Ammonia

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

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Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van ammonia op de vruchtbaarheid en het nageslacht, ook via de borstvoeding. 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.

Het advies is opgesteld door een vaste commissie van de Gezondheidsraad, de Subcommissie Classificatie Reproductietoxische Stoffen. Het is vervolgens getoetst door de Beraadsgroep Gezondheid en Omgeving van de raad.

Ik heb dit advies vandaag ook ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport en de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Met vriendelijke groet,

prof. dr. J.A. Knottnerus

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Ammonia

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. 2009/01OSH, The Hague, May 28, 2009

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Samenvatting

In het voorliggende advies heeft de Gezondheidsraad ammoniak onder de loep genomen. Tachtig procent van alle geproduceerde ammoniak wordt gebruikt als kunstmest. Een derde hiervan wordt direct op de bodem aangebracht, terwijl de rest als ammoniumverbindingen (voornamelijk zouten) in andere soorten kunstmest wordt verwerkt. Verder wordt ammoniak gebruikt voor de productie van synthetische vezels, plastics en explosieven. Tevens is het aanwezig in koelsystemen. Daarnaast wordt ammoniak in de vorm van ammoniumionen vaak toegevoegd aan huishoudproducten (schoonmaakmiddelen, ruitenreinigers) en wordt het gebruikt als vlugzout.

Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Subcommissie Classificatie Reproductietoxische Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Raad, hierna aangeduid als de commissie, kijkt naar effecten zowel op de vruchtbaarheid van mannen en vrouwen als op de ontwikkeling van het nageslacht. Bovendien worden effecten van blootstelling van de zuigeling via de moedermelk beoordeeld.

Op basis van Richtlijn 93/21/EEC van de Europese Unie doet de commissie een voorstel voor classificatie. Voor ammoniak komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie om ammoniak niet te classificeren wegens onvoldoende geschikte gegevens
- voor effecten op de ontwikkeling adviseert de commissie ammoniak niet te classificeren wegens onvoldoende geschikte gegevens
- voor effecten tijdens lactatie, adviseert de commissie om ammoniak niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report, the Health Council of the Netherlands reviewed ammonia. Eighty percent of all manufactured ammonia is used as fertilizer. A third of this is applied directly to soil as pure ammonia. The rest is used to make other fertilizers that contain ammonium compounds, usually ammonium salts. Ammonia is also used to manufacture synthetic fibres, plastics, and explosives. Furthermore, it is present in refrigeration systems. Many household cleaners and window-cleaning products also contain ammonia in the form of ammonium ions and it is further used in smelling salts.

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 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 the Directive 93/21/EEC of the European Union. For ammonia, they are:

- for effects on fertility, the committee recommends not classifying ammonia due to a lack of appropriate data

- for effects on development, the committee recommends not classifying ammonia due to a lack of appropriate data
- the committee is of the opinion that a lack of appropriate data precludes the labelling of ammonia for effects during lactation.

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. The classification is performed by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances, hereafter called the committee, according to the guidelines of the European Union (Directive 93/21/EEC). The committee'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 'toxic to reproduction' (class 1, 2 or 3) or labelled 'as may cause harm to breastfed babies' (R64).

1.2 Committee and procedure

The present document contains the classification of ammonia by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances. The members of the committee are listed in Annex A.

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

Classification and labelling was performed according to the guidelines of the European Union listed in Annex B.

Classification for fertility and development:	
Category 1	Substances known to impair fertility in humans (R60)
	Substances known to cause developmental toxicity in humans (R61)
Category 2	Substances which should be regarded as if they impair fertility in humans (R60)
	Substances which should be regarded as if they cause developmental toxicity in humans (R61)
Category 3	Substances which cause concern for human fertility (R62)
	Substances which cause concern for humans owing to possible developmental toxic effects (R63)
No classification for effects on fertility or development	
Labelling for lactation:	
	May cause harm to breastfed babies (R64)
	No labelling for lactation

In December 2008, the President of the Health Council released a draft of the report for public review. The individuals and organisations 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.

1.3 Additional considerations

The classification of compounds toxic to reproduction on the basis of the Directive 93/21/EEC 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 directive necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the directive, 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 progeny, the compound will be classified in category 1, irrespective the general toxic effects (see Annex B, 4.2.3.1 category 1).
- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.
- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g., wavy ribs, short rib XIII, incomplete ossification)

in offspring occur in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex B, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols* for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labelling for lactation

The recommendation for labelling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may 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, should be labelled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on a risk characterisation and therefore also includes consideration of the level of exposure of the breastfed child.

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

1.5 Data

Literature searches were conducted in the on-line databases Toxcenter, Medline, and Chemical Abstracts starting from 1985 up to April 2006. An additional search performed in PubMed in September 2008 did not result in relevant additional information. Literature was selected primarily on the basis of the text of

* Organisation for Economic Cooperation and Development

the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided into literature cited and literature consulted but not cited.

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

1.6 Presentation of conclusions

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

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

1.7 Final remark

The classification of compounds is based on hazard evaluation (Niesink *et al.*[20]) only, which is one of a series of elements guiding the risk evaluation process. The committee emphasises 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 organisations.

Chapter 2

Ammonia

2.1 Introduction

chemical name	:	ammonia
CAS number	:	7664-41-7
EINECS number	:	231-635-3
RTECS number	:	BO 0875000
synonyms	:	none
appearance	:	colourless gas
use	:	in the manufacture of nitric acid, explosives, synthetic fibres, fertilizers; in refrigeration systems; in chemical industry.
molecular formula	:	H_3N
structural formula	:	
molecular weight	:	17.03 g/mol
boiling point	:	-33.4°C
melting point	:	-77.7°C
vapour pressure	:	857 kPa at 20°C
vapour density (air=1)	:	0.59
solubility in water	:	531 g/L at 20°C
stability and reactivity	:	liquid ammonia explodes at temperature > 133°C
conversion factor (at 20°C, 101.3 kPa)	:	1 ppm = 0.71 mg/m³; 1 mg/m³ = 1.41 ppm
EU Classification	:	F, R10; T, R23; C, R34; N, R50

Data from WHO/IPCS[1], ECB[2], HSDB[3], Liesivuori[4]

Ammonia is a colourless gas with a distinctly pungent odour under normal conditions. Under high pressure, it can be compressed and become a liquid. It easily dissolves in water, where it forms and is in equilibrium with ammonium ions (NH_4^+). The equilibrium between ammonia (NH_3) and ammonium (NH_4^+) is pH dependent. Under normal physiological conditions, the predominant form will be NH_4^+.[4,5]

Ammonium salts will dissociate to yield an ammonium ion (NH_4^+) and a corresponding cation (e.g., Cl^-, SO_4^{2-}). Therefore, the committee will discuss data from studies with these salts as well, considering that they may be relevant with respect to the evaluation of the potential reproduction toxic effects of ammonia.

Unless otherwise stated, the term ammonia in this document refers to the sum of NH_3 and NH_4^+.

Ammonia plays a key role in the nitrogen metabolism in all mammalian species. It is a product of both protein and nucleic acid catabolism, and a precursor for non-essential amino acids and certain other nitrogen compounds. Approximately 4 grams of ammonia are produced in the gut by intestinal bacteria.[4,5] For comparison, the amounts of ammonia by daily intake through inhalation, ingestion (food and drink), and cigarette smoking (20/day) have been estimated to be approximately 18 mg, <1mg, and <1 mg, respectively.[1] The endogenously produced ammonia enters the portal circulation and is rapidly metabolized to urea and glutamine in the liver. Ammonia is primarily excreted as urea and urinary ammonium compounds through the kidneys.[5] Exhaled breath contains ammonia concentrations of 0.1-2.2 mg/m^3.[1,4] These values, which are higher than those expected from equilibrium with ammonia levels in plasma and lung parenchyma, are most likely due to the synthesis of ammonia from salivary urea by oral microflora.[4] Venous plasma levels of ammonia in healthy humans (‘reference intervals’ in clinical laboratory diagnostics) range from about 0.2 to 0.9 mg/L in adults and children older than 1 month. In newborns, these intervals are 0.3-2.1 mg/L and 0.45-1.1 mg/L for premature and mature babies, respectively.[6] Structural (congenital errors of metabolism, e.g., related to urea cycle enzymes) or functional (severe liver disease, e.g., cirrhosis) disturbances result in excess circulating ammonia levels (hyperammonaemia), which are associated with the clinical symptoms of hepatic encephalopathy in adults and decreased food intake, vomiting seizures, and lethargy in neonates and children.[6] Correlating ammonia blood levels are 1.5 mg/L[6] and 1.7-2.6 mg/L[7], respectively. Comatose states are usually not present until ammonia levels reach >3 mg/L.[6] The neurological effects are possibly induced by modulation of neurotransmission[8] or by cerebral metabolic disturbances.[9]

Depending on the route of exposure, the most important toxic effects of ammonia on humans and animals are irritation and corrosion of the respiratory tract, skin, eyes, and upper gastrointestinal tract. Hepatic and renal effects have also been reported, but ammonia does not appear to be a primary liver or kidney toxicant.[5]

Inhalation and dermal exposure will not always lead to increased ammonia concentrations in the systemic circulation. During short-term inhalation, increases in blood levels will be prevented by the high retention of ammonia in the upper respiratory tract and elimination in expired air. Both processes are dependent on exposure time (57-500 ppm for up to 120 seconds: 83-92% retention; 500 ppm for 10-27 minutes: 4-30 % retention, with 350-400 ppm eliminated in expired air by the end of the exposure period), suggesting an adaptive capability or saturation of the absorptive process. From a rat study, there are further indications that adaptive response mechanisms might be activated during inhalation with longer exposure times (increased blood concentrations of ammonia within 8 hours of exposure initiation returned to normal within 12 hours of continuous exposure and stayed so over the remaining of the 24-hour exposure period).[5] With respect to dermal exposure, no quantitative data were available, but data on toxic effects from dermal exposure did not suggest that ammonia enters the systemic circulation by this route.[5] However, dermal penetration may occur when spilling has caused skin damage.[4]

In cattle giving a high protein diet, increased ammonia and urea levels are found in blood and reproductive fluid (pre-ovulatory follicular fluid, uterine fluid). These elevated levels have been associated with reduced fertility[10] and reproductive inefficiency due to embryo toxicity[11], although the underlying toxic mechanism is not fully understood.[12,13]

To get insight in the effects of increased blood levels of ammonia on reproduction, some attention was paid to the above mentioned diseases and a high protein diet. However, since the relevance for ammonia exposure is unclear, effects are not further discussed. Below, the relevant data available for classification are evaluated.

2.2 Human studies

Fertility studies

There are no studies available regarding the effects of exposure to ammonia on human fertility.

Developmental toxicity studies

Xu *et al.*[14] conducted a retrospective epidemiological study in a large petrochemical complex in Beijing, China to assess the association between petrochemical exposure and spontaneous abortion. In total, 2853 non-smoking women who were 20-44 years of age and had been pregnant at least once participated in the study. Women and their husbands were interviewed collecting information on reproductive history, pregnancy outcomes, employment history, alcohol consumption, indoor air pollution, and demographic variables. Exposure during the pre-conception period and the first trimester of pregnancy was calculated from information on perceived ammonia exposure. Exposure during the first, second, and third trimesters was recorded separately for each pregnancy. Only results from the women's first pregnancies were analysed using multiple logistic regression. Ammonia was one of the specific chemicals to which 97 women (3.4%) were exposed. Data analyses did not show any effect on spontaneous abortion (Odds Ratio: 1.2; 95% CI: 0.5-2.6). From this study, indications were obtained that ammonia does not affect development, but actual exposure levels were unknown which limited the value of the study.

Lactation

There are no studies available regarding the effects of ammonia on human lactation.

2.3 Animal studies

Fertility studies

Inhalation
Diekman *et al.* (1989, 1993)[15,16] exposed cross-bred gilts* (n=40/dose) to 7±1 ppm (low) and 35±3 ppm (moderate) of aerial ammonia obtained respectively by flushing manure pits or by adding anhydrous ammonia to manure pits that were not flushed (see Annex D). No unexposed control groups were included. Effects in both groups were compared with each other. Per dose, half of the group (n=20) was continuously exposed from 6 weeks before breeding until slaughtering at day 30 of gestation. The other half (n=20) was killed after 6 weeks of exposure. Gilts of the moderate exposure group showed a lower mean daily weight gain in

* Young female pigs.

the first two weeks of exposure (n=40). After six weeks of exposure (n=40) and at day 30 of gestation (n=20), a reduction in body weight was found in this group (significant after 6 weeks). At both exposure levels, animals slaughtered after 6 weeks of exposure (n=20) showed lung lesions and moderate degeneration of nasal turbinates, while no significant differences in ovarian weight and uterine weight were observed. Six weeks of exposure did not affect age at puberty (day of breeding; low: 208 days; moderate: 205 days), and conception rate (low: 94.1%; moderate: 100%) as observed in the longer lived subgroups (n=20). However, weight at puberty was less for moderate ammonia-treated than low ammonia-treated gilts (low: 118.2 kg; moderate: 109.7 kg). In the longer lived subgroups, the implantation rate was not affected at day 30 of gestation. The value of this study is limited because of the limited number of parameters tested, the use of an uncommon animal species, and the absence of control animals. With respect to the latter, the moderate exposure group showed effects (decreases in body weight and body weight gain) not observed in the low exposure group indicating that ammonia affected the animals systemically at moderate concentrations.

Developmental toxicity studies

Inhalation

In the reproduction study on gilts of Diekman *et al.* (1989, 1993)[15,16], development was also investigated (see above and Annex D). At day 30 of gestation, in both exposure groups (7±1 ppm and 35±3 ppm), no significant differences in number of live foetuses and in foetal weight and length were found. However, the value of this study is limited because of the limited number of development parameters tested, the use of an uncommon animal species, and the absence of control animals.

Oral

Some minor indications that ammonia might affect development came from a study of Miñana *et al.* (1995)[17] investigating growth in relation to impaired NMDA receptor (i.e., a glutamate-binding receptor) function (see Annex D). Wistar rats (number not reported) were fed diets containing 0% and 20% (by mass) ammonium acetate* from gestation day 1 until lactation day 21. Thereafter, the pups exposed to ammonia *in utero* and during lactation were fed either a diet containing 20% ammonium acetate until post-natal day 120-150 (n=12 males; 9

* According to ATSDR equivalent to 4293 mg NH_4^+/kg bw/day.

females) or a normal diet (n=8 males; 13 females). Control pups (7 males; 8 females) never received ammonium acetate. The only parameter measured was pup growth during the exposure period. Furthermore, the effect of ammonia on the pup brain was investigated by measuring the function of the NMDA receptor on cerebellar neurons isolated from 7-8-day-old pups exposed or not exposed to ammonia during pregnancy and lactation. Body weight, food consumption, and health of the dams and food consumption of the pups were not reported. According to Miñana *et al.*, body weights of offspring exposed *in utero* and during lactation and fed a normal diet thereafter were significantly lower than those of controls until approximately day 60. Offspring similarly exposed but fed an ammonium acetate-containing diet after lactation had even greater decreased body weight gains at day 120 (data presented in a figure only). Cerebellar neurons isolated from 7-8-day-old pups exposed to ammonium acetate showed impaired NMDA receptor function. Functional NMDA receptors are necessary for normal growth of animals via regulation of growth hormone secretion. However, since data on food consumption in pregnant rats and offspring receiving ammonium acetate-containing diets were not given, the cause of the decreased body weights in these animals cannot be assessed.

In a study on Spraque-Dawley rats by Goldman and Yakovac (1964)[18], dams (n=10/dose) were exposed to drinking water containing 0 and 0.17 mol/L ammonium chloride (i.e., 3060 mg NH_4^+/L) from gestation day 7 (exposure time not specified, but presumably until the end of the experiment at day 20). Maternal and foetal mortality were recorded. Foetal growth was determined by measuring foetal weight and crown-rump length after delivery by Caesarean section on day 20. Furthermore, foetuses were examined grossly with special attention to dorsal-midline, ventral-midline, and eye defects. In addition, skeletal abnormalities were determined (alizarin red S. staining). Ammonium chloride only produced decreased foetal weights and crown-rump lengths. Maternal and foetal mortality were not affected. No gross pathology or skeletal abnormalities were found. Since maternal mortality was the only parameter measured for dams, it is unclear whether the exposure level was high enough to affect the foetus. Furthermore, drinking water consumption was not reported. For these reasons, the study can only be supplementary to other data.

Lactation

In the rat reproduction study of Miñana *et al.* (1995)[17] (see above), the effect of ammonia on the pups was reported in general as a reduced body weight gain dur-

ing the time period examined. Effects during lactation, however, were not specified and data during lactation were not given.

Moorby and Theobald (1999)[19] studied milk production on four multiparous Holstein-Friesian cows with rumen and duodenal cannulae. Two cows per treatment (body weight not given) were offered a constant diet based on grass silage continuously infused with ammonium acetate (2.0 M) or acetic acid (controls; 2.0 M) into the duodenum (via rumen and duodenal cannulae to avoid direct effects on rumen function) at the rate of approximately 200 mmol/h. Assuming a mean body weight of 500 kg, this rate corresponds with approximately 740 mg/kg bw/day ammonium acetate and 580 mg/kg bw/day acetic acid. These treatment diets were offered in a changeover design experiment with four periods consisting of six days of continuous infusion and ten days of rest. The only parameter affected was urinary N excretion (increase). No effects on milk yields or on milk constituent yields were observed.

2.4 *In vitro* studies

Many *in vitro* studies are available. However, since the results did not demonstrate specific ammonium reproductive toxicity, they are not presented and discussed in this report (see 'literature consulted but not cited').

2.5 Conclusion

There are no studies available regarding the effects of exposure to ammonia on human fertility.

In animals, only one study was available regarding the potential effect of ammonia on female fertility. In this study, the effect of inhaled ammonia on young female pigs was investigated. No unexposed control animals were available (low and moderate exposure groups were only compared with each other). The moderate group showed a lower mean daily weight gain resulting in a significant reduction in body weight. No significant differences in ovarian weight, uterine weight, age at puberty (day of breeding), and conception rate were observed and the implantation loss (rate) was not affected. The value of the study was limited because of the use of a non-standard test species, the absence of control animals, and the limited number of parameters measured.

No studies on male fertility were available.

Overall, the committee proposes not to classify ammonia for effects on fertility due to a lack of appropriate human and animal data.

Human data regarding development consisted of one epidemiological study of limited value only. In this study, no effects of ammonia on spontaneous abortion were observed in pregnant women working in a large petrochemical complex in Beijing. Actual exposure levels of ammonia, however, were unknown limiting the value of this study.

As to animals, no differences in number of live foetuses and in foetal weight and length were found in young female pigs exposed to ammonia by inhalation. The results, however, were difficult to interpret due to a number of flaws in study design (no unexposed controls, limited number of parameters tested, non-standard test animal). In rat offspring exposed *in utero* and during lactation, through their mothers which received a diet containing 20% ammonium acetate, and fed a normal diet thereafter, body weights were lower than those of controls until approximately day 60. Body weight, food consumption, and health of the dams throughout the study were not reported. Therefore, it cannot be excluded that the decrease in weight gain of the pups was caused by effects on the dams, especially since a high dietary ammonium acetate concentration of 20% (ca. 4300 mg NH_4^+/kg bw/day) was used. Pups similarly exposed and fed the same ammonium acetate diet as their dams after lactation had an even greater decrease in body weight gain. Further, NMDA receptor function, which is necessary for normal growth via regulation of growth hormone secretion, was impaired in isolated cerebellar neurons obtained from 7-8-day-old pups exposed to ammonia through their mothers' diet. However, since pup food consumption was not reported, the committee cannot assess the cause of the body weight decreases. Indications for foetal growth retardation were found in a study in which rats were exposed to ammonium chloride in drinking water (ca. 3000 mg NH_4^+/L). Ammonium chloride inhibited foetal growth without inducing skeletal and external effects. However, since drinking water consumption and maternal toxicity were not reported, the committee cannot draw conclusions from this study.

Overall, the committee proposes not to classify ammonia for effects on development due to a lack of appropriate human and animal data

There were no data on human lactation, while there were only limited data concerning animals.

The only study in which pups were exposed by lactation is the study in rats described above. In general, the effect of ammonium on the pups was reported as

a reduced body weight gain during the time period examined. However, effects on growth during lactation were not specifically reported.

In addition, a study was available directed on the effect of ammonia on cow's milk production. Ammonium absorption induced a loss of protein in the animal without resulting in reduced amino acid availability for lactation.

Overall, the committee proposes not labelling ammonia for effects during lactation because of a lack of appropriate data.

Proposed classification for fertility

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

Proposed classification for developmental toxicity

Lack of appropriate data precludes the assessment of ammonia for effects on development.

Proposed labelling for effect during lactation

Lack of appropriate data precludes the assessment of ammonia for effects during lactation.

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A The committee

B Directive (93/21EEG) of the European Community

C Comments on the public draft

D Fertility and developmental toxicity studies

Annexes

Annex **A**

The committee

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The first draft of the present document was prepared by J. Draaijer (National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands) by contract with the Health Council.

The Health Council and interests

Members of Health Council Committees – which also include the members of the Advisory Council on Health Research (RGO) since 1 February 2008 – 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 President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

Directive (93/21/EEC) of the European Community

4.2.3 Substances toxic to reproduction

4.2.3.1 For the purposes of classification and labelling and having regard to the present state of knowledge, such substances are divided into 3 categories:

Category 1:

Substances known to impair fertility in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility.

Substances known to cause developmental toxicity in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and subsequent developmental toxic effects in the progeny.

Category 2:

Substances which should be regarded as if they impair fertility in humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in impaired fertility on the basis of:

- Clear evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Substances which should be regarded if they cause developmental toxicity to humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of:

- Clear results in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

Substances which cause concern for humans owing to possible developmental toxic effects:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific conse-

quence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.

- Other relevant information.

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:
T; R60: May impair fertility

For substances that cause developmental toxicity:
T; R61: May cause harm to the unborn child

Category 2:

For substances that should be regarded as if they impair fertility in humans:
T; R60: May impair fertility

For substances that should be regarded as if they cause developmental toxicity in humans:
T; R61: May cause harm to the unborn child.

Category 3:

For substances which cause concern for human fertility:
Xn; R62: Possible risk of impaired fertility

For substances which cause concern for humans owing to possible developmental toxic effects:
Xn; R63: Possible risk of harm to the unborn child.

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

Reproductive toxicity includes impairment of male and female reproductive functions or capacity and the induction of non-inheritable harmful effects on the progeny. This may be classified under two main headings of 1) Effects on male or female fertility, 2) Developmental toxicity.

1) *Effects on male or female fertility,* includes adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response which

would interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.

2) *Developmental toxicity*, is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well as those manifested postnatally. This includes embrytoxic/fetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peri- postnatal defects, and impaired postnatal mental or physical development up to and including normal pubertal development.

Classification of chemicals as toxic to reproduction is intended to be used for chemicals which have an intrinsic or specific property to produce such toxic effects. Chemicals should not be classified as toxic to reproduction where such effects are solely produced as a non-specific secondary consequence of other toxic effects. Chemicals of most concern are those which are toxic to reproduction at exposure levels which do not produce other signs of toxicity.

The placing of a compound in Category 1 for effects on Fertility and/or Developmental Toxicity is done on the basis of epidemiological data. Placing into Categories 2 or 3 is done primarily on the basis of animal data. Data from *in vitro* studies, or studies on avian eggs, are regarded as 'supportive evidence' and would only exceptionally lead to classification in the absence of *in vivo* data.

In common with most other types of toxic effect, substances demonstrating reproductive toxicity will be expected to have a threshold below which adverse effects would not be demonstrated. Even when clear effects have been demonstrated in animal studies the relevance for humans may be doubtful because of the doses administrated, for example, where effects have been demonstrated only at high doses, or where marked toxicokinetic differences exist, or the route of administration is inappropriate. For these or similar reasons it may be that classification in Category 3, or even no classification, will be warranted.

Annex V of the Directive specifies a limit test in the case of substances of low toxicity. If a dose level of at least 1000 mg/kg orally produces no evidence of effects toxic to reproduction, studies at other dose levels may not be considered necessary. If data are available from studies carried out with doses higher than the above limit dose, this data must be evaluated together with other relevant data. Under normal circumstances it is considered that effects seen only at doses in excess of the limit dose would not necessarily lead to classification as Toxic to Reproduction.

Effects on fertility

For the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of

action, or chemical relationship to other known antifertility agents or other information from humans which would lead to the conclusion that effects would be likely to be seen in humans. Where there are studies in only one species without other relevant supporting evidence then classification in Category 3 may be appropriate.

Since impaired fertility may occur as a non-specific accompaniment to severe generalised toxicity or where there is severe inanition, classification into Category 2 should only be made where there is evidence that there is some degree of specificity of toxicity for the reproductive system. If it was demonstrated that impaired fertility in animal studies was due to failure to mate, then for classification into Category 2, it would normally be necessary to have evidence on the mechanism of action in order to interpret whether any adverse effect such as alteration in pattern of hormonal release would be likely to occur in humans.

Developmental toxicity

For classification into Category 2 there should be clear evidence of adverse effects in well conducted studies in one or more species. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections, and so on, it is important that the effects observed should occur in well conducted studies and at dose levels which are not associated with marked maternal toxicity. The route of exposure is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents, and the results of such studies must be interpreted with caution and on their own would not normally lead to classification.

Classification into Category 3 is based on similar criteria as for Category 2 but may be used where the experimental design has deficiencies which make the conclusions less convincing, or where the possibility that the effects may have been due to non-specific influences such as generalised toxicity cannot be excluded.

In general, classification in category 3 or no category would be assigned on an ad hoc basis where the only effects recorded are small changes in the incidences of spontaneous defects, small changes in the proportions of common variants such as are observed in skeletal examinations, or small differences in postnatal developmental assessments.

Effects during Lactation

Substances which are classified as toxic to reproduction and which also cause concern due to their effects on lactation should in addition be labelled with R64 (see criteria in section 3.2.8).

For the purpose of classification, toxic effects on offspring resulting *only* from exposure via the breast milk, or toxic effects resulting from *direct* exposure of children will not be regarded as 'Toxic to Reproduction', unless such effects result in impaired development of the offspring.

Substances which are not classified as toxic to reproduction but which cause concern due to toxicity when transferred to the baby during the period of lactation should be labelled with R64 (see criteria in section 3.2.8). This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk.

R64 would normally be assigned on the basis of:

a) toxicokinetic studies that would indicate the likelihood that the substance would be present in potentially toxic levels in breast milk, and/or
b) on the basis of results of one or two generation studies in animals which indicate the presence of adverse effects on the offspring due to transfer in the milk, and/or
c) on the basis of evidence in humans indicating a risk to babies during the lactational period. Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **C**

Comments on the public draft

A draft of the present report was released in 2008 for public review. The following persons and organisations have commented on the draft review:

- R.D. Zumwalde, National Institute for Occupational Safety and Health (NIOSH), Cincinnati, USA.

Annex **D**

Fertility and developmental toxicity studies

Table 1 Fertility studies in experimental animals.

authors	species	experimental period/design	dose/route	general toxicity	effects on reproductive organs and reproduction
Diekman *et al.* (1989, 1993)[15,16]	cross-bred gilts; n=40/group	half of the group (n=20) exposed continuously from 6 weeks before breeding until GD 30; other half (n=20) killed after 6 weeks of exposure. parameters measured: bw; lung and nasal turbinate lesions; ovarian and uterine weight; age at puberty; conception rate; foetus/corpus luteum ratio	7±1, 35±3 ppm (no control group); inhalation; ammonia obtained from manure, sometimes supplemented with anhydrous ammonia	at 7 and 35 ppm: after 6 weeks and at GD 30*:* lung and nasal turbinate lesions (similar mean score). at 35 ppm: first two weeks: lower mean daily weight gain; during the test period*:* reduced bw (sign. after 6 weeks; not sign. at GD 30	at 7 and 35 ppm: after 6 weeks: no sign. differences in ovarian weight, uterine weight, age at puberty, conception rate; at GD 30: no sign. differences in foetus/corpus luteum ratio

Table 2 Developmental toxicity studies in experimental animals.

authors	species	experimental period/design	dose/route	general toxicity	effects on development
Diekman *et al.* (1989, 1993)[15,16]	cross-bred gilts; n=40/ group	half of the group (n=20) exposed continuously from 6 weeks before breeding until GD 30; other half (n=20) killed after 6 weeks of exposure. parameters measured: live foetuses, foetal weight and length	7±1, 35±3 ppm (no control group); inhalation; ammonia obtained from manure, sometimes supplemented with anhydrous ammonia	at 7 and 35 ppm: after 6 weeks and at GD 30: lung and nasal turbinate lesions (similar mean score). at 35 ppm: first two weeks: lower mean daily weight gain; during the test period: reduced bw (sign. after 6 weeks; not sign. at GD 30)	foetuses: no sign. differences in number of live foetuses, and in foetal weight and length at GD 30 between the two groups
Goldman/ Yakovac (1964)[18]	female Sprague-Dawley rats; controls: n=15; exposed: n=10	from GD 7 onwards (exposure time and water consumption not reported) parameters measured: during test period: maternal en foetal mortality; after delivery by Caesarean section (GD 20): foetal growth (weight; crown-rump length), gross foetal examination (dorsal midline, ventral midline, eye defects; skeletal abnormalities - alizarin red S. staining)	0, 0.17 mol NH_4Cl /L drinking water (i.e., 3060 mg NH_4^+/L)		no sign. effect on maternal and foetal mortality; foetuses: inhibition of foetal weight and crown-rump length; no gross pathology; no skeletal abnormalities
Miñana *et al.* (1995)[17]	ammonia→ control group: n=8M+13F	males: not exposed females: from GD 1 until PND 21 pups: from PND 21 until PND 120-150 parameters measured: all groups: pup growth after birth; controls + exposed groups: function of NMDA receptor (= a glutamate binding receptor) measured *in vitro* on primary cultures of cerebellar neurons (n=4-5) isolated from 7-8-day-old control and exposed pups	0, 20% (by mass) ammonium acetate (AA) in the diet pups: unexposed controls: 0% AA (dams and pups); ammonia group: 20% AA (dams and pups); ammonia→control group: 20% AA (dams) and 0% AA (pups)	no information on maternal health, bw, food consumption reported.	pups: normal bw gain in controls; reduced bw gain during exposure in ammonia group (M: 40% ↓; F: 27% ↓) at PND 60; reduced bw gain during exposure in ammonia→ control group (M: 65% ↓; F: 53% ↓) at PND 60 impaired NMDA receptor function in ammonia group: reduced binding (60%↓ binding of [^{3}H]MK-801 antagonist); reduced function (no induction of aspartate aminotransferase by NMDA)

Table 3 Studies on lactation in experimental animals.

authors	species	experimental period/ design	dose/route	general toxicity	effects on lactation
Miñana *et al.* (1995)[17]	male, female Wistar rats; n= not reported pups: controls: n= 7M+8F; ammonia group: n=12M+9F; ammonia→control group: n=8M+13F	males: not exposed females: from GD 1 until PND 21 pups: from PND 21 until PND 120-150 parameters measured: all groups: pup growth after birth	0, 20% (by mass) ammonium acetate (AA) in the diet pups: unexposed controls: 0% AA (dams and pups); ammonia group: 20% AA (dams and pups); ammonia→control group: 20% AA (dams) and 0% AA (pups)	no information on maternal health, bw, food consumption reported.	pups: normal bw gain in controls ammonia and ammonia→ control group: generally, reduced male and female bw gain during the time period examined; however, effects during lactation were not specified and data were not given.
Moorby/ Theobald (1999)[19]	multiparous Holstein-Friesian cows n=2/group	4 exposure periods, each consisting of 6 days of continuous infusion and 10 days rest parameters measured: silage intake; rumen ammonia; pH; volatile fatty acid concentrations; urinary and faeces N excretion; whole body N balance; milk and milk constituent yields	constant diet based on grass silage continuously infused (approx. 200 mmol/h) with 2.0 M ammonium acetate or 2.0 M acetic acid (controls) into the duodenum via rumen and duodenal cannulae (to avoid direct effects on rumen function) (i.e., ca. 740 and 580 mg/kg bw/d of ammonium acetate and acetic acid, resp., assuming a bw of 500 kg (see section 2.3)	not reported	most parameters unaffected; only urinary N excretion increased; no effect on milk or milk constituent yields.