# Committee for Risk Assessment

# RAC

## Opinion

proposing harmonised classification and labelling
at EU level of

## lithium carbonate [1] lithium chloride [2] lithium hydroxide [3]

**EC Number: 209-062-5 [1] 231-212-3 [2] 215-183-4 [3]**
**CAS Number: 554-13-2 [1] 7447-41-8 [2] 1310-65-2 [3]**

CLH-O-0000007034-82-01/F

**Adopted**

**16 September 2021**

16 September 2021

CLH-O-0000007034-82-01/F

# OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

**Chemical name:** **lithium carbonate [1] lithium chloride [2] lithium hydroxide [3]**

**EC Number:** **209-062-5 [1] 231-212-3 [2] 215-183-4 [3]**

**CAS Number:** **554-13-2 [1] 7447-41-8 [2] 1310-65-2 [3]**

The proposal was submitted by **France** and received by RAC on **22 June 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

# PROCESS FOR ADOPTION OF THE OPINION

**France** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **3 August 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 October 2020**.

### ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Bjørge**

Co-Rapporteur, appointed by RAC: **Stine Husa**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2021** by **a simple majority of all members present and having the right to vote**.

**Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)**

| | Index No | Chemical name | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors and ATE | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitters proposal | TBD | lithium carbonate [1]<br>lithium chloride [2]<br>lithium hydroxide [3] | 209-062-5 [1]<br>231-212-3 [2]<br>215-183-4 [3] | 554-13-2 [1]<br>7447-41-8 [2]<br>1310-65-2 [3] | Repr. 1A | H360FD | GHS08<br>Dgr | H360FD | - | - | - |
| RAC opinion | TBD | lithium carbonate [1]<br>lithium chloride [2]<br>lithium hydroxide [3] | 209-062-5 [1]<br>231-212-3 [2]<br>215-183-4 [3] | 554-13-2 [1]<br>7447-41-8 [2]<br>1310-65-2 [3] | Repr. 1A<br>Lact. | H360FD<br>H362 | GHS08<br>Dgr | H360FD | - | - | - |
| Resulting Annex VI entry if agreed by COM | TBD | lithium carbonate [1]<br>lithium chloride [2]<br>lithium hydroxide [3] | 209-062-5 [1]<br>231-212-3 [2]<br>215-183-4 [3] | 554-13-2 [1]<br>7447-41-8 [2]<br>1310-65-2 [3] | Repr. 1A<br>Lact. | H360FD<br>H362 | GHS08<br>Dgr | H360FD | - | - | - |

# GROUNDS FOR ADOPTION OF THE OPINION

## RAC general comment

### *Read across*

The dossier submitter (DS) proposed to read across data between the inorganic lithium compounds lithium carbonate, lithium chloride and lithium hydroxide, based on the analogue approach (REACH Annex XI, 1.5 (2)). They can be considered to belong to the same category of substances due to the presence of common breakdown products produced via physical and biological processes which results in structurally similar chemicals.

Lithium carbonate, lithium chloride and lithium hydroxide dissociate to the lithium cation ($Li^+$) and the corresponding anions (carbonate ($CO_3^{2-}$), chloride ($Cl^-$) or hydroxide ($OH^-$) in aqueous solutions. These are physiological anions, which are naturally present in the body. They are rapidly integrated into the physiological pool of anions or neutralised in the body. The systemic toxicity is determined by the lithium cation and is not influenced by the anions. The lithium cation remains unchanged in the body, and due to similarities with sodium and potassium cations it uses the sodium ion channels to reach target organs. In the REACH registration dossier, the following conclusion was included for lithium hydroxide regarding read across for toxicity endpoints: "*Lithium hydroxide completely dissociates in water forming lithium cation and the corresponding hydroxide anion. Thus, lithium salts with different anion moieties and hydroxide compounds were found to be suitable candidates for read-across*".

During the stakeholder consultation, a question on the selection of these specific lithium salts was raised by a commenting Member State Competent Authority (MSCA), arguing that additional lithium salts could have been included to avoid regrettable substitution. The DS responded that the selection of lithium salts included in the CLH proposal was based on anions that are naturally occurring in the human body. For other lithium salts to be included, the toxicity of the respective anions would need further assessment.

Industry questioned the read across of data in support of classification for reproductive toxicity, especially from lithium carbonate/lithium chloride to lithium hydroxide since there was no data available for the latter on reproductive toxicity, and due to the corrosive nature and high pH of lithium hydroxide. Furthermore, industry commented that while substances used in e.g. pharmaceuticals have greater potential for direct, prolonged exposure to pregnant women and the developing foetuses, pregnant women are unlikely to be exposed to industrial products containing lithium hydroxide. In addition, lithium in industrial products is likely to enter the environment at a quantity or concentration substantially lower than oral administration of lithium carbonate to treat chronic illness. The DS responded that the read across between the different lithium salts followed the rules described in ECHA's Read-Across Assessment Framework (2017) and that the systemic toxicity of the lithium compounds included in the CLH report was determined by the lithium ion. Further, the DS responded that the CLH proposal was limited to three lithium compounds and that the use of the chemical was not taken into account in the classification.

RAC notes that read across from lithium carbonate to lithium hydroxide was relied on in the REACH registration dossier for lithium hydroxide. Furthermore, a testing proposal for reproductive toxicity of lithium hydroxide using lithium carbonate was submitted. However, the assessment of the testing proposal by ECHA was terminated since testing was already started by industry. RAC notes that corrosivity of the hydroxide anion does not prevent exposure to lithium cation from mixtures, especially if they have a lower pH. According to the REACH Guidance R.7.6.2.3.2 (v6.0), corrosive or highly irritating substances should be tested preferentially via

the oral route; however, it must be noted that *in vivo* testing with corrosive substances at concentration/dose levels causing corrosivity must be avoided. However, a vehicle with buffering capacity should be chosen to minimise gastrointestinal irritation, indicating that substances with corrosive properties are not excluded from testing for reproductive toxicity.

RAC notes that even very diluted solutions of lithium hydroxide have a high pH around 13.0 but that due to a very low alkaline reserved they are easily neutralised. This indicates that concentrations and pH values of lithium hydroxide solution can be obtained at which systemic effects, but not corrosive effects are observed, see table below.

RAC notes that the use of lithium hydroxide or conditions for exposure are not relevant for hazard classification, which is based on the intrinsic properties of the substance. In general, all routes and sources of exposure are considered relevant for lithium compounds and may contribute to lithium toxicity.

Overall, the read across approach between lithium carbonate, lithium chloride and lithium hydroxide based on the analogue approach proposed by the DS is supported by RAC. The compounds dissociate to lithium cation ($Li^+$) and the corresponding anions, carbonate ($CO_3^{2-}$), chloride ($Cl^-$) or hydroxide ($OH^-$), in aqueous solutions. The anions are rapidly integrated into the physiological pool of anions or neutralised in the body. Systemic toxicity is considered to be determined by the lithium cation and is not influenced by the anions. The lithium cation remains unchanged in the body, and due to similarities with sodium and potassium cations it uses the sodium ion channels to reach target organs. The corrosivity of lithium hydroxide would not prevent inclusion of this substance in the read across assessment from lithium carbonate and lithium chloride for the reproductive toxicity hazard class. Likewise, the corrosive nature of lithium hydroxide leading to low exposure to lithium cannot be used as an argument for excluding lithium hydroxide from read across since this would be related to risk assessment rather than hazard evaluation. RAC noted that no sub-acute or sub-chronic toxicity study with lithium hydroxide was included in the REACH registration or included in the CLH report by the DS, with which to compare potentially corrosive concentrations of lithium hydroxide. An oral acute toxicity study with lithium hydroxide monohydrate was included in the REACH registration (Bio-test, 1976). Due to questionable reliability, this study was not used for comparison of effects of lithium salts.

***Specific concentration limits***

It is noted that the potency considerations of a substance are not a part of the classification criteria for reproductive toxicity and lactation but could be considered for the setting of specific concentration limits (SCL). According to the ECHA guidance on the Application of the CLP criteria (CLP Guidance) (version 5.0, July 2017) "*in exceptional circumstances specific concentration limits may be set by the manufacturer, importer or downstream user where he has adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the concentrations set for the relevant hazard class*". For lithium hydroxide no studies with sub-acute or sub-chronic exposure were included in the REACH registration or available in the open literature. Further the CLP Guidance includes that, "*the classification of substances as reproductive toxicants may be based on information such as grouping and read across (Guidance IR&CSA, sections R.6 and R.7.2.3.1)*" and this is considered relevant for lithium hydroxide. However, the CLP Guidance says that "*in such cases, no direct estimate of the reproductive toxicity potency based on an ED10 value is possible*". Since the classification of the lithium salt is based on human data, the CLP Guidance states that "*the use of human data for ED10 calculation has several drawbacks including limited data on exposure, limited data on the size of the exposed population and limited information on whether the exposure included the window of sensitivity. For all these reasons, it is difficult to determine an*

*ED10 based on human data*". Therefore, RAC considers that setting of an SCL for lithium hydroxide based on a potentially lower potency is not justified.

# HUMAN HEALTH HAZARD EVALUATION

## RAC evaluation of germ cell mutagenicity

### Summary of the Dossier Submitter's proposal

The DS assessed a range of studies *in vitro* as well as in *vivo*, where lithium compounds were tested for mutagenicity, chromosome aberrations, sister chromatid exchanges, and DNA damage. Mainly negative results were obtained. Positive results were also reported; however, usually at cytotoxic dose levels. It was noted that lithium inhibits apoptosis by inhibiting the enzyme glycogen synthase kinase-3 (GSK3) and that the observed genotoxic effects at higher doses could be due to increased cell survival. Furthermore, the DS noted that despite the numerous negative in vitro and in vivo findings, an aneugenic potential of lithium salts cannot be formally excluded.

Overall, the DS did not propose a classification and argued that this endpoint could not be fully assessed due to lack of adequate data.

### Comments received during consultation

Comments were received from one MSCA and three Industry organisations, all supporting no classification for mutagenicity.

### Assessment and comparison with the classification criteria

Genotoxicity of lithium salts has been assessed in a range of different studies.

#### *Mutagenicity/genotoxicity studies in vitro*

Two bacterial reverse mutation assays according to OECD TG 471 were both negative. In the study by Anonymous (2000a), lithium hydroxide was tested up to concentrations of 5000 µg/plate in the absence and in the presence of S9-mix in two mutation experiments. The first mutation experiment was performed with *Salmonella typhimurium* TA 1535, TA 1537 and TA 98 and the second mutation experiment was performed with *Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100 and *E Coli* WP2uvrA. No precipitation was observed at any dose level. No cytotoxicity was observed at any dose level. This study was negative for genotoxic effects with and without metabolic activation. The study by Haworth et al. (1983) (tested *Salmonella typhimurium* TA 1535, TA1537, TA 98, TA 100 up to 10000 µg Li-chloride/mL with and without S9-mix. The results were negative for all strains tested.

Further, several tests on mammalian cells were assessed. Pastor et al. (2009) performed several assays (*in vitro* comet assay, anaphase anomaly study, *in vitro* micronucleus assay, and chromosome aberration assay) with lithium carbonate and lithium chloride. A dose-dependent cytotoxic effect was observed for lithium carbonate as well as for lithium chloride, with lithium carbonate (1-5 mM; $LD_{50}$ 5 mM) showing a higher cytotoxicity than lithium chloride (6 mM and above; $LD_{50}$ 15 mM), see figures from Annex I to the CLH report reproduced below.

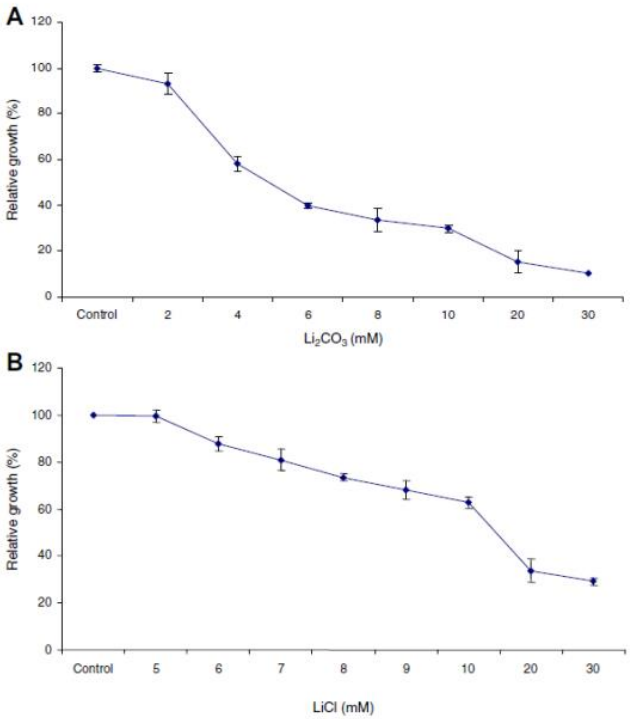



**Fig. 1.** Effectiveness of different concentrations of lithium salts to inhibit cell growth in the Chinese hamster fibroblast cell line AA8 as shown by the SRB assay. (A) $Li_2CO_3$, (B) LiCl. While $Li_2CO_3$ showed as cytotoxic at the lower concentrations (1–5 mM; $LD_{50}$: 5 mM), the negative effect for LiCl was only observed at higher concentrations (6 mM and higher; $LD_{50}$: 15 mM). Bars indicate percent survival from three independent experiments. Statistical analysis (Student's *t*-test) shows a significant difference ($p < 0.01$) for doses of $Li_2CO_3$ of 5 mM and higher as compared with control, while in the case of LiCl the dose was 10 mM.

In the Comet assay (non-guideline, not GLP) Pastor et al. (2009) treated Chinese hamster ovary cells (AA8 CHO cells) with lithium carbonate (2.2-10mM, 2.2-10 mM, equal to 163-739 µg/mL or 31-139 µg Li/mL) for 3 hours or 24 hours without S9-mix. In a second assay AA8 CHO cells were treated with lithium chloride (5-20 mM, equal to 212-848 µg/mL or 35-139 µg Li/mL) under similar conditions. Cytotoxic at concentrations ⩾ 70 µg Li/mL. Both the assays were negative. In the anaphase anomaly study (no guideline, not GLP) AA8 CHO cells were treated with lithium carbonate (2.2-10mM, equal to 163-739 µg/mL or 31-139 µg Li/mL) for 3 hours without S9-mix or lithium chloride (5-20 mM, equal to 212-848 µg/mL or 35-139 µg Li/mL) for 3 hours without S9-mix. After treatment the cell cultures were washed and maintained in fresh medium for 6 hours to recover. Cytotoxic was seen at concentrations ⩾ 70 µg Li/mL. Both lithium-compounds were positive and showed an increased frequency of anomalous anaphases. Multipolar anaphases (mostly tripolar) and lagging chromosomes were seen after treatment with lithium carbonate as well as lithium chloride. In a micronucleus assay (similar to OECD TG 487, not GLP, deviations: cell line not mentioned in TG, no positive control) AA8 CHO cells were treated with lithium carbonate as well as lithium chloride at doses of 2.2-10 mM (equal to 163-739 µg/mL or 31-139 µg Li/mL) and 5-20 mM (equal to 212-848 µg/mL or 35-139 µg Li/mL), respectively. Cytotoxic was seen at concentrations ⩾ 70 µg Li/mL. Micronuclei were induced in a dose-dependent manner for AA8 CHO cells treated with lithium carbonate as well as lithium chloride (see figures from Annex I to the CLH report reproduced below). It is however noted that this was in agreement with the observed cytotoxicity. Over 955 of the micronuclei were kinetochore-positive, which could indicate an aneuploid effect rather than a clastogenic effect.

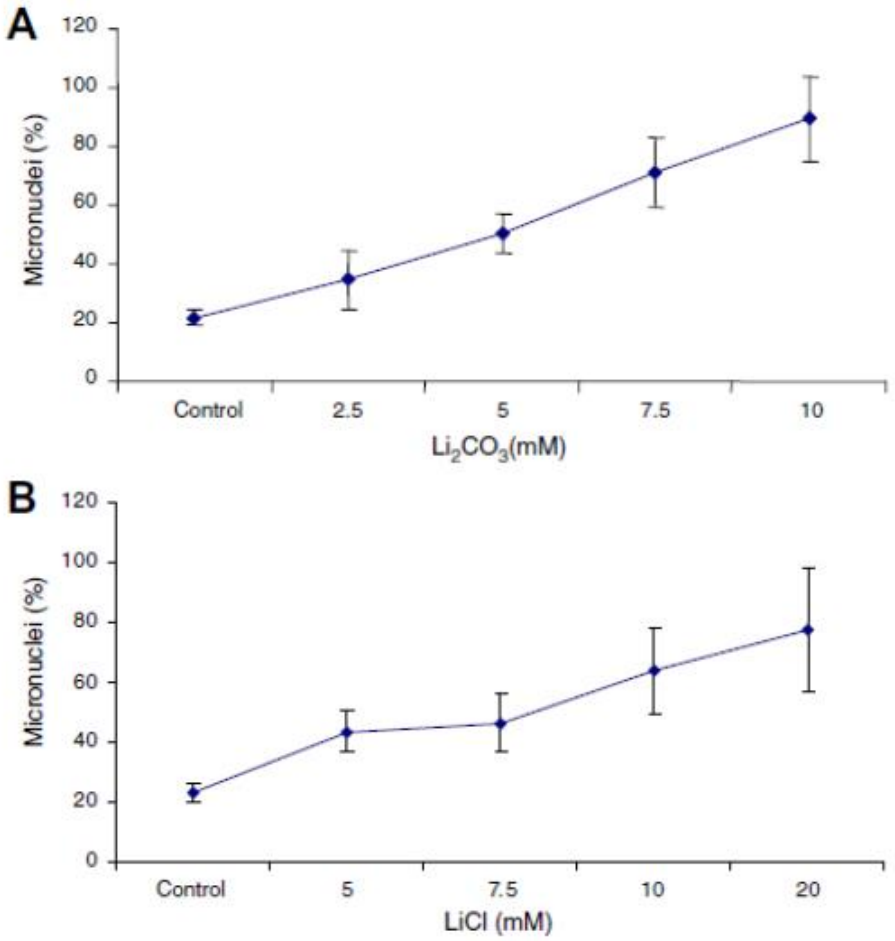



**Fig. 2.** Dose-dependent induction of micronuclei by both $Li_2CO_3$ (A), and LiCl (B) in AA8 CHO cells. All the doses tested showed a significant difference in the frequency of micronuclei as compared with control ($p < 0.01$; Student's *t*-test). As can be observed, $Li_2CO_3$ induced micronuclei with higher efficiency than LiCl.

The chromosome aberration assay by Pastor et al. (2009) (similar to OECD TG 473, not GLP) was negative (without S9-mix). In this assay AA8 CHO cells were treated with lithium carbonate (2.2-10 mM, equal to 163-739 µg/mL or 31-139 µg Li/mL) or lithium chloride (5-20 mM, equal to 212-848 µg/mL or 35-139 µg Li/mL) for 3 hours, followed by a 12- hour growth phase. Cytotoxicity was seen at concentrations ⩾ 70 µg Li/mL. Overall, Pastor et al. (2009) reported negative results for the induction of DNA strand-breaks in AA8 CHO cells. Further they indicated that lithium carbonate could interact with the spindle apparatus and also described a significant and dose-dependent increased numbers of micronuclei. However, this was reported alongside cytotoxicity which was already distinct/severe (40% growth reduction) at 4 mM and increasing with higher concentrations. It is noted that the micronucleus test is insufficiently reported (e.g. number of cells evaluated not provided), which limits the validity of these data.

Anonymous (2010a) performed a gene mutation study in mammalian cell according to OECD TG 476 and GLP. In this study, mouse lymphoma L5178Y cells were treated with 0, 12.5, 25, 50, 100 and 200 µg lithium hydroxide/mL for 3 hours with S9-mix, 3 and 24 h treatment without S9-mix. Cytotoxicity was observed at 200 µg/mL. The results were negative with respect to the mutant frequency in the L5178Y TK +/- mammalian cell mutagenicity test.

Anonymous (2000b) performed a chromosome aberration study in mammalian cells according to OECD TG 473 and GLP. Human lymphocytes from blood samples from one healthy male donor were exposed to lithium hydroxide. In a dose range finding test blood cultures were treated with

0, 10, 33, 100, 333 and 1000 µg lithium hydroxide/mL culture medium with and without S9-mix. Based on the result of the dose range finding, further experiments were performed. Experiment 1A: without S9-mix: 0, 100, 180, 333*, 420* and 560* µg lithium hydroxide/mL culture medium (3 h treatment time, 24 h fixation time) and with S9-mix: 0, 100, 333*, 420* and 560* µg/mL culture medium (3 h treatment, 24 h fixation time). Experiment 1B: with and without S9-mix: 0, 300, 350, 400, 450, 500 and 550 µg lithium hydroxide/mL culture medium (3 h treatment, 24 h fixation time). The experiment was not evaluated due to high cytotoxicity, and a third experiment (Experiment 1C) was performed: with and without S9-mix: 0, 275, 300, 325*, 350*, 375*, 400* (only with S9-mix), 425, 450, 475 and 500 µg lithium hydroxide/mL culture medium (3 h treatment, 24 h fixation time). Based on the results of the dose range finding test and experiments 1A, 1B and 1C the following dose levels were selected to perform an independent repeat (Experiment 2): Without S9-mix: 0, 275*, 300*, 325, 350*, 375, 400, 425 µg lithium hydroxide/mL culture medium (24 h treatment, 24 h fixation time); without S9-mix: 0, 275, 300, 325, 350*, 375*, 400*, 425 µg lithium hydroxide/mL culture medium (48 h treatment, 48 h fixation time);with S9-mix: 350, 375, 400*, 425*, 450*, 475, 500 and 525 µg lithium hydroxide/mL culture medium (3 h treatment time, 48 h fixation time). Test substance concentrations scored for chromosome aberration were selected based on precipitation and cytotoxicity. Lithium hydroxide did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations neither in the absence nor presence of S9-mix in experiments 1A and 1C. Experiment 2 was negative in the presence of S9-mix and lithium hydroxide did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations. In the absence of S9-mix, a statistically significant increase in chromosome aberrations were observed at the lowest (however within the historical control data (HCD)) and the highest concentration. It is noted that no dose response was observed, and the highest dose was a very cytotoxic concentration. Overall, no increase in structural chromosome aberrations in peripheral human blood lymphocytes were seen after a 3-h treatment with lithium hydroxide with concentrations up to 560 µg lithium hydroxide/mL.

Slamenova et al. (1986) performed a gene mutation assay (hypoxanthine-guanine phosphoribosyl transferase (HGPRT)) similar to OECD TG 476 (deviations: no positive control) with V79 cells at doses of 0, 1500, 2000, 2500 and 3000 µg/mL (282-564 ug Li/mL) with and without S9-mix. Without S9-mix the average number of 6-TG mutants per 100 000 cells at the respective dose groups were 0.2, 0.3, 1.1, 0.4, 0.4, a weak effect at 2000 µg/mL, a very weak effect at 2500 µg/mL and no effect at the other dose levels. Similarly, with S9-mix the average number of 6-TG mutants per 100 000 cells at the respective dose groups were 0.4, 0.2, 0.1, 0.9, 0.8, a very weak increase in mutations were observed at 2500 µg/mL with S9-mix; however, no effect was observed at the other dose levels. No clear dose response relationship was observed. Cytotoxicity was reported at the highest concentration tested. See table below from Slamenova et al. (1986), reproduced from Annex I to the CLH report.

TABLE 1

THE OCCURRENCE OF 6-TG$^r$ MUTATIONS IN V79 CELLS TREATED WITH Maz (120 min), Li carb (60 min), Drop (120 min) and B(a)P (120 min) IN THE PRESENCE OR IN THE ABSENCE OF S9 FRACTION

| Treatment without S9 | | | | | | Treatment with S9 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Samples (μg/ml) | Expression time | | A* | B** | Degree of effect | Samples (μg/ml) | Expression time | | A* | B** | Degree of effect |
| | 6 days | 8 days | | | | | 6 days | 8 days | | | |
| Control | 0.2 | 0.2 | 0.2 ± 0.3 | 1 | | Control | 0.4 | 0.5 | 0.4 ± 0.4 | 1 | |
| Maz 500 | 0.1 | 0.1 | 0.1 ± 0.5 | 0.53 | No | Maz 500 | 1.5 | 0.6 | 1.0 ± 0.6 | 2.09 | Very weak |
| Maz 600 | 0.2 | 0.8 | 0.5 ± 0.4 | 1.96 | Very weak | Maz 600 | 0.1 | 0.1 | 0.1 ± 0.0 | 0.28 | No |
| Maz 1000 | 3.5 | 5.5 | 4.5 ± 1.4 | 16.84 | Medium | Maz 1000 | 2.7 | 1.6 | 2.1 ± 0.7 | 4.20 | Weak |
| Li carb 1500 | 0.1 | 0.5 | 0.3 ± 0.2 | 1.19 | No | Li carb 1500 | 0.3 | 0.1 | 0.2 ± 0.1 | 0.52 | No |
| Li carb 2000 | 1.9 | 0.4 | 1.1 ± 1.0 | 4.40 | Weak | Li carb 2000 | 0.1 | 0.1 | 0.1 ± 0.0 | 0.28 | No |
| Li carb 2500 | 0.1 | 0.8 | 0.4 ± 0.4 | 1.81 | Very weak | Li carb 2500 | 1.2 | 0.6 | 0.9 ± 0.4 | 1.81 | Very weak |
| Li carb 3000 | 0.2 | 0.5 | 0.4 ± 0.1 | 1.51 | No | Li carb 3000 | 1.1 | 0.5 | 0.8 ± 0.4 | 1.62 | No |
| Drop 2000 | 0.2 | 0.1 | 0.1 ± 0.0 | 0.68 | No | Drop 2000 | 0.1 | 0.1 | 0.1 ± 0.0 | 0.25 | No |
| Drop 5000 | 0.1 | 0.2 | 0.1 ± 0.0 | 0.73 | No | Drop 5000 | 0.1 | 0.1 | 0.1 ± 0.0 | 0.22 | No |
| B(a)P | 0.2 | 0.5 | 0.4 ± 0.2 | 2.68 | Very weak | B(a)P | 15.0 | 28.0 | 21.5 ± 9.1 | 41.46 | Strong |

A*, average numbers of 6-TG$^r$ mutants per 100000 cells ± standard deviation.
B**, ratio of induced to spontaneous mutations.
Degree of effect: B**, 1 ± 0.7 = No; B**, 1.7–3 = Very weak; B**, 3–10 = Weak; B** 10–30 = Medium; B**30–100 = Strong; B** > 100 = Very strong.

Further, they investigated DNA strand breaks (alkaline elution) in heterodiploid human EUE cells at dose levels of 150, 250 and 500 µg lithium carbonate/mL (28-94 ug Li/mL). A positive effect was reported at the highest concentration tested (500 µg/mL). In addition, they reported that high concentrations of lithium carbonate (3000 µg/mL) slightly inhibited DNA synthesis in human EUE fibroblasts, an effect which was decreased by the addition of S9-mix.

De La Torre et al. (1976) reported positive results in a chromosome aberration assay with lithium chloride in human peripheral blood lymphocytes. They performed a chromosome aberration test (similar to OECD TG 473, deviations: no positive control) with lithium chloride at dose levels of 0, 50, 100, 150 µg lithium chloride/mL (8.2-25 µg Li/mL) on Phytohemagglutinin-stimulated lymphocyte cultures from a healthy human donor. They observed an increase in breaks (7.9%, 4.5%, 10.9% compared to 1.2% in the control) and gaps (14.4%, 14%, 20.5% compared to 0.8% in the control) in all groups. Further, increased deletion and translocations were observed from 100 µg/mL (2.2%, 4.2% and 0.6%, 1.7%, respectively).

Timson and Price (1971) reported no increase in structural chromosome aberrations in peripheral human blood lymphocytes after treatment with lithium carbonate for 72 h with concentrations equivalent to 0.1, 1.0 and 10 g lithium carbonate distributed in the body of a 70 kg person. The study was not according to test guideline or GLP and was disregarded by the DS.

***In vivo studies in mammalian somatic or germ cells***

Two *in vivo* studies in mammalian somatic cells or germ cells were included by the DS. The studies were all disregarded. Sobti et al. (1989) performed one *in vivo* chromosome aberration assay and one *in vivo* sister chromatid assay with lithium carbonate (0, 1.2, 12, 120 mg/kg bw / 0, 0.23, 2.3, 23 mg Li/kg bw) and lithium chloride (0, 0.212, 2.125, 21.25 mg/kg bw / 0, 0.035, 0.35, 3.5 mg Li/kg bw) in mice exposed by single gavage application 72 h before bone marrow preparation. The chromosome aberration assay was positive, however, without a dose response. The frequency of various types of aberrations and the number of cells studied were not provided, no positive control and with negative control values higher than in other published reports (Lagerkvist and Lindell, 2002). The sister chromatid exchange assay was negative. For lithium carbonate there was a slight increase compared to control, however, not statistically significant. There was no information on number of cells studied, no positive control and the negative control values were higher than in other published reports.

Bille et al. (1975) studied chromosome aberrations in female rats exposed to lithium (86 mg/d) by three days intraperitoneal (i.p.) exposure and sacrificed 12 and 24 hours after the last injection. The result reported were negative; however, it is noted that details on the method used is limited and no control was included.

***Human data***

Several studies where chromosome aberrations were investigated in human are available for assessment. In these studies, mainly negative results were described. No cytogenetic effect of lithium treatment was observed in several studies with patients:

Turecki et al. (1994) studied chromosome aberrations in peripheral blood lymphocytes from 8 patients treated with lithium for at least one year (mean dose 768.75 ± 139.05 mg/d compared to 10 psychiatrically healthy drug-free controls matched for sex and age. No increase of chromosomal lesions was observed.

Similarly, Matsushima et al. (1986) studied chromosome aberrations in peripheral blood lymphocytes from 13 patients treated with lithium carbonate (serum Li levels 0.02-1.54 mEq/L) for 4 months to 7 years. No increase of chromosomal lesions was observed.

De La Torre et al. (1976) measured chromosome aberrations in peripheral blood lymphocytes in 10 patients (5 males, 5 females) dosed with 800-2400 mg lithium carbonate/d (equivalent to Li serum levels of 0.55-1.25 mEq/L) and 3 control patients. The patients were treated from 2 to 36 months. A slight increase in chromosome aberrations was observed, however, without a clear dose response.

Bille et al. (1975) measured chromosome aberrations in bone marrow cells in 7 psychiatric patients (males, 28-66 years) receiving daily doses of 900-1500 mg for 2-10 years. No cytogenetic changes were observed.

Jarvik et al. (1971) measured chromosome aberrations in peripheral blood leucocytes in 16 manic depressive patients treated with lithium carbonate at daily doses of 900-1800 mg for 2 weeks to over 2 years. No aberrations were observed.

Friedrich and Nielsen (1969) performed a chromosome analysis in lymphocytes from 3 psychiatric patients treated with lithium, (highest daily dose: 600, 600, 900 mg; total dose: 1234, 3632, 50 g; treatment period: 147, 134, 2 months, respectively) and reported an increase in mean chromosome breaks (not statistically significant) and hypodiploid cells (statistically significant). However, since insufficient number of patients were analysed and reporting of methods and number of cells investigated are lacking, the results are not further considered for classification.

In a review Aral and Vecchio-Sadus (2008) reported another study (Genest and Villeneuve, 1971) that found no aberrations in 19 lithium-treated manic-depressive patients compared to 23 controls. However, they reported that the mitotic index was significantly reduced.

**In summary**, a classification for germ cell mutagenicity in Category 1A is based in positive evidence from human epidemiological studies. The human data show no increase in chromosome aberrations, except for the study by De La Torre et al. (1976) which observed a slight increase in chromosome aberrations, however, without a clear dose response and the study by Friedrich and Nielsen (1969) which reported an increase in mean chromosome breaks (not statistically significant) and hypodiploid cells (statistically significant). The study by Friedrich and Nielsen (1969) is, however, not considered further for classification due to insufficient number of patients and lack of detail on the method used and number of cells investigated. On this basis, classification in Category 1A is not appropriate.

A classification for germ cell mutagenicity in Category 1B requires either positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. The available *in vivo* mammalian somatic or germ cells studies are all disregarded due to low quality of the studies, and a classification in Category 1B is not appropriate.

A classification for germ cell mutagenicity in Category 2 requires positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments. Negative results were mostly obtained with lithium carbonate, hydroxide, and chloride in the bacterial reverse mutation assay, the *in vitro* chromosome aberration assay and the gene mutation assays, both in the presence and absence of metabolic activation. The study by Pastor et al. (2009) showed a dose-related increase in micronuclei in CHO cells, however, cytotoxicity was observed in this study. The aneugenic potential of lithium salt cannot be completely ruled out, however, there is a lack of micronucleus test *in vivo* to further investigate this. Since there are no other evidence of mutagenicity in from *in vitro* acceptable test in somatic cells or bacteria and no evidence of mutagenicity from *in vivo* acceptable tests in somatic cells, Category 2 is not appropriate.

In conclusion, RAC is of the opinion that based on the data available **no classification for germ cell mutagenicity is warranted**.

## RAC evaluation of carcinogenicity

### Summary of the Dossier Submitter's proposal

No carcinogenicity or long-term animal studies according to current guidelines were available to the DS. The DS included four tumour promotion studies and one repeated dose toxicity study. Three studies from the same group did not indicate any tumour promoting activity. In contrast, a fourth study described an increased urinary bladder tumour rate in animals exposed to lithium carbonate and N-butyl-N-(4-hydroxybutyl)-nitrosamine in comparison to animals only treated with N-butyl-N-(4-hydroxybutyl)-nitrosamine. This study could, however, not be adequately assessed due to lack of information. In an insufficiently documented 2-year rat toxicity study with exposure to lithium chloride, no occurrence of tumours was described.

As regards human data, six epidemiological studies were included in the assessment. Overall, the DS was of the opinion that these studies could not establish an association between lithium exposure and an increased incidence of tumours.

The DS concluded that no classification could be justified based on lack of data of adequate quality.

### Comments received during consultation

One commenting MSCA supported no classification for carcinogenicity.

### Assessment and comparison with the classification criteria

#### *Animal data*

No reliable carcinogenicity studies in animals exposed to lithium are available. However, four tumour promotion studies and one repeated dose toxicity study has been assessed.

Ziche et al. (1980) studied tumour promotion in female Buffalo/N-rats and female Sprague-Dawley rats. In the first study, 24-27 animals per dose group were treated once with N-nitrosourea (i.v.) and afterwards exposed to lithium carbonate (0, 0.5, 1 or 10mM, equivalent to 0, 0.69, 1.38, 13.8 mg Li/kg bw/d) in drinking water for three months. Controls received sodium carbonate. No significant effect on tumour incidence was observed. In the second study, 5 animals per dose group were treated three times with N-nitrosourea (i.v.) and afterwards exposed to lithium carbonate (0, 10 or 20 mM, equivalent to 0, 13.8 or 27.6 mg Li/kg bw/d) in drinking water for two months. Controls received sodium carbonate. Lithium carbonate treatment did not influence mammary tumour development or size of tumours induced by N-nitrosourea. In the third study, female Sprague-Dawley rats were treated once with 20 mg 7,12-dimethylbenzanthracene (by gavage). 120 days after 7,12-dimethylbenzanthracen exposure, 480 animals had developed tumours of the mammary gland. The 120 animals without any tumours were exposed to 0, 1 or 10 mM lithium carbonate (0, 1.38, 13.8 mg Li/kg bw/d) via drinking water for 3 months. The additional treatment with lithium carbonate did not result in a higher tumour incidence.

Frolov and Pliss (1991; 1992) performed a tumour promoting study with lithium carbonate in rats. Animals received N-butyl-N-(4-hydroxybutyl)-nitrosamine prior to exposure to lithium carbonate. Urinary bladder tumour rate was increased about 6 times after exposure to lithium carbonate compared to exposure only to N-butyl-N-(4-hydroxybutyl)-nitrosamine, the effect was strongest 3-6 months after exposure. The DS noted that the original publications were written in Russian and were cited from Hartwig (2014) who indicated that these studies were insufficiently documented and could not be used for the evaluation of a possible tumour promoting activity of lithium carbonate.

Trautner et al. (1958) described no increased tumour incidence in a 2-year study in rats ingesting lithium chloride in drinking water (0, 10, 20, 30 and 50 mM corresponding to approx. 0, 85, 170, 250 and 425 mg lithium chloride/kg bw/d and 0, 14.2, 28.3, 41.7 and 70.8 mg Li/kg bw/d). At 20 mM, no effects on health or behaviour were observed except slight, transitory initial disturbances. At 30 mM lithium chloride weight loss and mortality were observed. At the high dose (50 mM) death of the animals occurred within 2-3 weeks. The details provided on the protocol and the results reported were, however, too sparse to use this study for the assessment of carcinogenicity following exposure to lithium chloride.

***Human data***

Several epidemiological studies have assessed the cancer risk in patients treated with lithium.

Ambrosiani et al. (2018) investigated the correlation between treatment with lithium and occurrence of thyroid or renal tumours. This were performed by:

- A retrospective analysis of the clinical records in the lithium clinic database
- An analysis of cause of death of patients visiting the lithium clinic at least once between 1980 and 2013.
- An analysis of adverse reactions to lithium reported to the European and WHO pharmacovigilance databases.
- A review of thyroid and renal tumours in lithium-treated patients reported in literature.

Overall, they could not confirm an association between lithium treatment and increased risk of thyroid or renal cancers.

Martinsson et al. (2016) investigated the cancer incidences in two cohorts of patients with bipolar disorder in a prospective cohort study. One cohort consisted of 3049 patients (age 50-84 years) without lithium treatment and the other consisted of 2393 patients (age 50-84 years) treated with lithium (at least one prescription of lithium per year) between 1 July 2005 and 31 December 2009. Cancer incidences in persons with bipolar disorders were compared to data of the general

population (about 2 600 000 men and women). The incidence rate ratios, adjusted for age and gender, of first cancer and site-specific cancer diagnosis between 1 July 2005 and 31 December 2009 were calculated. They concluded that the overall cancer risk was not increased in patients with bipolar disorder. Neither bipolar disorder (IRR = 1.03, 95% CI: 0.89-1.19) nor lithium treatment of bipolar disorder (IRR = 1.04, 95% CI: 0.89-1.23) was associated with increased incidence of unspecified cancers. It is noted that the risk of respiratory, gastrointestinal, and endocrine cancer was increased in patients without lithium treatment, but not in patients with lithium treatment.

Pottegård et al. (2016a) found no association between long term (5 years) lithium use and increased risk of colorectal adenocarcinoma. In this case-control study the cases included patients diagnosed with incident colorectal adenocarcinoma during 2000-2012 (n=36248) while the controls were ten matched cancer free controls per case. Long-term use of lithium was similar among cases (0.22%) and controls (0.20%), yielding an odds ratio of 1.13 (95% confidence interval (CI), 0.89–1.43) for colorectal adenocarcinoma. The odds ratio for colorectal adenocarcinoma in cases were 1.13 (95% CI: 0.89-1.43) while odds ratios for different subsides were for proximal colon: 1.01 (95% CI: 0.66-1.55), distal colon: 1.52 (95% CI: 1.05-2.20) and rectum: 0.80 (95% CI: 0.50-1.30). It is noted that this study lacks data on life-style habits, including smoking, obesity and alcohol consumption in addition to exact data on lithium consumption.

Pottegård et al. (2016b) found no association between long term (5 years) lithium use and increased risk of upper urinary tract cancer. In this case-control study the cases included patients diagnosed with upper urinary tract cancer during 2000-2012 (n=6477) while the controls were age and sex matched cancer free patients (n= 259 080). Long-term use of lithium was observed among 0.22% of cases and 0.17% of controls. The adjusted odds ratio for upper urinary tract cancer was 1.3 (95% CI: 0.8-2.2) for upper urinary tract cancer associated with long term use of lithium.

Kessing et al. (2015) found no association between continued treatment with lithium and increase in renal and upper urinary tract tumours in a retrospective population based longitudinal cohort study. In this study, cohort I consisted of:

(i) randomly selected sample of 1,500,000 out of all persons registered in Denmark on 1 January 1995
(ii) all patients having their first psychiatric contact ever in the period from 1994 to 2012 and receiving a main diagnosis of a single manic episode or bipolar disorder
(iii) all persons exposed to either lithium or anticonvulsants.

Cohort II were a sub-cohort of cohort I and included 9651 patients with a main diagnosis of a single manic episode/bipolar disorder diagnosed between 1995-2012.

There was no increased rate of renal and upper urinary tract tumours in lithium-treated patients (hazard rate ratios malignant or benign: 0.67-1.18, range of different exposure groups according to number of prescription).

Zaidan et al. (2014) concluded in a retrospective cohort study that the frequency of renal cancer was significantly higher among lithium-treated patients with cystic kidney disease than among lithium-free patients with cystic kidney disease (4.1% vs 0.3%, P=0.002). They also reported an increased incidence ratio of renal tumours in lithium-treated patients with cystic kidney disease compared to French general population (7.51 (95% CI:1.51–21.95) and 13.69 (95% CI: 3.68–35.06) in men and women, respectively). In this study the incidence ratio of renal tumours in 170 lithium-treated patients with cystic kidney disease was compared to the French general population and 340 patients with cystic kidney disease without lithium therapy.

Licht et al. (2014), however, questioned the results of Zaidan et al. (2014) as the influence of confounders was not appropriately checked. Moreover, this study could have been subject to selection/inclusion bias because it was conducted in a specialised nephrology department and the limited number of cases did not allow detailed statistics. This study suggests an association between lithium and renal cancer; however, causation criteria are not meet and the results should be supported by other studies.

Further, the Zaidan et al. (2014) was also questioned by Pottegård et al. (2016b) arguing that the precise methodology in this study was somewhat unclear and that the high relative risk estimate was based on only seven invasive cases of renal cell cancer. In addition, they pointed out that it was not taken into account that the lithium users in this study population were referred to renal imaging, and that the underlying reason for such imaging *per se* is likely associated with in increased cancer risk.

It is noted that the European Medicine Agency (EMA) in 2015 adopted the following recommendation: "in light of the data available, the PRAC (Pharmacovigilance Risk Assessment Committee) has agreed that the evidence is sufficient to conclude that long-term use of lithium may induce microcysts, oncocytomas and collecting duct renal carcinomas". PRAC later noted that the effects were only investigated in patients with severe renal impairment. The recommendation by EMA was in part based on the study by Zaidan et al. (2014) which was later questioned by Licht et al. (2014) and also by Pottegård et al. (2016b).

**In summary,** there are no carcinogenicity or long-term animal studies according to acceptable test guidelines available. One insufficiently documented 2-year study in rats did not show an increase in tumours after exposure to lithium chloride. Four non-guideline tumour promotion studies were assessed; three of these studies did not show any tumour promoting activity, while the fourth study indicated an increase in urinary bladder tumour. It is, however, noted that this study cannot be adequately assessed.

Several epidemiological studies did not reveal any association between lithium exposure and increased tumour incidence. One study, however, found an increase in renal tumours in patients with cystic kidney disease treated with lithium. It is noted that this study has been criticised for methodological deficiencies including confounders not appropriately checked and selection/inclusion bias.

In conclusion, RAC is of the opinion that based on the data available **no classification for carcinogenicity is warranted**.

## RAC evaluation of reproductive toxicity

### Summary of the Dossier Submitter's proposal

#### *Effects on sexual function and fertility*

For the assessment of adverse effects on sexual function and fertility the DS included a 2-generation reproductive toxicity study performed according to OECD TG 416 and GLP (Anonymous, 2012, Van Deun et al. 2021) and several non-test guideline studies assessing the effect of exposure to lithium salts on male reproductive tract. In the 2-generation study, no reproductive effect was observed. However, various studies consistently indicated that lithium affected the male reproductive system and the effects included impaired spermatogenesis and morphological changes of the reproductive organs. Further in one of the studies (90-d/mating study) a decrease in the fertility index was reported (Thakur et al., 2003).

Human data, consisting of a few case reports, were assessed by the DS. However, the case reports were not considered sufficient to serve as a basis for a classification for effects on sexual function and fertility.

The DS concluded that despite of the overall negative findings in the 2-generation study, there were high consistency of the effects on male reproduction reported in recent and robust studies showing a clear evidence of effects on fertility. Therefore, a classification as **Repr. 1B** for lithium carbonate, lithium chloride and lithium hydroxide was proposed by the DS for adverse effects on sexual function and fertility.

***Developmental toxicity***

The studies performed on animals were considered inconclusive by the DS, due to the heterogeneity of the results and the overall quality of the dataset.

Further, reported findings in some of the human studies were not seen in the animal studies (no increase in cardiac malformation seen in animal studies), which could be considered related to difference in mechanism of action between rodents and humans. However, the DS considered that the human data, and particularly the homogeneity of recent robust human studies were sufficient by themselves as evidence of developmental effects following exposure to lithium.

Medical data were not available in the framework of the dossier, but the DS noted that for lithium-based drug labels, it is clearly stated that an increase in the overall rate of malformations has been observed in children exposed *in utero* to lithium and that discontinuation of treatment should be considered until the 9th week of amenorrhea.

The existing epidemiological studies were rather contradictory, of various quality, and was summarised chronologically by the DS:

In the 1970s, retrospective studies, based on the Registry of Lithium Babies, i.e. children from women who had been treated with lithium during the first trimester of pregnancy (Giles and Bannigan, 2006; Schou et al., 1973; Weinstein, 1976; Weinstein and Goldfield, 1975), pointed to an increased risk of malformations in babies exposed to lithium during gestation.

However, later, valid case-control studies did not identify an association between congenital, especially cardiovascular, malformations, and lithium exposure during gestation (Correa-Villasenor et al., 1994; Edmonds and Oakley, 1990; Kallen et al., 1988; Sipek et al., 1989; Zalztein et al., 1990). Cohort studies on the other hand provided contradictory results, and case reports pointed to perinatal complications due to gestational exposure to lithium.

In recent publications, the DS considered that a more precise pattern emerged regarding the effects of lithium on development. Authors of a review (Yacobi et al., 2008), a meta-analysis (McKnight et al., 2012) and a cohort study (Patorno et al., 2017) came to very similar conclusions, i.e., that the evidence between lithium exposure during pregnancy and cardiac malformation was weak but that there was an association, but with a magnitude lower than previously reported. In particular, Patorno et al. pointed to a risk of cardiac malformations particularly at high therapeutic doses, with a clear dose-response relationship. The DS noted that the relatively weak association could be influenced by the higher rate of spontaneous or therapeutic abortions of woman under lithium therapy, which was not taken into consideration by authors of these publications and could lead to an underestimation of developmental effects of lithium. The DS therefore concluded that lithium should be classified as Repr. 1A; H360D for development.

***Lactation***

The DS considered that there was no doubt that lithium can be transferred to infants via breast milk. However, the existing data did not clearly indicate that severe toxic effects were induced in infants exposed to lithium via breast milk. In experimental animal studies, effects observed

could not clearly be distinguished from effects caused by gestational exposure, and there was no evidence that neonates were more sensitive than adults. There was one case report indicating that maternal serum levels in the toxic range could also lead to toxic effects in the infant. However, the DS concluded that the database was not sufficient for a classification for effects on or via lactation.

## Comments received during consultation

*Effects on sexual function and fertility:* Comments from an International NGO and two MSCAs supported the classification as Repr. 1B. One MSCA considered that a classification as Repr. 2 was more appropriate since no adverse effects on sexual function and fertility up to doses inducing some systemic toxicity was observed in the OECD TG 416 study (according to GLP). Fertility effects were indicated in various other non-guideline studies. However, the MSCA considered that the quality of evidence was less convincing due to deficiencies in the studies, e.g. substance purity information missing, no information on systemic effects/absence of systemic effects.

Four comments from Industry or Trade Associations and four comments from Company-Importers/Company-Manufacturers did not support the classification as Repr. 1B since no effects were reported on reproduction in the OECD TG 416 study performed according to GLP. Additional data were provided on sperm parameters from the OECD TG 416 2-generation study (Anonymous, 2012, Van Deun et al. 2021) showing no effect. The other studies investigating the reproductive toxicity in rats and mice were considered to have many deficiencies especially regarding information on systemic toxicity, overdosing and purity of the test compound. They were not conducted under GLP and some of the studies used unrealistic exposure routes (intraperitoneal injections, i.p. and subcutaneous exposure, s.c.).

*Developmental toxicity:* One MSCA supported the classification as Repr. 1A for development and informed about a recent prospective population-based mother-child cohort study by Harari et al. (2015) investigating the effects of environmental exposure of lithium on pregnant women residing in Argentina Andes (n=194). The study provided indications that lithium exposure through drinking water might be associated with impaired foetal size that seemed to be initiated in early gestation.

A second MSCA supported the classification as Repr. 1A for development. They commented that experimental data on developmental toxicity of lithium was inconclusive. However, recent human data provide sufficient evidence to suspect developmental effects upon lithium exposure.

A third MSCA did not support a classification as Repr. 1A and considered Repr. 2 to be more appropriate. The MSCA considered the epidemiological data to be of varying quality and rather contradictory. Confounding factors and limited statistical power led to quite weak evidence. Further, no developmental toxicity was reported in the OECD TG 414 (GLP) study and the reliability of the non-guideline studies was questionable due to limited reporting of maternal toxicity. They also missed medical data, since medical product leaflets state that an increase in the overall rate of malformations was observed in children exposed *in utero* to lithium, indicating that a clinical/medical database exists. The DS replied that the French Agency for the Safety of Health Products gave access to the archives of regulatory affairs (marketing authorisation provided by laboratories). However, no useful data related to classification could be retrieved.

One International NGO supported the classification as Repr. 1A for development.

Comments from five Industry or Trade Organisations (same comment from one Company-Manufacture) did not support the classification as Repr. 1A for development. They questioned the validity of the studies included in the CLH report since results of a recent study performed under GLP showed no lithium related effects on development. The foetal effects reported in the

non-test guideline studies were considered most likely secondary to excessive toxicity or extreme conditions. As regards the human data, epidemiological studies from therapeutic use of lithium showed equivocal and often contradictory results and should not be used for classification for development. Further, animal studies were carried out on healthy animals and epidemiological studies were carried out on humans with neurological diseases, so how this impacts the interpretation of the data should have been included.

One comment from a Company-Manufacturer did not support the classification as Repr. 1A for development. They informed about a study by Andrews et al. (2019) addressing lithium carbonate and lithium chloride written by representatives from the US-FDA, Health Canada, Brazil-ANVISA, Netherlands-CBG-MEB and a number of global pharmaceutical companies. This study included that lithium was not added to the "known" human teratogen list because the human data was inconsistent, and effects were lacking in the animal model. It is noted that the full reference to the study was not provided by the Company-Manufacturer.

Two comments from Company-Importers did not agree to the classification as Repr. 1A based on a weight of evidence of the available human and animal data. The animal data showed no evidence of teratogenicity or cardiac malformations in developing foetuses even at high maternally toxic doses. The human epidemiological studies reported equivocal and often contradictory evidence of foetal cardiovascular abnormalities at low incidence following therapeutic use of lithium during pregnancy. In addition, one of the commenting Company-Importers considered that labelling of drugs can be used as Precautionary Principle in case of a suspected human reproductive toxicant, but it cannot be considered as clear evidence or criteria for classification in Category 1A under CLP. The other Company-Importer commented that there is little evidence from occupational use studies to indicate significant risk associated with exposure to lithium during gestation.

*Lactation:* One MSCA supported that the animal studies did not show any clear effects on pups via lactation. However, they noted that lithium treatment was contraindicated during breastfeeding in several international treatment guidelines and on the leaflets of some lithium-based medicines. The MSCA included also in their comments that a potential mechanism could be explained by the immature excretory systems of infants that increase the possibility of adverse reactions, since lithium is eliminated via renal excretion. These reactions included cardiac arrhythmia, goiter, electrolyte imbalance, hypothyroidism, tremor, muscle weakness, gastrointestinal problems and nephrotoxicity and had been reported in nursing infants (Chaudron and Jefferson, 2000). This concern was increased based on experimental animal studies showing lithium-induced severe renal structural changes in the developing rat kidney (Christensen et al. 1982). An additional concern was the potential of lithium to accumulate in the developing bone of the infant, thus causing a decrease in bone calcium (Chaudron and Jefferson, 2000).

One MSCA commented that the experimental animal studies on toxicological effects of lithium carbonate exclusively due to exposure via lactation were limited and of insufficient quality, however, noting that there is no doubt that lithium can be detected in breast milk and can be transferred to infants via breast milk. However, according to the CLP criteria, lithium should be present in breastmilk at "potentially toxic levels". In the CLH report it is described that the serum levels in infants are approximately one fourth of the maternal serum levels upon exposure via breast milk. Further, one case study reports toxic effects in a breast-fed child, but these symptoms could be traced back to extremely high (16 mM) maternal lithium concentrations (Health Council of the Netherlands (HCN), 2000).

The DS responded that they still consider the available data as not sufficient to support a classification for lactation but agreed that it is an open point and should be discussed in RAC.

## Assessment and comparison with the classification criteria

### *Adverse effects on sexual function and fertility*

Animal data

*Studies in rats*

In a 2-generation reproductive toxicity study performed according to OECD TG 416 and GLP Wistar rats (25/sex/group) were exposed to 0, 5, 15 and 45 mg lithium carbonate/kg bw/d by gavage corresponding to 0, 0.9, 2.8, 8.5 mg Li/kg bw/d (Anonymous, 2012, Van Deun et al., 2021). In the **P0 generation** no effects on reproductive function were reported up to the highest dose tested. The assessment included oestrous cyclicity, pre-coital time, gestation length, pup survival, mating, fertility, and fecundity. Further, no effects were reported on the weight and histopathology of the reproductive organs or on sperm parameters up to 45 mg/kg bw/d (sperm morphology and motility, testicular spermatid count and epididymal sperm count), see table below provided during stakeholder consultation.

***Table:*** *Sperm parameters in the P0 and F1 generation*

| **Dose (mg(kg)** | **0** | **5** | **15** | **45** |
|---|---|---|---|---|
| **P0 generation** | | | | |
| Motile sperm (%) | 84.9 | 85.3 | 87 | 87.4 |
| Progressively motile sperm (%) | 61.2 | 62.5 | 66.0 (+) | 65.7 (+) |
| Sperm morphology (% normal sperm) | 96.8 | 97.9 | 99.2 | 98.6 |
| No. sperm per g cauda epididymis (x106) | 858.56 | NE | NE | 887.91 |
| No. spermatids per g of parenchyma (x106) * | 114.64 | NE | NE | 113.87 |
| **F1 generation** | | | | |
| Motile sperm (%) | 87.9 | 85.8 | 87.0 | 87.0 |
| Progressively motile sperm (%) | 66.5 | 63.0 | 63.3 | 64.8 |
| Sperm morphology (% normal sperm) | 99.7 | 99.3 | 99.4 | 99.6 |
| No. sperm per g cauda epididymis (x106) | 754.56 | NE | NE | 766.21 |
| No. spermatids per g of parenchyma (x106) * | 108.02 | NE | NE | 109.40 |

NE = not evaluated; (+) or (-): significantly higher/lower than vehicle control group * Detergent and Homogenization Resistant Testicular Spermatid Counts

General toxicity was only reported in the P0 generation in the high dose group and included an increase in the net weight gain (up to 16.6%) and food intake (up to 12.7%) in males and females and water intake (up to 40%) only in males compared to the control animals. In the liver morphological changes evident as a higher incidence of increased cytoplasmic rarefaction was reported in males, and in females a higher incidence of focal basophilic hepatocytes and hepatocellular hypertrophy was reported. In the kidneys, higher incidences with minimal severity of dilated tubules in males and females (11/25 and 3/25, respectively) were reported. In the thyroid an increase in colloid in the follicular lumen was reported in females. In the **F1 and F2 generation** no reproductive and foetal toxicity was reported. It was noted that no information on lithium serum concentrations was provided. RAC notes that higher doses of lithium carbonate could have been tested in the study since only mild general toxicity was reported in the P0 generation and no general toxicity in the F1 and F2 generation.

Several studies assessing effects of lithium on the male reproductive tract was included by the DS, however, these studies were not performed according to OECD TG or GLP.

In the study by Thakur et al. (2003) male Wistar rats, 20/group, were exposed to 0, 500, 800, 1100 mg/kg lithium carbonate (purity not provided ) in the diet for 90 days corresponding to approximately 0, 20, 32, 44 mg lithium carbonate/kg bw/d, (calculated using the ECHA guidance on information requirements and chemical safety assessment R8, 2012, table R8-17), and 0, 3.8, 6.0, 8.3 mg Li/kg bw/d. RAC used this study in the overall weight of evidence assessment for effects on sexual function and fertility. The study included 3 experiments: **Experiment 1:** The animals were exposed to lithium carbonate for 90 days and sacrificed. General toxicity measured

as decreased body weight (body weight calculated based on the testis weight per body weight (%), see table below) was in the 0, 500, 800 and 1100 mg/kg bw/d 461 g, 460 g (0% decrease), 387 g (16% decrease) and 408 g (10% decrease), respectively. The reproductive tract was histologically analysed, and sperm parameters and testosterone measured. At doses at and above 800 mg/kg diet a statistically significant reduction in the absolute weight of testes (up to 36%), epididymis (up to 27%) and accessory sex organs (up to 38%) was reported (see table below).

***Table:*** *Weight of male reproductive organs. Values presented are mean ± SD (Table AI – 3 from Annex 1 to the CLH report)*

| | 0 mg/kg diet | 500 mg/kg diet | 800 mg/kg diet | 1100 mg/kg diet |
|---|---|---|---|---|
| Testis (g) | 3.28 ± 0.15 | 3.31 ± 0.24 | 2.67 ± 0.31** | 2.08 ± 0.39** |
| Testis weight per body weight (%) | 0.71 ± 0.06 | 0.72 ± 0.07 | 0.69 ± 0.10 | 0.51 ± 0.10 |
| Epididymis (g) | 1.06 ± 0.16 | 1.15 ± 0.14 | 0.89 ± 0.15* | 0.77± 0.14** |
| Epididymis weight per body weight (%) | 0.22 ± 0.04 | 0.26 ± 0.04 | 0.21 ± 0.04 | 0.19 ± 0.04 |
| Seminal vesicles (g) | 1.10 ± 0.20 | 1.06 ± 0.18 | 0.90 ± 0.19* | 0.78 ± 0.16** |
| Seminal vesicles weight per body weight (%) | 0.24 ± 0.04 | 0.23 ± 0.04 | 0.21 ± 0.06 | 0.19 ± 0.06 |
| Prostate (g) | 0.78 ± 0.19 | 0.74 ± 0.19 | 0.61 ± 0.16* | 0.48 ± 0.14** |
| Prostate weight per body weight (%) | 0.13 ± 0.03 | 0.14 ± 0.02 | 0.14 ± 0.05 | 0.12 ± 0.06 |

* Significant difference at $p < 0.05$, ** significant difference at $p < 0.01$ compared with control.

The relative organ weights were not affected. However, it is noted that according to the OECD Guidance document on Mammalian Reproductive Toxicity Testing and Assessment "*Both absolute and relative weights of the male reproductive organs should be considered as a decrease in absolute weight may occur and may not necessarily be related to a reduction in body weight gain. However, care should be taken in interpreting data where a substantial bodyweight effect is evident. Since there is low inter-animal variability in testis weight, a significant change in absolute testis weight (increase or decrease) can indicate an adverse effect*". The reduced absolute testes weight is therefore by RAC considered as an effect that should be taken into account in the classification.

Further, a dose-dependent effect on sperm number from cauda epididymis (up to 47% reduction) and the daily sperm production (up to 71% reduction), serum testosterone (up to 65% reduction) and testicular interstitial fluid volume (up to 50% reduction) were statistically significant from the mid dose group. The number of abnormal spermatozoa was already statistically significantly increased from the lowest dose (10.9%). In the high dose group, severe degenerative changes were reported in the testes and accessory reproductive organs with a milder degree in the mid dose group.

***Table:*** *Effects on testosterone levels and interstitial fluid volume (IFV). Values presented are mean ± SD (Table AI – 4 from Annex 1 to the CLH report)*

| | 0 mg/kg diet | 500 mg/kg diet | 800 mg/kg diet | 1100 mg/kg diet |
|---|---|---|---|---|
| Testosterone (ng/mL) serum | 2.2 ± 1.1 | 1.7 ± 1.1 | 1.2 ± 0.7* | 0.77 ± 0.56** |
| IFV (µg/ g testis) | 40 ± 7.2 | 34.5 ± 8.5 | 25.5 ± 6.2** | 20 ± 5.6*** |

* Significant difference at $p < 0.05$, ** significant difference at $p < 0.01$, ***significant difference at $p < 0.001$ compared with control. Results taken fromThakur et al. (2003)

***Table:*** *Effects on sperm parameters. Values presented are mean ± SD. (Table AI – 5 from Annex 1 to the CLH report)*

| | 0 mg/kg diet | 500 mg/kg diet | 800 mg/kg diet | 1100 mg/kg diet |
|---|---|---|---|---|
| Number of animals | 20 | 20 | 20 | 20 |
| Sperm number from cauda epididymis ($x10^6$) | 325 ± 75 | 300 ± 61 | 203 ± 42** | 172 ± 52** |
| Daily sperm production ($x10^6$) | 45.6 ± 6.2 | 37.5 ± 7.2 | 18.2 ± 5.6** | 13.1 ± 4.9** |
| Sperm transit rate (days) | 7.1 ± 1.5 | 8.00 ± 3.6 | 11.15 ± 2.7** | 13.12 ± 3.00** |
| Percent of abnormal sperm | 7.3 ± 3.3 | 10.9 ± 4.0* | 12.4 ± 3.5** | 14.1 ± 2.1** |

* Significant difference at $p < 0.05$, ** significant difference at $p < 0.01$ compared with control.

**Experiment 2:** The animals were treated as in experiment 1 and after 90 days they were caged with unexposed females to determine the fertility index. A statistically significant decrease in male fertility index was reported in the mid and high dose group (90%, 80%, 60%, 40% at 0, 500, 800, 1100 mg/kg in the diet). The mating index was not affected. **Experiment 3:** The animals were treated as in experiment 1 for 90 days. After a recovery period of 30 days, they were caged with unexposed females to determine the fertility index. A statistically significant decrease in the male fertility index was reported in the mid and high dose group (90%, 80%, 70%, 50% at 0, 500, 800, 1100 mg/kg in the diet). The mating index was not affected.

***Table:*** *Effects on fertility parameters from Experiment 2 and 3. (Table AI – 6 from Annex 1 to the CLH report)*

| | 0 mg/kg diet | 500 mg/kg diet | 800 mg/kg diet | 1100 mg/kg diet |
|---|---|---|---|---|
| **Experiment 2: Fertility parameters** | | | | |
| Mating index (%) | 85 | 95 | 90 | 95 |
| Male fertility index (%) | 90 | 80 | 60** | 40** |
| **Experiment 3: Fertility parameters** | | | | |
| Mating index (%) | 90 | 90 | 95 | 95 |
| Male fertility index (%) | 90 | 80 | 70** | 50** |

** Significant difference at $p < 0.01$ compared with control. Results taken fromThakur et al. (2003)

It is noted that no information was provided on systemic toxicity and lithium plasma or serum levels in the study by Thakur et al. (2003). However, the study is considered to cover an important and relevant dose range close to the doses used in the OECD TG 416 study where maternal toxicity at top dose in the P0 generation was very mild and no general toxicity was

reported in the F1 and F2 generation investigating endpoints relevant for male fertility in 20 male rats/group with sufficient details. In addition, this is the only study analysing and reporting an impact on fertility of the observations made on sperm and hormonal parameters in several studies. RAC therefore considers the study as relevant for classification noting that the 2-generation study was performed with gavage dosing whereas the Thakur et al. (2003) study administrated lithium carbonate via the diet.

In the study by Zarnescu and Zamfirescu (2006) mature male Wistar rats (4-6 months old but could have been 4-6 weeks due to the weight of 100-120 g at the initiation of the study), were exposed to 0 (4 rats) or 35 mg lithium carbonate/kg bw/d (10 rats) corresponding to 6.6 mg Li/kg bw/d for 21 days via gavage (purity not provided). RAC used this study in the overall weight of evidence assessment for effects on sexual function and fertility. Following exposure, the ultrastructure of the seminiferous tubules was examined by electron microscopy. Only microscopic pictures were included in the publication with no quantitative analysis. Results: Ultrastructural changes were induced following lithium carbonate exposure in both germinal cells and Sertoli cells. In the treated rats abnormal or degenerated spermatids and structural abnormalities like loss of germ cell attachment or expanded intercellular spaces between spermatogonia and spermatocytes were reported. Furthermore, round spermatids were shown to have abnormal acrosomes. RAC considers that despite the limited study with only one dose tested, low number of animals and that information on lithium plasma levels as well as systemic effects was not reported, these results are considered to be in agreement with the findings reported in the study by Thakur et al. (2003).

In the study by Toghiani et al. (2012) hormonal measurement and histological examination of the testicular tissue was performed in male Wistar rats (6/dose group) following exposure for 48 days via gavage to 0, 10, 20, 30 mg lithium carbonate/kg bw/d corresponding to 0, 1.9, 3.8, 5.6 mg Li/kg bw/d (purity not provided, purchased from Tehran Darou Co, Pharmaceutical company). RAC used this study in the overall weight of evidence assessment for effects on sexual function and fertility. Results: A dose-dependent and statistically significant decrease in the relative testicular tissue weight (0.55 g, 0.43 g, 0.32 g and 0.25 g in 0, 10, 20 and 30 mg/kg bw/d, respectively), germ and somatic cells in seminiferous epithelium (spermatogonia (up to 42% in high dose group), primary spermatocytes (up to 53% in high dose group), spermatids (up to 57% in high dose group), spermatozoids (up to 70% in high dose group), Sertoli cells (up to 19% in high dose group) and Leydig cells (up to 37% in high dose group)) was reported, see table below:

***Table:*** *Effects on sperm cells, Sertoli cells and Leydig cells (from Annex I to the CLH report)*

| Groups | Spermatogonia (Count± SD) | Primary spermatocyte (Count± SD) | spermatid (Count± SD) | Spermatozoa (Count± SD) | Sertoli (Count± SD) | Leydig (Count±SD) |
|---|---|---|---|---|---|---|
| A | 119.6667±11.9443 | 357.000±36.33180 | 173.0000±12.3288 | 2550.5000±123.0670 | 11.5000±1.04881 | 8.66±2.42212 |
| B | 86.0000±4.42719 | 295.8333±22.89469 | 108.8333±7.5476 | 1087.1667±294.307 | 10.5000±0.8366 | 6.33±1.63299 |
| C | 75.1667±4.44597 | 265.0000±15.49193 | 98.5000±5.75326 | 1030.3300±70.5256 | 10.000±1.0236 | 6.05±0.30767 |
| D | 69.3333±6.40833 | 168.5000±3.67423 | 74.667±6.9761 | 772.+-61231 | 9.3±1.0366 | 5.47±1.75119 |

Further, a dose-dependent and statistically significant decrease in blood concentrations of LH (up to 61% in high dose group), FSH (up to 53% in high dose group) and testosterone (up to 81% in high dose group) was reported. No information was available regarding systemic toxicity, but the results are considered to be consistent with those of Thakur et al. (2003) and Zarnescu and Zamfirescu (2006).

With a similar study design (seems to be the same study as Toghiani et al., 2012, but with other parameters included) (48 days exposure via gavage to 0, 10, 20, 30 mg lithium carbonate/kg bw/d corresponding to 0, 1.9, 3.8, 5.6 mg Li/kg bw/d) Toghyani et al. (2013) analysed sperm in the epididymis in 6 Wistar male rats/dose group. Results: A dose-dependent reduction in numbers of normal sperm (97%, 88%, 88% and 70% in the 0, 10, 20 and 30 mg/kg bw/d dose group, respectively), sperm motility (96%, 68%, 48% and 39% in the 0, 10, 20 and 30 mg/kg

bw/d dose group, respectively), and number of sperm cells in cauda epididymis (2.19 x $10^8$, 1.42 x $10^8$, 1.21 x $10^8$ and 1.12 x $10^8$ in the 0, 10, 20 and 30 mg/kg bw/d dose group, respectively) was reported. No other parameters were assessed, and few details were provided limiting the acceptability of this study. However, the results are in agreement with the effects reported in the studies by Thakur et al. (2003), Zarnescu and Zamfirescu (2006) and Toghiani et al. (2012).

In the study by Allagui et al. (2006) Wistar rats (12/sex/dose were sacrificed on day 7, 14, 21 and 28, total number of animals 144/sex) were exposed for up to 28 days to 0, 2000 or 4000 mg lithium carbonate/kg diet corresponding to approximately 200 or 300 mg/kg bw/d and 37.6 or 56.4 mg Li/kg bw/d in the low and high dose group, respectively. The mean serum lithium concentrations were measured and were as follows: low dose group: 0.443, 0.621, 1.797, 1.475 mmol/L on days 7, 14, 21, 28, respectively, high dose group: 0.646, 1.219 mmol/L on days 7, 14, respectively. In comparison, humans treated for bipolar disorders have serum concentrations of 0.4-0.8 mM. In the high dose group, treatment was stopped after 14 days due to 60% mortality. Results: In both dose groups a statistically significant dose-related decrease in serum concentrations of testosterone was reported, see table below.

***Table:*** *Weekly serum concentrations of testosterone (ng/mL)*

| **Testosterone ng/mL** | **Day 7** | **Day 14** | **Day 21** | **Day 28** |
|---|---|---|---|---|
| **Control** | 1.23 ± 0.33 | 1.40 ± 0.37 | 3.07 ± 0.68 | 1.96 ± 0.32 |
| **200 mg/kg bw/d** | 0.89 ± 0.36 (-28%) | 1.14 ± 0.40 (-19%) | 1.53 ± 0.54* (-50%) | 0.84 ± 0.32** (-57%) |
| **300 mg/kg bw/d** | 0.55 ± 0.10* (-55%) | 0.53 ± 0.09* (-62%) | - | - |

*$P$<0.05, **$P$<0.01 student's $t$-test

The high dose group was terminated at day 14 due to high mortality reaching 50-60% in male and female rats. Effects on spermatogenesis was reported at day 28 in the low dose group including 73±2% of azoospermia and 70±5% deprived of flagella when compared to control animals. Further a statistically significant dose-related decrease in serum levels of triiodothyronine (free T3) and thyroxine (free T4) was reported in male and female rats. Serum oestradiol concentrations were increased by 54% and 91% at days 21 and 28 in the low dose group, and disturbances of the oestrus cycle was reported. Most control animals were in oestrus or post-oestrus phases, while rats exposed to 200 mg/kg bw/d lithium were mostly in post-oestrus on days 7 and 14 and in dioestrus on days 21 and 28. Similarly, on day 14, rats at high dose group (300 mg/kg bw/d) were mostly in post-oestrus or dioestrus. General toxicity included polydipsia, polyuria and diarrhoea and a dose-dependent decrease in food consumption and body weight, see table below.

***Table:*** *Weekly estimation of food consumption and weight changes (g/rat)*

| **Days** | **Males** | | | **Females** | | |
|---|---|---|---|---|---|---|
| | control | 200 mg/kg bw/d | 300 mg/kg bw/d | control | 200 mg/kg bw/d | 300 mg/kg bw/d |
| **1-7** | | | | | | |
| Weight changes | +19.60 | -8.90 | -21.75 | +14.30 | -6.25 | -21.80 |
| Food consumption | 97.00 | 85.00 | 49.20 | 97.00 | 75.00 | 43.00 |
| **7-14** | | | | | | |
| Weight changes | +24.75 | -12.20 | -19.90 | +8.70 | -5.25 | -24.70 |
| Food consumption | 105.40 | 58.00 | 42.20 | 101.65 | 57.00 | 37.60 |
| **14-21** | | | | | | |
| Weight changes | +13.30 | +1.00 | - | +10.00 | -10.75 | - |
| Food consumption | 125.10 | 60.00 | | 119.95 | 49.00 | |
| **21-28** | | | | | | |
| Weight changes | +9.34 | +3.70 | - | +9.00 | +3.10 | - |
| Food consumption | 159.50 | 63.00 | | 125.00 | 56.00 | |

Statistical significance of the effects reported were mainly reached when serum lithium levels were in the upper range of therapeutic doses or even exceeded the therapeutic range, i.e. in the

low dose group on observation days 21 and 28 and in the high dose group on observation day 14. RAC notes the mortality reported in the high dose group, however, considers the effects observed in the low dose group as related to exposure to lithium and used the study in the overall weight of evidence assessment for effects on sexual function and fertility.

*The two following studies have several limitations. Therefore, only a brief summary is included since these studies are not considered important for the classification of lithium for effects on sexual function and fertility:*

Gralla and McIlhenny (1972) investigated effects of lithium carbonate following oral exposure via gavage on fertility and general reproductive performance in Charles River rats (20/sex/group). Females were treated 14 days before cohabitation with approximately 50, 150 and 300 mg lithium carbonate/kg bw/d corresponding to 0, 9.3, 28.1, 56.2 mg Li/kg bw/d. The plasma concentration was 1.4 mmol/L after daily exposure to 300 mg/kg bw/d for 3 days. Males were treated 70 days before cohabitation with approximately 20, 50 and 100 mg lithium carbonate/kg bw/d corresponding to 3.8, 9.3, 18.7 mg Li/kg bw/d. One half of the treated females were sacrificed on GD 13 and the number and distribution of implantation sites were recorded. The remaining females were allowed to deliver and nurse their offspring to PND 21. Results: Two pregnant rats died unexpectedly for unknown reasons, no further information in which dose group. No effects on reproduction were reported (no further information), and no effects in offspring observed (details not reported).

Trautner et al. (1958) investigated the effects of lithium chloride exposure via drinking water on pregnancy in Wistar rats (52 exposed/100 controls). The rats were exposed to 66 mg lithium chloride/kg bw/d corresponding to 11 mg Li/kg bw/d (plasma Li levels of 1.5-2.0 mmol/L) starting 10-14 days before mating for males and 3-7 weeks before mating for females till the end of pregnancy or lactation. Results: No effects on pregnancies were reported including incidence and progress of pregnancy, birth and lactation, and the health and progress of the offspring. No malformations or other defects in the lithium-exposed litters were reported. Weight gain and growth were retarded in offspring of dams exposed to lithium chloride during pregnancy and lactation (no further details were provided). In another group (6/sex) exposed to 83 mg lithium chloride/kg bw/d corresponding to 14 mg/Li/kg bw/d for 17 days before mating, a reduction in the number of pregnancies was reported. No information on systemic toxicity available.

*Studies in mice*

In the study by Banerji et al. (1986) female fertility was studied in adult virgin C57BL/6 mice exposed 15 days to 0.4% lithium chloride in diet corresponding to 520 mg lithium chloride/kg bw/d and 86.6 mg Li/kg bw/d calculated according to ECHA Guidance R8 table R8-17 (20 females/dose). For the assessment of oestrous cycle, vaginal smears were examined each day. Results: No irregularity in oestrous cycle was observed in the first 3 days of treatment. From the fourth day, 30% of the mice showed irregularity, displaying a constant dioestrus. This percentage increased on days 5, 6 and 7 up to 100% on day 8 and until the end of the study (15 days). Limited information was available especially regarding systemic toxicity. RAC notes that disturbances of the oestrus cycle were also reported in rats in the study by Allagui et al. (2006). RAC considers the study as supportive for a classification for effects on sexual function and fertility.

In the study by Mroczka et al. (1983) CFW mice mating pairs were exposed to drinking water containing lithium chloride concentrations of 0, 10, 20, 30, 50, 100 or 200 mM corresponding to approx. 0, 85, 170, 250, 425, 850, 1700 mg lithium chloride/kg bw/d and 0, 14.2, 28.3, 41.7, 70.8, 141.6 and 282.2 mg Li/kg bw/d starting about 2 or 5 weeks before mating. Results: Mice in the highest dose group refused to drink and died within one week. Mice in the 100 mM group survived but did not reproduce (no further information). Mice in the 425 mg/kg bw/d group (with corresponding plasma concentrations in the therapeutic range 0.67 mM), had fewer litters of

normal size at birth, prolonged intervals between litters and increased postnatal mortality, including loss of entire litters, whereas no effects were observed in the three lowest dose groups. Due to insufficient reporting (only results of 425 mg lithium chloride/kg bw/d group documented, no data on general toxicity and number of animals per group) this study was considered not reliable and RAC did therefore not include this study in the weight of evidence assessment.

*Studies in mice and rats with exposure routes not considered relevant for classification*

Several studies were included by the DS and are shortly described in the BD.

Human data

The human data available assessing effects of lithium on male fertility are restricted to a few case reports, which are not considered sufficient to serve as basis for a classification.

Blay et al. (1982) reported two human cases indicating that lithium could impair male fertility. Two male patients (n=2) treated with lithium (serum lithium levels 0.5-0.9 mM) complained about reduced libido and erectile dysfunction. After termination of treatment or replacing lithium by a placebo, recovery of normal sexual functions was reported.

In the Aizenberg et al. (1996) study the results of a sexual function questionnaire in 35 bipolar and schizoaffective men, aged 43.3 +/- 9.6 years was reported. Eleven patients (31.4%) reported sexual dysfunction on at least two items. However, there was no difference in serum lithium levels in patients with and without sexual dysfunction and no statistical correlation between sexual function scores and serum lithium levels.

Levin et al. (1981) analysed semen from 9 patients treated 3 weeks with lithium carbonate, at dosage sufficient to maintain a plasma concentration of 0.6 to 1.4 mEq/L. When comparing the semen before and after treatment, they reported that lithium produced a significant decrease in the percentage of sperm viability, from 70% to 55%. However, the sperm count, and motility were not affected by lithium treatment.

The effect of lithium therapy on human PRL levels were studied in several studies and summarised in HCN, (2000). Four studies did not report any effect of lithium treatment on plasma PRL levels; however, a fifth study reported an increase in PRL levels during lithium therapy. HCN concluded that due to these contradictory results no final conclusions could be drawn.

HCN (2000) also reported a reduced sperm viability but no effects on sperm count or motility in 4 patients under lithium carbonate therapy. Further, *in vitro* investigations with human sperm showed a negative effect of lithium on motility at concentrations comparable with those reported in semen after oral administration of lithium.

Summary of the human and animal data

Ten experimental animal studies investigated the effects on sexual function and fertility following exposure to lithium salts in rodents. No toxicologically significant effects on fertility were observed in the most recent 2-generation OECD TG 416 study with less than optimal dose selection (Anonymous, 2012, Van Deun et al. 2021) and in two insufficiently reported rat fertility studies with a much lower weight of evidence (Gralla and McIlhenny, 1972; Trautner et al., 1958).

Studies investigating effects of lithium carbonate on the male rat reproductive tract consistently showed significant effects on sperm number (decrease up to 70%) or production (decrease up to 70%), sperm function, and/or male reproductive organ structure, as well as on testosterone levels (decrease up to 81%). All five studies on male reproduction were performed with the identical rat strain as used in the 2-generation study (Wistar rats) and four of them used doses in the same range as the 2-generation study (Thakur et al., 2003; Zarnescu and Zamfirescu, 2006; Toghyani et al., 2013; Toghiani et al., 2012). However, it is noted that in the 2-generation study oral administration by gavage was used (Anonymous, 2012, Van Deun et al. 2021) whereas

in the Thakur et al. (2003) study lithium carbonate was given in the diet. Further, in the 90-d study (Thakur et al., 2003) with subsequent mating, effects were reported on male fertility, evident as a reduction in male fertility index (from 90% in control group to 40% in the high dose group), confirming the consequence of the effects reported on the reproductive organs. Only the study by Allagui et al. (2006) used higher, partially lethal doses, and the results on sperm parameters and testosterone levels should be interpreted with caution. RAC notes that a complete study report of the 2-generation OECD TG 416 study was not available, but based on information provided on sperm parameters, reasons for the contradictory findings on sperm parameters and male reproductive organs are not known. In a fertility study with lithium chloride in mice, reduced fertility was also observed. However, this study was disregarded due to the high doses used in the study as well as the limited information provided (Mroczka et al., 1983).

RAC notes that the reported effects on the male reproductive tract were confirmed by mechanistic studies; however, these were performed with less realistic routes of exposure (intraperitoneal or subcutaneous) showing comparable effects (Ali et al., 2008; Ghosh et al., 1990b; 1991b). Even if differences in kinetics are expected, they can be used in a weight of evidence assessment, supporting the reproductive effects reported following exposure by the oral route.

The reproductive effects were also supported by the results reported on biochemical measurements performed in various studies including a decreased level of testosterone, FSH, LH and PRL, as well as on key enzymes in androgen biosynthesis.

Some effects were noted in the female reproductive system, including irregularity in oestrous cycle (Banerji et al., 1986; Allagui et al., 2006). However, RAC considers that these data are not sufficient by themselves for classification for effects on sexual function and fertility.

Human data were restricted to a few case reports, which were not sufficient by themselves to serve as basis for a classification for effects on sexual function and fertility.

**In conclusion:** No effects on sexual function and fertility were reported in the OECD TG 416 2-generation study, however RAC notes that higher doses of lithium could have been used in this study due to limited general toxicity in the top dose. On the other hand, consistent findings on the male reproductive tract in the 90-d/mating study as well as in studies on male reproductive organs were reported and considered to be induced in the absence of marked systemic toxicity. These studies are considered by RAC as valid and relevant for classification, and show clear evidence of effects on sperm number (decrease up to 70%) or production (decrease up to 70%), sperm function, and/or male reproductive organ structure, as well as on testosterone levels (decrease up to 81%) Further, in the 90-d study with subsequent mating, effects were reported on male fertility, evident as a reduction in male fertility index (from 90% in control group to 40% in the high dose group), confirming the consequence of the effects reported on the male reproductive organs. Based on the weight of evidence, RAC considers that a classification as Repr. 1B; H360F for the three lithium compounds is justified.

***Developmental toxicity***

Animal data

For the assessment of developmental toxicity, the DS included 8 studies in rats, 5 studies in mice, 1 study in rabbit, 1 in monkey and 1 in pig.

*Studies in rats*

In the key developmental toxicity study female Crl CD (SD) rats (25 rats/dose) were exposed to 0, 10, 30 or 90 mg lithium carbonate/kg bw/d by gavage from GD 6 to 19 corresponding to 0, 1.88, 5.64, 16.91 mg Li/kg bw/d (purity 99.6%) (Anonymous, 2010b, Van Deun et al., 2021). The study was similar to OECD TG 414 (deviation was exposure from GD 6 instead of GD 5) and according to GLP. Serum analysis revealed a clear dose-related systemic exposure to lithium

(0.24, 0.52, 1.39 mM Li). Maternal toxicity: In the high dose group a slight but statistically significant reduction in body weight gain (66.9% increase in bw from GD 1 to laparotomy compared to 74.4% in controls), and in food intake (up to 18.3% below controls) and water intake was reported. Further, piloerection was reported in a few dams. Foetal toxicity: No foetal malformations, and no test item related increase in the incidence of external/internal, skeletal or soft tissue variations or skeletal retardations was reported.

*The following studies in rats are by RAC regarded as supportive of classification.*

In the prenatal and postnatal study by Teixeira et al. (1995) pregnant female Wistar rats received either tap water ad libitum or tap water with 10 mM lithium chloride (purity not provided) corresponding to 53 mg lithium chloride/kg bw/d or 8.83 mg Li/kg bw/d from GD 1 to the end of lactation, or were water restricted until weaning of pups. Following birth, pups were fostered to form five experimental groups: a) control water restricted (21 litters), b) Li+ during prenatal and lactating periods (18 litters), c) Li+ during prenatal period only and then water restricted (22 litters), d) water restricted prenatally and Li+ during lactating period (25 litters), and e) Control, no treatment, not water restricted (13 litters). Maternal toxicity: No information included. Foetal toxicity: No malformations, stillborn or differences between litter size were reported at birth. A reduction in the number of pups with normal righting reflex at birth in both the water restricted (78.5%) and lithium-treated litters (70.5%) compared to the control group (94.2%) was reported; however, the lithium-treated group had also a reduced correct righting reflex compared to the water restricted group. A statistically significant delay in the day of eye opening and in the avoidance of visual cliff was reported following lithium exposure and in the water restricted group compared to the control group. Further, a statistically significant lower body weight was reported at PND 21 in pups exposed to lithium during lactation; however, lithium exposure prenatally did not have any effects on the body weight compared to the water restricted group.

In the prenatal and postnatal developmental toxicity study by Ibrahim and Canolty (1990) female Sprague-Dawley rats (11 or 15/dose group) were exposed in the diet to 0 or 50 mg lithium carbonate/kg bw/d corresponding to 9.4 mg Li/kg bw/d from GD 1 to the end of gestation (purity not provided). At parturition the litters were adjusted to 6 pups/litter. Then the diet was switched for half of both groups, and exposure continued to LD 21. Maternal toxicity: Decreased body weight gain (38%) and feed intake (20%) in exposed animals compared to control animals at parturition. In the end of lactation, a decrease in body weight was only reported in groups exposed to lithium during the entire study (10% reduction) or only during lactation (11.5% reduction). The group exposed to lithium only during gestation showed no difference in the end of lactation. The absolute liver weight, and the relative organ weights of the heart, kidneys and liver was decreased. Foetal toxicity: no gross malformation was reported in the pups. At birth, mean pup weight was significantly lower in the exposed group (6.3 g and 5.7 g in control and lithium groups, respectively) and the litter size was reduced (12.5 and 9.4 in control and lithium groups, respectively). At the end of lactation, the mean pup weight was significantly decreased in group exposed during lactation only (58 g and 44 g in control and lithium groups, respectively). Heart weight (0.31 g and 0.24 g in control and lithium-exposed group, respectively) and spleen weight (0.23 g and 0.17 g in control and lithium-exposed group, respectively) was decreased in the group exposed to lithium carbonate during lactation only. These effects are considered to be related to exposure to lithium during lactation.

In the prenatal developmental toxicity study by Fritz (1988) the effects of lithium carbonate (purity not provided) on the developing kidney were investigated in three experiments. **experiment 1**: Female Tif:RAIf rats (Sprague Dawley-derived) (14-19/dose group) were exposed by gavage to 100 mg lithium carbonate/kg bw/d corresponding to 0 and 18.79 mg Li/kg bw/d from GD 6-10, GD 11-15 or GD 16-20 and examination on GD 21. Maternal toxicity: Reduced body weight gain and feed consumption, polyuria. In GD 16-20 exposed dams, 7 died one day before expected delivery (no gross pathological findings). Foetal toxicity GD 6-10:

Embryonic and foetal deaths (3.8% of the implantation sites), dilatation of renal pelvis with obsolete or missing papillae: 0/67 in foetuses. Foetal toxicity GD 11-15: Embryonic and foetal deaths (7.0% of the implantation sites), dilatation of renal pelvis with obsolete or missing papillae (3/75, 4% of foetuses). Foetal toxicity GD 16-20: Increased prenatal mortality, embryonic and foetal deaths (38.5% of the implantation sites), dilatation of renal pelvis with obsolete or missing papillae (7/41, (17%) in foetuses, 4/14 litters). The normal range of embryonic and foetal death was 3-6% (standard-range derived from series of an untreated control population, HCD not provided).

In **experiment 2,** Fritz (1988) female rats (20/group) were exposed to 0 or 100 mg lithium carbonate/kg bw/d corresponding to 0 or 18.79 mg/lLi/kg bw/d during GD 16-20 with examination on GD 21 and PND 11-19. Maternal toxicity: Reduced body weight gain in exposed rats (11.5% vs 21.5% weight gain in controls), mortality (2/20), polyuria and increased water consumption. Foetal toxicity: Dilatation of renal pelvis with obsolete or missing papillae: (20/93 foetuses, 22% vs 0/133 foetuses, 0% in controls, GD 21), mortality in treated animals (half of the animals died PND 1-4 with dilatation of renal pelvis), surviving animals without nephrotoxicity.

Further, in **experiment 3,** Fritz (1988) exposed female rats (28/group) to 0 or 60 mg lithium carbonate/kg bw/d corresponding to 0 or 11.3 mg/lLi/kg bw/d during GD 16-20 with examination on PND 35-40. Maternal toxicity: Reduction in body weight gain (12.9% vs 19% weight gain in controls) and food consumption (20% compared to controls) as well as polyuria. The kidneys were normal by macroscopical examination. Foetal toxicity: No renal toxicity was reported in the offspring; however, a reduction in the litter size was seen (10.9 ±5.8, vs 16.0 ±2.1 in controls). The results from the study indicated that since maternal toxicity was reported following exposure to 60 and 100 mg/kg bw/d whereas renal toxicity in the offspring was only reported at 100 mg/kg bw/d, it could be considered that the renal toxicity was not secondary to maternal toxicity; however, it is noted that the maternal toxicity was more severe following exposure to 100 mg lithium chloride/kg bw/d compared to 60 mg/kg bw/d. RAC notes that effects on the developing kidney in rats was reported in the study by Christensen et al. (1982).

During the stakeholder consultation, information on a study by Christensen et al. (1982) was submitted. In this study functional and structural changes caused by lithium in the developing kidney in rats was studied following exposure to lithium chloride. Female Wistar rats (6/group) were exposed to 40 mmol/kg lithium in the diet for 4 weeks, followed by a dose of 60 mmol/kg lithium for another 4 weeks before mating with non-treated males for 2 weeks. The plasma lithium levels were 0.62 mmol/L the 4$^{th}$ week of pre-treatment, 1.15 - 1.47 mmol/L the 3$^{rd}$ week after delivery and 0.66 mmol/L the 5$^{th}$ week after delivery. In offspring the levels were from 0.5-0.85 mmol/L 3 and 8 weeks postnatally. After mating the dams were kept with their litters for 4 weeks after birth. After birth the dams were divided into 4 groups (3 dams with their litters/group): Half of control dams and their litters were given control diet (group C/C), and the other half 40 mmol Li/kg diet (group C/Li). Half of the lithium-treated mothers and their litters were given control diet (group Li/C), and the other half continued with lithium (group Li/Li). The pups were examined at 8 weeks of age. Lithium plasma levels were 0.5-1.0 mM/L 3 and 8 weeks postnatally. Maternal effects: During premating, water intake was statistically significantly increased in mother (9 and 50 mL/100 g/24 h in control and lithium-exposed group, respectively). During pregnancy, maternal body weight gain was decreased in exposed rats (64 g vs 98 g in controls). However, neither the litter size (10.8 versus 11.1) nor the average weight of the pups (6.2 versus 6.5 g) was significantly changed by maternal lithium exposure. No information was provided on the maternal body weight in the C/Li group. Foetal toxicity: In the C/Li and Li/Li groups severe structural changes, consisting of up to 3 mm cortical cysts, extensive interstitial fibrosis with cell infiltration, and atrophy of the cortical collecting ducts was reported. The kidneys from C/C and Li/C groups were normal. Functionally, in the C/Li and Li/Li groups growth retardation, polyuria with lowering of renal concentration ability, and uremia associated with as

much as 80% lowering of the normal glomerular filtration rate (GFR) was reported. However, in the Li/C group no effects on the concentration ability were reported. The study concluded that postnatal development of the rat kidney was particularly sensitive to nephrotoxic effects of lithium.

In the prenatal developmental toxicity screening study by Marathe and Thomas (1986) female Wistar rats were exposed by gavage to 0 (20 rats), 50 (11 rats), 100 (13 rats) mg lithium carbonate/kg bw/d corresponding to 0, 9.4, 18.79 mg Li/kg bw/d, once daily from GD 6 to GD 15 with examination on GD 20 (purity not provided). Maternal toxicity: No information available. Foetal toxicity: High dose group: reduced pup body weight, reduced implantations, increase in number of resorptions, reduced number of pups alive; see table below:

***Table:*** *Effect of exposure on rat foetuses, values expressed as mean±SD (Table AI – 13 from Annex 1 to the CLH report)*

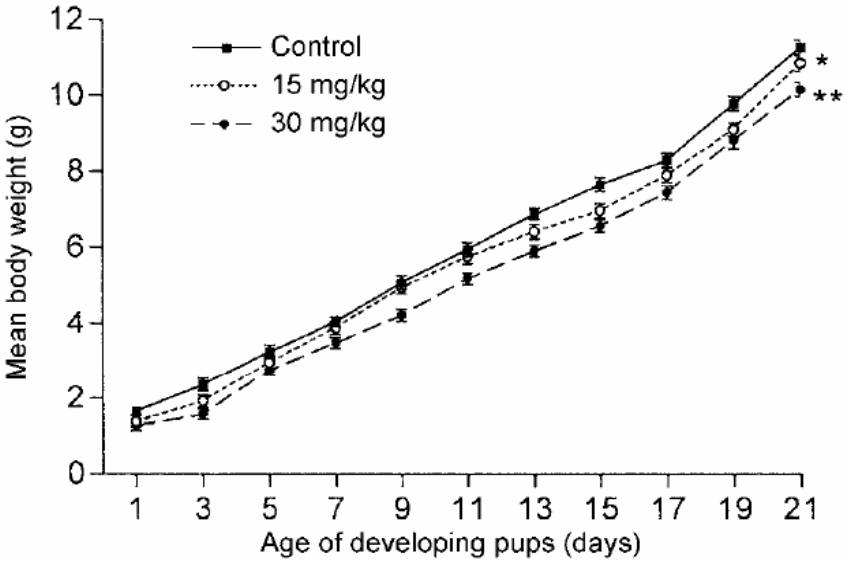
| **mg/kg bw/d** | **Implantations** | **Live pups** | **Resorptions** | | **Weight of pups (g)** | |
|---|---|---|---|---|---|---|
| | | | **Early** | **Late** | **Male** | **Female** |
| **0 (n=20)[a]** | 10.25±0.68 | 9.00±0.68 | 1.00±0.24 | 0.25±0.10 | 3.65±0.07 | 3.32±0.10 n=10 |
| **50 (n=13)** | 8.69±0.78 | 8.23±0.78 | 0.31±0.17 | 0.15±0.10 | 3.33±0.10 | 3.28±0.08 |
| **100 (n=11)** | 7.91**±0.71 | 4.73*±1.29 | 1.91±0.97 | 1.18±0.67 | 2.42*±0.40 n=8 | 2.24*±0.37 n=9 |

*p<0.01, **p<0.05; [a], number of dams

Following examinations of 95 foetuses in the control group, 107 foetuses in the 50 mg/kg bw/d dose group and 54 foetuses in the 100 mg/kg bw/d dose group, the following skeletal abnormalities were reported in the high dose group with no increase in the low- and mid-dose groups; incomplete ossification of sternebrae (39% vs 11% in control), shortening of several bones [radius and ulna (37% vs 0% in controls), humerus (37% vs 0% in controls), tibia and fibula (33% vs 0% in controls) and femur (41% vs 0% in control)], malformations of scapula (37% vs 0% in control) and pelvic bone (33% vs 0% in control). No HCD were provided. However, these findings could be related to retardation of ossification due to the decreased foetal pup body weight. Since no information regarding maternal toxicity was provided there are uncertainties as to whether maternal toxicity occurred. However, it is noted that in the developmental guideline study (Anonymous, 2010b, Van Deun et al. 2021) only slight maternal toxicity was observed at 90 mg/kg bw/d, a dose similar to the highest dose tested in the study by Marathe and Thomas, 1986.

*Studies in mice*

In a neurodevelopmental study comparable to OECD TG 426, Swiss Webster mice (at least 7 pregnant/dose) were exposed via drinking water to lithium chloride (analytical grade). The doses were 0, 90 and 180 mg lithium chloride/kg bw/d corresponding to 0, 15, 30 mg Li/kg bw/d from GD 1 to PND 15 with neurobehavior examination until PND 21. The lithium doses were calculated based on average total volume of drinking water consumed by the animals in 24 h and, the lithium doses per day dissolved in the water. However, no analytical verification of the doses was included (Abu-Taweel, 2012). Three pups/litter were examined/test. No information was included with regard to maternal toxicity and with regard to foetal toxicity, a dose-dependent statistically significant decrease in body weight gain from PND 1 to PND 21 was reported, see figure below from Abu-Taweel, 2012:




Further, a statistically significant delayed eye opening and appearance of body hair was reported; however, pups were also exposed during lactation when these endpoints were assessed. A decrease in sensory motor reflexes (righting reflex, rotating reflex and cliff avoidance) were seen in both sexes and was statistically significant from PND 1. In addition, in males (10/group, females not analysed), a statistically significant reduction in locomotor activity was reported in both doses tested at PND 22 evident as number of squares crossed (254, 115*, 79* in control, 90 and 180 mg/kg bw/d dose group), wall rears (26, 13*, 8* in control, 90 and 180 mg/kg bw/d dose group), rears (9, 6*, 2* in control, 90 and 180 mg/kg bw/d dose group), locomotion duration (239, 129 and 114 sec. in control, 90 and 180 mg/kg bw/d dose group). Further, the immobility duration was increased at weaning (61, 131, 186 sec. in in control, 90 and 180 mg/kg bw/d dose group). Biochemical parameters were studied in males (1 male/litter) at weaning and included a statistically significant dose-dependent decrease in liver acid phosphatase, liver alkaline phosphatase and brain acetylcholine esterase. The decrease in liver acid phosphatase and alkaline phosphatase may have led to variations in the phosphate pool of the pups during early development leading to a disturbed energy source available to the animals. This may have led to the reported disturbed physical maturation (body weight gain, eye opening and body hair appearance) sensory motor reflexes and behaviour activities.

The decrease in brain acetylcholine esterase may have led to disturbances in the behaviour of the animals. The authors of the study included that lithium is considered to affect neuronal communication (Phiel et al., 2001) and affect nerve excitation through the synthesis, activation, and inactivation of various neurotransmitters (Casado et al., 1989; Ghoshdastidar, 1999). Therefore, lithium exposure in the present study, could have produced developmental abnormalities in the brain of the exposed offspring leading to the observed teratological and behavioural effects reported in the offspring. However, it was noted that assessment of other neurotransmitters like catecholamines (serotonin, norepinephrine, dopamine, etc.) in the brain was not included which would be considered to give a better explanation for effects on behavioural. This study indicates that lithium chloride can induce neurodevelopmental toxicity following exposure during gestation and lactation. However, the study used few animals (7/dose group compared to OECD TG 426 with 20/dose group), included no neuropathology, and with no information on maternal toxicity.

In the prenatal and postnatal developmental toxicity study, albino mice (5 females/group) were exposed to lithium chloride 0 ans 1 mEq in drinking water corresponding to 0 and 10 mg lithium chloride/kg bw/d corresponding to 1.7 mg Li/kg bw/d from mating until end of weaning (Messhia, 1986). Maternal toxicity: no information. Foetal toxicity: 14 days after weaning a significant

decrease in brain weight in males and females, kidney weight in females, and testis weight in males was reported. The decrease in brain weight was confirmed after postnatal exposure, suggesting an effect on lactation.

In the prenatal developmental toxicity screening study in 129 Sv/SL mice, 16 female/dose, with no control group were exposed to 2 mg lithium carbonate/mL via drinking water corresponding to ca. 400 mg lithium carbonate/kg bw/d, and ca. 75 mg Li/kg bw/d. The mice were exposed from GD 1-18, and examination was on GD 17 or 18 (Smithberg and Dixit, 1982). The serum levels were 0.5-1.0 mmol/L. Results: Reduced number of litters (only 2/16 pregnant rats) with 60% resorptions, no further information. No external or skeletal malformations, visceral malformations no examined.

In a dose-range finding prenatal developmental toxicity study HaM/ICR mice (3-4 females/dose, no control group) were exposed by gavage to 200, 300, 465 mg lithium carbonate/kg bw/d corresponding to 37.6, 56.4, 87.4 mg Li/kg bw/d (purity not provided) from GD 6-15 with examination on GD 18 (Szabo, 1970). Maternal toxicity: No information. Foetal toxicity: High dose group; prenatal mortality (26%) and cleft palate in 11/37 mice (30%) in 3/4 litters. Mid dose group; cleft palates in 3/50 mice (6%) in 1/4 litters. Low dose group; no adverse effects reported.

In the main prenatal developmental toxicity study by Szabo (1970), HaM/ICR mice (15-20 females/dose, 16 females in control group) were exposed by gavage to lithium carbonate 0, 200, 465 mg lithium carbonate/kg bw/d corresponding to 0, 37.6, 87.4 mg Li/kg bw/d from GD 6-15 with examination on GD 18. Maternal toxicity: Mortality (37%) was reported in the high dose group. Foetal toxicity: High dose group; dead foetuses and resorption (32% vs 12.3% in controls), cleft palate (12/121 foetuses (10%) in 7/15 litters (control: 0/181, historical control: 6/2881 (0.2%)). Low dose group; cleft palate in 1/243 foetuses in 1/20 litters. The effects reported in the high dose group were considered to be secondary to maternal toxicity.

It is noted that induction of cleft palate in mice has also been reported by Loevy and Catchpole, 1973. In this study, CD1 mice received 15.5 mg of lithium chloride/mouse (about 620 mg lithium chloride/kg bw/d corresponding to 100 mg Li/kg bw/d) in sterile water by subcutaneous injection on 2 or 3 days from GD 11 to GD 13. The mice were sacrificed on day 17 of pregnancy. Maternal toxicity: No effects were reported. Foetal toxicity: An increased incidence of resorptions (11 – 21 vs. 4 in controls) as well as cleft palates were reported in the offspring injected on days 11, 12, and 13 (15.1%); on days 12 and 13 (7.2%); and on days 11 and 12 (3.4%).

Studies with two different strains of mice and different route of exposure (oral and subcutaneous) indicate that cleft palate may be induced following *in utero* exposure to lithium carbonate or lithium chloride (Loevy and Catchpole, 1973; Szabo, 1970). However, the finding was not confirmed in the study in A/J mice by Smithberg and Dixit (1982) following i.p. exposure to lithium carbonate, a strain especially sensitive for the induction of cleft palate; see further information below on studies with i.p. exposure. RAC notes the limited information regarding maternal toxicity and considers that the studies were of insufficient reliability. So, whether the induction of cleft palates was secondary to maternal toxicity or whether the inconsistent findings in the different strains were due to different dose levels of lithium is considered to be unclear, and RAC considers that limited weight should be given regarding the induction of cleft palate in mice.

*Studies in mice with i.p. route of exposure*

In a developmental toxicity study, 10 swiss albino mice/group were exposed by i.p. route to 25 mg lithium carbonate/kg bw/d from GD 10 to the end of lactation (Mostafa et al., 2010). Maternal toxicity: No information. Offspring toxicity: Increased body weight and diameter of seminiferous

tubules, decrease of primary spermatocytes count, nuclear diameter of Leydig cells, diameter of epididymis ductulus and testosterone level.

In a developmental toxicity study, A/J mice and 129SV mice were exposed to lithium carbonate by i.p. injections on single or repeated days of gestation. The doses were 0, 0.8, 1.6, 3.2, 5.0 mg lithium carbonate per animal (0, 32, 64, 128, 200 mg lithium carbonate/kg bw/d corresponding to 0, 6.0, 12.0, 24.0 and 37.5 mg lithium/kg bw/d) (Smithberg and Dixit, 1982). Serum measurements revealed that 0.8 mg lithium carbonate per animal by i.p. injection resulted in serum concentrations similar to the therapeutic range (0.5-1.0 mmol/L). Maternal toxicity: Not reported. Foetal toxicity: No effects up to 128 mg/kg bw/d. In the high dose group (about six-fold higher than therapeutic serum concentrations), an increased incidence of malformations (fused ribs, and/or vertebral defects and exencephaly) were reported, especially after exposure on GD 9 (19.3%, 41.6%, 17.1% malformation after exposure on GD 8, 9, 10, respectively). Results obtained in A/J mice were similar to the effects seen in 129 SV mice. The study has shortcomings including that control animals were only exposed on GD 9 and visceral malformations were not examined.

In a study CD-1 mice were exposed by single i.p. injection to 300 mg lithium carbonate/kg bw corresponding to 54.4 mg Li/kg bw/d (Giles and Bannigan, 1997). No information was available regarding the day of exposure. Maternal toxicity: No maternal effects reported. Foetal toxicity: An increased incidence of resorptions (19%) and a 2% increased incidence of open cranial neural tube defects was reported compared to controls (0.5% and 0%, respectively). According to Jurand (1988) similar findings were reported in JBT/JD mice after single i.p. injection with concentrations above 330 mg lithium carbonate/kg bw on GD 9. However, these data are not regarded as relevant for the classification, because according to the authors high peak serum levels of 9.8 mM were reached one hour after i.p. treatment.

Overall, RAC considers that studies with i.p. exposure to lithium carbonate during pregnancy leading to high peak exposure, and resulting in developmental toxic effects, including neural tube defects and exencephaly are not regarded as relevant for the assessment of developmental toxicity following exposure to lithium chloride.

*Study in New Zealand white rabbits*

In a prenatal developmental toxicity study, New Zealand white rabbits (10 females/dose) were exposed via oral capsule to 0, 0.675, 1.08 mmol lithium carbonate/kg bw/d corresponding to 0, 49.51, 79.8 mg lithium carbonate/kg bw/d or 0, 9.30, 14.99, mg Li/kg bw/d, once daily from GD 5 to 18, with examination on GD 28. The plasma concentration in the highest dose group was 1.5-2.4 mM (Gralla and McIlhenny, 1972). Maternal toxicity: Mortality in high dose group (3/10) and 1 dam in low dose group died for unknown reasons. Foetal toxicity: No effects reported on number of implantation sites, mean litter size and body weight. No grossly visible internal or skeletal effects reported.

*Study in pigs*

In a pre- and postnatal toxicity study, pigs (12 females/group) were exposed in the diet to 0 and 3000 mg lithium carbonate/kg diet corresponding to approximately 0 and 40 mg lithium carbonate/kg bw/d and approximately 0, 7.5 mg Li/kg bw/d, from GD 30-114, with observation until PND 21. The serum lithium concentrations on GD 60 were 1.4 mEq/L and on GD 110 2.3 mEq/L (Kelley et al., 1978). Maternal toxicity: Decreased body weight gain, significant at GD 110 (23% reduction, no further information available). Five out of 12 exposed pigs did not complete pregnancy. Offspring toxicity: Prenatal mortality increased (adjusted mean number of live piglets/litters: 9.6 in exposed vs 11.3 in control, adjusted mean number of stillbirths and mummies/litter: 2.1 in exposed vs 0.6 in control), reduced litter birth weight (11.1 vs. 15.4 kg in exposed and control, respectively), reduced survival of offspring during lactation (6.5 vs. 8.0

in exposed and control, respectively). RAC considers that due to the design (only one dose) and limited reporting the study is of limited validity, but is considered to support the finding in Wistar rats including that gestational exposure to lithium carbonate might cause severe developmental effects (e.g. increased number of stillbirths, and reduced postnatal survival reported in the study by Fritz, 1988 and Marathe and Thomas, 1986).

*Study in Rhesus monkey*

In a prenatal developmental toxicity study, Rhesus monkeys (6 females in exposure group, 5 females in control group) were exposed via oral capsule to 0 or 0.67 mmol lithium carbonate/kg bw/d corresponding to 0 or 49.51 mg lithium carbonate/kg bw/d or 0 or 9.30 mg Li/kg bw/d, once daily from GD 14 to 35 (purity not provided). Observation until PND 30 and up to 12-15 month of age (Gralla and McIlhenny, 1972). Maternal toxicity: No effects reported. Offspring toxicity: No adverse effects reported on development: no visible malformations and no signs of functional neurological defects. Normal growth and no physical defects clinically were reported at 12-15 month of age.

**Summary of the animal study findings**: In the OECD TG 414 study in rats no developmental toxicity was reported but the highest dose tested induced only slight maternal toxicity. However, some of the developmental toxicity studies, although not performed according to OECD TG 414, indicated that lithium may induce developmental toxicity, including reduced pup body weight, decreased litter size, neurobehavioral effects and nephropathy as well as delayed ossification and malformations, however, often reported at doses which were potentially maternally toxic. Few studies provide sufficient information on maternal toxicity and/or lithium plasma concentrations, which therefore limits the interpretation of these studies. Therefore, in these studies it is not possible to conclude if the effects seen in pups were secondary, non-specific effect of maternal toxicity. Studies in rats indicated an effect on the kidney in the offspring; however, effects on the kidney were reported at doses inducing marked maternal toxicity including mortality. On the other hand, an effect on the kidney could be considered as substance-related since the kidneys is one of the target organs of lithium toxicity. Studies in mice point to a neurotoxic effect following *in utero* exposure to lithium chloride, however, with no information on maternal toxicity and few animals/dose groups. RAC notes that no effects on neurodevelopment were reported in two epidemiological studies following exposure to lithium during pregnancy. However, the epidemiological studies investigating neurodevelopmental effects were limited and of questionable quality.

Overall, the experimental animal database, which is limited with respect to the quality of the studies, does not provide clear and consistent evidence of developmental toxicity following *in utero* exposure to lithium. Especially, the OECD 414 TG study did not report developmental toxicity, but the highest dose tested induced only slight maternal toxicity. However, RAC notes that there are some concerns for neurodevelopmental effects reported in rats and mice as well as decreased pup body weight and litter size. In addition, in several studies it was not possible to conclude if the effects seen in pups were a secondary, non-specific effect of maternal toxicity. Therefore, RAC considers that the results from the animal data could support a classification for developmental toxicity.

Human data

*Cardiac malformations*

The concern for potential teratogenic effects following exposure to lithium during pregnancy emerged from studies published around 1970. Retrospective studies, based on the Register of Lithium Babies founded in 1968 (Weinstein, 1976) included children from women who had been treated with lithium during the first trimester of pregnancy (Schou et al., 1973; Weinstein, 1976; Weinstein and Goldfield, 1975).

The Schou et al. (1973) study, retrospectively included information from 118 children born to mothers given lithium treatment during the first trimester of pregnancy (data from the Register of Lithium Babies). Out of the 118 children included in the study, five were stillborn and seven died within the first week of life; six of these 12 children were malformed. The total number of malformed children was nine with 2 having cardiac malformations of Ebstein's type. One of these 2 mothers were treated with another drug. However, the study authors noted that if a baby exposed to lithium during pregnancy is stillborn, malformed or dies after birth it is more likely to be reported to the Register than if it is alive and normal. Further, little attention may be paid to drugs taken during pregnancy by mothers giving birth to normal children, whereas birth of an abnormal child leads to more complete reporting. Incomplete and selective reporting is therefore probable. It was therefore likely that the reported frequencies of stillbirth, congenital malformations, and other anomalies were higher than the true frequencies among children of lithium-treated women.

A later study by Weinstein (1976), also including data from the same international Register of Lithium Babies, collected data from cases of lithium treatment during pregnancy from USA, UK, Canada, Denmark, Sweden and Switzerland. The criteria for inclusion in the Register of Lithium Babies was (a) that there was exposure to lithium in the first trimester, and (b) that the conceptus was available for morphological examination. 166 mother-child pairs meeting the criteria were included in the study and 18 malformed children were reported with 13/18 children having malformations in the cardiovascular system including 4 cases with Ebstein's anomaly (it is assumed that these numbers include the data from the study by Schou et al., 1973). Six of the malformed infants had been exposed only to lithium. It is noted that abnormal babies were more assiduously reported than normal babies and that serious abnormalities (such as Ebstein's anomaly) were reported more completely than less serious defects. The author concluded that the maximum frequency of congenital malformations reported to the Register (10.8%) did not substantially exceed the expected incidence of such malformations in the general (non-lithium-treated) population.

Later, case-control studies did not identify an association between congenital, especially cardiovascular malformations, and lithium exposure (Correa-Villasenor et al., 1994; Edmonds and Oakley, 1990; Kallen et al., 1988; Sipek et al., 1989; Zalztein et al., 1990). It is noted that the studies were small and lithium exposure is relatively rare among pregnant women. Consequently, these studies either had only a few or did not have any lithium-exposed women among cases and controls and the power to detect an effect was limited. Cohort studies provided contradictory results, and case reports pointed to perinatal complications due to gestational exposure to lithium.

Below is a description of more recent epidemiological studies examining the developmental toxicity (including teratogenicity and miscarriage) following lithium exposure of pregnant women. Most of the studies are case reports. However, some cohort and case control studies are available, with most of them being retrospective studies, only a few of them are prospective studies.

A meta-analysis combining data from 6 cohort studies with a total of 727 pregnancies with lithium exposure and 21 397 reference pregnancies in mothers with a mood disorder, but without lithium exposure, was performed by Munk-Olsen et al. (2018). The cohorts included pregnancies resulting in live-born singleton deliveries from 1997 to 2015, where health-related information was available both for the mother and for the infant. The analysis was performed following a shared protocol established a priori to minimise heterogeneity related to selection criteria, exposure, outcome and covariate definitions, and statistical methodology. The odds ratios (OR) were adjusted for maternal age at delivery (in years), primiparity, calendar year of birth, and treatment with any other psychotropic medication during pregnancy. Furthermore, pregnancies in which mothers were prescribed known teratogenic medications in pregnancy were altogether excluded from the analysis. The study showed that lithium treatment during pregnancy was not

associated with preeclampsia, foetal distress, or postpartum haemorrhage. Further, no difference between groups were observed for caesarean section, preterm birth, low birth weight, or small for gestational age. Lithium exposure during the first trimester (654 out of 727 children (90%) was exposed during the first trimester) was associated with a statistically significantly increased risk of major malformations such as cardiovascular defects, neural tube defects, hypospadias and epispadias (7.4% vs 4.3%; pooled adjusted OR = 1.71, 95% CI: 1.07-2.72), but not for major cardiac malformations (2.1% vs 1.6%, pooled adjusted OR = 1.54, 95% CI: 0.64-3.70). No Ebstein's anomaly was reported in the meta-analysis. It is noted that 74.8% in the lithium-treated group and 61.2% in the reference group used other psychotropic drugs, but as explained the ORs were adjusted for the use of such drugs. The authors concluded that their results suggest an association between lithium exposure and major malformation, but that this association was much smaller than previously reported. The study is considered as a good quality study with robust methodology; however, no serum lithium levels were measured, so no dose-response analysis of the effects could be performed.

A cohort study including 1 325 563 pregnancies between 2000 and 2010 delivering live-born infants included 663 women exposed to lithium during the first trimester of pregnancy (Patorno et al., 2017). Exposure was defined based on the prescription of lithium during the first trimester. The outcomes investigated were cardiac malformation, major congenital malformation, and noncardiac congenital malformation. The authors considered the following covariates as potential confounders: maternal age at delivery, race or ethnic group, year of delivery, smoking status, maternal psychiatric disorders and medical conditions, concomitant medication use, and general markers of the burden of disease, including the Obstetric Comorbidity Index and measures of the intensity of health care use. These parameters were included in the statistical analysis. Women with exposure to known teratogens during pregnancy were excluded from the study. Comparison of lithium-exposed mothers was made separately to two reference groups: (1) all pregnancies and (2) pregnancies of mothers with bipolar disorder treated with lamotrigine. Patients who were exposed to both lithium and lamotrigine during the first trimester (67 patients) were excluded. Cardiac malformation was reported in 16 of the 663 infants exposed to lithium (2.41%) and 15 251 of the 1322 955 nonexposed infants (1.15%). A correlation between lithium exposure early in pregnancy and cardiac malformation was reported. This was similar to the frequency of cardiac malformations in lithium-exposed infants reported in the study by Munk-Olsen et al. (2018) (2.1%). The adjusted risk ratio for cardiac malformations among infants exposed to lithium as compared with unexposed infants was 1.65 (95% CI = 1.02-2.68).

When calculated for each dosage band, the risk ratio (RR) was

1.11 (95% CI = 0.46-2.64) for a daily dose of 600 mg or less,

1.60 (95% CI = 0.67- 3.80) for a daily dose of 601 to 900 mg, and

3.22 (95% CI = 1.47-7.02) for more than 900 mg daily,

This shows a clear dose-response relationship. The prevalence of right ventricular outflow (RVO) tract obstruction defects (including Ebstein's anomaly) was 0.60% among lithium-exposed infants versus 0.18% among unexposed infants (adjusted RR: 2.66; 95% CI, 1.00 to 7.06 with daily doses of more than 600 mg lithium). Results were similar when lamotrigine-exposed infants were used as the reference group. The authors concluded that maternal use of lithium during the first trimester was associated with an increased risk of cardiac malformations, including Ebstein’s anomaly, also showing that this association was dose-dependent. However, because of the previously postulated association with lithium and cardiac malformations, cardiac malformations may have been misclassified and/or preferentially investigated in infants exposed to lithium during pregnancy, resulting in an overestimated true effect of lithium exposure. The authors took this possibility into account and used a validated definition of major cardiac malformations. The outcome definition focused on major cardiac defects that were likely to be clinically consequential

for the infant and was further restricted to malformations that were recorded several times or had required surgery. On the other hand, an underestimation of the cardiac malformations is also possible since the cohort was restricted to live births, and spontaneous abortions or planned terminations due to congenital malformations that were diagnosed early in pregnancy were missed. It has been shown that therapeutic abortions may be 5-10% higher among pregnant women treated with lithium than among pregnant women not treated with lithium (Poels et al., 2020; Diav-Citrin et al., 2014; Jacobson et al., 1992). The association between lithium exposure and cardiac malformation was smaller in Patorno et al. (2017) than had been previously postulated. The study is considered as a good quality study based on a substantial cohort.

In a prospective, comparative, observational cohort study 183 lithium-exposed pregnancies of women who contacted the Israeli Teratology Information Service were followed and compared to 72 disease-matched and 748 non-teratogenic-exposed pregnancies (Diav-Citrin et al., 2014). The rate of total major congenital anomalies excluding chromosomal or genetic conditions did not differ significantly between the three groups (6.5% lithium-exposed group, 3.3% bipolar disorders group, 2.7% non-teratogenic exposure group). Cardiovascular anomalies were reported more frequently in infants exposed to lithium during the first trimester including one case of Ebstein's anomaly when compared to the non-teratogenic exposure group, but not so clear when compared to the bipolar disorder group (4.1% in lithium-exposed group, 3.3% in bipolar disorders group and 0.6% in non-teratogenic exposure group). However, when excluding spontaneously resolved cases of cardiovascular anomalies, the frequencies were: 2.4% in lithium-exposed group, 1.6% in bipolar disorders group and 0.3% in the non-teratogenic exposure group. The odds ratios (OR) were adjusted for pregnancy order, smoking and the existence of bipolar disorder. For cardiovascular malformations the adjusted OR was

4.75 (95% CI = 1.11-20.36) in the lithium-treated group and

5.43 (95% CI = 0.93-31.90) in the bipolar disorder group.

The increase in cardiovascular anomalies in the lithium group was only statistically significant if both the persistent and spontaneously resolving cardiovascular anomalies were taken into account. The incidence of miscarriage was also assessed in this study with an increase of 16.4% reported in lithium-treated mothers compared to 8.3% in the bipolar disorder group and 5.7% in the non-teratogenic exposure group. It is noted that about 58% of the lithium-exposed mothers were treated with a mean dose of lithium of 906 mg throughout pregnancy and not only during first trimester. Concurrent psychiatric medications were taken by 66.1% of the cohort. Further, the study relies on maternal interview and their recollection of medical records in most cases. This study is considered as a supportive study due to the limited number of pregnancies exposed to lithium included.

During the stakeholder consultation information on a retrospective cohort study by Poels et al. (2020) was submitted which also investigated miscarriage due to lithium exposure during pregnancy. The odds ratio adjusted for the age at conception and the clustering of pregnancies per woman was calculated. In the crude unadjusted analysis, miscarriages occurred in 20.8% of the lithium-exposed pregnancies compared to 10.9% in the unexposed pregnancies with an OR = 2.14; (95% CI: 1.13–4.06, p = 0.018). After adjusting for the age at conception and the clustering of pregnancies per woman, the odds ratio of miscarriage after lithium use during pregnancy was OR = 2.94; 95% (CI: 1.39–6.22, p = 0.005). It is noted that lifetime valproate or carbamazepine use was present in 26% of the lithium-exposed pregnancies and in 21% of the pregnancies not exposed to lithium. Furthermore, pregnancy outcome and medication were collected retrospectively by questionnaire and no information regarding smoking, alcohol and substance use at the time of miscarriage was available. This study is considered as a supportive study on miscarriage following *in utero* exposure to lithium but has no reporting on the induction of cardiovascular malformations.

In a review and meta-analysis including cohort studies, case-control studies and case reports, McKnight et al. (2012) studied the lithium toxicity profile published between 1966 and 2010. Out of 385 studies, 62 publications assessed the teratogenic potential of lithium (7 cohort studies, 7 case-control studies and 48 case reports). Six case-control studies (n=264) assessed the association between Ebstein's anomaly and *in utero* exposure to lithium. The study recorded no significant increase of congenital malformations. The number of Ebstein's anomaly did not differ significantly from controls; however, estimates were considered as uncertain due to the low number of events (OR = 2.0; 95% CI (0.20-20.6). In one case-control study 10 698 infants born with any major congenital abnormality and 21 546 healthy controls showed no significant association between *in utero* lithium exposure and congenital abnormalities (OR= 2.62; CI 95% (0.74-9.20)). However, the number of infants exposed to lithium was low in cases (6 of 10 698) and controls (5 of 21 546). It is noted that many of the studies were also discussed in Yacobi et al. (2008), see below, with some differences between the selections, not only due to timeline. The conclusion of the authors was nevertheless very similar with Yacobi and Ornoy (2008): The evidence that lithium is teratogenic is quite weak, and the findings showed that the risk has been previously over-estimated. However, due partly to heterogeneity in the results, uncertainty remains, the risk cannot be ruled out, and lithium have to be avoided during pregnancy according to the authors. This study is considered as a supportive study due to the limited number of pregnancies exposed to lithium included.

In a review by Yacobi and Ornoy (2008) human studies published between 1969 and 2005 assessing the teratogenic and embryotoxic effects of gestational exposure to lithium were included. The review included 24 case reports where 9 of the infants were born to mothers treated with lithium only (1/9 was stillborn). The other 15 pregnant women also received other drugs. Cardiac anomalies were reported in 2/8 lithium only exposed liveborn children, one with Ebstein's anomaly and one with patent ductus arteriosus. However, no information was included regarding the number of lithium-treated women with normal children. The study also reported perinatal toxicity in 78% (19/23) of the liveborn children exposed to lithium including higher rate of pre-maturities, higher birth weight, goitre, respiratory distress, cyanosis, hyporeflexia and diabetes insipidus. However, most of these effects were transitory and lasting from hours to a few weeks, partly due to the prolonged half-life of lithium in the new-born's serum. These effects were in several cases reported when serum lithium levels in the new-borns were below 1 mM/L, however, in many cases it was much higher, with the highest level being 46 mM/L. This study is considered to be of limited value due to the low number of pregnancies with lithium exposure included and that no information regarding the number of lithium-treated women with normal children was provided.

Källen and Tandberg (1983) performed a retrospective cohort study from 1973-1979. The study included 350 mother-child pairs with mothers having a previous history of inpatient ward treatment due to manic depressive disease. Maternity health care records were available for 82% of the pairs and were used to divide them into groups of (1) no indication of psychiatric illness before pregnancy (N=110), (2) psychiatric illness but no indication of drug use (N=80), (3) psychotropic drug use, but not lithium (N=38), (4) lithium use only (N=41) and (5) lithium and other psychotropic drug use (N=18). In women treated only with lithium 4/41 had neonatal deaths, 5/41 had malformed infants, 2/41 had dead and malformed infants and 3/41 had heart defects (no Ebstein’s anomaly). The numbers of same outcomes were reported for the other groups mentioned above, but no statistical testing was performed to compare the groups. Heart defects were also observed in the no drug group (2/80, of which one was related to a Down’s syndrome) and in the group exposed to both lithium and other drugs (1/18). There were differences in prevalence of smoking between the groups (those with lithium or other drug treatment tended to be more often smokers). However, as there was no statistical testing or risk estimation overall between the groups, also the potential confounding effect of smoking or any other potential confounders was not analysed. It was reported separately that when the entire

cohort was compared to all Swedish pregnancies, adjusted for age, parity and hospital district, the frequency of heart defects was increased just reaching statistical significance, but no numerical values were presented. Such a comparison was not reported for the lithium-exposed part of the cohort, while the authors noted that 4 of the 6 cardiac defects occurred among infants exposed to lithium, either alone or together with other drugs. This study is considered to be of limited value due to the lack of proper comparison between lithium-exposed and unexposed groups with relatively small numbers of cases as well as lack of control for potential confounding by other factors.

In a later note entitled *"Comments on teratogenic update: Lithium"* a possible association with lithium exposure during pregnancy and Ebstein's anomaly was studied in a joint case-control study on infants with Ebstein's anomaly or tricuspid atresia with two controls without cardiac defects for each case, matched for age and parity (Källen, 1988). Information was obtained from four programs in countries where lithium was used to treat manic-depressive illness (New Zealand, Hungary, Sweden and Denmark) and included 69 cases and 128 controls. Seven of the 69 case infants and two of the 128 controls also had extracardiac malformations. Drug exposures were identified by interviews or, in Sweden, from prospectively collected information on drug use during early pregnancy. Lithium exposure during pregnancy was not reported among the cases. This was supported by adding 15 cases of Ebstein's anomaly from a French monitoring system where drug exposure was reported. However, also none of these 15 cases reported exposure to lithium during pregnancy. When considering use of any of the drugs investigated, there was no statistically significant association between the drug use and Ebstein's anomaly (OR = 0.93; CI 95% (0.5-1.8)). This study is considered to be of limited value due to lack of reporting of confounding factors.

**Overall,** the epidemiological studies concluded that lithium therapy adds only a small risk for cardiovascular malformations including Ebstein's anomaly and does not increase the general rate of major anomalies. Furthermore, when reviewing the data accumulated until today the risk is lower than previously anticipated from the Register of Lithium babies. Although the association is weak, RAC still considers the increase in cardiac malformations to be reliable evidence, since these are rare malformations and the population at risk, the pregnant women under lithium therapy will never contribute to a high number of cases. However, the impact of lithium exposure during pregnancy may on the other hand have been underestimated since a higher rate of therapeutic abortions has been reported in lithium-treated pregnant women (10% vs 6% in Jacobson et al., 1992). RAC also notes that no cardiovascular malformations were reported in the experimental developmental toxicity studies in animals.

*Neurodevelopmental effects*

Two studies focused on neurodevelopmental effects in infants exposed *in utero* to lithium. In a systematic review and meta-analysis focusing on neurodevelopmental effects following intrauterine exposure to lithium including 7 preclinical studies, 3 cohort studies, and 5 case studies no effect on neurodevelopment was reported. However, the study had many confounding factors including among others that exposed children were compared to non-exposed children with no history of psychiatric illness (Poels et al., 2018). Analysis of human studies lead to the conclusion that "*In humans, the existence and nature of any effects remains poorly determined. At present, there is insufficient evidence to estimate the neurodevelopmental effects of intrauterine exposure to lithium.*" However, it is noted that studies investigating neurodevelopment are limited and of questionable quality.

In a cohort study by Van der Lugt et al. (2012) the long-term effect of lithium on children neurodevelopment was studied. Fifteen children born between 1994 and 2007 that were exposed to lithium *in utero*, but not breastfed, were studied at 3-15 years of age. The mother's serum lithium levels were 0.6-0.8mmol/L during pregnancy. Tests on neurological or cognitive

development as well as the parent's response on the child's behaviour checklist was included. No adverse effects on growth, neurological, cognitive and behavioural development was reported. However, the group of children studied was small, with no appropriate control group, and other medication besides lithium was used by the pregnant mothers.

*Summary epidemiological studies*

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

The available human epidemiological studies are considered to be of variable quality; however, some of the studies are considered as well conducted.

In the seventies, retrospective studies, based on the Registry of Lithium Babies, i.e. children from women who had been treated with lithium during the first trimester of pregnancy, indicated an association between first trimester lithium exposure and cardiac malformations (Schou et al., 1973; Weinstein and Goldfield, 1975; Weinstein, 1976). However, the studies are not considered robust enough to prove this association. The studies had methodological deficiencies and the results are therefore difficult to interpret. They did not assess if cardiovascular anomalies occurred more frequently in infants exposed to lithium during pregnancy compared to non-exposed infants. Further, in several cases malformations were reported in infants exposed *in utero* to other drugs in addition to lithium.

In more recent robust studies, a more precise pattern of the effects in infants exposed to lithium during pregnancy has emerged. The review by Yacobi et al. (2008), meta-analyses by McKnight et al. (2012) and Munk-Olsen et al. (2018) and cohort study by Patorno et al. (2017) lead to very similar conclusions, i.e., that the association between lithium exposure during pregnancy and cardiac malformation was quite weak. They found an association, however, with a risk lower than previously reported. Especially, Patorno et al. point to a risk of cardiac malformation at high therapeutic doses, with a clear dose-response relationship. The relatively weak association could be influenced by the higher rate of spontaneous or therapeutic abortions of woman under lithium treatment, which was not taken into consideration by the authors of these studies and could lead to an underestimation of developmental effects of lithium.

***Table**. Summary of malformations observed in the most recent review and meta-analysis (McKnight) and the original studies published after that. The risk estimates in bold are statistically significant.*

| Malformation type | | | McKnight et al. (2012) | Patorno et al. (2017) | Munk-Olsen et al. (2018) | Diav-Citrin et al. (2014) |
|---|---|---|---|---|---|---|
| | | | OR | RR | OR | OR |
| All | | | 2.62 (0.74–9.20) | **1.37** (1.01–1.87) | **1 ·71** (1·07–2·72) | - |
| | Non-cardiac | | - | 1.22 (0.81–1.84) | - | - |
| | Cardiac | | - | **1.65** (1.02-2.68) [1.1 (0.46-2.64) daily dose of 600 mg or less, 1.60 (0.67- 3.80) daily dose of 601 to 900 mg **3.22** (1.47-7.02) daily dose of more than 900 mg] | 1·54 (0·64–3·70) | **4.75** (1.11–20.4) |
| | | Right ventricular outflow tract obstruction defect (RVO) | - | **2.66** (1.00–7.06) | - | - |
| | | Other than RVO | - | 1.46 (0.84–2.57) | - | - |
| | | Ebstein | 2.0 (0.20 – 20.6) | Not reported [1] | - | - |

[1] RVOs were not coded as Ebstein, however most of them were compatible with the characteristics of Ebstein

In the prospective cohort study by Diav-Citrin et al. (2014) and the retrospective cohort study by Poels et al. (2020) an increase in miscarriage was reported in lithium-treated mothers compared to mothers with bipolar disorders. However, limited information was available regarding possible confounding factors in the studies.

In the systematic review and meta-analysis by Poels et al. (2018) and cohort study by van der Lugt et al. (2012) assessing neurodevelopmental effects following *in utero* exposure to lithium, no clear association was found. However, the studies investigating neurodevelopmental effects are limited and of questionable quality.

It is noted that on lithium-based drug labels, it is clearly stated that an increase in the overall rate of malformations has been observed in children exposed *in utero* to lithium and that discontinuation of treatment should be considered until the 9th week of amenorrhea.

**In conclusion,** RAC is of the opinion that in a weight of evidence assessment, and taking into account that classification is hazard based, the epidemiological studies showing weak evidence of an increase in rare cardiac malformations in infants exposed to lithium during the first trimester of pregnancy should be considered for the classification of lithium. Especially, the study by Patorno et al. (2017) including appropriate controls, a balanced assessment, and due consideration of bias or confounding factors is considered robust and relevant for classification. The study concluded that maternal use of lithium during the first trimester is associated with an increased risk of the cardiac malformation evident as right ventricular outflow tract obstruction defect, compatible with Ebstein's anomaly, also showing that this association is dose-dependent. This is supported by the reported findings of the other recent and robust epidemiological studies, especially the large study by Munk-Olsen et al. (2018) and also the smaller study by Diav-Citrin et al. (2014). It is noted that the earlier studies described above and included in the analyses by Yacobi et al. (2008) and McKnight et al. 2012 have methodological deficiencies and are difficult to interpret quantitatively, but do not contradict the observations of the more robust studies. Cardiac malformations are considered as serious, although a rare malformation. It is noted that there is a limited number of pregnancies where lithium has been used during the first trimester. Therefore, the finding of cardiac malformations should not be dismissed. RAC notes that a classification for developmental toxicity is supported by experimental animal studies where some concerns for neurodevelopmental effects in rats and mice as well as decreased pup body weight

and litter size were reported. RAC is of the opinion that a **classification as Repr. 1A; H360D for the three lithium compounds is warranted** based on the human epidemiological data.

***Adverse effects on or via lactation***

Experimental animal data:

In Sprague Dawley rats, Ahmed et al. (2018) observed the presence of lithium in breast milk and in the mammary gland in female rats exposed to lithium (n=6, one lactating rat and 5 virgin rats).

Very few studies have investigated effects of lithium exposure exclusively via lactation. In the study in rats by Teixeira et al. (1995) described in the developmental toxicity section, a statistically significant lower body weight was reported at PND 21 in pups where dams were exposed to 53 mg/kg bw/d lithium chloride or 8.83 mg Li/kg bw/d only during lactation. The study also reported a delay in the critical day of maturation in groups exposed during lactation (day of eye opening and avoidance of visual cliff) compared to the control group. However, as this effect was also observed in the group exposed only during gestation, and in the water-deprived group, it is difficult to assess if the effect was related to *in utero* or lactational exposure to lithium.

In the study by Ibrahim and Canolty (1990), rats were exposed to 50 mg lithium carbonate/kg bw/d corresponding to 9.4 mg Li/kg bw/d, with one group only receiving lithium carbonate during lactation (5-7 rats). The study is also described in the developmental toxicity section. At the end of lactation, the mean pup weight was significantly decreased in pups exposed during lactation only (58 g and 44 g in control and lithium groups, respectively). Heart weight (0.31 g and 0.24 g in control and lithium-exposed group, respectively) and spleen weight (0.23 g and 0.17 g in control and lithium-exposed group, respectively) was also decreased in the pups exposed to lithium carbonate during lactation only. There is no information available to reveal if the organ weights were absolute or relative to body weight. However, maternal toxicity observed as decrease in body weight gain and food consumption, decreased absolute liver weight and relative liver, kidney and heart weight were reported in dams exposed to lithium during the entire study or only during lactation.

In a third prenatal and postnatal developmental toxicity study also described in the developmental toxicity section, albino mice (5 females/group) were exposed to 0, 10 mg/kg bw/d lithium chloride corresponding to 1.7 mg Li/kg bw/d in drinking water from mating until end of weaning (Messhia, 1986). The study had few animals/dose group and limited details were provided by the authors. No information on maternal toxicity was given. In pups, a significant decrease in brain weight in males and females, kidney weight in females, and testis weight in males was reported 14 days after weaning. The decrease in brain weight was confirmed after postnatal exposure, suggesting an effect on lactation.

During stakeholder consultation, information on a study by Christensen et al. (1982) was included. In this study, functional and structural changes caused by lithium in the developing rat kidney was studied. Further description of the study design is included in the developmental toxicity section. In the groups exposed to lithium pre- and postnatally or only postnatally, structural changes consisting of up to 3 mm cortical cysts, extensive interstitial fibrosis with cell infiltration, and atrophy of the cortical collecting ducts was reported, whereas the control group and the group exposed to lithium only prenatally was normal. Functionally, in the groups exposed to lithium pre- and/or postnatally growth retardation, polyuria with lowering of renal concentration ability, and uremia associated with an 80% lowering of the normal glomerular filtration rate was reported. However, in the group exposed to lithium only prenatally no effects were reported. The

study concluded that the postnatal development of the rat kidney was particularly sensitive to nephrotoxic effects of lithium.

The study by Hsu et al. (1978) was mentioned in several reviews, but not available to the DS. In this study, 13 pregnant McCollum strain rats were exposed to 20 mM lithium in drinking water. The corresponding plasma levels were assumed to be 1.5 to 2.0 mM, based on Trautner et al. (1958) who used the same species, dose, and exposure route. At birth, three pups each from three control litters were switched to dams on lithium treatment, and three pups treated with lithium prenatally were switched to control dams. The study reported that postnatal lithium exposure delayed development, measured by age at eye opening and weaning weight. Maternal toxicity was reported in dams exposed to lithium postpartum as decreased water consumption and weight gain. Two tests of learning and memory, performed after lithium treatment ended, showed a decrease in performance in rat pups that had either prenatal or postnatal exposure to lithium.

In a recent study by Ahmed et al. (2021), provided after the stakeholder consultation, effects on the developing pups were studied following exposure to lithium carbonate only during lactation (from PND 4 to PND 21). The pups were assessed on PND 18, 25 and 60. Nursing Sprague-Dawley rats (11 rats/group) received lithium at human therapeutic levels ~0.5 mmol/L (1000 mg/12 h/50 kg corresponding to 40 mg lithium carbonate/kg bw/24h or 7.2 mg Li/kg bw/24h). The control group received water. The pups (12 pups/litter) were studied for effects on pup body weight, kidney function (assessed as BUN (blood urea nitrogen)), and thyroid function (assessed as levels of T4, free T4, free T3, TSH), shortly after, and long after breast-feeding. Further, pups were behaviourally assessed using a forced-swim test on PND 18. The level of lithium in plasma was measured in the pups at PND 18. Results: Lithium was transmitted through breast milk and was measured in the pup plasma (0.075 mmol/L). At PND 18 the pups showed increased body weight (42.5 vs 40.31 g in control pups, $p < 0.05$), increased TSH (1.38 vs 0.94 mlU/min in control pups $p<0.05$) reduced blood thyroxine (T4) (67.2 vs 93.41 nmol/L in control pups, $p < 0.05$), and elevated blood urea nitrogen (BUN) levels (6.71 vs 5.25 nmol/L in control pups, $p < 0.05$), indicating thyroid and kidney impairment, while mothers had low therapeutic blood lithium levels (in the human therapeutic ranges). The swim time measured at PND 18 was slower in lithium-exposed pups, however, not reaching statistically significance (447 vs 492 sec. in controls). A transient increase in BUN was observed, suggesting reduced kidney function that resolved shortly after weaning and lithium clearance. In the thyroid, exposed pups had higher TSH and reduced blood T4, and these changes were indicated to be related to hypothyroidism and persisted after weaning, and after lithium was cleared from the blood. Thyroid iodine uptake was similarly reduced during breast-feeding and shortly after.

Other experimental animal studies with exposure during both gestation and lactation (described in the developmental toxicity section) do not allow to draw conclusions regarding effects on or via lactation. Further, no effects on the offspring were reported in a 2-generation rat study (Anonymous, 2012, Van Deun et al. 2021) performed according to OECD TG 416.

Human data

Studies with mother-infant pairs clearly confirmed that lithium is transferred to breast milk and via breast milk to infant's serum.

In a clinical study with 10 mother-child pairs, the mothers were treated with lithium (600-1200 mg lithium/d during pregnancy and lactation) and the sampling of the children were between 1 and 52 weeks of age (Viguera et al., 2007). In this study, the breast milk concentrations were between 11-56% of serum levels. Maternal serum, breast milk, and infant serum concentrations

of lithium averaged 0.76, 0.35, and 0.16 mEq/L, respectively. No serious adverse effects were reported.

In another clinical study with 3 mother-child pairs, the mothers were treated with 600-900 mg lithium/d during pregnancy and lactation and the children were 1 month of age at the time of sampling (Bogen et al., 2012). The maternal serum levels were: 0.12-0.97 mM lithium, children serum levels: 0.08-0.11 mM (corresponding to 10-17% of maternal levels).

In a single case study, toxic effects were reported in a breast-fed child (cyanosis, electrocardiographic changes, floppy muscles). The lithium serum concentrations in the mother were extreme, 16 mM, and serum level in the infant also very high (6 mM). The symptoms resolved after the discontinuation of breastfeeding (HCN, 2000).

During stakeholder consultation, information was included regarding a study investigating the effect on infants exposed to lithium during breastfeeding (Chaudron and Jefferson, 2000). In this study, 11 cases with lithium exposure during breast-feeding were included (lithium serum levels in infants were measured in 7/11 infants). In one case, a cyanotic episode and lethargy was reported, and in another case signs of "lithium toxicity". It is noted that the infants were also exposed to lithium during pregnancy, so the infant serum concentration from *in utero* exposure combined with lithium exposure from breastfeeding adds up in infants due to the immature excretory systems and increase the possibility of adverse reactions since lithium is eliminated via renal excretion.

Based on available data it is estimated that lithium concentrations in breast milk are about half of the concentration found in maternal serum, and concentrations in infant's serum are about half the concentration in breast milk.

Summary: According to the CLP criteria*: "Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites)* ***in breast milk in amounts sufficient to cause concern for the health of a breastfed child****, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the":*

*(a) human evidence indicating a hazard to babies during the lactation period; and/or*
*(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or*
*(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.*

It has been clearly shown from humans and animals that lithium can be transferred to infants via breast milk. However, the limited existing data on lithium do not clearly indicate that severe toxic effects are induced in infants exposed to lithium via breast milk. In experimental animal studies effects observed, including reduced pup body weight, could in most of the studies not clearly be distinguished from effects caused by gestational exposure or the effects could be secondary due to maternal toxicity.

However, information provided during stakeholder consultation described a potential mechanism of toxicity in infants. Due to an immature excretory system in infants, there is an increased possibility of adverse reactions since lithium is eliminated via renal excretion. It is noted that the infants were also exposed to lithium during pregnancy, so the infant serum concentration from *in utero* exposure combined with lithium exposure from breastfeeding adds up due to the immature excretory systems of infants. Further, it is noted that in experimental animal studies, lithium induced severe renal structural changes in the developing rat kidney (Christensen et al.,

1982; Fritz, 1988, noting that in the study by Fritz, 1988, the renal effects in the offspring could be secondary to maternal toxicity). In a study in rats provided after stakeholder consultation, where pups were exposed to lithium via breastmilk from dams exposed to therapeutic levels of lithium, reduced blood T4, increased TSH levels and elevated blood urea nitrogen levels were reported, indicating effects on thyroid and kidney function following exposure to lithium during lactation.

**In conclusion,** RAC considers that based on the presence of lithium in human breast milk and infant serum, and the potential for a slower excretion of lithium in infants due to the immature excretory system, together with the reported effects in rats on kidney and thyroid functions in offspring exposed to lithium only during lactation, there is a concern for the health of children breast-fed to mothers on lithium therapy. This is considered to be in accordance with the CLP criteria: "*However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child shall be classified and labelled to indicate this property hazardous to breastfed babies".*

RAC is of the opinion that a **classification for effects on or via lactation as Lact.; H362 (May cause harm to breast-fed children) for the three lithium compounds is warranted**.

## Additional references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**ANNEXES:**

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Annex 3 Records of the targeted consultation to take account of a new study in relation to effects on or via lactation made available by the Dossier Submitter.