in liver weight in pups of all dose groups (p<0.01 or p<0.05); no differences in pup weight or body length; no significant differences in developmental signs (pinnae unfolding, lower incisor eruption, eye opening)
Sánchez et al., 2006	uranyl acetate dihydrate	adult female Sprague Dawley rats	for 4 weeks before mating with untreated males, as well as during pregnancy and lactation	at doses of 0, 40 and 80 mg $UO_2(CH_3COO)_2$* $2H_2O$ /kg bw/day in the drinking water	no statistically significant effects on maternal body weight gain	no statistically significant effects on number of implants (total, viable and non viable)/litter. No statistically significant effects on body weight pups at postnatal day 1, decrease in body weight pups (male and female) on postnatal day 4 (at 40 mg/kg bw/day, p<0.05), 12 and 21 (at 40 and 80 mg/kg bw/day, p<0.05)
Arneault et al., 2008	uranyl nitrate	female C57Bl×CBA mice	group 1: 15 weeks, directly followed by analysis group 2: 15 weeks, just before gestation. Analysis after 3 months	5, 50 or 400 mg/L $UO_2(NO_3)_2$*$6H_2O$ in drinking water (~ 1.25, 12.5 and 100 mg U/kg bw/day)	no effects on body weight, kidney weight and behavior	female pups: fewer large antral follicles (Ø >200µm) than controls (p<0.05). No monotone dose-response relationship. Normal amount of secondary and early preantral follicles (Ø 70-110 µm)

bw=body weight(s); gd=gestational day(s); pnd=post-natal day(s); U=uranium; UN=uranyl nitrate hexahydrate.

Table 3 Developmental studies (other) in animals.

authors	compound	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs and reproduction
Bosque et al., 1993a	uranyl acetate dihydrate	Swiss mice (16-22 dams/ group)	gd 6-15	SC injections at dose levels of 0, 0.5, 1, and 2 mg $UO_2(CH_3COO)_2$* $2H_2O$ /kg bw/day	dose-related deaths (0, 1, 2, and 7 deaths at 0, 0.5, 1 and 2 mg/kg bw/day, respectively) and decreased weight gain during gestation (at all dose levels, (p<0.001, p<0.01 or p<0.05) and decreased body weight at termination (1 and 2 mg/kg bw/day, p<0.01 and p<0.001, respectively); maternal liver weight (absolute and relative) and kidney weight (absolute) were decreased in the 1and 2 mg/kg bw/day dose groups (p<0.001 or p<0.05)	at ≥ 0.5 mg/kg bw/day: increased percentage of early resorptions (p<0.05) and post-implantation loss (p<0.01); reduction in foetal body weight at 1 and 2 mg/kg bw/day and by a dose-dependent increase in the number of total internal (renal hypoplasia) and total skeletal (decreased ossification) defects, (statistically significant at all doses: p<0.001, p<0.01 or p<0.05). increase in malformations (cleft palate and bipartite sternebrae) at 1 and 2 mg/kg bw/day (p<0.001, p<0.01 or p<0.05).
Bosque et al., 1993b	uranyl acetate dihydrate	Swiss mice (12 dams/ group)	gd 10	single SC injection of 4 mg $UO_2(CH_3COO)_2$* $2H_2O$ /kg bw	death (20%, compared to 0% in controls), lethargy and reduced body weight gain (p<0.01)	significantly increased percentage of resorptions and dead foetuses (together 36.3 vs 2.21%, p<0.001); decreases in foetal body weight (p<0.001) and increases in the percentage of skeletal anomalies (78.6 vs 10.7% foetuses affected in treated vs control group, p<0.05); skeletal alterations including reduced ossifications, wavy ribs and dorsal hyperkyphosis

Albina et al., 2003	uranyl acetate dihydrate	Sprague Dawley rats (11-12/ group)	gd 6-15. Cesarean sections on gd 20	SC injections of 0.415 and 0.830 mg $UO_2(CH_3COO)_2$* $2H_2O$ /kg bw/day	at 0.830 mg/kg bw/day: decreased body weight gain, decreased gravid uterine weight, decreased absolute and relative kidney weight (p<0.05)	at 0.415 and 0.830 mg/kg/day by reductions in foetal body weight (dose-dependent, p<0.05) and increases in the percentage of skeletally affected foetuses (16, 55 and 88% in controls, at 0.415 and at 0.830 mg/kg/day, respectively (p<0.003)). The skeletal abnormalities observed were delayed ossifications.

bw=body weight(s); gd=gestational day(s); pnd=post-natal day(s); U=uranium; UN=uranyl nitrate hexahydrate.

Health Council of the Netherlands

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