Lithiumcarbonate and Lithiumchloride

Evaluation of the effects on reproduction, recommendation for classification

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp	:	aanbieding advies
Uw kenmerk	:	DGV/BMO-U-932542
Ons kenmerk	:	U 949/AB/jt/543-Z2
Bijlagen	:	1
Datum	:	3 mei 2000

Mijnheer de Staatssecretaris,

Bij brief van 3 december 1993, nr. DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid om naast het afleiden van gezondheidskundige advieswaarden ook te adviseren over andere onderwerpen ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In 1995 heeft de Staatssecretaris van Sociale Zaken en Werkgelegenheid besloten tot het opstellen van een zogenaamde niet-limitatieve repro-tox-lijst. Op deze lijst komen stoffen die volgens de richtlijnen van de Europese Unie ingedeeld moeten worden in categorie 1 of 2 wat betreft effecten op de voortplanting. De Gezondheidsraad is verzocht om voor stoffen een classificatie volgens de EU-criteria voor te stellen.

In 1996 heb ik hiervoor de Commissie Reproductietoxische stoffen ingesteld.

Hierbij bied ik u - gehoord de Beraadsgroep Gezondheid en Omgeving - de publikatie van de commissie aan over lithiumcarbonaat en lithiumchloride. Deze publikatie heb ik heden ter kennisname aan de Minister van Volksgezondheid Welzijn en Sport en aan de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer gestuurd.

Hoogachtend,
w.g.
prof. dr JJ Sixma

Lithiumcarbonate and Lithiumchloride

Evaluation of the effects on reproduction, recommendation for classification

Committee for Compounds toxic to reproduction,
a committee of the Health Council of the Netherlands

to

the Minister and State Secretary of Social Affairs and Employment

No. 2000/06OSH, The Hague, 3 May 2000

Preferred citation:
Health Council of the Netherlands: Committee for Compounds toxic to reproduction. Lithiumcarbonate and Lithiumchloride; Evaluation of the effects on reproduction, recommendation for classification. The Hague: Health Council of the Netherlands, 2000; publication no. 2000/06OSH.

ISBN: 90-5549-313-9

Contents

Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de Raad, adviseert een classificatie voor reproductietoxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie lithiumcarbonaat en lithiumchloride onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit adviseert de commissie om lithiumcarbonaat en lithiumchloride in categorie 3 (*Stoffen die in verband met hun mogelijke voor de vruchtbaarheid van de mens schadelijke effecten reden geven tot bezorgdheid*) te classificeren en met R62 (*Mogelijk gevaar voor verminderde vruchtbaarheid)* te kenmerken.
- Voor ontwikkelingsstoornissen, adviseert de commissie om lithiumcarbonaat en lithiumchloride in categorie 1 *(Stoffen waarvan bekend is dat zij bij de mens ontwikkelingsstoornissen veroorzaken)* te classificeren en met R61 *(kan het ongeboren kind schaden)* te kenmerken.
- Voor effecten tijdens de lactatie, adviseert de commissie om lithiumcarbonaat en lithiumchloride tevens met R64 (*Kan schadelijk zijn via de borstvoeding*) te kenmerken.

Executive summary

On request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the effects on the reproduction of substances at the workplace. The Health Council's Committee for Compounds Toxic to Reproduction recommends a classification for compounds toxic to reproduction according to Directive 93/21/EEC of the European Union. In the present report the committee has reviewed lithiumcarbonate and lithiumchloride.

The committee's recommendations are:

- For effects on fertility, the committee recommends to classify lithiumcarbonate and lithiumchloride in category 3 (*Substances which cause concern for human fertility*) and to label the compounds with R62 (*Possible risk for impaired fertility*).
- For developmental toxicity, the committee recommends to classify lithiumcarbonate and lithiumchloride in category 1 (*Substances known to cause developmental toxicity in humans*) and to label the compounds with R61 (*May cause harm to the unborn child*).
- For effects during lactation, the committee recommends that lithiumcarbonate and lithiumchloride should be labelled with R64 (*May cause harm to breastfed babies).*

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 recommend a classification of compounds toxic to reproduction. The classification is performed according to the guidelines of the European Union (Directive 93/21/EEC) by the Health Council's Committee for Compounds Toxic to Reproduction. 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 and 2) of the European Union.

1.2 Committee and procedure

The present document contains the classification of lithiumcarbonate and lithiumchloride by the Health Council's Committee for Compounds Toxic to Reproduction. The members of the committee are listed in Annex A. The first draft of this report was prepared by Mrs ir IDH Waalkens-Berendsen at the Department of Neurotoxicology and Reproduction Toxicology of the TNO Nutrition and Food Research Institute, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility, development and lactation of the above mentioned compound.

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

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 September 1999, 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 B. 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 C, 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 C, 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 compound as potentially toxic to the breastfed child when exposure to this compound via the milk results in an intake exceeding 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 Toxline and Medline, starting from 1966 up 1995. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection

* Organisation for Economic Cooperation and Development

of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited. Before finalising the public draft the committee performed an additional literature search in Medline and Toxline for the period 1995 to 1997. The results of this search were no reason for the committee to adjust the recommendations.

The committee chose to describe human studies in the text (summarised in Annex D), starting with review articles. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

Animal data are described in the text and summarised in Annex D.

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 characterisation, human exposure assessment and recommendations of other organisations.

* for definitions see Tox95

Chapter 2

Lithiumcarbonate and lithiumchloride

2.1 Introduction

Name	:	lithium carbonate
CAS-no	:	554-13-2
Use	:	as alloying agent, as catalyst, as lubricant, as component of pottery glazes, in psychiatric therapy
Mol weight	:	73.89
Chem formula	:	Li_2CO_3

Name:	:	lithium chloride
CAS-no	:	7447-41-8
Use:	:	at manufacturing mineral waters, at soldering aluminium, in psychiatric therapy
Mol weight:	:	42.39
Chem formula:	:	LiCl

Lithium (Li^+) is the active ingredient in the two substances mentioned above. The substances will therefore not be discussed separately in the following text.

2.2 Human studies

Exposure of man to lithium predominantly occurs as a therapy for manic depression, often in combination with other therapeutic substances. Lithium was registered for acute mania in 1960 (dose 1800 mg/day) and for maintenance therapy since 1970 (dose 900-1200 mg/day) (Moo95). The committee concluded that in the earlier studies with lithium, higher dosages were used and therefore these studies might be more useful for the hazard-based classification purposes.

The human studies are summarised in Tables 1 and 2 (Annex D).

Fertility

Blay *et al* (1982) reported 2 cases of male sexual dysfunction associated with lithium therapy. After 1 month of therapy (serum levels were kept at 26-33 mg/l) the first patient, age 42 years, reported loss of libido and impaired erection. After 3.5 months of treatment he was switched to a placebo treatment in a blind fashion for 2 weeks. On the second day of placebo treatment, his serum lithium level decreased to zero and there was normalisation of libido and sexual behaviour. The second patient, age 58 years, complained about decreased libido and difficulty obtaining erection after start of treatment with lithium; serum lithium levels were kept at 18 mg/l. 2 Months after ceasing the lithium treatment, full remission occurred (Bla82).

Levin *et al* (1981) studied the effects of lithium carbonate (serum levels of 22-52 mg/l, dose unknown) on sperm function in 4 patients suffering from clinical depression and 9 controls. Three weeks of continuous therapy with lithium resulted in a significant decrease in sperm viability but no significant change in sperm count or motility were observed (Lev81).

Lithium is supposed to cause hyperprolactinemia, thought to be associated with male infertility; for that reason the committee paid special attention to studies in which prolactin levels were measured.

Lanng Nielsen *et al*. (1977) studied plasma prolactin (PRL) levels in a longitudinal study for 90 days. Manic-depressive patients (n=9) were examined before lithium treatment started and at various times during treatment (Lan77). No significant changes were found, nor could any correlation be established between lithium treatment and plasma prolactin. In a transversal study, plasma PRL levels were determined in 26 patients during long-term (3 months to 20 years) lithium-treatment and in 16 control patients. The plasma PRL levels were not elevated in the treated patient compared with the controls.

Ghadirian *et al*. (1992) studied the effects of lithium and lithium in combination with benzodiazepines in bipolar patients, 45 men and 59 women (Gha92). No relationship was found between serum lithium and plasma PRL or serum lithium levels and sexual dysfunction scores.

In a study of Czernik (Cze79), levels of gonadotropin hormone (GH), thyroid stimulating hormone (TSH), PRL, follicle stimulating hormone (FSH), luteinising hormone (LH) and cortisol were measured in manic-depressive patients treated with lithium and in healthy patients before and after a combined treatment with insulin, thyroid releasing hormone (TRH) and luteinising hormone releasing hormone (LHRH). Baseline serum prolactin levels in lithium-treated patients were lower than in the controls, but the difference was not statistically significant.

Tanimoto *et al*. (Tan81) studied the effect of TRH (0.5 mg/person) once administered before treatment with lithium and once administered at the end of a 3-4 weeks lithium carbonate treatment period (600-1800 mg lithiumcarbonate /day) on plasma TSH and PRL levels in patients with manic states and in controls. Basal plasma PRL concentrations did not differ between the two groups and was not affected by lithium administration to either group. In the controls and male patients, lithium had no effect on TRH-induced PRL response. In the female patients, lithium increased the TRH-induced PRL response.

Goodnick *et al*. (Goo83) studied the effect of chronic lithium carbonate treatment (patients were treated with lithium until an adequate therapeutic lithium level of 30-55 mg/l plasma was established) on apomorphine*-induced changes in serum prolactin and growth hormone following establishment of appropriate steady state lithium plasma levels in patients with primary affective disorder or schizoaffective illness. Baseline serum prolactin levels before and after lithium treatment were not significantly different (14.6 ± 3.7 before and 16.4 ± 4.6 ng/ml after lithium treatment).

Grof *et al*. (Gro85) studied the PRL response to insulin hypoglycemia in bipolar patients treated with lithium and in healthy volunteers on and off lithium. No effect of lithium was observed on base-line prolactin response.

Joffe *et al*. (Jof86) studied the responses to sequential stimuli with arginine (500 mg/kg bw) and 90 minutes later with LHRH (100 µg/person) and TRH (500 µg/person) in 8 patients who were depressed before and after lithium carbonate treatment (1230 ± 247 mg/day for at least 6 weeks). Plasma levels of LH, FSH, TSH, GH and prolactin were measured. Baseline prolactin plasma levels were increased by Li treatment and Li augmented the prolactin response to TRH. The authors discussed that their results about the effect of Li on base-line prolactin levels are in contrast to the results of 5 other stu-

---

* a dopamine agonist

dies (4 of these are described in this paper) which found unaltered baseline prolactin levels with lithium treatment.

Yatham (Yat94) studied the effect of buspirone (0.5 mg/kg orally), a 5HT agonist, on prolactin release in 11 manic patients and 11 healthy controls. Six of the manic patients were treated with lithium (600-1500 mg/day for 3 weeks) where after the buspirone challenge was repeated. The baseline prolactin levels in manic patients were not different compared to the controls and lithium treatment did not affect baseline prolactin levels.

The studies concerning prolactin levels in lithium-treated men were ambiguous. Based on these studies no conclusions concerning decreased human fertility can be drawn.

In vitro studies with human sperm were performed by Raoof *et al* (1989) and Shen *et al* (1992) (Rao89) (She92). Both detected a negative effect on motility at concentrations comparable with those reported in semen after oral administration.

Developmental studies

Lithium, administered as Li_2CO_3, was used in a 2-year study as a therapeutic and prophylactic drug in 25 manic-depressive patients (male and female) in the Douglas Hospital, Quebec, Canada in a dosage ranging from 300 to 1800 mg per day (blood level: 18-37 mg/l). In the first month blood levels were determined weekly, thereafter bimonthly. Apart from beneficial effects, diverse adverse effects were observed, among them tremors, diarrhoea, skin rash, alopecia, mental confusion. One patient was pregnant during treatment and delivered a child with congenital malformations, bilateral club feet and lumbar meningomyelocele (Vac70).

Weinstein (1976) reported in the International Register of Lithium Babies about 166 patients who used lithium during pregnancy; 18 of these patients delivered malformed children. The malformations were, among others, cardiovascular defects, including the rare Ebstein's anomaly. Most of the pregnant patients, however, also received other drugs for treatment. Moreover, the incidence of congenital malformations did not substantially exceed the expected incidence in a non-lithium population (Wei76).

A medical birth registry program in Sweden identified 350 manic-depressive women who had born a child in the period of 1973-1979. The number of perinatal deaths and the incidence of heart defects was higher, gestational length shorter and the birth weight lower than expected in a control population. 41 Mothers of these children had used only lithium during pregnancy. The incidence of heart defects was high within this group; Ebstein's anomaly, however, was not among the defects (Käl83).

In 1977, the Danish Lithium Baby Register included 183 infants who had been exposed to lithium during the first trimester of gestation. Eleven percent of these had major congenital anomalies, the majority of which consisted of cardiovascular defects, including a few cases of Ebstein's anomaly (4% of the total) (War88).

A study on 50 lithium-using pregnant women in San Diego, USA, showed upon delivery no cardiovascular malformations; one child had a lumbar meningomyelocele, and another had unilateral inguinal hernia (Cun89).

The possibility of the use of lithium during pregnancy causing foetal hypothyroidism was reported in a Letter to the Editor (Rob90).

An epidemiological study carried out between 1979 and 1991 in Canada and the USA. revealed no differences in the occurrence of congenital anomalies, particularly of cardiac malformations, between a lithium-exposed cohort and an unexposed cohort (Jac92).

The teratogenic risk of therapeutic use of lithium was evaluated by Cohen *et al* (1994) who concluded that "the teratogenic risk of first-trimester lithium exposure was lower than previously suggested. The clinical management of women with bipolar disorder who have childbearing potential should be modified with this revised risk estimate" (Coh94).

Lactation

Scialli (1992) reported excretion of lithium in breast milk, resulting in a milk lithium concentration of 40% of the maternal plasma levels (Sci92).

Sykes *et al*. (1976) and Schou *et al*. (1973) noted concentrations of lithium in infant serum which were 10 to 50% of that found in maternal serum (Syk76, Sch73). The effect of exposure to this amount of lithium in a neonate is minimal.

Tunnessen and Hertz (1972) described a breast fed baby with serum lithium level of 44 mg/l and a mother’s serum level of 111 mg/l manifesting cyanosis and floppy muscles along with electrocardiographic changes e.g.. T-wave inversions (Tun72). The symptoms resolved after the discontinuation of breastfeeding, however the authors did not specifically mention a repeat ECG.

2.3 Animal studies

Tables 3 and 4 (Annex D) summarise the reproduction and developmental toxicity studies with lithium in experimental animals.

Fertility

Banerji *et al*. (Ban83) injected adult male Sprague-Dawley rats intraperioneally (ip) with LiCl (100 mg LiCl/kg bw, twice daily for 2 or 7 days) and examined the effect (by radioimmunoassay) on the levels of LH, FSH and PRL in plasma and pituitary homogenates. No effect was observed on the concentrations of LH, FSH and PRL in the pituitary homogenates after treatment with LiCl. Plasma LH was increased after 2 days and decreased after 7 days of LiCl treatment. No effect of LiCl treatment was observed on FSH-plasma levels as well. Two days of LiCl treatment had no effect on PRL-plasma levels whereas the level of PRL in plasma was statistically significantly decreased after 7 days of LiCl treatment compared to the control group (7 days ip injections of saline). In the control group, the PRL-levels in plasma after 7 days were higher than after 2 days of treatment (comparable to pituitary) whereas in the LiCl-treated group no increase was observed. Four out of 20 animals that received lithium for 7 days died on the 6th day; one rat of the controls died during treatment. A number of rats that received lithium for 7 days showed signs of polydipsia and polyuria.

In a study of McIntyre *et al*., male Sprague-Dawley rats received saline or lithium (ip, 2 mg/kg) once or for 14 consecutive days. No effect of Li was observed on PRL-levels whereas PRL-levels after 14 days of saline or Li treatment were higher than those in rats receiving only one ip injection (McI83).

In a series of studies of Ghosh *et al*., the effect of LiCl on testicular activity was investigated (Gho91a, Gho91b). In the first study (Gho91a), immature Wistar rats (35 days old) were treated for 15, 20 and 25 days with LiCl (subcutaneously, 2.0 mg/kg, 8 rats per group) or distilled water (control group, 8 rats per group). After 15 days of treatment, spermatogenesis was inhibited at stage VII of the seminiferous epithelial cycle and serum levels of FSH, LH, PRL and testosterone were decreased. Longer treatment resulted in a decrease of the weights of the accessory sex organs as well. In a second comparable experiment, rats were treated with PRL just after LiCl treatment. This resulted in a recovery of above described events (Gho91b). In both studies no effect on body weight was observed. The serum lithium concentration were 20 mg/l, which is comparable with the therapeutic level in man.

Groups of 10 female Charles River albino rats were gavaged daily with doses of lithium carbonate solutions at levels of 0, 25, 75 or 150 mg/kg bw/day for 14 days before mating. They were mated with male rats of the same strain which were treated with 0, 10, 25 or 50 mg/kg bw/day for 70 days. Mating pairs were formed within the low-, mid- or high-dose groups. The pregnant females were allowed to litter and raise offspring up to postnatal day 21, when they were sacrificed. Two high-dose females died during pregnancy. No differences were found in general maternal health or body weight; no differences were found in litter size, pup viability or in mean pup weight, except for postnatal

day 21, when the high-dose pups body weights were statistically significantly lower than in the other groups (Gra72).

Groups of 11-12 swine were fed a diet containing 0 or 3000 mg Li_2CO_3/kg diet (~120 mg/kg bw) during gestation. They were followed during gestation and up to day 21 of lactation. Maternal body weight was reduced from day 60 of gestation and onwards, reaching the level of statistical significance on gestation day 110. Postpartal body weights were also reduced when compared to the controls. The number of pregnancies and total number of piglets born per litter were slightly reduced; the mean number of live born piglets, mean birth weight and survival at 21 day were statistically significantly reduced, and the number of mummies and stillborns was statistically significantly increased (Kel78).

Mating pairs of 10 CFW (Carworth Farms) mice/group were offered drinking water containing 0, 2000, 4000 or 8000 mg LiCl/l, starting from either 3 weeks or 6-8 weeks of age. They were mated when they were 8-10 weeks. The mice offered 8000 mg/l did not drink and died soon. Mice offered 4000 mg/l did drink, but less than the controls and did not reproduce. Mice maintained on 2000 mg/l starting from 6-8 weeks of age did not show adverse effects, but their reproduction capacity was reduced i.e.. the average time between litters reduced and the total number of litters was reduced when compared to the control pairs. Litter size was not affected, but preweaning pup mortality was statistically significantly increased. In the mating pairs that were exposed to lithium from 3 weeks of age the same effects were observed. Moreover, a statistically significantly high number of pups showed delayed growth and development (Mro83). General toxicity and number of animals per group was not described.

Groups of 6-9 pregnant female Sprague-Dawley rats (Taconic Farms) were exposed to 0, 80 or 160 mg natural lithium salts/kg bw/day via the drinking "water" (50% orange juice) for 10 days after which they were mated with untreated males. Exposure continued during gestation and lactation up to postpartal day 28. The dams were monitored for behaviour (nesting, nursing, grooming). Maternal behaviour was altered in both groups: no grooming, little and short nursing; they did not cannibalise dead pups (Sec86).

Groups of 5 pregnant Sprague-Dawley albino mice were offered drinking water containing 0 or 40 mg LiCl/l from mating up postpartal day 23, when the pups were weaned and maintained on normal drinking water for a subsequent 14 days; then they were sacrificed. In a similar experiment, exposure started immediately after delivery. In a third experiment, female mice were exposed to 0 or 40 mg/l in drinking water for 2 weeks prior to conception and during gestation. The new-borns were killed about one day after delivery. A reduction was recorded in the weights of the brain (significant), kidney (females: significant), spleen (females only) and testis of the pups from experiments 1 and 2 (Mes86).

Developmental toxicity

Groups of pregnant HaM/ICR mice (Charles River, Mass.) were treated with 200, 300 or 465 mg Li_2CO_3/kg bw/day by gavage from day 6 to 15 of gestation. They were killed on gestation day 18. In the low-dose treated animals, no effects were observed. Treatment with 300 mg/kg bw/day caused cleft palate in 11% of the animals. Treatment with 465 mg/kg bw/day resulted in a statistically significant increase in maternal and foetal death and in 30% of the survivors cleft palate was found (Sza70).

Groups of 20 pregnant Charles River albino rats were administered 0, 25, 75 or 150 mg Li_2CO_3/kg bw/day by gastric intubation from gestation day 5 through 15. They were sacrificed on gestation day 20. Groups of 10 pregnant New Zealand White rabbits were dosed orally with capsules containing 0, 25 and 40 mg Li_2CO_3/kg bw/day from gestation day 5-18. On gestation day 28 the rabbits were killed. Six pregnant rhesus monkeys were dosed with 0 or 25 mg Li_2CO_3/kg bw/day in capsules during organogenesis, from day 14 through 35 of gestation. The females were either delivered by caesarian section around day 160 or they were allowed to litter naturally. All offspring was examined thoroughly, the surviving monkeys also for postnatal (behavioural) development. Two high-dose rats died, 3 high-dose rabbits refused to eat and eventually died, one low dose group rabbit died. No differences were observed between control and dose groups in maternal fertility, body weight gain, number of implantation sites, litter size, litter weight, foetal mortality and gross visceral or skeletal malformations or (for the monkeys) postnatal development (Gra72).

Groups of 15 - 25 malformation-prone strain 129 Sv/SL or A/J mice were injected intraperitoneally with 0, 0.8, 1.6, 3.2 (or 5.0: Sv/SL mice only) mg Li_2CO_3/mouse on gestation day 8, 9 or 10, or on gestation day 12, 13 or 14, respectively. They were killed on gestation day 18-19. The Sv/SL mice only showed malformations in the high dose group (41.6%). These malformations consisted of fused ribs, vertebral defects, or exencephaly. Most effects were found in animals exposed on day 9. The foetuses of the A/J mice showed a high incidence of cleft lip and palate (16.4% in the control group). The incidence of cleft lip and cleft palate in the lithium treated groups was 3.1-21.2%; no relationship with the concentration of lithium dosed per animal was observed. Furthermore, the day of administration did not influence these effects. (Smi82)

Another group of 16 pregnant Sv/SL mice was offered drinking water containing 2 mg Li_2CO_3/ml from gestation day 1 though 18; they were killed at gestation day 18. The chronically exposed mice produced only 2 litters, and a high percentage of resorptions. The 6 resulting foetuses were grossly normal (Smi82).

Groups of 11-20 pregnant Wistar rats were administered by gavage doses of 0, 50 or 100 mg Li_2CO_3/kg bw in agar from gestation day 6 to 15. They were killed on gestation day 20. Effects were found on the number of implantation sites, live foetuses and

foetal body weights which were slightly reduced in the low dose group and statistically significantly in the high dose group. The high dose group foetuses had congenital malformations such as reduced size, shortened limbs, and other deformities of the skeleton. No differences in skeleton were observed in the low-dose group; no defects were observed at visceral examination in any of the foetuses (Mar86).

Groups of pregnant albino rats were given intragastrically 0 or 7 mg Li_2CO_3/kg bw/day from gestation day 0 - 10. They were killed at term. The following defects were observed in the exposed offspring: growth retardation, cleft palate, brain liquification, hepatomegaly, digital abnormalities, hydrocephaly, cardiomegaly and syndactyly (Sha86).

Groups of 6-9 female Sprague Dawley rats (Taconic Farms) were exposed to 0, 80 or 160 mg natural lithium salts/kg bw/day via the drinking "water" (50% orange juice) for 10 days after which they were mated with untreated males. Exposure continued during gestation and lactation up to postpartal day 28. The pups were followed for effects on body weight, physical landmarks, development of reflexes, and they were tested for open field activity. Adverse effects were present in both dose groups, the effects in the high dose group being more pronounced: pup body weight was decreased, the physical landmarks eye opening and pinna unfolding were delayed, as was the development of depth perception. In an open field activity test they showed lower spontaneous activity than the control offspring: the exposed pups were lethargic at 4 months of age (Sec86).

Groups of 3-22 pregnant JBT/Jd mice were injected intraperitoneally with 0, 250, 300, 310, 315, 320, 325, 330, 335, 340, 350 or 400 mg Li_2CO_3/kg bw on gestation day 9. They were killed on gestation day 13. The defects found were various, and depended on dose level. They included a dose-related increase in number of dead foetuses, number of abnormalities and retardations. The abnormalities consisted a.o. of exencephaly, craniorachischisis, kinking of the spinal cord and dilatation of the 4^{th} brain ventricle. These anomalies were concluded to be the result of the neurotropic effect of the drug (Jur88).

2.4 Overall conclusion

Some case-reports suggest that Li induces libido and erection disturbances. In *in vitro* studies with human sperm, decreased motility was observed at the same concentration as is found in the semen of patients. Studies of plasma PRL levels in lithium-treated men were ambiguous. The committee concludes that the amount of evidence and the quality of the human studies is insufficient for a classification of Li for fertility.

Fertility studies in animals were often carried out in such a way that the serum level of lithium in the tested animals was similar to that of treated human patients. Differences in sensitivity amongst species, however, resulted in general toxicity in most of the animals, whereas at the same serum levels in man no signs of overt toxicity were observed.

Exposure below these levels hardly caused any effects in animals except for a reduction in reproduction capacity in one study in the mice (Mro83). Ghosh *et al*. (Gho91a and b) detected that the spermatogenesis was inhibited at stage VII of the seminiferous epithelial cycle after subcutaneous injection of 2 mg LiCl/kg bw/day for at least 15 days.

Based on the evidence in animal studies (Gho91a and b) the committee recommends to classify lithiumcarbonate and lithiumchloride for fertility in category 3 ('substances which cause concern for human fertility') and to label lithiumcarbonate and lithiumchloride with R62 (Possible risk of impaired fertility).

Women exposed to lithium during pregnancy in early studies were reported to give birth to children with congenital malformations of extremities of limbs and central nervous system or of the cardiovascular system in combination with hypothyroidism (Vac70, Wei76, Käl83, War88). A more recent epidemiological study (Jac92), however, indicated no differences in the occurrence of congenital malformations between a Li-exposed and a control cohort. The committee is of the opinion that this discrepancy might be explained by relatively lower dosages used in the later studies when lithium was mainly used for maintenance therapy (900-1200 mg/day) instead of treatment for acute mania (1800 mg/day). Therefore, the absence of a teratogenic epidemiological risk found in studies of a later date probably reflects the grown awareness of the clinicians and the restrictive prescription of lithium at high doses during the first trimester of pregnancy.

Lithium predominantly caused developmental effects when female animals were exposed during the first part of pregnancy, before and/or during organogenesis, but exposure before conception or after organogenesis also had effects.

In view of the amount of evidence in earlier human studies, the committee recommends to classify lithiumcarbonate and lithiumchloride in Category 1 (substances known to cause developmental toxicity in humans) and to label lithiumcarbonate and lithiumchloride with R 61 (may cause harm to the unborn child).

Lithium ingested during lactation is found in breast milk in sufficient amounts to warrant concern for the infant's development. Therefore the committee recommends to label lithiumcarbonate and lithiumchloride with R 64 (May cause harm to breastfed babies).

Proposed classification for fertility

Category 3, R62.

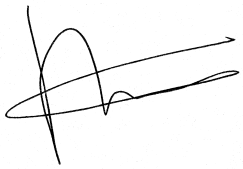
Proposed classification for developmental toxicity

Category 1, R61.

Proposed labelling for effects during lactation

R64.

For the committee,
The Hague, 3 May 2000



dr ASAM van der Burght,
scientific secretary

chairman

References

Ban83 Banerji TK, Parkening TA, Collins TJ, Rassoli A. Lithium-induced changes in the plasma and pituitary levels of luteinizing hormone, follicle stimulating hormone and prolactin in rats. Life Sciences 1983; 33: 1621-1627.

Bla 82 Blay SL, Feraz MP, Calil HM. Lithium-induced male sexual impairment: two case reports. J Clin Psychiatry 1982; 43: 497-498.

Coh94 Cohen LS, Friedman JM, Jefferson JJ, Johnson EM, Weiner ML. A reevaluation of risk of in utero exposure to lithium. JAMA 1994; 271: 146-150.

Cun89 Cunniff CM, Sahn DJ, Reed KL, Chambers CC, Johnson KA, Jones KL. Pregnancy outcome in women treated with lithium. Teratology 1989; 39: 447-448.

Cze79 Czernik A, Kleesiek K. Neuroendokrinologische Veränderungen unter Langzeitbehandlung mit Lithiumsalzen. Pharmakopsychiatr Neuropsychopharmacol 1979; 12: 305-312.

Gha92 Ghadirian AM, Annable L, Bélanger RN. Lithium, benzodiazepines, and sexual function in bipolar patients Am J Psychiatry 1992; 149: 801-805.

Gho91a Ghosh PK, Biswas NM, Ghosh D. Effect of lithium chloride on testicular steroidogenesis and gametogenesis in immature male rats. Acta Endocrinologica 1991; 124: 76-82.

Gho91b Ghosh D, Biswas NM, Ghosh PK. Studies on the effect of prolactin treatment on testicular steroidogenesis and gametogenesis in lithium-treated rats. Acta Endocrinologica 1991; 125: 313-318.

Goo83 Goodnick PJ, Meltzer HY. Effect of subchronic lithium treatment on apomorphine-induced change in prolactin and growth hormone secretion. J Clin Psycopharmacol 1983;4: 239-243.

Gra72 Gralla EJ, McIlhenny HM. Studies in pregnant rats, rabbits and Monkeys with Lithium carbonate. Toxicol Appl Pharmacol 1972; 21: 428-433.

Gro85 Grof E, Grof P, Brown GM. Effects of long-term lithium treatment on prolactin regulation. Adv Biochem Psychopharmacol 1985; 40: 81-87.

Jac92 Jacobson SJ, Jones K, Johnson K, Ceolin L, Kaur P *et al.* Prospective multicenter study of pregnancy outcome after lithium exposure during the first trimester. Lancet 1992; 339: 530-533.

Jof86 Joffe RT, Post RM, Ballenger JC, Rebar R, Gold PW. The effects of lithium on neuroendocrine function in affectively ill patients. Acta Psychiatr Scand 1986; 73: 524-528.

Jur88 Jurand A. Teratogenic activity of lithium carbonate: an experimental update. Teratology 1988; 38: 101-111.

Käl83 Källén B, Tandberg A. Lithium and pregnancy. Acta Psychiatr Scand 1983; 68:134-139.

Kel78 Kelly KW, McGlone JJ, Froseth JA. Lithium toxicity in pregnant swine. Proc Soc Exp Biol Med 1978; 158: 123-127.

Lan77 Lanng Nielsen J, Amdisen A, Darling S, Pedersen EB. Plasma prolactin during lithium treatment. Neurophychobiology 1977; 3: 30-34.

Lev81 Levin RM, Amsterdam JD, Winokur A, Wein AJ. Effects of psychotropic drugs on human sperm motility. Fertil Steril 1981; 55: 503-506.

Mar86 Marathe MR. Thomas GP. Embryotoxicity and teratogenicity of lithium carbonate in Wistar rat. Toxicol Lett 1986; 34: 115-120.

McI83 McIntyre IM, Kuhn C, Demitriou S, Fucek FR, Stanley M. Modulating role of lithium on dopamine turnover, prolactin release and behavioral supersensitivity following haloperidol and reserpine. Psychopharmacology 1983; 81: 150-154.

Mes86 Messiha FS. Lithium and the neonate: development and metabolic aspects. Alcohol 1986; 3: 107-112.

Moo95 Moore JA and an IEHR Expert Scientific Committee. An assessment of lithium using the IEHR evaluative process for assessing human developmental and reproductive toxicity of agents. Repro Toxicol 1995; 9 (2): 175-210.

Mro83 Mroczka DL, Hoff KM, Goodrich CA, Baker PC. Effect of lithium on reproduction and postnatal growth of mice. Biol Neonate 1983; 43: 287-296.

Rao89 Raoof NT, Pearson RM, Tuerner P. Lithium inhibits human sperm motility in vitro. Br J Pharmac 1989; 28: 715-717.

Rob90 Robert E, Francannet C. Comments on "Teratogen update on lithium" by J. Warkany Teratology 1990; 42: 205.

Sch73 Schou M, Amdisen A. Lithium and pregnancy. III: lithium ingestion by children breast-fed by women in lithium treatment. BMJ 1973; 2 (5859):138

Sci92 Scialli AR. Breast milk. A clinical guide to reproductive and developmental toxicology 1992; 193-208.

Sec86 Sechzer, JA, Lieberman KW, Alexander GJ, Weidman D, Stokes PE. Aberrant parenting and delayed offspring development in rats exposed to lithium. Biol Psychiatry 1986; 21: 1258-1266.

Sha86 Sharma A, Rawat AK. Teratogenic effecs of lithium and ethanol in the developing fetus.Alcohol 1986; 3: 101-106.

She92 Shen MR, Yang R-C, Chen SS. Effects of lithium and haloperidol on human sperm motility in-vitro. J Pharm Pharmacol 1992; 44: 534-536.

Smi82 Smithberg M, Dixit PK. Teratogenic effects of lithium in mice. Teratology 1982; 26: 239-246.

Syk76 Sykes PA, Quarrie J, Alexander FW. Lithium carbonate and breast feeding. BMJ 1976; 2(6047):1299.

Sza70 Szabo KT. Teratogenic effect of lithium carbonate in the foetal mouse. Nature 1970; 225: 73-75.

Tan81 Tanimoto K, Maeda K, Yamaguchi N, Chihara K, Fujita T. Effect of lithium on prolactin responses to thyrotropin releasing hormone in patients with manic state. Psychopharmacology 1981; 72: 129-133.

Tox95 Niesink RJM, de Vries J, Hollinger MA, eds. Toxicology, Principles and Applications, Boca Raton: CRC Press, 1995:385

Tun72 Tunnessen WW, Hertz CG. Toxic effects of lithium in newborn infants. A commentary. J Pediatr 1972; 81:804-807.

Vac70 Vacaflor L, Lehman HE, Ban TA. Side effects and teratogenicity of lithium carbonate treatment. J Clin Pharmacol J New Drug 1970; 10(6): 387-389.

War88 Warkany J. Teratogen update: lithium. Teratology 1988; 38: 593-596.

Wei76 Weinstein MR. The international register of lithium babies. Drug Info J 1976; 10: 94-100. Yat94:Yatham LN. Buspirone induced prolactin release in mania. Biol Psychiatry 1994; 35: 553-556.

Literature consulted but not cited

Ana93 Ananth J. Lithium during pregnancy and lactation. Lithium 1993; 4:231-237.

Bow98 Bowden CL. Key treatment studies of lithium in manic-depressive illness: efficacy and side effects. J Clin Psychiatry 1998; 59S: 13-19, (comments on pages 35-36)

Chi 97 Chisholm CA, Kuller JA. A guide to the safety of CNS-active agents during breastfeeding. Drug Saf 1997; 17(2):127-142.

Dil97 Dillon AE, Wagner CL, Wiest D, Newman RB. Drug therapy in the nursing mother. Ostet Gynecol Clin N Am 1997; 24: 675-696.

Gho91c Ghosh PK, Biswas NM, Ghosh D. Effect of lithium chloride on spermatogenesis and testicular steroidogenesis in mature albino rats: duration dependent response. Life Sciences1991; 48: 649-657.

Mar92 Marken PA, Haykal RF, Fisher JN. Management of psychotropic-induced hyperprolactinemia. Clin Pharm 1992: 11: 851-856.

Pow93 Power AC, Dorkins CE, Cowen PJ. Effect of lithium on the prolactin response to D-fenfluramine in healthy subjects. Biol Psychiatry 1993; 33: 801-805.

Sch90 Schou M. Lithium treatment during pregnancy, delivery, and lactation: an update. J Clin Psychiatry 1990; 51(10): 410-413.

Sin73 Singer I, Rotenberg D. Mechanisms of lithium action. New Engl J Medicine 1973; 289: 254-260.

Sch79 Schneider HPG, Leyendecker G. The normal and dysregulated human menstrual cycle. Adv Steroid Biochem Pharmacol 1979; 7: 23-50

Annexes

Annex **A**

The committee

- BJ Blaauboer, *chairman*
  Toxicologist; Research Institute of Toxicology, Utrecht
- JN van den Anker
  Professor of pediatrics and Neonatology; Erasmus University, Rotterdam
- AM Bongers, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- HFP Joosten
  Toxicologist; NV Organon, Department of Toxicology and Drug Disposition, Oss
- D Lindhout
  Professor of Clinical Genetics/Teratology; Erasmus University, Rotterdam
- JHJ Copius Peereboom-Stegeman
  Toxicologist; Catholic University Nijmegen, Nijmegen
- AH Piersma
  Reproductive toxicologist; National Institute of Public Health and the Environment, Bilthoven
- A Stijkel
  Toxicologist; Environmental Awareness Foundation, ‘s-Graveland
- PJJM Weterings
  Toxicologist; Weterings Consultancy BV, Rosmalen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague

The first draft of the present document was prepared by IDH Waalkens-Berendsen, from the TNO Nutrition and Food Research Institute in Zeist, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: E Vandenbussche-Parméus.
Lay-out: J van Kan.

Annex **B**

Comments on the public draft

A draft of the present report was released in 1999 for public review. No organisations or persons have commented on the draft document.

Annex **C**

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 resuts 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 consequence 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 in-

terfere 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 **D**

Fertility and developmental toxicity studies

Table 1 Effects of lithium on human fertility.

authors	type of study/ no. of subjects/age	dose/ Route of exposure	experimental period	findings	remarks
Lev81	male patients; n=4 controls:volunteers; n=9	serum Li 22-52 mg/l *(oral)*	3 weeks	decreased sperm viability no effect on motility and count of sperm	
Tan81	2 male and 4 female patients (22-35 years old); 4 male and 4 female controls (24-38 years old)	600-1800 mg Li2CO3/day per os for 3-4 weeks	3-4 weeks	No effect of Li on PRL plasma levels	
Bla82	2 case reports, males age 42 years and 58 years	serum Li case 1: 2.6-3.3 mg/l case 2: 1.8 mg/l *(oral)*	case 1: 3.5 months case 2: 7 months	case 1: after 1 month of Li use loss of libido and impaired erection; after receipt of a blind placebo rapid normalization of libido and sexual behaviour case 2: after start of the Li treatment loss of libido and difficulty in obtaining erection; after 2 months of use spontaneously recovery from symptoms	
Goo83	9 males (mean age 28.3 years) and 9 females (mean age 33.3 years)	Li_2CO_3 until plasma level of 3-5.6 mg/l (iv)		Baseline serum PRL levels were not affected by Li treatment	
Gro85	12 control and 12 patients	Li until plasma level was 2.2-3.3 mg/l		No effect of Li on base-line PRL levels	
Jof86	8 patients (mean age 39.4 years)	1230 mg Li_2CO_3 /d	at least 6 weeks	Li increased base-line levels of PRL and augmented a TRH-induced PRL response	
Gha92	45 men and 59 women (mean age 45, range 20-68)	300-2100 Li mg/day (orally)	mean: 6.7 years Li use	No relationship between serum Li or plasma PRL levels and sexual dysfunction scores	
Yat94	9 male and 2 female patients and 11 controls	lithium, 600-1500 mg/d in six patients	3 weeks	Li had no effect on base-line PRL levels	

Table 2 Effects of lithium on human development.

authors	type of study/no. of subjects/age	dose/Route of exposure	experimental period	findings	remarks
Vac70	uncontrolled clinical 2-year study in 25 patients male and female; 1 female became pregnant	300-1800 mg/day *(oral)*	pregnancy	bilateral club feet and meningomyocele in lumbar region	
Wei76	Birth Register of lithium babies n=166	at least sometime during first trimester *(oral)*	first trimester pregnancy	congenital malformations (11/166): 13 of these involved the cardiovascular system and of which 4 concern Ebstein's anomaly	
Käl83	cohort study female n=350	*(oral)*	pregnancy	perinatal death, heart defects; Ebstein anomaly not detected	
Cun89	female n=72	*(oral)*	pregnancy	4 spontaneous, 6 abortus provocatus, 1 still born, 1 lumbar meningocele and 1 unilateral inguinal hernia	
Rob90	female case report n=1	750-1000 mg/day *(oral)*	first trimester pregnancy	hypothyroidism, lingual thyroid ectopy associated with atrial septal defect	
Jac92	n=138/n=148	*(oral)*	pregnancy	no increased risk of congenital defects	

Table 3.1 Fertility studies with lithium in animals.

authors	species	route	experimental period	dose	findings	remarks
Ban83	adult male Sprague Dawley rats (n=20); age 50 days	ip	twice daily for 2 or 7 days	0 and 100 mg LiCl/kg bw	no effects LH, FSH, PRL in pitiuitary, after 2 days plasma LH increased after 7 days plasma decreased no effect on plasma FSH after 2 and 7 days after 2 days no effect on plasma PRL level after 7 days decreased plasma PRL level	4 out of 20 animals that received lithium for 7 days died on the 6th day; one control animal died during treatment. A number of rats that received lithium for 7 days showed signs of polydipsia and polyuria
McI83	male Sprague Dawley (n=6-9); 230-260 grams	ip	once or 14 days	0 or 2 mg Li/kg bw	no effect on serum PRL levels	general toxicity not described
Gho91a	Wistar rats (n=8) age 35 days	sc	15, 20 or 25 days	0 and 2 mg LiCl/kg bw/d; serum lithium concentration 20 mg/l	after 15 days: spermatogenesis was inhibited at stage VII of the seminiferous epithelial cycle, serum FSH, LH, PRL and testosterone levels were decreased after 20 and 25 days: spermatogenesis was inhibited at stage VII of the seminiferous epithelial cycle, serum FSH, LH PRL and testosterone levels were decreased decreased weight of accessory sex organs	no effect on body weights
Gho91b	Wistar rats (n=8) age 90 days	sc	21 days	0 and 2 mg LiCl/kg bw/d, 0.25 m/kg PRL treatment just after lithium treatment	PRL injection just after treatment with lithium resulted in a recovery of effects mentioned in Gho91a	no effect on body weights

Table 3.2 Fertility studies with lithium in animals

authors	species	route	experimental period	dose	findings	remarks
Gra72	Charles River rat (males and females)	gavage females; diet males	administration females: 14 d premating administration males: 70 d premating sacrifice females: lactation d 21	females: 0, 25, 75 or 150 mg Li_2CO_3/kg bw/d, males: 0, 10, 25 or 50 mg Li_2CO_3/kg bw/d	high dose group: 2 females died during gestation; d 21 pup body weight: stat.sign. reduction	
Kel78	swine (females)	diet	administration: gestation	0, 3000 mg Li_2CO_3/ kg diet = ~120 mg/kg bw/d	reduction maternal body weight from gestation d 60, stat.sign. from d 110. Stat.sign. reduction of no. of live born, birth weight, survival d 21; stat.sign. increase mascerated foetuses and stillborn pups	
Mro83	CFM mouse (males and females)	drinking water	administration: from 3 or 6-8 w and onwards	0, 2000, 4000 or 8000 mg LiCl/l	8000 mg: mice did not drink, died 4000 mg: mice drank little, did not reproduce 2000 mg from 6-8 w: time between litters stat.sign. increased, no. of litters decreased; stat.sign. increase preweaning pup mortality 2000 mg from 3 w: same as above + stat.sign. delay pup growth and development	general toxicity and number of animals per dose group were not described
Sec86	Sprague-Dawley rat (females)	drinking water	administration: 10 d premating through postpartal d 28	0, 80 or 160 mg natural Li salts/kg/d	80 or 160 mg: altered maternal behaviour: no grooming, little and short nursing; dead pups were not cannibalized	
Mes86	Sprague-Dawley mouse (females)	drinking water	administration: from mating (exp.1) or delivery (exp.2) through postpartal d 23 sacrifice: PP d 37, or (exp.3): administration 2 w premating through d 1 after delivery; sacrifice: d 1 after delivery	0 or 40 mg LiCl/l	exp.1+2 females: stat.sign. reduction brain and kidney weight, reduction spleen weight; pups: stat.sign. reduction brain, reduction kidney and testis weight, induction L-ADH, L-C-ALDH, (exp.1 only:) inhibition L-M-T-LDH exp.3 pups: inhibition L-MT-LDH	

Table 4.1 Developmental toxicity studies with lithium in animals.

authors	species	route	experimental period	dose	findings	remarks
Sza70	Charles River mouse	gavage	administration: gestation d 6-15 sacrifice: gestation d 18	200, 300, 465 mg Li_2CO_3/kg/d	465 mg: stat.sign. increase in maternal and foetal death, 30% cleft palate 300 mg: 11 % cleft palate 200 mg: no effects	
Gra72	Charles River rat New Zealand white rabbit rhesus monkey	gavage oral (capsules) oral (capsules)	rat administration: gestation d 5-15, sacrifice: d 20 rabbit administration: gestation d 5-18, sacrifice: d 28 monkey administration: gestation d 14-35, sacrifice: d 160	rat: 0, 25, 75 or 150 mg Li_2CO_3/kg bw rabbit: 0, 25 or 40 mg/kg bw/d Li_2CO_3/kg bw monkey: 0 or 25 mg Li_2CO_3/kg bw Li_2CO_3/kg/d	high doses: 2 rats and 3 rabbits died low doses: 1 rabbit died No differences with controls in maternal fertility, bw gain, implantation sites, litter size and weight, foetal mortality and visceral or skeletal malformations	
Smi82	129 Sv/SL or A/J mouse	i.p. injection	SV/SL: gestation d 8, 9 or 10 A/J: gestation d 12, 13 or 14 sacrifice: gestation d 18-19	1x0, 0.8, 1.6 or 5.0/3.2 mg Li_2CO_3/mouse	SV/SL: stat.sign. increase malformations in high dose group only (fused ribs, vertebral defects, exencephaly). Most effects upon exposure d 9 A/J mice: not sign. increase in higher dose groups, no effects resulting from day of exposure	possible maternal effects unknown
idem	129 Sv/SL mouse	drinking water	administration: gestation d 1-18 sacrifice: d 18	2 mg Li_2CO_3/ml	2 out of 16 females had viable foetuses; all 6 foetuses were malformed	

Table 4.2 Developmental toxicity studies with lithium in animals.

authors	species	route	experimental period	dose	findings	remarks
Mar86	Wistar rat	gavage	administration: gestation d 6-15 sacrifice: gestation d 20	0, 50, 100 mg Li_2CO_3/kg/d	low dose: slight effects on no. implantation sites, live foetuses, foetal bw high dose: stat.sign. effects on no. implantation sites, live foetuses, foetal bw. High incidence of skeletal anomalies and malformations	possible maternal effects unknown
Sha86	rat	gavage	administration: gestation d 0-10 sacrifice: at term	0, 7 mg Li_2CO_3/kg/d	high incidence (>10%) of growth retardation, cleft palate, brain liquification, hepatomegaly, digital anomalies lower incidence >10%) of hydrocephaly, cardiomegaly	possible maternal effects unknown
Sec86	Sprague-Dawley rat	drinking water	administration: 10 d premating through postpartal d 28	0, 80 or 160 natural Li salts/kg/d	Maternal effects related to behaviour Dose-related decrease in pup body weight, eye-opening, pinna unfolding, development in depth perception, spontaneous activity	
Jur88	JBT/Jd mouse	ip. injection	administration: gestation d 9sacrifice: gestation d 13	0, 250-400 mg Li_2CO_3/kg	minimal maternal lethal dose: 300 mg/kg LD50: 440 mg/kg Dose-related effects on no. of dead foetuses, retardations, abnormalities (exencephaly, craniorachischisis, dilation of 4th ventricle)	possible other maternal effects unknown

d = day; i.p. = intraperitoneal

Annex **E**

Abbreviations

Abbreviations used

bw	body weight
d	day
F	female(s)
FSH	follicle stimulating hormone
GH	gonadotropin hormone
i.p.	intraperitoneal
i.v.	intravenous
L-ADH	hepatic alcohol dehydrogenase
L-C-ALDH	hepatic cytoplasmatic aldehyde dehydrogenase
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
L-MT-LDH	hepatic mitochondrial lactate dehydrogenase
M	male(s)
n	number
NOAEL	no adverse effect level
OECD	Organisation for Economic Cooperation and Development

PN	postnatal
PP	post partum
PRL	prolatine
s.c.	subcutaneously
TRH	thyroid releasing hormone
TSH	thyroid stimulating hormone