Ribavirin

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Ribavirine*
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Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van ribavirine op de vruchtbaarheid en het nageslacht; het betreft ook effecten die optreden na blootstelling 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.

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

Met vriendelijke groet,

prof. dr. J.A. Knottnerus

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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. 2010/03OSH, Den Haag, February 11, 2010

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Samenvatting

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 werknemers tijdens hun 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, adviseert een classificatie van reproductietoxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie ribavirine onder de loep genomen. Ribavirine is een antiviraal geneesmiddel dat werkzaam is tegen DNA- en RNA-virussen.

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

- Voor effecten op de fertiliteit adviseert de commissie om ribavirine niet te classificeren wegens onvoldoende geschikte gegevens.
- Voor effecten op de ontwikkeling adviseert de commissie ribavirine in categorie 3 *(stoffen die in verband met hun mogelijke voor de ontwikkeling schadelijke effecten reden geven tot bezorgdheid voor de mens)* te classificeren en met Xn; R63 te kenmerken.
- Voor effecten tijdens lactatie, adviseert de commissie om ribavirine niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report the Health Council of the Netherlands reviewed ribavirin. Ribavirin is an antiviral drug, active against DNA and RNA viruses. This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at 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 effects of exposure on lactation are considered.

According to the Directive 93/21/EEC of the European Union, the committee recommends a classification. The committee's recommendations for ribavirin are:

- For effects on fertility, the committee is of the opinion that due to a lack of appropriate data ribavirin should not be classified.
- For developmental toxicity, the committee recommends classifying ribavirin in category 3 *(substances which cause concern for humans owing to possible developmental toxic effects)* and to label ribavirin with Xn; R63.
- For effects during lactation, the committee is of the opinion that due to a lack of appropriate data ribavirin should not be labeled.

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 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, 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

This document contains the classification of ribavirin 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 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

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

In 2009, 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 at 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 if this concentration leads to exceeding the 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 Toxline Plus starting from 1985 up to 2001, and Medline starting from 1966 through 2005. Literature

* OECD: Organisation for Economic Cooperation and Development.

was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, the literature database of the Teratology Information Service of the National institute for public health and the environment was consulted as well as handbooks and a collection of most recent reviews. References are divided in literature cited and literature consulted but not cited.

A literature search in January 2009 did not reveal any additional relevant references.

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. Of each study, the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of 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 preclude 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* 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.

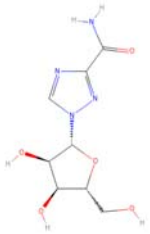
* for definitions see Niesink *et al*[1]

Chapter 2

Ribavirin

2.1 Introduction

Name	:	Ribavirin
CAS-no	:	36791-04-5
RTECS-no	:	XZ4250000
Synonyms	:	1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide 1-[3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,2,4-triazole-3-carboxamide
Use	:	Antiviral drug. Ribavirin is applied both orally and by inhalation.
Mol weight	:	244.2
Chemical formula	:	$C_8H_{12}N_4O_5$
Structural formula		



General remark

Ribavirin is an antiviral drug with *in vitro* activity against both DNA and RNA viruses. Ribavirin is a synthetic nucleoside analogue. It is a member of the nucleoside antimetabolite drugs that interfere with duplication of viral genetic mate-

rial. Ribavirin is a pro-drug, meaning that it is a chemical precursor for the actual pharmacologically active molecule. Ribavirin is activated by cellular kinases which change it into the 5' triphosphate nucleotide. In this form, it interferes with aspects of RNA metabolism related to viral reproduction.

2.2 Human studies

Fertility

Mishkin and Deschênes[2] reported on a 29-yr-old woman, diagnosed with chronic hepatitis C. The patient became pregnant 3.5 months after discontinuing treatment with ribavirin (1000 mg/day for 7 weeks) and interferon-α-2b. Treatment was stopped because of severe haemolysis. A healthy child was born.

Development

Maternal exposure

Maddrey[3] performed randomized, double blind, placebo-controlled studies with 2,089 hepatitis C patients receiving interferon-α-2b in combination with or without ribavirin. Although stringent study entry criteria were used to minimize the potential for patients or the partners of patients to become pregnant, 10 female and 15 male chronic hepatitis C patients became pregnant or fathered a child during treatment. The patients received 1,000-1,200 mg ribavirin daily for 24-48 weeks in combination with interferon-α-2b. Among the pregnant women, 4 terminated pregnancy voluntarily, 4 miscarried and 2 were lost to follow-up. Among the pregnant partners of treated men, there were 2 women who delivered healthy babies, 2 women terminated pregnancy voluntarily, 4 miscarried and 7 were lost to follow-up.

Atmar *et al.*[4] reported 9 cases of pregnant women with severe measles who were treated with ribavirin aerosol therapy (20 mg/ml for 18 hours per day for 2-6 days) during the second half of pregnancy (weeks 23-38 of gestation). All women gave birth to healthy neonates. Two of these women gave birth prematurely (weeks 34 and 35 of gestation), which was considered to be associated with the measles infection. Four of the infants were followed up to 3-7 months and healthy at that time.

Shek *et al.*[5] reported 5 cases of pregnant women with severe acute respiratory syndrome (SARS) who received ribavirin intravenously (in one case 400 mg every 8 hours for 4 days, for other cases dosage not indicated) in late pregnancy

(gestation weeks 26-32). In all cases, the women received also other medication, ie. hydrocortisone, methylprednisolone or both. In three women, caesarean section was performed in the acute phase of the disease, because of deteriorating maternal condition. In these cases, the mothers received ribavirin 3-4 days before delivery. Two of the babies of these 3 women developed abdominal distension, pneumoperitoneum, and respiratory distress syndrome as well as necrotising enterocolitis with bowel perforation. The third baby was healthy, apart from a fever on day 12. The other 2 women continued their pregnancies delivering growth retarded babies at weeks 33 and 37. None of the infants had congenital malformations. It is not possible to determine whether the growth retardation was attributable to ribavirin as opposed to other factors such as the poor maternal condition during the acute phase of the illness or to high-dose corticosteroids. The babies were not infected with SARS.

Rezvani and Koren[6] reported a case of a woman who was 7 weeks pregnant when she was treated for suspected SARS, intramuscularly with 3 injections of 200 mg ribavirin within 3 days. At term, she delivered a healthy baby. At 8 months of age, the child was still reported to be healthy.

Paternal exposure

Hegenbarth *et al.*[7] reported 2 cases of paternal exposure to ribavirin. Both men received 800 and 1200 mg ribavirin daily, in combination with interferon-α-2a. They had been treated with ribavirin for 5 and 4.5 months respectively, when their wives became pregnant. Both women delivered healthy children.

Bianca and Ettore[8] reported one case of paternal periconceptional exposure. The man started ribavirin (1,000 mg/day) and interferon-α-2b treatment 4 weeks before the last menstrual cycle of his wife and continued after the pregnancy was discovered. A healthy baby was born.

De Santis *et al.*[9] reported 7 pregnancies with paternal ribavirin exposure (600-1,000 mg/day). In 6 cases, the men were also treated with interferon-α-2b. In 5 cases, ribavirin treatment ended 1-3 months before conception. Four out of these 5 pregnant women delivered healthy babies. One woman had a very early miscarriage at 5 weeks; her partner stopped ribavirin treatment 1 month before conception. In one case, the partner of a pregnant woman was exposed to ribavirin between week 2 and 6 of gestation. In another case, the partner was treated with ribavirin between 11 months before conception and 12 weeks of gestation. In both cases, healthy babies were delivered.

Lactation

No studies were found concerning the excretion of ribavirin in human breast milk.

2.3 Animal studies

Fertility studies

Oral administration

Johnson[10] described and re-evaluated a study performed by the Industrial Bio-Test Laboratories Inc. in 1973 and reported to ICN Nucleic Acid Research Institute. Male Charles River rats were treated orally by gavage from 60 days before mating up to and including the breeding period. The male rats were mated with female Charles River rats which were treated from 2 weeks prior to breeding up to gestational day 14 (half of the females were sacrificed) or up to postnatal day 21 at which the pups were weaned. Dosages were 0, 30, 60 or 90 mg/kg/day. At gestational day 14, females were investigated for corpora lutea and implantation sites. There was no effect on fertility parameters in male or female adult rats.

Intraperitoneal administration

Hoffmann *et al*.[11] performed a dominant lethal study in CD rats. Males were treated intraperitoneally with ribavirin, ethyl methanesulphonate (positive control) or a vehicle (negative control) for 5 consecutive days (n=20 rats per treatment group). At first, 2 preliminary studies were performed to establish suitable dose levels. Male rats were treated with 250, 300 or 350 mg/kg/day (first preliminary study) or with 50, 150 or 250 mg/kg/day (second preliminary study) followed by one mating. In both preliminary studies, animals died (first study: 2 low dose, 2 mid-dose, and 4 high-dose; second study: 1 high-dose). In the main study, doses of 50, 100 and 200 mg/kg/day ribavirin were used. Following treatment, male rats were mated weekly with 8 consecutive groups of females, with 2 females per individual male. Females were sacrificed approximately 2 weeks after mating. The uteri of the females were examined for live implantations, early deaths, late deaths and corpora lutea graviditatis. There was no mortality in the main study.

The proportion of pregnant females was slightly lower in females that mated with high dose males (average 87%) than in the control group (average 96%).

This slight reduction was significant in week 2 (80%, $p < 0.05$) and approached significance in week 4 (85%, $p = 0.06$). There was no effect on the number of corpora lutea per pregnant female, the number of implants per pregnancy, and the number of late deaths. On two occasions there were marginally, but statistically significantly increased incidences of early deaths (in weeks 3 and 8, in the low and high dose groups). In week 6, there were significantly reduced proportions of early deaths in all treated groups. Overall, there was no evidence for a biologically significant effect on the number of early deaths.

Narayana *et al.*[12] administered ribavirin intraperitoneally for 5 consecutive days to male albino Wistar rats at doses of 0, 20, 100, or 200 mg/kg/day. Cyclophosphamide was used as a positive control. Animals were sacrificed at 14, 28, 35, 42, and 70 days after the last administration. Sperm was investigated (1,000 sperm cells per animal) and classified into normal and different abnormal types. Ribavirin caused at all doses as well as cyclophosphamide head and tail anomalies of sperm up to and including 42 days after treatment, but not at 70 days after treatment.

Narayana *et al.*[13] administered ribavirin intraperitoneally to male Wistar rats for 5 consecutive days at doses of 0, 20, 100 or 200 mg/kg/day. Animals were sacrificed at 14, 28, 35, 42, 70 or 105 days after the last treatment. The epididymis from one side was removed and sperm was counted. Sperm counts were decreased, at 100 and 200 mg/kg/day from day 14 and at 20 mg/kg/day from day 28. At 20 and 100 mg/kg/day, sperm counts were recovered at 70 days after treatment. At 200 mg/kg/day, recovery was also observed, although not entirely complete at 105 days after treatment.

Developmental toxicity

Oral administration

Ferm *et al.*[14] administered single oral doses of ribavirin to female hamsters on gestational day 8 (2.5, 3.75 or 5.0 mg/kg bw) and to rats on gestation day 9 (25.0 or 37.5 mg/kg bw). Hamsters were sacrificed on day 13-15 and rats on day 16-17. Ferm *et al.*[14] also described experiments with intraperitoneal and intravenous administration to hamsters and rats (see below). Foetuses were examined for gross external malformations and rib anomalies. The authors mentioned that no maternal toxicity was observed in all experiments, although no specific data were given. Number of malformations were increased at all dosages. Observed malformations were central nervous system defects (especially encephalocoeles and exencephaly), eye defects, rib defects, limb defects and tail abnormalities in

hamsters and brain, eye, tail and facial defects in rats. The number of resorptions was increased in hamsters at 3.75 mg/kg bw. At 5.0 mg/kg bw, only 2 out of 25 hamster embryos survived.

Johnson[10] described and re-evaluated several (unpublished) experiments in Charles River rats, New Zealand White rabbits (performed by Industrial Bio-Test Laboratories Inc. in 1973 and reported to ICN Nucleic Acid Research Institute) and baboons (performed by Inveresk Research International in 1977 and reported to ICN Pharmaceuticals Inc.).

In a teratology study[10], rats were treated orally by gavage (doses 0, 0.1, 1.0 or 10 mg/kg bw/day) during gestation days 6-15 and were sacrificed on gestational day 20. Foetuses were weighed, investigated for external and skeletal malformations, and cross sections of the foetuses were examined. At 10 mg/kg, the number of resorptions was slightly increased, foetal weight was slightly below that of controls, and the percentage of abnormal foetuses was increased, in particular through skeletal malformations. However, according to Johnson, it was difficult to fully attribute these effects to the test agent in the absence of statistical analysis and historical control data from the laboratory. There was maternal toxicity at the high dose, consisting of a decreased weight gain during and after treatment.

In a reproduction study, male Charles River rats were treated orally by gavage from 60 days before mating up to and including the breeding period. The male rats were mated with female Charles River rats which were treated from 2 weeks prior to breeding up to gestation day 14 (half of the females were sacrificed) or up to postnatal day 21 at which the pups were weaned. Dosages were 0, 30, 60 or 90 mg/kg bw/day. No effects were reported on the male and female adult rats. At gestation day 14, females were investigated. At 90 mg/kg bw, the number of resorptions was increased. Since both males and females were treated, it is unknown whether the increased number of resorptions was due to a paternal or a maternal effect or both. The number of viable foetuses at gestation day 14 was decreased, as well as the number of viable pups that were delivered, probably the consequence of the increased number of resorptions. Postnatal survival also showed a decrease starting at 60 mg/kg bw, although not statistically significant at this dosage. At doses ≥ 60 mg/kg bw, the percentage of malformed pups was increased as well. However, Johnson could not confirm the reported number of foetuses and the percent malformed from the data.

Rabbits were treated orally by capsules (doses 0, 0.1, 0.3, or 1 mg/kg bw/day) during gestation days 6-18 and were sacrificed on gestation day 29. Foetuses were weighed and investigated for external and skeletal malformations. No effects were observed on foetuses or dams.

Seven pregnant baboons were treated orally via bananas containing a corn oil suspension of ribavirin for 4 consecutive days during gestation days 20-23 (2 animals, receiving either 60 or 120 mg/kg bw/day), 24-27 (1 animal, 120 mg/kg bw/day), 28-31 (1 animal, 120 mg/kg bw/day), 32-35 (2 animals, either 60 or 120 mg/kg bw/day), or 36-39 (1 animal, 120 mg/kg bw/day). The animals were sacrificed on gestation day 100. Foetuses were weighed and investigated externally and for skeletal malformations. One animal, receiving 60 mg/kg bw during gestation days 32-35, aborted (it was not indicated on what day). No effects were observed on the foetuses. Doses were mentioned to be non-maternally toxic, but no data on maternal weight gain were provided.

Clark *et al.*[15] exposed female C3H/HeJ and CBA/J mice to ribavirin (via drinking water), starting just after mating DBA/2 male mice (denoted as day 0.5 of gestation). The purpose of the study was to investigate causes of implantation failure. By mating CBA/J mice with DBA/2 mice, there is a high frequency of abortion because of trophoblast failure. Because it was suggested that infection may play a role, the authors investigated the influence of, among other factors, the antiviral agent ribavirin. Treatment with ribavirin continued up to sacrifice at various times between days 6.5 and 14.5 of gestation. Ribavirin was dissolved in the drinking water at 0.15 mg/ml and total dosages were calculated over the whole treatment period, based on average water consumption estimates. In addition, there was a control group, drinking plain water. Total dosages were therefore dependent on the duration of treatment and were calculated as varying between 0.52-23 μg. No data on the body weights of the mice were provided, but assuming an average body weight of 20 g, total dose in mg/kg bw varied from 0.026-1.15 mg/kg. After sacrifice, number and size of viable and dead implantations were noted and tissues were studied histologically. Resorption rate was increased following ribavirin treatment, with a total dose of 2.2-3.4 μg for CBA/J mice and with a total dose of 3.5-5.6 μg for C3H/HeJ mice. Histological investigation of a 8.5 day embryo revealed that embryo development was retarded, since it progressed only to a stage expected for a day 7.5 embryo.

Intraperitoneal injection

Kilham and Ferm[16] injected LVG hamsters intraperitoneally on gestation day 8 with 1.25, 2.1, 2.5, 3.1, 4.2 or 6.25 mg/kg bw ribavirin. The animals were sacrificed and examined on gestation day 14. Dose related increases in resorptions and malformations were observed. The most observed defects were of the limbs, eyes, central nervous system and ribs. No negative control group was used.

Ferm *et al.*[14] injected hamsters and rats intraperitoneally with a single dose of ribavirin of 2.5, 3.75 or 5.0 mg/kg bw on gestation day 8 (hamster) or 25.0, 37.5 or 50 mg/kg bw on gestation day 9 (rat). Saline was injected intraperitoneally as negative control. Hamsters were sacrificed on day 13-15 and rats on day 16-17. Number of malformations was increased at all dosages. Observed malformations were central nervous system defects (especially encephalocoeles and exencephaly), eye defects, rib defects, limb defects and tail abnormalities in hamsters and brain and eye defects as well as cleft lips in rats. The number of resorptions was increased at all dosages in hamsters and at 50 mg/kg in rats.

Kochhar *et al.*[17] injected ICR mice intraperitoneally as a single dose with 0 or 10 – 200 mg/kg bw ribavirin between gestation days 10 and 13. The animals were sacrificed on gestation day 18. Foetuses were weighed and examined for gross external and skeletal malformations. At 10 mg/kg bw, no effects on development were observed. At doses ≥ 25 mg/kg bw, an increased number of resorptions, a decreased foetal body weight compared to the controls, and a dose dependent increase in malformations was observed. Observed malformations were cleft palate, several skeletal defects, spina bifida, limb defects and digital defects. It was not mentioned in the publication whether there was maternal toxicity. It may be assumed, that there were no signs of overt maternal toxicity at the dosages used. In a separate experiment described by Kochhar *et al*., ICR mice received a single dose of 0, 10, 50, 100 or 200 mg/kg ribavirin intraperitoneally on gestation day 11 and were sacrificed 2 or 24 hours later. At 2 hours, a dose dependent decrease in DNA synthesis, measured by [^{3}H]thymidine-incorporation, was still observed. It was remarkable that at 10 mg/kg, a 20% inhibition of DNA synthesis was observed, while at the same dose, no malformations or foetal body weight reductions were observed in the first experiment of Kochhar *et al*. After 24 hours, DNA synthesis was not decreased, but increased at dose levels ≥ 50 mg/kg.

Intravenous injection

Ferm *et al.*[14] injected hamsters intravenously with a single dose of ribavirin of 5.0 mg/kg on gestation day 8. Saline was injected intravenously as negative control. Hamsters were sacrificed on day 13-15. Malformations and resorptions were increased. Observed malformations were central nervous system defects (especially encephalocoeles and exencephaly), eye defects, rib defects, limb defects and tail abnormalities.

Lactation

Hoffmann *et al*.[18] exposed female Sable or Siamese ferrets and their young of 10-13 days old to aerosolized ribavirin (0, 162, 355 or 620 mg/m^3) for 6 h per day for 10 or 30 consecutive days. The low dose (originally targeted to be 200 mg/m^3) was based on the concentration used for patient exposures (190 mg/m^3). The authors calculated that the low dose corresponded to approximately 37-58 mg/kg/day for ferret young, whereas the human dose corresponded to approximately 10 mg/kg/day. For the mid and high dose, this was 81-127 mg/kg/day and 145-222 mg/kg/day, respectively. The young were observed for mortality, body weight and clinical signs and at weaning (age 40 days) and at puberty (age 160 days), the following observations were made: pulmonary function studies, gross necropsy, terminal body and lung weights, histopathology (lungs, tracheas, and tissues with gross lesions), lung lavage studies, alveolar size determinations and DNA content of lung tissue. Body weight gain was significantly decreased during treatment in mid and high dose young. In some high dose groups there was even body weight loss. Mortality was also increased in mid and high dose young. Decreased body weight gain and mortality were obviously related to lactation failure that was observed in mid and high dose female ferrets. All the young found dead or killed moribund were thin and their gastrointestinal tracts were empty. No gross lesions were observed in the young and no treatment related effects were observed on lung function, lung growth, histopathology of lungs and trachea, and DNA content of lungs. After 30 days of exposure, mild increases of alveolar diameter were observed in some treated groups. The toxicological significance of these increases was not clear.

2.4 Conclusion

Concerning the human data, only one case report was available describing the effect of ribavirin on human fertility.[2] Therefore, the committee is of the opinion that the available human data are insufficient for conclusions regarding the effects on fertility.

In rats, intraperitoneally administered ribavirin caused an increased number of sperm cell abnormalities and a decreased sperm count. However, no information regarding general toxicity was available in these studies and the exposure route was less relevant for occupational exposure.[12,13] In a one-generation study in rats (oral gavage) described by Johnson[10], no effect on fertility parameters was observed. The dominant lethal test performed in rats by Hoffman *et al*.[11] was

negative. In this study, the number of pregnant females was slightly decreased at a dose that produced also general toxicity (200 mg/kg/day).

In conclusion, the committee recommends not classifying ribavirin for effects on fertility due to a lack of appropriate human data and data in experimental animals.

Human data regarding the effects on development consisted of only case reports of pregnancies conceived during the use of ribavrin as well.[3,7-9] [4-6] Therefore, the committee concluded that the available human data are not sufficient to draw a conclusion regarding the effects on human development.

In a review, Johnson *et al.* described and re-evaluated studies concerning effects on development after treating rats orally by gavage. Effects included a decreased number of live foetuses at GD 14, at term, and at PN-day 1 and 21 and an increased number of resorptions at gestation day 14. However, Johnson *et al.*[10] did neither find effects in rabbits after oral administration (only tested at very low dosages), nor in baboons after oral administration (with only a small number of animals studied and only treated a short treatment period).[10] In addition, effects on development were also observed in hamsters, rats and mice, after oral and intraperitoneal administration.[14,16,17] Increased numbers of resorptions were observed in hamsters, rats and mice, increased foetal death in hamsters and decreased foetal body weight in mice.[14-17] Reliable information regarding maternal toxicity was often lacking. In most studies maternal effects were either not mentioned[16,17] or only a general statement was made.[10,14] It is likely, that in these studies, animals were only followed with respect to the obvious clinical effects. Therefore, maternal effects on weight gain might have been missed. Only Johnson reported maternal weight data of rabbits and rats.[10] Furthermore, the committee noticed that in none of the studies foetal visceral tissues were investigated. Therefore, the effects of ribavirin on organ development remains largely unknown. Finally, Kochhar *et al.* demonstrated DNA inhibition in mice after intraperitoneal injection.

In conclusion, the committee is of the opinion that the human data are insufficient for drawing conclusions regarding effects of ribavirin on development. Animal data show effects on development in several animal species and after relevant exposure route (oral administration). Furthermore, the mechanism of action of ribavirin (inhibition of DNA and RNA replication) indicates that effects on development are plausible. Taking into account the limited availability of studies, the insufficient data on maternal toxicity and the observed interspecies

differences with no effects in human primates (baboons), the committee recommends classifying ribavirin in category 3 for effects on development.

There are no human data regarding the excretion of ribavirin in human breast milk. In experimental animals, there are no data regarding the excretion of ribavirin in milk. In suckling ferret young, decreased body weight gain and increased mortality were observed after exposure to high concentrations of ribavirin. The ferret young were however, not only exposed to ribavirin via mother's milk, but they were exposed by inhalation together with the female ferrets.[18]

In conclusion, the committee is of the opinion that a lack of appropriate data precludes assessment of ribavirin for effects during lactation.

Proposed classification for fertility

A lack of appropriate data precludes the assessment of ribavirin for effects on fertility.

Proposed classification for developmental toxicity

Category 3, Xn; R63

Proposed labelling for effect during lactation

A lack of appropriate data precludes the assessment of ribavirin for effects during lactation.

Additional comment

The committee is aware of the fact that based on the same data concerning the effects of ribavirin on reproduction, the Food and Drug Administration (US FDA) in the United States recommended to classify ribavirin differently. The US FDA classified ribavirin in pregnancy category X.

This category is defined as:

studies in animals or humans have demonstrated fetal abnormalities and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risk involved in use of the drug in pregnant women clearly outweigh potential benefits.

However, in accordance with EU Directive 93/21/EEC (see Annex B), the committee concluded that the limited information available and the absence of information on maternal toxicity prevents classifying ribavirin in category 2.

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A	The committee
B	Directive (93/21/EEG) of the European Community
C	Comments on the public draft
D	Fertility and developmental toxicity studies
E	Abbreviations

Annexes

Annex **A**

The committee

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The first draft of the present document was prepared by ir. I.E.M. Bosselaers, from the RIVM in Bilthoven, by contract with the Ministry of Social Affairs and Employment.

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 2009 for public review. The following persons and organisations have commented on the draft review:

- R.D. Zumwalde, Department of Health and Human Services, National Institute for Occupational Safety and Health (NIOSH), United States of America.

Annex **D**

Fertility and developmental toxicity studies

Table 1 Fertility study (oral) in animals with ribavirin.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproductive organs/effects on reproduction	Remarks
Johnson (1990)	Charles river rats (n= 7-10 per group)	Treatment ♂: 60 d before up to mating, ♀: 2 wk before up to sacrifice gd 14 or weaning pups pnd 21	0, 30, 60 or 90 mg/kg gavage	No paternal or maternal toxicity	No effects on fertility	

n=number of animals; d=days; wk=weeks; gd=gestation day; pnd=postnatal day

Table 2 Fertility studies (intraperitoneal) in animals with ribavirin.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproductive organs/effects on reproduction	Remarks
Hoffmann *et al.* (1987)	CD rats, male (n=20 per group, positive control n=10)	Males mated weekly with 8 consecutive groups of females, following treatment of 5 days; 2 females per male. Sacrifice of females 2 wk after mating	0, 50, 100 or 200 mg/kg ip	Dose-related reduced weight gain	200 mg/kg: slightly reduced % pregnant females No significant effect on early deaths or corpora lutea	
Narayana *et al.* (2002)	Wistar albino rats, male (n=5 per group)	Treatment for 5 days. Sacrificed at 14, 28, 35, 42 and 70 days. Sperm investigated for abnormalities	0, 20, 100 or 200 mg/kg ip	Not mentioned	Sperm abnormalities observed at all doses and all time points except 70 days	

Narayana *et al*. (2002)	Wistar rats male (n=5 per group)	Treatment for 5 days. Sacrificed at 14, 28, 35, 42, 70 and 105 days. Sperm cells counted	0, 20, 100 or 200 mg/kg ip	Not mentioned	Reversible decrease of sperm count at all doses	

n=number of animals; d=days; wk=weeks; gd=gestation day; pnd=postnatal day; ip=intraperitoneal

Table 3 Developmental toxicity studies (oral) in animals with ribavirin.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproduction	Remarks
Ferm *et al.* (1978)	LGV hamsters (n=5-6 per group)	Single dose gd 8 Sacrifice gd 13-15	2.5, 3.75 or 5.0-mg/kg oral-(gavage or diet not indicated)	No maternal toxicity observed	≥ 2.5 mg/kg: malformations ≥ 3.75 mg/kg: resorptions ↑ 5.0 mg/kg: foetal death ↑	Only external malformations investigated Only general statement on maternal toxicity
Ferm *et al.* (1978)	CD-1 rats (n=6-7 per group)	Single dose gd 9 Sacrifice gd 16-17	25.0 or 37.5 mg/kg oral (gavage or diet not indicated)	No maternal toxicity observed	Malformations increased at both dosages	Only external malformations investigated Only general statement on maternal toxicity
Johnson (1990)	Charles River rats (n=14-19 per group)	Treatment gd 6-15 Sacrifice gd 20	0, 0.1, 1.0 or 10 mg/kg gavage	10 mg/kg: maternal weight gain during and after treatment ↓	10 mg/kg: resorptions slightly ↑, foetal weight slightly ↓, % abnormal foetuses ↑	Foetuses weighed and investigated for external and skeletal malformations and cross sections
Johnson (1990)	New Zealand White rabbits (n=10-13 per group)	Treatment gd 6-18 Sacrifice gd 29	0, 0.1, 0.3, or 1 mg/kg oral capsules	No maternal toxicity observed	No effects	Foetuses weighed and investigated for external and skeletal malformations
Johnson (1990)	Baboons (n= 1 per group)	Treatment for 4 days during gd 20-39 Sacrifice gd 100	60 or 120 mg/kg oral (bananas containing suspension)	No maternal toxicity observed, no data on maternal weight	No effects	Foetuses weighed and investigated for external and skeletal malformations
Johnson (1990)	Charles river rats (n= 7-10 per group)	Treatment ♂ 60 d before up to mating, ♀ 2 wk before up to sacrifice gd 14 or weaning pups pnd 21	0, 30, 60 or 90 mg/kg gavage	No paternal or maternal toxicity	≥ 60 mg/kg: postnatal survival ↓, % malformed ↑ 90 mg/kg: resorptions ↑, viable foetuses gd 14 ↓, viable pups delivered ↓	Only external malformations investigated
Clark *et al.* (1993)	CBA/J, C3H/HeJ, and DBA/2 mice (n=?)	Female C3H/HeJ or CBA/J mice mated with male DBA/2 treatment after mating up to sacrifice gd 6.5-4.5	Total dose 0 or 0.52-23.0 µg Via drinking water	Mice appeared healthy, further no data	Resorptions ↑ from 2.2-3.5 µg total dose Retardation of development (dose not indicated)	

n=number of animals; d=days; wk=weeks; gd=gestation day; pnd=postnatal day;

Table 4 Developmental toxicity studies (intraperitoneal) in animals with ribavirin.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproduction	Remarks
Kilham & Ferm (1977)	LVG hamsters (n=22 per group)	Single dose gd 8 Sacrifice gd 14	1.25, 2.1, 2.5, 3.1, 4.2, or 6.25 mg/kg ip	Not mentioned	Dose related increases in resorptions and malformations (limb, eye, CNS and rib)	No control group was used
Ferm *et al.* (1978)	LGV hamsters (n=8-14 per group)	Single dose gd 8 Sacrifice gd 13-15	0, 2.5, 3.75 or 5.0 mg/kg ip	No maternal toxicity observed, only general statement	All dosages: malformations and resorptions	Only external malformations investigated
Ferm *et al.* (1978)	CD-1 rats (n=7-14 per group)	Single dose gd 9 Sacrifice gd 16-17	0, 25.0, 37.5 or 50 mg/kg ip	No maternal toxicity observed, only general statement	≥ 25 mg/kg: malformations 50 mg/kg: resorptions ↑	Only external malformations investigated
Kochhar *et al.* (1978) (abstract)	ICR mice (n=?)	Single dose gd 10-12 Sacrifice gd?	10, 50, 100, 150 or 200 mg/kg ip	Not mentioned	≥ 50 mg/kg resorptions and skeletal malformations	Preliminary study Only skeletal malformations recorded
Kochhar *et al.* (1980)	ICR mice (n=95)	Single dose gd 10-13 Sacrifice gd 18	0 and 10-200 mg/kg ip	Not mentioned	≥ 25 mg/kg increases in resorptions, decreased foetal weight and malformations (cleft palate, spina bifida, skeletal defects, limb defects)	Foetuses weighed and investigated for external and skeletal malformations
Kochhar *et al.* (1980)	ICR mice (n=29)	Single dose gd 11, sacrifice gd 11, 2 or 24 h after injection, DNA synthesis in embryos determined	0, 10, 50, 100 or 200 mg/kg ip	Not mentioned	Dose-dependent but transient inhibition of DNA synthesis in embryos of treated dams	

n=number of animals; d=days; wk=weeks; gd=gestation day; pnd=postnatal day; ip=intraperitoneal

Table 5 Developmental toxicity study (intravenous) in animals with ribavirin.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproductive organs/ effects on reproduction	Remarks
Ferm *et al.* (1978)	LGV hamsters (n=9 per group)	Single dose gd 8 Sacrifice gd 13-15	0 or 5.0 mg/kg iv	No maternal toxicity observed	Increased resorptions and malformations	Only external malformations investigated Only general statement on maternal toxicity

n=number of animals; d=days; wk=weeks; gd=gestation day; pnd=postnatal day; iv=intravenous

Table 6 Study on lactation (inhalation) in animals with ribavirin.

Authors	Species	Experimental period/design	Dose and route	Maternal effects	Effects on suckling young	Remarks
Hoffmann *et al*. (1987)	Litters of female Sable or Siamese ferrets (n= 6-12 young/ sex/group)	Exposure of jills + litters of 10-13 days old for 10 or 30 days, 6h/day Sacrifice at age 40 or 160 days	0, 162, 355 or 620 mg/m³) by inhalation	≥ 355 mg/m³: lactation failure	≥ 355 mg/m³: bw gain ↓, mortality ↑	

n=number of animals; d=days; wk=weeks; gd=gestation day; pnd=postnatal day; bw= body weight

Annex **E**

Abbreviations

Abbreviations used:

bw	body weight
d	day
F	female(s)
i.p.	intraperitoneal
i.v.	intravenous
M	male(s)
n	number
NOAEL	no adverse effect level
OECD	Organisation for Economic Cooperation and Development
PN	postnatal