CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

[1] Lithium carbonate; [2] lithium chloride; [3] lithium hydroxide

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

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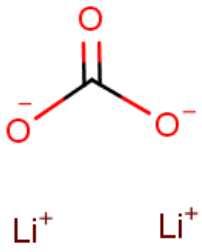
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substances

1.1.1 Lithium carbonate

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Lithium carbonate, Dilithium carbonate
Other names (usual name, trade name, abbreviation)	Lithium carbonate, Carbonic acid lithium salt (Li2CO3), Carbolithum, Priadel, Theralite, Plenur, Phasal, Manialith, Maniprex, Eskalith, Camcolit
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	209-062-5
EC name (if available and appropriate)	Lithium carbonate
CAS number (if available)	554-13-2
Other identity code (if available)	ICSC number: 1109, RTECS number: OJ5800000
Molecular formula	Li_2CO_3
Structural formula	
SMILES notation (if available)	C(=O)([O-])[O-].[Li+].[Li+]
Molecular weight or molecular weight range	73.888 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	No impurities or additives relevant for classification

1.1.2 Lithium chloride

Table 2: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Lithium chloride; Lithium Chloride, Anhydrous; lithium(1+) chloride
Other names (usual name, trade name, abbreviation)	Lithium chloride; Chloride Lithium Anhydrous
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	231-212-3
EC name (if available and appropriate)	Lithium chloride
CAS number (if available)	7447-41-8
Other identity code (if available)	/
Molecular formula	ClLi
Structural formula	
SMILES notation (if available)	[Li+].[Cl-]
Molecular weight or molecular weight range	42.394 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	No impurities or additives relevant for classification

1.1.3 Lithium hydroxide

Table 3: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Lithium hydroxide
Other names (usual name, trade name, abbreviation)	Lithium hydroxide
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	215-183-4
EC name (if available and appropriate)	Lithium hydroxide
CAS number (if available)	1310-65-2
Other identity code (if available)	/
Molecular formula	LiOH or HLiO
Structural formula	
SMILES notation (if available)	[Li+].[OH-]
Molecular weight or molecular weight range	23.947 g/mol (anhydrous) 41.962 g/mol (monohydrate)
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	No impurities or additives relevant for classification

1.2 Composition of the substance

Table 4: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
Lithium carbonate	Mono-constituent	-	Joint entries (306 notifiers): Acute Tox. 4 (H302) Eye Irrit. 2 (H319) Several other classifications : Acute Tox. 4 (H302) Skin Irrit. 2 (H315) Eye Dam. 1 (H318) STOT SE 3 (H335, respiratory system) Repr. 1B (H360, may damage the unborn child)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self- classification and labelling (CLP)
			STOT RE 1 (H372, central nervous system, kidney)

Table 5: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self- classification and labelling (CLP)
Lithium chloride	Mono-constituent	-	Joint entries (303 notifiers): Acute Tox. 4 (H302) Eye Irrit. 2 (H319) Skin Irrit. 2 (H315) Several other classifications : Acute Tox. 4 (H302) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Aquatic Chronic 2 (H411) Eye Dam. 1 (H318) STOT SE 3 (H335, respiratory tract) Acute Tox. 4 (H332) Acute Tox. 4 (H312) Repr. 1A (H360, Df) Lact (H362) STOT SE 1 (H370, nervous system) Carc. 1A (H350) STOT RE 2 (H373, Heart and Kidney)

Table 6: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self- classification and labelling (CLP)
Lithium hydroxide	Mono-constituent	-	Joint entries (400 notifiers): Acute Tox. 4 (H302) Skin Corr. 1B (H314) Eye Dam. 1 (H318) Several other classifications : Skin Corr. 1A (H314) Skin Corr. 1C (H314) Aquatic Chronic 3 (H412) Aquatic Chronic 2 (H411) Acute Tox. 3 (H301) Acute Tox. 3 (H331) Repr. 1A (H360) Lact (H362) Met. Corr. 1 (H290)

Table 7: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No data available				

Table 8: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No data available					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 9:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	Not available										
Dossier submitters proposal	TBD	lithium carbonate [1] lithium chloride [2] lithium hydroxide [3]	209-062-5 [1] 231-212-3 [2] 215-183-4 [3]	554-13-2 [1] 7447-41-8 [2] 1310-65-2 [3]	Repr. 1A	H360FD	GHS08 Dgr	H360FD			
Resulting Annex VI entry if agreed by RAC and COM	TBD	lithium carbonate [1] lithium chloride [2] lithium hydroxide [3]	209-062-5 [1] 231-212-3 [2] 215-183-4 [3]	554-13-2 [1] 7447-41-8 [2] 1310-65-2 [3]	Repr. 1A	H360FD	GHS08 Dgr	H360FD			

Table 10: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	data not sufficient for classification	Yes
Carcinogenicity	data conclusive but not sufficient for classification	Yes
Reproductive toxicity	harmonised classification proposed: Repr. 1A – H360FD	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Lithium carbonate, lithium chloride and lithium hydroxide have not been classified according to the Classification and Labelling of the Dangerous Substance Directive (Dir. 67/548/EEC) and have no entry in Annex VI Tables 3.1 and 3.2 of the Regulation (EC) No. 1272/2008 (CLP Regulation) (ECHA, 2020).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level for CMR endpoints.

5 IDENTIFIED USES

Lithium carbonate is the starting material for the production of lithium salts. It is used in the manufacture of aluminium and as a flux in the glass, enamel and ceramic industries, and in the construction industry. Further, it is applied in the prophylaxis and treatment of affective disorders.

Lithium chloride is used to absorb moisture in air conditioning systems and in batteries and in welding and brazing fluxes in the production of lightweight alloys.

Lithium hydroxide (monohydrate) is used in alkaline storage batteries and for manufacturing of lithium soaps. Lithium hydroxide (anhydrous) is used as an additive to potassium hydroxide in big industrial batteries and in the production of lithium stearate. (Lagerkvist and Lindell, 2002; Montelius, 2003).

According to ECHA website, lithium carbonate is manufactured and/or imported in the European Economic Area at 10 000 - 100 000 tons per year and lithium chloride and lithium hydroxide at 1 000 - 10 000 tons per year.

6 DATA SOURCES

Starting point for data searches for this report have been recent reviews and monographs with toxicological risk assessments on lithium and lithium compounds. Most relevant reviews used are Hartwig (2014), Lagerkvist and Lindell (2002), Montelius (2003), and HCN (Health Council of the Netherlands) (2000).

Furthermore, REACH registration dossiers (last modified: 25 October 2016) for lithium carbonate, lithium chloride and lithium hydroxide available from ECHA's disseminated database (ECHA, 2020) have been analysed for study references, which then have been considered as data sources for this CLH report.

Calculation of doses, if not provided in the specific references, have been performed according to and using the default values provided in the ECHA 'Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health' (ECHA, 2012).

Furthermore, ECHA guidance documents on the application of CLP criteria and on the preparation of dossiers for harmonised classification and labelling were used to compile this report (ECHA, 2014; 2017).

Systematic searches for publications and other relevant data were performed based on the following databases until December 2018:

- U.S. National Library of Medicine, Pubmed.gov
- TOXNET, ChemIDplus, IPCS, eChemPortal
- Chemical Abstracts (at host STN International Europe)
- SciSearch, Biosis, CAB Abstracts, Embase (at host Deutsches Institut für Medizinische Dokumentation und Information, DIMDI)

As lithium has pharmaceutical use, the French Agency for the Safety of Health Products (Agence Nationale de Sécurité du Médicament et des Produits de Santé, ANSM) was contacted and gave access to the archives of regulatory affairs.

All data sources used in this report are also listed in section 15 or Annex II (references).

7 PHYSICOCHEMICAL PROPERTIES

Table 11: Summary of physicochemical properties for lithium carbonate

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	White solid, granular or power form	(ECHA, 2020)	
Melting/freezing point	722 °C	(ECHA, 2020)	Measured at 1013.25 hPa
Boiling point	No data	(ECHA, 2020)	Study technically not feasible
Relative density	2.1 g/cm³	(ECHA, 2020)	
Vapour pressure	No data	(ECHA, 2020)	Study technically not feasible
Surface tension	No data	(ECHA, 2020)	
Water solubility	8.4 g/L	(ECHA, 2020)	Measured at 20 °C
Partition coefficient n-octanol/water	No data	(ECHA, 2020)	
Flash point	No data	(ECHA, 2020)	
Flammability	Non flammable	(ECHA, 2020)	
Explosive properties	No data	(ECHA, 2020)	
Self-ignition temperature	No data	(ECHA, 2020)	
Oxidising properties	No data	(ECHA, 2020)	
Granulometry	D50: 5.47 µm D10: 1.76 µm D90: 12.1 µm	(ECHA, 2020)	
Stability in organic solvents and identity of relevant degradation products	No data	(ECHA, 2020)	
Dissociation constant	No data	(ECHA, 2020)	
Viscosity	No data	(ECHA, 2020)	

Table 12: Summary of physicochemical properties for lithium chloride

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	white, odourless, crystalline solid	(ECHA, 2020)	
Melting/freezing point	608.52°C	(ECHA, 2020)	Measured at 1013.25 hPa
Boiling point	1360 - 1383°C	(ECHA, 2020)	Study technically not feasible

Property	Value	Reference	Comment (e.g. measured or estimated)
Relative density	2.1 g/cm³	(ECHA, 2020)	
Vapour pressure	No data	(ECHA, 2020)	Study technically not feasible
Surface tension	No data	(ECHA, 2020)	
Water solubility	569 g/L	(ECHA, 2020)	Measured at 20 °C
Partition coefficient n-octanol/water	No data	(ECHA, 2020)	
Flash point	No data	(ECHA, 2020)	No justified
Flammability	Non flammable	(ECHA, 2020)	
Explosive properties	No data	(ECHA, 2020)	
Self-ignition temperature	No data	(ECHA, 2020)	
Oxidising properties	No data	(ECHA, 2020)	
Granulometry	D10: 229 µm, D50: 383 µm, D90: 549 µm	(ECHA, 2020)	
Stability in organic solvents and identity of relevant degradation products	No data	(ECHA, 2020)	
Dissociation constant	2.256	(ECHA, 2020)	
Viscosity	No data	(ECHA, 2020)	

Table 13: Summary of physicochemical properties for lithium hydroxide

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Crystalline white, odourless solid	(ECHA, 2020)	
Melting/freezing point	lithium hydroxide anhydrous : 422.83°C lithium hydroxide monohydrate : 423.93°C	(ECHA, 2020)	Measured at 1013.25 hPa
Boiling point	No data	(ECHA, 2020)	Study technically not feasible
Relative density	1.5 g/cm³	(ECHA, 2020)	
Vapour pressure	No data	(ECHA, 2020)	Study technically not feasible
Surface tension	No data	(ECHA, 2020)	
Water solubility	Lithium hydroxide anhydrous : 71 g/L - 125 g/L at 20 °C Lithium hydroxide monohydrate : 189 g/L - 223 g/L at 10 °C	(ECHA, 2020)	
Partition coefficient n-octanol/water	No data	(ECHA, 2020)	
Flash point	No data	(ECHA, 2020)	

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	Non flammable	(ECHA, 2020)	
Explosive properties	No data	(ECHA, 2020)	
Self-ignition temperature	No data	(ECHA, 2020)	
Oxidising properties	No data	(ECHA, 2020)	
Granulometry	**Lithium hydroxide anhydrous** D10: 4 µm D50: 14 µm D90: 32 µm in absence of ambient air D10: 190 µm D50: 391 µm D90: 631 µm in presence of ambient air **Lithium hydroxide monohydrate** D10: 202 um D50: 440 um D90: 570 um in absence of ambient air D10: 43 um D50: 150 um D90: 634 um in presence of ambient air	(ECHA, 2020)	
Stability in organic solvents and identity of relevant degradation products	No data	(ECHA, 2020)	
Dissociation constant	Lithium hydroxide anhydrous pKa = 13.8 - 14.18 at 20°C	(ECHA, 2020)	
Viscosity	No data	(ECHA, 2020)	

8 EVALUATION OF PHYSICAL HAZARDS

Not performed for this substance

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 14: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Conclusions from studies are summarised in section 9.1			

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The present proposal for harmonised classification and labelling covers several existing entries in Annex VI of the Regulation (EC) No 1272/2008 (CLP Regulation). Read-across between the three concerned lithium compounds (carbonate, hydroxide and chloride) and data on lithium ion are used in this report. A detailed justification for this approach is provided in Annex II Read Across Justification Document.

Further assays performed with different lithium salts like sulfate, citrate, hypochlorite are summarised in several reviews, for example by Hartwig (2014), Lagerkvist and Lindell (2002), Aral and Vecchio-Sadus (2008). Only results obtained with lithium carbonate, chloride and hydroxide are discussed in this document and considered equivalent.

Except for absorption, only toxicokinetic information for lithium in ionic form is reported in this section.

a) Absorption

Soluble lithium salts are readily absorbed from the gastrointestinal tract. Solubility of the lithium salt determines the time to peak and plateau concentrations. Peak plasma concentrations occurred 1-4 hours after a single oral dose of lithium carbonate tablets in humans and complete absorption was observed within ca. 8 hours (Lagerkvist and Lindell, 2002). An oral absorption of lithium from lithium carbonate of about 20% was described in the registration dossier without further information (ECHA, 2020).

After a single oral dose of lithium chloride or carbonate in rats an increase in plasma levels during the first 15-30 minutes followed by a plateau for 12-24 hours, depending on the dose, was observed (ECHA, 2020; Hartwig, 2014).

In vitro investigations indicate that lithium is transported through the intestinal mucosa by passive diffusion via the leaky epithelium of the small intestine (Lagerkvist and Lindell, 2002).

Absorption data after inhalation exposure of intensive care patients who were mechanically ventilated with lithium chloride coated heat and moisture exchangers for at least 5 days revealed that lithium is also absorbed via inhalation (up to 0.1 mM serum lithium concentrations) (Lagerkvist and Lindell, 2002). Lagerkvist and Lindell (2002) concluded that lithium may be extensively absorbed via the lung.

Dermal absorption of lithium is regarded as low. Examinations with healthy volunteers who were exposed to lithium chloride via bath water (40 mg Li/L, 20 min per day, 4 days per week) did not indicate any differences in serum concentrations before and after bathing (Lagerkvist and Lindell, 2002).

b) Distribution

Human and animal studies reveal that lithium ions do not bind to plasma or tissue proteins to a great extent. The final volume of distribution is similar to that of the total body water. After distribution in the extracellular fluid it accumulates to various degrees in different organs. Lithium entry into the cells is probably via sodium or potassium transport proteins. In comparison to serum concentration at steady state lower concentrations are observed in liver, erythrocytes, and cerebrospinal fluid, and higher concentrations are reached in e.g. kidneys, thyroid, bone, muscles and certain brain regions. Most studies indicate that in

brain lithium concentrations show later peaks and slower rates of elimination than in serum (Hartwig, 2014; Lagerkvist and Lindell, 2002).

Lithium crosses the placenta freely. Lithium serum levels of mothers and their child were comparable at birth (Moore, 1995). Lithium is also excreted into breast milk with lithium concentrations in the breast milk of about one half of the serum concentration (see section 10.10.7) (Lagerkvist and Lindell, 2002; Moore, 1995).

c) Metabolism

Lithium is not metabolized to any appreciable amount in the human body (Hartwig, 2014; Lagerkvist and Lindell, 2002).

e) Elimination

Both, in humans and animals, lithium is mainly excreted via the kidneys through glomerular filtration. A considerable fraction of the filtered lithium (about 80%) is subsequently reabsorbed in the proximal tubules. Lithium clearance is closely related to the sodium balance and the risk of lithium intoxication is conversely correlated with sodium intake.

In humans, over 95% of a single oral dose of lithium ion is excreted unchanged through the kidneys. During a 6-12 hours initial phase about one to two thirds of the dose are excreted. This phase is followed by a slow excretion phase over the next 10-14 days. Less than 1% of a single dose are excreted via faeces and about 4-5% via sweat. In case of repeated administration lithium excretion increases during the first 5-6 days until a steady state is reached. Lithium elimination half-time in humans is 12-27 hours after a single dose. In elderly or persons with chronic lithium intake half-time increases up to 58 hours (Hartwig, 2014; Lagerkvist and Lindell, 2002).

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Evaluation not performed for this substance

10.2 Acute toxicity - dermal route

Evaluation not performed for this substance

10.3 Acute toxicity - inhalation route

Evaluation not performed for this substance

10.4 Skin corrosion/irritation

Evaluation not performed for this substance

10.5 Serious eye damage/eye irritation

Evaluation not performed for this substance

10.6 Respiratory sensitisation

Evaluation not performed for this substance

10.7 Skin sensitisation

Evaluation not performed for this substance

10.8 Germ cell mutagenicity

Table 15: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference Reliability
Tests on bacteria				
Bacterial gene mutation Ames Test OECD TG 471 Deviations: no GLP: yes	Lithium hydroxide (purity 92.9%)	*Salmonella typhimurium* TA 1535, TA1537, TA 98, TA 100 and *E.coli* WP2 uvrA 0, 3, 10, 33, 100, 333, 1000, 3330 and 5000 µg Li-hydroxide/plate Tested up to limit concentration Vehicle: Milli-Q-water +/- S9 mix Positive controls: yes	Negative (+/- S9 mix) for all strains tested Not cytotoxic	Anonymous, 2000a from (ECHA, 2020) Klimisch score : 1 key study
Bacterial gene mutation Ames Test Similar to OECD TG 471 Deviations: yes GLP: no	Lithium chloride (purity not provided)	*Salmonella typhimurium* TA 1535, TA1537, TA 98, TA 100 Up to 10000 µg Li-chloride/mL +/- S9 mix Positive controls: yes	Negative (+/- S9 mix) for all strains tested	Haworth et al., 1983 Klimisch score : 2 Supportive study
Test on mammalian cells				
Gene mutation study in mammalian cells OECD TG 476 Deviations: no GLP: yes	Lithium hydroxide (purity 57.8 wt%)	Mouse lymphoma L5178Y cells Target gene: Thymidine kinase (TK) 0, 12.5, 25, 50, 100 and 200 µg/mL (3 h treatment with S9 mix, 3 and 24 h treatment without S9 mix) Test substance concentrations were selected based on cytotoxicity +/- S9 mix Vehicle: water Positive controls: yes	Negative (+/- S9 mix) Cytotoxic at 200 µg/mL	Anonymous, 2010a from (ECHA, 2020) Klimisch score : 1 key study
Chromosome aberration study in mammalian cells OECD TG 473	Lithium hydroxide (purity 92.9%)	Human lymphocytes Dose range finding: 0, 10, 33, 100, 333 and 1000 µg/mL culture medium Experiment 1A: Without S9 mix: 0, 100, 180, 333*, 420* and 560* µg/mL culture medium (3 h treatment time, 24 h fixation time); With S9-mix: 0, 100, 333*, 420* and	Negative in experiment 1A and 1C (+/- S9 mix) Experiment 2 : negative with S9 mix without S9 mix, statistically significant at	Anonymous, 2000b from (ECHA, 2020) Klimisch score : 2 Supportive

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference Reliability
Deviations: no GLP: yes		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); not evaluated due to high cytotoxicity Experiment 1C: 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) 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 CA (*) were selected based on precipitation and cytotoxicity +/- S9 mix of Aroclor 1254 induced rat liver Vehicle: DMSO Positive controls: yes	the lowest (but within the HCD) and the highest concentration. No dose response, very toxic response at the highest dose : not relevant.	study
In vitro comet Assay (DNA strand breaks) Guideline: no GLP: no Performed according to the original protocol of Singh et al. (1988)	Lithium carbonate (purity not provided)	AA8 CHO cells 1-30 mM (73. 9-2217 µg/mL; 13.9-417 µg Li/mL), 3 h or 24 h treatment - S9 mix Untreated and positive controls included. Result expressed as tail moment.	Negative (- S9 mix) Cytotoxic at concentrations ≥ 70 µg Li/mL	Pastor et al., 2009 Klimisch score : 2 Supportive study
	Lithium chloride (purity not provided)	AA8 CHO cells 1-30 mM (42.4-1272 µg/mL; 7-209 µg Li/mL), 3 h or 24 h treatment - S9 mix Untreated and positive controls included. Result expressed as tail moment.	Negative (- S9 mix) Cytotoxic at concentrations ≥ 70 µg Li/mL	
Anaphase anomaly study Guideline: no	Lithium carbonate (purity not provided)	AA8 CHO cells, up to 10 mM (739 µg/mL; 139 µg Li/mL), 3 h treatment - S9 mix	Positive (- S9 mix): anomalous anaphases: multipolar anaphases and lagging chromosome Cytotoxic at	Pastor et al., 2009 Klimisch score : 2

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference Reliability
GLP: no After treatment, cells in anaphase were analysed for any alterations of normal chromosome segregation.		Negative control included.	concentrations ≥ 70 µg Li/mL Details of concentrations not provided, number of cells evaluated not provided	Supportive study
	Lithium chloride (purity not provided)	AA8 CHO cells, no further details, 3 h treatment - S9 mix Negative control included.	Positive (- S9 mix): anomalous anaphases: multipolar anaphases and lagging chromosome Cytotoxic at concentrations ≥ 70 µg Li/mL Details of concentrations not provided, number of cells evaluated not provided	
Micronucleus assay *in vitro* Similar to OECD TG 487 Deviations: yes (cell line used not mentioned in the TG, no positive control) GLP: no	Lithium carbonate (purity not provided)	AA8 CHO cells 2.2-10 mM (163-739 µg/mL; 31-139 µg Li/mL), 3 h treatment - S9 mix Untreated controls (Cytochalasin B) and vehicle controls (DMSO) included.	Positive (- S9 mix) at concentrations ≥ 31 µg Li/mL, mostly kinetochore positive Cytotoxic at concentrations ≥ 70 µg Li/mL	Pastor et al., 2009 Klimisch score : 2 Supportive study
	Lithium chloride (purity not provided)	AA8 CHO cells 5-20 mM (212-848 µg/mL; 35-139 µg Li/mL), 3 h treatment - S9 mix Untreated and vehicle controls included.	Positive (- S9 mix) at concentrations ≥ 35 µg Li/mL, mostly kinetochore positive Cytotoxic at concentrations ≥ 70 µg Li/mL	
Chromosome aberration assay Similar to OECD TG 473 Deviations: yes (substance used as positive control not mentioned in TG, only 200 metaphases examined) GLP: no	Lithium carbonate (purity not provided)	AA8 CHO cells, 1-30 mM (73.9-2217 µg/mL; 13.9-417 µg Li/mL), 3 h treatment and 12 h growth phase - S9 mix Negative and positive controls: yes	Negative (- S9 mix) Cytotoxic at concentrations ≥ 70 µg Li/mL	Pastor et al., 2009 Klimisch score : 2 Supportive study
	Lithium chloride (purity not provided)	AA8 CHO cells 1-30 mM (42.4-1272 µg/mL; 7-209 µg Li/mL), 3 h treatment and 12 h growth phase - S9 mix Negative and positive controls: yes	Negative (- S9 mix) Cytotoxic at concentrations ≥ 70 µg Li/mL	

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference Reliability
Gene mutation assay (HGPRT) Similar to OECD TG 476 Deviations: yes (no positive control) GLP: no	Lithium carbonate (purity not provided)	V79 cells 0, 1500, 2000, 2500, 3000 µg/mL (282-564 µg Li/mL) +/- S9 mix Negative control included; comparison to mutagenic activity of B(a)P	(- S9 mix): average number of 6-TG mutants per 100000 cells partially increased at the respective dose groups: 0.2, 0.3, 1.1, 0.4, 0.4 (+ S9 mix): average number of 6-TG mutants per 100000 cells partially increased at the respective dose groups: 0.4, 0.2, 0.1, 0.9, 0.8 Cytotoxic at highest concentration (564 µg Li/mL)	Slameňová et al., 1986 Klimisch score : 2 Supportive study
DNA strand breaks (alkaline elution) Guideline: no GLP: no	Lithium carbonate (purity not provided)	Human EUE cells 150, 250, 500 µg/mL (28-94 µg Li/mL), 1 h treatment - S9 mix	Positive (- S9 mix) at highest concentration (94 µg Li/mL)	Slameňová et al., 1986 Klimisch score : 2 Supportive study
Chromosome aberration test Similar to OECD TG 473 Deviations: yes (no positive control) GLP: no	Lithium chloride (purity not provided)	Phytohemagglutinin-stimulated lymphocyte cultures of a healthy human donor 0, 50, 100, 150 µg lithium chloride/mL (8.2-25 µg Li/mL) Untreated control included.	Positive 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. Increased deletion from 100 µg (2.2%, 4.2%) and translocation (0.6%, 1.7%)	De La Torre et al., 1976 Klimisch score : 2 Supportive study
Chromosome aberration test Guideline: no GLP: no	Lithium carbonate (purity not provided)	Peripheral blood lymphocytes Dose equivalent to 0.1, 1.0 and 10 g lithium carbonate distributed in the body of a 70 kg person (no further information)	Negative	Timson and Price, 1971 Klimisch score : 4 Disregarded study

Table 16: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference Reliability

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference Reliability
In vivo chromosome aberration assay; Guideline: no GLP: no	Lithium carbonate (purity not provided)	Mouse (Lacca strain) Bone marrow cells 0, 1.2, 12, 120 mg/kg bw (0, 0.23, 2.3, 23 mg Li/kg bw) single gavage application (vehicle olive oil) 72 h before bone marrow preparation	Positive, no clear dose response (mean CAs 5.66, 14.0, 13.0, 20.33 in the respective dose groups) Number of cells studied not provided. No positive control included and negative control values higher than in other published reports.	Sobti et al., 1989 Klimisch score : 3 Disregarded study
	Lithium chloride (purity not provided)	Mouse (Lacca strain) Bone marrow cells 0, 0.212, 2.125, 21.25 mg/kg bw (0, 0.035, 0.35, 3.5 mg Li/kg bw) single gavage application (vehicle olive oil) 72 h before bone marrow preparation	Positive (mean CAs 2.66, 9.0, 10.0, 14.66 in the respective dose groups) Number of cells studied not provided. No positive control included and negative control values higher than in other published reports.	
In vivo sister chromatid exchange assay; Guideline: no GLP: no	Lithium carbonate (purity not provided)	Mouse (Lacca strain) Bone marrow cells 0, 1.2, 12, 120 mg/kg bw single gavage application (vehicle olive oil) 72 h before bone marrow preparation	Negative Slight increase compared to control, but not statistically significant. Number of cells studied not provided. No positive control included and negative control values higher than in other published reports.	Sobti et al., 1989 Klimisch score : 3 Disregarded study
	Lithium chloride (purity not provided)	Mouse (Lacca strain) Bone marrow cells 0, 0.212, 2.125, 21.25 mg/kg bw (0, 0.035, 0.35, 3.5 mg Li/kg bw) single gavage exposition (vehicle olive oil) 72 h before bone marrow preparation	Negative Number of cells studied not provided. No positive control included and negative control values higher than in other published reports.	
Chromosome aberrations Guideline: no GLP: no	Lithium (no other information)	Female Lister rats Bone marrow cells 86 mg/day Three days intraperitoneal exposure. Sacrifice 12 and 24 hours after the last injection	Negative Very few details given on method used, no control.	Bille et al., 1975 Klimisch score : 3 Disregarded study

Table 17: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Chromosome aberrations in peripheral blood lymphocytes	Lithium (no further information)	8 patients, mean dose 768.75 ±139.05 mg/day for at least one year 100 cells per subject Compared with 10 psychiatrically healthy drug-free controls matched for sex and age.	Negative	Turecki et al., 1994
Chromosome aberrations in peripheral blood lymphocytes	Lithium carbonate (purity not provided)	13 psychiatric patient (5 males, 8 females), serum Li level 0.02-1.54 mEq/L, treatment between 4 month and 7 years	Negative	Matsushima et al., 1986
Chromosome aberrations in peripheral blood lymphocytes	Lithium carbonate (purity not provided)	10 patients (5 males, 5 females, 19-61 years), doses 800-2400 lithium carbonate/kg bw/day, blood levels 0.60-1.25 mmol/L 3 control patients included.	Negative (increased CA, gaps and breaks in some patients, no clear dose response)	De La Torre et al., 1976
Chromosome aberrations in bone marrow cells	Lithium (no further information)	7 male psychiatric patients (between 28 and 66 years), daily doses of 900-1500 mg for two to ten years Lithium level in serum: 0.8-1.2 mmol/L 50 cells per subject	Negative (hyperdiploid cells and structural aberrations)	Bille et al., 1975
Chromosome aberrations in peripheral blood leukocytes	Lithium carbonate (purity not provided)	16 manic-depressive patients Exposure to lithium carbonate from 2 weeks to more than 2 years (seven of them for more than one year), blood concentrations between 0.6 and 2.1 mEq/L, daily doses between 900 and 1800 mg	Negative	Jarvik et al., 1971
Chromosome analysis (cells not mentioned)	Lithium (no further information)	3 psychiatric patients (2 females, 1 male), highest daily dose: 600, 600, 900 mg; total dose: 1234, 3632, 50 g; treatment period: 147, 134, 2 months, respectively	Gaps not significantly increased: mean: 13.5% (3.2, 11.6, 27.6% in the individual patients) vs. 10.3 % in 11 controls hypodiploid cells significantly increased: mean 16.3% (n.d., 31.6, 8.6% in the individual patients) vs. 6.9% in controls	Friedrich and Nielsen, 1969

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Genotoxicity of lithium salts has been investigated in different test systems and assays. Guideline studies presented (bacterial reverse mutation, *in vitro* chromosome aberration, gene mutation in a mouse lymphoma assay) were only performed with lithium hydroxide.

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.

Increased mutation rates reported for lithium carbonate in the HGPRT-assay by Slameňová et al. (1986) were not associated with a clear dose response relationship.

Contradictory results were obtained on the induction of DNA strand-breaks. Whereas Slameňová et al. (1986) reported a positive effect for lithium carbonate at the highest concentration tested (500 µg/mL) in human EUE cells, negative results were reported by Pastor et al. (2009) for lithium carbonate and chloride at even higher concentrations, up to 2217 µg/mL and 1272 µg/mL, respectively, in AA8 CHO cells. Further, Slameňová et al. (1986) 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.

Results obtained with human cells *in vitro* are contradictory. De La Torre (1976) reported positive results in a chromosome aberration assay with lithium chloride in peripheral blood lymphocytes. However, no increase in structural chromosome aberrations in peripheral human blood lymphocytes were seen 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 (no information on number of cells screened, no positive controls) (Timson and Price, 1971), or in a 3 h treatment with lithium hydroxide with concentrations up to 560 µg lithium hydroxide/mL (Anonymous, 2000b).

Further investigations of Pastor et al. (2009) pointed to an interaction of lithium carbonate with the spindle apparatus. They described significant and dose dependent increased numbers of micronuclei, effects seen in all dose groups. However, cytotoxicity was already distinct/severe (40% growth reduction) at 4 mM and even higher at increasing concentrations. Moreover, the study is insufficiently reported (e.g. number of cells evaluated not provided), which limits the validity of these data.

Per oral administration of lithium carbonate or chloride to mice resulted in a significant increase of bone marrow chromosome aberrations but not in a significant elevation of sister chromatid exchanges (Sobti et al., 1989). However, the frequency of various types of aberrations and the number of cells studied were not provided. Further, no positive controls were included and the negative control values were higher than in other published reports (Lagerkvist and Lindell, 2002), therefore the study is not regarded as reliable.

In humans mainly negative results were described. No cytogenetic effect of lithium treatment were observed in several studies with patients: Jarvik et al. (1971) did not find aberrations in the lymphocytes of 16 manic-depressive patients who got lithium carbonate for periods between 2 weeks and more than 2 years (seven of them for more than one year). No increase of chromosomal lesions were observed in 8 patients who had been receiving continuous lithium therapy (mean 768.75 +/- 139.05 mg/day) for at least one year in comparison to 10 controls (Turecki et al., 1994). No cytogenetic changes were observed in bone marrow cells from seven lithium treated patients (daily dose of 900-1500 mg; serum concentration 0.8-1.2 mmol/L) treated with lithium salts (Bille et al., 1975). Negative results were also obtained in 13 psychiatric patients treated with lithium carbonate for up to 7 years (Matsushima et al., 1986). De La Torre et al. (1976) observed a slight increase in chromosome aberrations in peripheral lymphocytes of 10 psychiatric patients (3 controls), but without clear relation of response with dose or time. Aral and Vecchio-Sadus (2008) reported another study comprising 19 lithium treated manic-depressive patients and 23 controls with negative results.

Friedrich and Nielsen (1969) reported an increase in mean chromosome breaks and hypodiploid cells in 3 psychiatric patients treated with lithium. However, hypodiploid cells were only increased in another patient with the highest total dose. Insufficient number of cells and number of patients were analysed. Investigations with human lymphocytes treated *in vitro* did not cause chromosome damage except at toxic concentrations. Due to the small number of patients and in light of the overall negative findings these results are not regarded as relevant for classification.

According to Aral and Vecchio-Sadus (2008) lithium could have several ways of acting on DNA: Lithium binds selectively to DNA and competes with magnesium (2+) and may therefore impair DNA synthesis and DNA repair. But, existing *in vitro* and *in vivo* investigations do not clearly indicate genotoxicity of lithium carbonate. Additionally, inhibition of unscheduled DNA synthesis induced by N-methyl-N’-nitro-N-

nitrosoguanidine in rats was observed if lithium carbonate was administered via drinking water at 500 ppm for 3, 6 or 12 months (Šrám et al., 1990).

In summary, lithium compounds have been tested for mutagenicity, chromosome aberrations, sister chromatid exchanges, DNA damage in a number of *in vitro* and *in vivo* studies. Mainly negative results were obtained, but positive results were also reported, usually at high cytotoxic doses. According to Lagerkvist and Lindell (2002) a possible explanation for the observation of genotoxic effects at higher doses may be increased cell survival, since lithium inhibits apoptosis by inhibiting the enzyme glycogen synthase kinase-3 (GSK3). However, an aneugenic potential of lithium salts could not be excluded considering positive results obtained in *in vitro* micronucleus test associated with an increase of kinetochore positive micronuclei and an increase of damage mitosis. Moreover, no micronucleus test was performed *in vivo* to investigate this aneugenic potential.

10.8.2 Comparison with the CLP criteria

For potential classification on germ cell mutagenicity, criteria from CLP-Regulation/guidance (ECHA, 2017) were applied.

- *'The classification in Category 1A is based on positive evidence from human epidemiological studies' (ECHA, 2017).*

There is no positive evidence from human epidemiological studies, as existing data on mutagenic effects in patients are mainly negative.

- *The classification in Category 1B is based on:*
  - *positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or*

There are no *in vivo* heritable germ cell mutagenicity tests in mammals available for lithium compounds.

  - *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. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells;*

In vivo somatic cell mutagenicity tests in animals provide contradictory results. However, the tests are mainly regarded as not reliable due to methodological shortcomings. Investigations with humans yielded mainly negative results, so that this criterion is not fulfilled.

  - *positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.*

No data on human germ cells provides positive results. Therefore, this criterion is not fulfilled.

- *The classification in Category 2 is based on:*
  - *Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:*
    - *Somatic cell mutagenicity tests in vivo, in mammals; or*
    - *Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.*

The only evidence of mutagenicity from *in vitro* test in somatic cells is the increase of micronuclei from Pastor et al. (2009). However, cytotoxicity was also observed in this study. There is no other evidence of mutagenicity from *in vitro* acceptable test (Klimisch score 1 or 2) in somatic cells or bacteria. Additionally, as outlined above, there is also no evidence of mutagenicity from *in vivo* acceptable tests (Klimisch score 1 or 2) in somatic cells. Therefore, this criterion does not apply.

However, it can be noted that, considering:

- results from Pastor et al. (2009), suggesting a damage of spindle apparatus and increased number micronuclei *in vitro*;
- toxic effect on germ cells *in vivo* (see section 10.10);
- the absence of a robust micronuclei assay *in vivo;*

the aneugenic potential of lithium cannot be clarified with data available.

In conclusion, the quality of the database is questionable, and despite the numerous negative *in vitro* and *in vivo* findings, an aneugenic potential of lithium salts cannot be formally excluded. It is therefore not possible to conclude on genotoxic potential of lithium salts. No classification is proposed.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification is proposed because data of adequate quality are lacking and therefore this endpoint could not be fully assessed.

10.9 Carcinogenicity

Table 18: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Tumour promotion Guideline: no GLP: no Female Buffalo/N-rats 24-27 animals per dose group	Lithium carbonate (purity not provided) Animals were treated once i.v with N-nitrosourea. Afterwards animals were exposed to 0, 0.5, 1 or 10 mM lithium carbonate (ca. 0.69, 1.38, 13.8 mg Li/kg bw/d) [a]. for 3 months via drinking water Control animals received sodium carbonate	The additional exposure to lithium carbonate for 3 months had no significant effect on the tumour incidence. Animals in the high dose group rapidly lost weight and were killed in week 2. No further information available	Ziche et al., 1980 Klimisch score : 2 Weight of Evidence
Tumour promotion Guideline: no GLP: no Female Buffalo/N-rats 5 animals per dose group	Lithium carbonate (purity not provided) Animals were treated three times i.v with N-nitrosourea and were ovariectomised after development of mammary tumours. Afterwards animals were exposed to 0, 10 or 20 mM lithium carbonate (0, 13.8 or 27.6 mg Li/kg bw/d) [a]. for 2 months via drinking water Control animals received sodium carbonate	Rats developed mammary tumours after N-nitrosourea treatment; tumour volume in lithium carbonate exposed animals were not increased at the end of the exposure period.	Ziche et al., 1980 Klimisch score : 2 Weight of Evidence
Tumour promotion Guideline: no GLP: no Female Sprague-	Lithium carbonate (purity not provided) Animals were treated once with 20 mg 7,12-dimethylbenzanthracene/animal via gavage. Afterwards animals were exposed to 0,	120 days after 7,12-dimethylbenzanthracen exposure, 480 animals had developed tumours of the mammary gland. The 120 animals without any tumours received lithium carbonate for 3	Ziche et al., 1980 Klimisch score : 2 Weight of

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Dawley 600 animals in total	1or 10 mM lithium carbonate (0, 1.38 or 13.8 mg Li/kg bw/d) [a]. for 3 months via drinking water Control animals received sodium carbonate	months. The additional treatment with lithium carbonate did not result in a higher tumour incidence.	Evidence
Tumour promotion experiment Guideline: no GLP: no No further information available	Lithium carbonate (purity not provided in the English abstract, study in Russian) Animals were treated with N-butyl-N-(4-hydroxybutyl)-nitrosamin. Afterwards animals were exposed to lithium carbonate (no further information) Control animals only received N-butyl-N-(4-hydroxybutyl)-nitrosamin	In animals treated with lithium carbonate, urinary bladder tumour rate was increased about 6 times compared to animals only treated with N-butyl-N-(4-hydroxybutyl)-nitrosamin. After 3-6 month exposure this effects was strongest.	Frolov and Pliss, 1991; 1992 Klimisch score : 4 Disregarded study
Repeated dose toxicity Guideline: no GLP: no Rats Wistar Males and females Details provided on the protocol and the results too sparse	Lithium chloride (purity not provided) 0, 10, 20, 30 and 50 mM lithium chloride in drinking water 2 years	10 mM: no effects 20 mM: no effects on health or behaviour except slight, transitory initial disturbances 30 mM : weight loss and mortality observed. 50 mM : Death of the animals occurred within 2-3 weeks. No carcinogenicity reported	Trautner et al., 1958 Klimisch score : 3 Disregarded study

a: calculation of dose reported from (Hartwig, 2014)

Table 19: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Review/Retrospective analysis	Lithium therapy (no further information)	Investigate the possible correlation between lithium treatment and thyroid or renal tumors. (1) retrospective analysis of the clinical records in the lithium clinic database; (2) analysis of the causes of death of the patients who had been visited at least once at the lithium clinic between 1980 and 2013; (3) analysis of the reports of lithium adverse reactions to the European and the WHO pharmacovigilance	Not possible epidemiologically to confirm an increased risk of thyroid or renal cancers associated with lithium. However, the association between lithium treatment and occurrence of renal neoplasm still need to be assessed.	Ambrosiani et al., 2018

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		databases; (4) review of the literature on thyroid and renal tumors in patients treated with lithium		
Sweden prospective cohort study patients with bipolar disorder with or without lithium treatment vs. general population	Lithium therapy (no further information) at least one lithium prescription in the period from 1 July 2005 to 31 December 2009	One subcohort without (n= 3049) and one subcohort with lithium treatment (n=2393); age group 50-84, comparison to general population (about 2 600 000 men and women), calculation of incidence rate ratios (IRR), adjusted for age and gender, of first cancer and site-specific cancer Assessment of occurrence of any cancer between 2005-2009	Overall cancer risk 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; increased risk of respiratory, gastrointestinal, and endocrine cancer in patients without lithium treatment, but not in patients with lithium treatment.	Martinsson et al., 2016
Denmark Case-control study	Lithium therapy (no further information)	Cases: patients diagnosed with incident colorectal adenocarcinoma during 2000-2012 (n=36 248), controls: ten matched cancer free controls per case; analysis for possible association between long-term (5 years) lithium use and colorectal adenocarcinoma; similar long term lithium use in cases (0.22%) and controls (0.20%)	No association between long term lithium use and increased risk of colorectal adenocarcinoma; odds ratio for colorectal adenocarcinoma in cases of 1.13 (95% CI: 0.89-1.43); different odds ratios for different subsides: 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)	Pottegård et al., 2016a
Denmark Case-control study	Lithium therapy (no further information)	Cases: patients diagnosed with upper urinary tract cancer during 2000-2012 (n=6477), controls: age and sex matched cancer free controls (n= 259 080); analysis for possible association between long-term (5 years) lithium use and upper urinary tract cancer; similar long term lithium use in cases (0.22%) and controls (0.17%)	No association between long term lithium use and risk for upper urinary tract cancer (adjusted odds ratio 1.3 (95% CI: 0.8-2.2), no significant increases in the OR for localized disease, renal pelvis or ureter cancer	Pottegard et al., 2016b
Denmark Retrospective population-based longitudinal cohort study	Lithium therapy (no further information)	Cohort I: (i) randomly selected sample of 1,500,000 out of all persons registered in Denmark on 1 January1995; (ii) all	Lithium treated patients: no increased rate of renal and upper urinary tract tumours (hazard rate ratios malignant or benign: 0.67-1.18, range of different exposure groups according to number of prescription)	Kessing et al., 2015

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		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: subcohort of cohort I, patients with bipolar disorder diagnosed between 1995-2012		
France Retrospective cohort study	Lithium therapy (no further information)	170 patients with cystic kidney diseases under lithium therapy; compared to general French population and 340 lithium free patients with cystic kidney disease without lithium therapy	Standardized Incidence Ratio of renal cancer was significantly higher in lithium-treated patients compared with the general population: 7.51 (95% CI: 1.51–21.95) and 13.69 (95% CI: 3.68–35.06) in men and women, respectively. No adequate control for possible confounders	Zaidan et al., 2014

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

There are no reliable carcinogenicity studies in animals with lithium compounds, as considered in this CLH report. As no robust key study could be identified, these studies are used in a weight of evidence approach.

For lithium carbonate, four studies in experimental animals were identified, which investigated the tumour promoting effects of this substance. These studies are summarised in Table 18.

Ziche and coworkers (1980) exposed female Buffalo/N-rats, which have been treated previously once or for three-times with N-nitrosourea (intravenous route), to drinking water containing up to 20 mM lithium carbonate. Lithium carbonate treatment did not influence breast tumour development or size of tumours induced by N-nitrosourea.

Lithium carbonate exposure via drinking water (1 or 10 mM) did also not influence breast tumour development in Sprague-Dawley rats, which had received a single exposure to 7,12-dimethylbenz[a]anthracene by gavage 120 days prior to lithium carbonate treatment and had not developed breast tumours at the start of lithium carbonate treatment.

In contrast, a tumour promoting effect of lithium carbonate is described in rats, which received N-butyl-N-(4-hydroxybutyl)-nitrosamine (Frolov and Pliss, 1991; 1992). In animals treated with lithium carbonate, urinary bladder tumour rate was increased about 6 times compared to animals only treated with N-butyl-N-(4-hydroxybutyl)-nitrosamine. After 3-6 month exposure this effect was strongest. The original publications are written in Russian and were cited from secondary literature (Hartwig, 2014). According to Hartwig (2014) these studies could not be used for the evaluation of a possible tumour promoting activity of lithium carbonate due to insufficient documentation.

In a 2 year study in rats ingesting lithium chloride in drinking water (20 mM) no effects on health or behaviour were observed except slight, transitory initial disturbances. Plasma lithium levels were 1.5 to 2

mM. At 30 mM lithium chloride toxic effects including weight loss and mortality were observed. At higher concentration (50 mM) food and water intake fell within a few days and animals showed clinical effects like staggering gait and fine muscular tremor. Death of the animals occurred within 2-3 weeks. Lithium plasma concentration was 3 mM when behavioural changes occurred, rose to 7 mM during the second week of treatment and exceeded 8 mM just before death. No increased incidence of tumours was described. The details provided on the protocol and the results were however too sparse to use this study for the assessment of carcinogenicity effects (Trautner et al., 1958).

Several publications assessed the association between lithium treatment and excess of cancer in patients.

In 2015, the European Medicine Agency (EMA) 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"* with a precision on leaflet that those effects have been reported in patients with severe renal impairment. This new recommendation was in part based on a French retrospective cohort study published few month earlier (according to the authors, actually the study is not a typical cohort), reporting a frequency of renal cancer significantly higher among lithium-treated patients than among lithium-free patients (4.1% vs 0.3%, P=0.002) and an increased incidence ratio of renal tumours in lithium-treated patients with cystic kidney disease compared to French general population (Zaidan et al., 2014). The results of this study were questioned, as the influence of confounders was not appropriately checked (Licht et al., 2014). Moreover, this study could have been subject to selection/inclusion bias because it has been conducted in a specialized nephrology department and the limited number of cases did not allow detailed statistics. This study suggest an association between lithium and renal cancers, however causation criteria are not meet and should be supported by other studies.

After the publication of this document, several publications investigated the association between lithium exposure and renal cancers.

In a prospective Swedish cohort study, cancer incidences in patients with bipolar disorder were investigated in two cohorts, one without (n=3049) and one with lithium treatment (n=2393, i.e. at least one lithium prescription in the years 2005 to 2009). Cancer incidences in persons with bipolar disorders were compared to data of the general population (about 2 600 000 men and women). 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. The overall cancer risk was not increased in patients with bipolar disorder. Neither bipolar disorder nor lithium treatment of bipolar disorder was associated with an increased incidence of unspecified cancer. An increased risk of respiratory, gastrointestinal, and endocrine cancer was observed in patients without lithium treatment, but not in patients with lithium treatment (Martinsson et al., 2016).

A Danish nationwide case-control study assess possible association between long-term (5 years) lithium use and upper urinary tract cancer. Cases were patients diagnosed with upper urinary tract cancer during 2000-2012 (n=6477) and controls were age and sex matched cancer free patients (n= 259 080). Authors did not identify an association between long term lithium use and an risk for upper urinary tract cancer (adjusted OR 1.3 (95% CI: 0.8-2.2)) (Pottegard et al., 2016b).

Also, in a population-based longitudinal cohort study from Denmark, no association between continued treatment with lithium and an increased rate of renal or upper urinary tract tumours was observed (Kessing et al., 2015).

In 2018, Ambrosiani et al. tried to investigated the correlation between lithium treatment and thyroid or renal tumor, by different meaning: (1) a retrospective analysis of the clinical records in the lithium clinic database; (2) an analysis of the causes of death of the patients who had been visited at least once at the lithium clinic between 1980 and 2013; (3) an analysis of the reports of lithium adverse reactions to the European and the WHO pharmacovigilance databases; (4) a review of the literature on thyroid and renal tumors in patients treated with lithium. They concluded that even if it has not been possible epidemiologically to confirm an increased risk of thyroid or renal cancers associated with lithium, the association between lithium treatment and occurrence of renal neoplasm still need to be assessed, considering particularly the seriousness of the alert of the PRAC.

In a further Danish nationwide case-control study, no association was found between lithium use and an overall increased risk of colorectal adenocarcinoma. However, the study shows some weaknesses like lack of data on life-style habits, including smoking, obesity and alcohol consumption or no exact data on lithium consumption (Pottegård et al., 2016a).

10.9.2 Comparison with the CLP criteria

For potential classification on carcinogenicity, criteria from CLP-guidance (ECHA, 2017) were applied.

- *"Category 1A, known to have carcinogenic potential for humans: The classification in Category 1A is based on strength of evidence together with additional considerations. Such evidence may be derived from: human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen)" (ECHA, 2017).*

Epidemiological studies mainly did not found an association between lithium exposure and an increased incidence of tumours. Only one study described an increased risk of renal tumors in lithium-treated cystic kidney disease patients (Zaidan et al., 2014). These findings were criticised due to methodological deficiencies like inappropriate control for confounders.

In addition, no other epidemiological study supported these observations. Overall, epidemiological studies do not point to an association between lithium exposure and tumor development. Thus, criteria for a category 1A are not fulfilled.

- *"Category 1B, presumed to have carcinogenic potential for humans: The classification in Category 1B is based on strength of evidence together with additional considerations. Such evidence may be derived from: animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen)" (ECHA, 2017).*
- *"The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies."*

There are no carcinogenicity or long-term animal studies according to current guidelines available. In a 2-year rat toxicity study, which is insufficiently documented, no occurrence of tumours was described with lithium chloride. Lithium carbonate tumour promoting activity has been investigated in several non-guideline studies. Three experiments from one working 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 (Frolov and Pliss, 1991; 1992). However, the details available do not allow an adequate assessment of this study. Thus, criteria for a category 1B or 2 are not fulfilled.

Overall,

- in the absence of an association between lithium treatment and an increased tumour incidence in most of the epidemiological studies;
- in the absence of carcinogenic effect / tumour promotion in most of the experimental studies;
- taking into account the limitations of the few studies showing potential carcinogenic / tumour promotion effect and the overall limitation of the database;
- taking into account the negative conclusion from mutagenicity assessment,

it is concluded that existing data do not allow classifying lithium compounds as carcinogenic according to CLP criteria and that a classification is therefore not recommended.

However, considering the doubt about the aneugenic potential of lithium and the conclusion from Ambrosiani et al. (2018) ("*the association between lithium treatment and occurrence of renal neoplasm still need to be assessed, considering particularly the seriousness of the alert of the PRAC*") a carcinogenic effect of lithium salts cannot be formally excluded.

10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification is proposed because data of adequate quality are lacking and therefore this endpoint could not be fully assessed.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 20: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two-Generation Reproduction Toxicity Study OECD TG 416 Deviations: no GLP: yes Male/female Wistar rats 25 animals per sex and dose for the parental and F1 generation.	Lithium carbonate (information on purity: see confidential annex) 0, 5, 15 and 45 mg lithium carbonate/kg bw/ day, 0, 0.9, 2.8, 8.5 mg Li/kg bw/ day (P) Treatment started from the age of 9 weeks and continued throughout the treatment period until F1 litters were weaned. (F1) Treatment started for F1 generation from the time of weaning and continued until F2 were weaned and sacrificed. Exposition via gavage	<u>P0:</u> No effects on reproductive function, weight and histopathology of reproductive organs or sperm parameter observed LOAEL: 45 mg/kg bw/d: increase of 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 control, morphological changes in liver (higher incidence of increased cytoplasmic rarefaction in males; in females, higher incidences of focal basophilic hepatocytes and hepatocellular hypertrophy), kidneys (higher incidences with minimal severity of dilated tubules in males and females (11/25 and 3/25, respectively), and thyroid (increased colloid in the follicular lumen in females) NOAEL: 15 mg/kg bw/day for male and female rats <u>F1:</u> NOAEL: 45 mg/kg bw/day based on lack of reproductive and foetal toxicity <u>F2:</u> NOAEL: 45 mg/kg bw/day based on lack of reproductive and foetal toxicity No information on lithium serum concentrations, detailed sperm parameters not given	Anonymous, 2012 Klimisch score : 1 Key study
Electron microscopic examination of rat testes after 21 days of exposure Guideline: no GLP: no male Wistar rats n=10 dose group n=4 control group	Lithium carbonate (purity not provided) 0, 35 mg lithium carbonate/kg bw/day, 0, 6.6 mg Li/kg bw/day, For 21 days via gavage	Structural changes in tunica propria of seminiferous tubules, germ cells and Sertoli cells. Loss of germ cell attachment and appearance of expanded intracellular spaces between spermatogonia and spermatocytes, round spermatids with abnormally shaped acrosomes and dilation of subacrosomal space. Degenerated late spermatids with random orientation, spermatids with alterations in the F-actin ectoplasmic specialization LOAEL = 35 mg lithium carbonate/kg bw/day	Zarnescu and Zamfirescu, 2006 Klimisch score : 1 Key study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Effect of subchronic exposure (90 days) on reproductive organs of male rats Guideline: no GLP: not specified Wistar rats <u>1. Experiment:</u> n=20 per dose group, male animals sacrificed after 90 d of exposure <u>2. Experiment</u> n=20 per dose group, male animals (treated for 90 days with lithium carbonate) caged with untreated female animals to determine fertility index <u>3. Experiment</u> male animals (treated for 90 days with lithium carbonate and a 30 days recovery period) caged with untreated female animals to determine fertility index	Lithium carbonate (purity not provided) 0, 500, 800, 1100 mg/kg diet for 90 days, 0, 20, 32, 44 mg lithium carbonate/kg bw/day, 0, 3.8, 6.0, 8.3 mg Li/kg bw/day	<u>1. Experiment</u> ≥800 mg/kg diet: significantly reduced absolute weight of testes (up to 36%), epididymis (up to 27%) and accessory sex organs (up to 38%)observed, relative organ weights not affected. Dose dependent effects (reduced sperm number from cauda epididymis (up to 47%) and the daily sperm production (up to 71%), reduced serum testosterone (up to 65%) and testicular interstitial fluid volume (up to 50%)) observed, significant at 800 mg/kg diet. Number of abnormal spermatozoa already significantly increased at lowest dose (up to 93%). In the highest dose, severe degenerative changes observed in the testes and accessory reproductive organs. Effects also observed at 800 mg/kg diet to a milder degree. <u>2. Experiment</u> Significantly decreased male fertility index at 800 mg/kg diet and above (90%, 80%, 60%, 40% at 0, 500, 800, 1100 mg/kg diet), mating index not affected <u>3. Experiment</u> Significantly decreased male fertility index at 800 mg/kg diet and above (90%, 80%, 70%, 50% at 0, 500, 800, 1100 mg/kg diet), mating index not affected NOAEL = 20 mg lithium carbonate/kg bw/day LOAEL = 32 mg lithium carbonate/kg bw/day	Thakur et al., 2003 Klimisch score : 1 Key study
Hormonal measurement and histological examination in testicular tissue Guideline: no GLP: not specified Male Wistar rats n=6 per dose group	Lithium carbonate (purity not provided) 0, 10, 20, 30 mg lithium carbonate/kg bw/day, 0, 1.9, 3.8, 5.6 mg Li/kg bw/day, For 48 days via gavage	Dose dependant and statistically significant (from the lowest dose group) decrease of testicular tissue weight (from 0.55 in the control group to 0.25 in the high dose group), germ and somatic cells in seminiferous epithelium (spermatogonia (up to 42%), primary spermatocytes (up to 53%), spermatids (up to 57%), spermatozoids (up to 70%), Sertoli (up to 19%) and Leydig cells (up to 37%)). Dose dependent and statistically significant decreased of blood concentrations of LH (up to 61%), FSH (up to 53%) and testosterone (up to 81%) LOAEL = 10 mg lithium carbonate/kg bw/day	Toghiani et al., 2012 Klimisch score : 2 Supportive study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Influence of 28 day lithium exposure on thyroid and sex hormone levels Guideline: no GLP: no Wistar rats n=14 animals per sex per dose	Lithium carbonate (purity not provided) 0, 2000, 4000 mg/kg diet/day Low dose group: males: 189-246 mg lithium carbonate/kg bw/day; females: 164-217 mg lithium carbonate/kg bw/day 28 days exposure High dose group: males: 303-306 mg lithium carbonate/kg bw/day; females: 271-306 mg lithium carbonate/kg bw/day exposure high dose group terminated after 14 days due to 50-60% mortality	High dose group: 60% mortality in high dose, treatment stopped on day 14, Low dose group: growth arrest and subsequent weight loss (8.9 g/rat during the first 7d, 12.23 g/rat during the second 7d), diarrhea and polydipsia, significant inhibition of testosterone synthesis (-50% and -57% at day 21 and 28) and spermatogenesis (at 28d, 73±2% of azoospermia was found and 70±5%), significant increased serum estradiol concentrations (+54% and +91% at 21d and 28d), disturbance of estrous cycle. Mean serum lithium concentrations low dose group: 0.443, 0.621, 1.797, 1.475 mmol/L on days 7, 14, 21, 28, respectively Mean serum lithium concentrations high dose group: 0.646, 1.219 mmol/L on days 7, 14, respectively LOAEL ca. 200 mg lithium carbonate/kg bw/day	Allagui et al., 2006 Klimisch score : 2 Supportive study
Mouse, female fertility study Guideline: no GLP: no C57BL/6 mice N = 20 females per group	Lithium chloride (purity not provided) 0.4% lithium chloride in diet Exposure period : 15 days	No irregularity in oestrous cycle observed in any mice in the first 3 days of treatment. From the fourth day, 30% of the mice showed irregularity (constant diestrous). This percentage increased on days 5, 6 and 7 until 100% on day 8 and after.	Banerji et al. 1986 Klimisch score : 2 Supportive study
Sperm analysis in epididymis Guideline: no GLP: no Male Wistar rats n=6 per dose group	Lithium carbonate (purity not provided) 0, 10, 20, 30 mg lithium carbonate/kg bw/day, 0, 1.9, 3.8, 5.6 mg Li/kg bw/day, For 48 days via gavage	Dose dependent reduction in number of normal sperm (97%, 88%, 88%, 71% in control, low, mid, high dose group, respectively), sperm motility (96%, 68%, 48%, 39% in control, low, mid, high dose group, respectively) and number of sperms in cauda epididymis (2.19×10^8, 1.42×10^8, 1.21×10^8, 1.12×10^8 in control, low, mid, high dose group, respectively) LOAEL = 10 mg lithium carbonate/kg bw/day	Toghyani et al., 2013 Klimisch score : 4 Not assignable study
Mouse, fertility study Guideline: no GLP: no CFW mice Males and females, number not provided	Lithium chloride (purity not provided 0, 10, 20, 30, 50, 100 or 200 mM lithium chloride in drinking water 0, 423.94, 847.88, 1271.82, 2119.7, 4239.4, 8478.8 mg lithium chloride/L, about 0, 85, 170, 250, 425,	Animals of the highest dose group died within 1 week, animals of the second highest dose group did not reproduce (no details provided), 50 mM group (425 mg lithium chloride/kg bw/day): only results for this group provided; fewer litters of normal size at birth, prolonged intervals between litters and increased postnatal mortality, including loss of entire litters, increased relative heart weights, reduced relative litter weights No toxic effects in the three lowest dose groups (no	Mroczka et al., 1983 Klimisch score : 3 Disregarded study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Only results of 425 mg lithium chloride/kg bw/day group documented, no data on general toxicity and number of animals per group	850, 1700 mg lithium chloride/kg bw/day, about 0, 14, 28, 42, 70, 140, 280 mg Li/kg bw/day Exposure not clearly described, started about 2 or 5 weeks before mating	details provided) NOAEL = 250 mg lithium chloride/kg bw/day Plasma lithium levels after 2 weeks of exposure: 0.09 mM – 0.67 mM in the 10 and 50 mM lithium chloride group, respectively	
Rat, effects on fertility Guideline: no GLP: no Exposure before cohabitation, one half of females sacrificed on GD 13 (number and distribution of implantation sites examined), other dams allowed to deliver and nurse till PND21 (necropsy of dams and litters) n=20 per sex per dose Insufficient reporting	Lithium carbonate (purity not provided) <u>Females</u>: 0, 0.675, 2.025, 4.05 mmol lithium carbonate/kg bw/day 0, 49.51, 149.63, 299.25 mg lithium carbonate/kg bw/day, 0, 9.30, 28.11, 56.22 mg Li/kg bw/d, 14 days before cohabitation <u>Males</u>: 0.27, 0.67, 1.35 mmol lithium carbonate/kg bw/day, 19.95, 49.51, 99.75 mg lithium carbonate/kg bw/day, 3.75, 9.30, 18.74 mg Li/kg bw/day, 70 days before cohabitation Exposition via gavage	No effects on reproduction observed (no further information), no effects in offspring observed (details not reported) <u>NOAEL fertility:</u> Males: 99.75 mg lithium carbonate/kg bw/day Females: 299.25 mg lithium carbonate/kg bw/day Plasma concentration 1.4 mM after daily treatment with 4.05 mmol lithium carbonate/kg bw/day for 3 days.	Gralla and McIlhenny, 1972 Klimisch score : 3 Disregarded study
Rat, fertility study Guideline: no GLP: no Wistar rat Males and females, n=100 controls (20 mM group) n=52 treated (20 mM group) n=6 per sex per group in 25 mM group and control	Lithium chloride (purity not provided) 0, 20, 25 mM lithium chloride in drinking water, 0, 850, 1062.5 mg lithium chloride/L, 0, 66, 83 mg lithium chloride/kg bw/day, 0, 11, 14 mg Li/kg bw/day, Treatment of 20 mM started 3-7 weeks before mating till end of pregnancy or lactation;	<u>25 mM</u>: reduced number of pregnancies (no information on other effects provided) <u>20 mM dams:</u> no effect on reproduction, no toxic signs or behavioural changes (no details provided) <u>20 mM offspring:</u> slower weight gain and growth, differences no longer seen after 2-3 month of growth NOAEL unclear due to limited reporting Plasma lithium levels at 20 mM lithium chloride in drinking water: 1.5-2 mM	Trautner et al., 1958 Klimisch score : 3 Disregarded study

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
group Insufficient reporting	animals in 25 mM group only exposed for 17 days before mating		

Table 21: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Questionnaire analysis	Lithium therapy (no other medication, no further information)	Sexual function questionnaire in 35 bipolar and schizoaffective men, aged 43.3 +/- 9.6 years	Eleven patients (31.4%) reported sexual dysfunction on at least two items. Reduction in frequency of sexual thoughts and loss of erection during sex in 23 and 20% of patients, respectively, difficulties in achieving and maintaining erections in 14% of patients No difference in serum lithium levels in patients with and without sexual dysfunction, no statistical correlation between sexual function scores and serum lithium levels	Aizenberg et al., 1996
Two case reports	Lithium therapy (no further information)		Reduced libido and impaired erection, effects reversible after termination of lithium therapy and reoccurring after restarting lithium therapy in one subject and spontaneously remitting after continuation of lithium therapy	Blay et al., 1982
Semen analysis on patients	Lithium carbonate	3 weeks therapy Dosage sufficient to maintain a plasma concentration of 0.6 to 1.4 mEq/liter	Lithium carbonate therapy produced a significant reduction in sperm viability but no change in sperm count or motility.	Levin et al., 1981

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Reproductive toxicity of lithium carbonate was investigated in a two-generation study with Wistar rats performed according to OECD TG 416 and GLP (Anonymous, 2012). This is the only study in experimental animals on lithium effects on fertility performed in accordance with guidelines and GLP. Animals were treated by gavage with 0, 5, 15, 45 mg lithium carbonate/kg bw/day.

There were no relevant treatment-related changes in oestrous cyclicity, pre-coital time, gestation length, pups survival, mating, fertility, and fecundity or sperm parameters (sperm morphology and motility, testicular

spermatid count and epididymal sperm count) in both generations when dose response and historical control ranges were taken into account, except slightly higher post-implantation loss at 45 mg/kg bw/day dose in the P generation, which subsequently led to lower mean litter size. There were no treatment-related changes in reproductive organ weights and gross findings of parents or weanlings in both generations. Systemic effects were observed in the highest dose group: increased body weights and net body weight gains in males of P generation and increased water intake in both P and F1 generations in males. In both P and F1 generations premating females showed higher net body weight gains.

At post-mortem examination in P generation a higher body weight in males, a significant increase in the absolute and relative liver weight in males and in the relative liver weight in females was observed. Additionally, a marginal increase in absolute and relative adrenal weight and an increase in absolute, but not in relative weight of thyroid in males only was noted.

In F1 generation, the terminal body weight was not affected in any of the female and male dose groups. Absolute and relative liver weights were significantly increased in males of the highest dose group only.

Microscopic analysis of P0 in the highest dose group revealed increased cytoplasmic rarefaction of hepatocytes in liver in males, hepatocellular hypertrophy of minimal severity and focal basophilic hepatocytes in females. Adrenals of males of the high dose group showed higher incidences of cortical vacuolation. Thyroid follicles of females showed increased colloids. The test item related microscopic changes observed in adrenals of males and thyroid of females in P generation were not evident in F1 generation parental animals. F1 parental animals also revealed increased cytoplasmic rarefaction of hepatocytes in males and focal basophilic hepatocytes in the liver of females. Pronounced and severely dilated tubules of kidneys were observed in both generations.

In mid-dose animals, slightly dilated tubules of kidneys were seen microscopically in both generation males and females. These effects were discussed by the authors to be an adaptation to the pharmacological effect of lithium carbonate (vasopressin-downregulation) and therefore not considered as a toxicological effect.

No test item related microscopic findings were observed in both male and female pups of F1 and F2 litters.

Based on these findings, the LOAEL and no-observed-effect level (NOAEL) for systemic toxicity are 45 and 15 mg/kg bw/day, respectively. The NOAEL for fertility is 45 mg/kg bw/day based on no adverse effects reported. It has to be noted that lithium serum concentrations were not provided.

This is a robust study compliant with OECD guideline and GLP (Anonymous 2012).

Allagui et al. (2006) exposed Wistar rats (14/sex/dose) with 0, 2000 or 4000 mg lithium carbonate/kg diet (about 200 or 300 mg/kg bw/day in the low and high dose group, respectively). In high dose group, treatment was stopped at day 14 due to 60% mortality. In both groups, authors reported inhibited testosterone synthesis and spermatogenesis in Wistar rats. Further a reduction of serum levels of tri-iodothyronine and thyroxine were observed. Serum estradiol concentrations were increased. A dose-dependent loss of appetite and a decrease in growth rate as well as polydipsia, polyuria and diarrhea were observed. Statistical significance was 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.

The relevance of this study is questionable considering the high doses used, which is confirmed by the mortality at the high dose group.

Numerous studies only focused on the effect of lithium on male reproductive tract.

Thakur et al. (2003) exposed 20 male Wistar rats/group to 0, 500, 800, 1100 mg lithium carbonate/kg diet (about 0, 20, 32, 44 mg lithium carbonate/kg bw/day) for 90 days. Effects seen in rats of the mid and high dose groups were statistically significant reduced absolute (but not relative) testes, epididymis seminal vesicle and prostate weights. A statistically significant reduced sperm numbers, sperm production, or increase abnormal sperm percentage was also observed at the two highest doses. A statistically significant decrease in serum testosterone levels and testicular interstitial fluid volume and a degeneration of testicular

structures were also noted at the same doses. In consequence, authors reported a dose dependant reduced male fertility index at the two highest doses, even after 30 days of withdrawal of lithium carbonate treatment.

Because no information were provided on systemic toxicity and lithium plasma or serum levels this study was disregarded in the registration dossier. However, as it covers an important and relevant dose range, close to the doses used in the OECD 416 study, and investigates and reports endpoints relevant for male fertility on a substantial number of animals with sufficient details, it is regarded as relevant for classification. Moreover, this is the only study assessing and showing an impact on the fertility of the observations made on sperm and hormonal parameters in numerous studies.

Zarnescu and Zamfirescu (2006) exposed mature male Wistar rats to 0 or 35 mg lithium carbonate/kg bw/day for 21 days per gavage and examined the ultrastructure of the seminiferous tubules at the end of the treatment period. Treated rats showed abnormal or degenerated spermatids and structural abnormalities like loss of germ cell attachment or expanded intercellular spaces between spermatogenic cells.

This study was disregarded in the registration dossier due to “limited investigation depth and reporting deficiencies”. Despite the limitation that only one dose was investigated and that information on lithium plasma levels as well as systemic effects is missing, these results are in good agreement with the findings of Thakur et al. (2003).

Toghiani et al. 2012 exposed male Wistar rats (6/group) to 0, 10, 20, 30 mg of lithium carbonate/kg bw/day for the 48 days of spermatogenesis by gavage. The authors found a dose dependent and statistically significant decreased up to the lowest dose of all cells count in seminiferous tubule : spermatogonia, primary spermatocytes, spermatids, spermatozoa, and Sertoli and Leydig cells. They also performed an hormonal measurement with the same conclusions for LH, FSH and testosterone. A LOAEL of 10 mg of lithium carbonate/kg bw/day can be derived from this study.

No information is available on systemic toxicity, but again results are consistent with those of Thakur et al. (2003) and Zarnescu and Zamfirescu (2006).

In a similar protocol, Toghyani et al. 2013 observed a dose dependent reduction in numbers of normal sperm, sperm motility, and number of sperms in cauda epididymidis was observed, with the same LOAEL of 10 mg of lithium carbonate/kg bw/day.

No other parameters were investigated and few details are provided by the authors precluding this study for being acceptable, even if results are in good agreement with previous studies.

One study assesses specifically effects of lithium chloride on female fertility:

Banerji et al. (1986) exposed female C57BL/6 mice 15 days to 0.4% lithium chloride in diet for 15 days. In order to evaluate effects on reproduction and particularly on oestrous cycle, vaginal smears were examined each days. No irregularity in oestrous cycle was observed in any mice in the first 3 days of treatment. From the fourth day, 30% of the mice showed irregularity, displaying a constant diestrous. This percentage increased on days 5, 6 and 7 until 100% on day 8 and after.

This study give little information on the protocol and the results, particularly, there is no exploration of systemic toxicity.

Other studies exposed rodents via other route with less relevance, but still provide information.

Ali (2008) reported that a cumulative dose of 23.25 mg lithium carbonate/animal (about 22 mg/kg bw/day) administered intraperitoneally over a period of 35 days to adult male Swiss mice (n=20 per group) resulted in decreased testes and body weights and histopathological changes of the testes including disappearance of spermatogonia, decreased number of spermatocytes, Sertoli and Leydig cells with vacuolated cytoplasm and

hypertrophied nuclei, inter- and intracellular vacuoles of germinal cells, widening of the ductus epididymis, increase of abnormal sperms and reduction of serum testosterone levels. The reliability of this study is limited, as the total dose as provided by the authors does not correlate to the dosing regimen, which would result in a total dose of 26.25 mg lithium carbonate/animal, no information on systemic toxicity is provided and because application was intraperitoneally.

Mechanistic aspects of lithium exposure on male fertility were investigated in a series of assays by Ghosh and colleagues.

In the study of Ghosh et al. (1990b) 8 adult male Wistar rats per group were subcutaneously injected with 1, 2 or 4 mg lithium chloride/kg bw/d for 21 days. Spermatogenesis was inhibited in treated animals as revealed by the decreased number of spermatogonia A and step 7 spermatids at the two highest concentrations. This effect was not observed on preleptotene spermatocytes and midpachytene spermatocytes. Moreover, at the two highest concentrations, serum FSH, LH, prolactin (PRL) and testosterone plasma levels were significantly decreased in treated animals as well as testicular 3-beta-hydroxysteroid dehydrogenase and 17-beta-hydroxysteroid dehydrogenase, two key enzymes in androgen biosynthesis.

In a study with a similar protocol, the same authors (Ghosh et al. 1991b) exposed sexually immature male rats (35 days old) subcutaneously with 2 mg lithium chloride/kg bw/day (0.33 mg Li/kg bw/day) for 15, 20, or 25 days with very similar results. Spermatogenesis was inhibited in treated animals. Additionally, serum FSH, LH, PRL and testosterone levels were significantly decreased in treated animals as well as testicular 3-beta-hydroxysteroid dehydrogenase and 17-beta-hydroxysteroid dehydrogenase, two key enzymes in androgen biosynthesis. Additionally, administration of lithium chloride for 20 and 25 days decreased the testicular, prostatic and seminal vesicular weights significantly. Serum lithium levels were about 0.5 mmol. Lithium effects were partially reversible by prolactin application as shown by the same group in a second comparable experiment where rats were treated with prolactin 8 hours after Li-chloride treatment (Ghosh et al., 1991a). Animals revealed a significant restoration of testicular weight in comparison to lithium-treated animals not receiving prolactin. Body weights of the lithium-treated animals in all groups did not differ from that in controls.

Inhibition of 3-beta-hydroxysteroid dehydrogenase and 17-beta-hydroxysteroid dehydrogenase activity by lithium chloride in testes was confirmed *in vitro* (Ghosh et al. 1990a). Incubation of the whole organ in the presence of 0.5, 2.5 or 5 mM lithium inhibited the enzyme activity in a dose dependent manner at concentrations of 2.5 mM or above. These findings support the results obtained in *in vivo* studies.

Banerji et al. (1983) reported that FSH plasma levels of adult male Sprague-Dawley rats were not influenced by intraperitoneal treatment with 100 mg lithium chloride/kg bw twice daily for 2 or 7 days. Plasma LH was increased after 2 days and decreased after 7 days of treatment. Plasma PRL was decreased after 7 days of treatment, but no effect was observed after 2 days of treatment. Two of 20 animals out of the 7 day group died on the 6th day, and a number of rats of this group showed signs of polydipsia and polyuria. Pituitary LH, FSH and PRL-levels were not affected by lithium treatment.

The same group of authors studied the effect of acute intraperitoneal injection of 34.7 mg/kg (5 mEq/kg) lithium chloride in proestrus female C57BL/6 mice (3 injections the same day). Animals were sacrificed on the evening. They observed a statistically significant reduction of plasma LH, but as in the previous experiment, no change in pituitary LH. According to the authors, this could be explained by lithium interfering with the process of secretion, rather that the process of synthesis of LH. Plasma levels of FSH showed a significant increased, as for pituitary levels (Banerji et al., 1986).

In a similar study of the same group of investigators as Allagui et al. (2006), male mice (n=6 per group) were exposed intraperitoneally to 0, 20, 40, 80 mg Li carbonate/kg bw/day for 14 or 28 days (Nciri et al., 2009). Mice revealed increased weight gain and polydipsia and reduced serum testosterone levels. Further, increased lipid peroxidation levels and superoxide-dismutase and glutathione-peroxidase activity were recorded. According to the authors albino Wistar mice were exposed. This inaccuracy is a shortcoming of the publication and questions the reliability of the data. This study is therefore regarded as non-valid.

In another study described in section 10.10.4 (Mostafa et al. 2010), authors observed increased diameter of seminiferous tubules and decrease of primary spermatocytes count, nuclear diameter of Leydig cells,

diameter of epididymis ductules and testosterone level on offspring after intraperitoneal exposure of dams during gestation and lactation.

Studies were performed subcutaneously of intraperitoneally on females rats with various duration of exposure. Lithium exposure in OVX rats for 3 and 7 days resulted in a significant reduction in plasma LH and FSH levels (Sheikha et al., 1989). As in the study of Barneji et al. (1986), it was observed that duration of the oestrous cycle was increased in lithium-treated rat with longer metestrous and diestrous phases (Jana et al., 2001). Also, it was showed that lithium induced follicular atresia, significant decreases in serum progesterone concentration and ovarian weight (Mirakhori et al., 2013; Khodadadi et al., 2013).

Finally, other available experimental studies are considered not reliable.

Mroczka et al. (1983) exposed mice mating pairs to drinking water containing Li-chloride concentrations of 0, 10, 20, 30, 50, 100 or 200 mM lithium chloride (about 0, 85, 170, 250, 425, 850, 1700 mg lithium chloride/kg bw/day). Animals of the highest dose group refused to drink and died within one week. Animals of the 100 mM group survived, but did not reproduce (no further information). Animals of the 50 mM 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/day group documented, no data on general toxicity and number of animals per group) this study was considered not reliable.

Gralla and McIlhenny (1972) investigated effects of lithium carbonate on fertility and general reproductive performance in Charles River albino rats. Females (n=20 per dose group) were treated per oral gavage for 14 days before cohabitation with 49.51, 149.63, 299.25 mg lithium carbonate/kg bw/day, the control group received tap water. Males (n=20 per dose group) were treated per oral gavage for 70 days before cohabitation with 19.95, 49.51, 99.75 mg lithium carbonate/kg bw/day. Males and females of respective high, intermediate and low dose groups were mated. One half of the treated females were sacrificed on GD13 and the number and distribution of implantation sites were recorded. The remaining females were allowed to deliver and nurse their offspring to PND 21, at which time both dams and offspring were sacrificed and examined for gross internal and external physical defects (no further information). Authors also performed a teratology studies, detailed below. No adverse effects on reproduction were observed (no further information). Two pregnant female rats died unexpectedly for unknown reasons (not clear in which of the three rat studies reported by the authors mortality of the two rats occurred). Plasma concentration was 1.4 mmol Li/L after daily treatment with 299.25 mg lithium carbonate/kg bw/day for 3 days. The NOAEL for fertility in this study is 299.25 and 99.75 mg lithium carbonate/kg bw/day in females and males, respectively, based on no effect.

Due to insufficient reporting this study was considered not reliable.

Trautner et al. (1958) studied the effects of lithium chloride exposure via drinking water on pregnancy in rats (52 treated rats and 100 controls). The animals were administered lithium chloride in a concentration of 66 mg lithium chloride/kg bw/day producing plasma Li levels of 1.5-2.0 mmol. According to the authors normal pregnancies of lithium-treated females and controls were recorded (with respect to incidence and progress of pregnancy, birth and lactation, and the health and progress of the young). No malformations or other defects in the lithium-exposed litters were recorded. Weight gain and growth were retarded in offspring of dams, which were continuously exposed during pregnancy and lactation (no details provided). Reduced numbers of pregnancies resulted from treatment with about 83 mg lithium chloride/kg bw/day (no information on plasma concentration or systemic toxicity).

Due to insufficient reporting this study was considered not reliable.

Human data for lithium effects on male fertility are restricted to few case reports, which remain not sufficient to serve as basis for a classification.

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

Aizenberg et al. (1996) reported the results of a sexual function questionnaire in 35 bipolar and schizoaffective men, aged 43.3 +/- 9.6 years. 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) performed an analyse of semen of 9 patients treated for 3 weeks with lithium carbonate, at dosage sufficient to maintain a plasma concentration of 0.6 to 1.4 mEq/liter. Comparing before and after treatment, they found that lithium produces significant decrease in the percentage of sperm viability, from 70% to 55%. Sperm count and motility were not affected by lithium treatment.

Effects of lithium therapy on human PRL levels were investigated in several studies and summarised in HCN (2000). Whereas 4 studies did not observe any effect of lithium treatment on plasma PRL level, a fifth study reported an increase in PRL levels under lithium therapy. HCN concluded that due to these contradictory results no final conclusions could be drawn.

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

Altogether, existing fertility studies seem not consistent. The only guideline study available does not indicate effects of lithium treatment on fertility up to systemic toxic doses (45 mg/kg bw/day).

In addition to these investigations on fertility several recent investigations of lithium effects on male reproductive tract are available.

Most of them were performed with doses (10-44 mg/kg/d) similar to the doses used in the two generation study (5-45 mg/kg/day) and are very consistent with each other (decreased of testes weight, germ and somatic cells, serum testosterone levels, histopathological changes…). However, information on systemic toxicity and lithium plasma level is missing in most of those studies, as the focus of these studies were effects on sperm parameters or histopathological differences between male reproductive organs of control and treated group (Thakur et al., 2003; Zarnescu and Zamfirescu, 2006; Toghiani et al., 2012; Ali et al., 2008).

However, as stated in the CLP guidance "*Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes. There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity. However, mating behaviour can be influenced by parental effects not directly related to reproduction (e.g. sedation, paralysis), and such effects on mating behaviour may not warrant classification.*", for fertility, the influence of systemic toxicity is marginal compared with developmental toxicity. Therefore, even if there is no information on systemic toxicity in these studies, no marked toxicity is anticipated regarding dose range used by the authors in the light of the results of other studies on lithium compound. In particular, no excessive toxicity was reported in the OECD 416 study performed at doses up to 45 mg/kg bw/day, in the same strain of rats, with the same route of exposure and during longer exposure duration. Confirming these effects, the only robust study with subsequent mating shows consequences on male fertility, with dose dependant reduced fertility index.

Some other studies are not relevant or had to be disregarded due to several reasons:

- Negative findings were reported with lithium carbonate in rats and lithium chloride in mice but the studies are old and give no details on protocol and results (Banerji et al., 1986; Gralla and Mcllhenny, 1972);
- Investigations with lithium chloride in rats observed effects on fertility at very high doses, which prevent a proper investigation of fertility effects (Mroczka et al., 1983).

In summary, no reproductive effect is observed in the OECD 416 study. However, various data consistently indicate that lithium affect the **male reproductive system**, impairing spermatogenesis and causing morphological changes of the reproductive organs, up to a concrete effect on fertility with a decrease of fertility index.

- A decreased fertility index is reported in one study (Thakur et al. 2003), considered as a key study with Klimisch score 1.
- Effects on male reproductive system (histopathological findings and alteration of sperm parameters) are consistently noted in different species (rats and mice), by different routes of exposure (gavage, diet, intraperitoneal and subcutaneous administration). This is supported by the mechanistic investigations of several authors (Allagui et al. 2006, Ghosh et al., 1990a, 1990b, 1991b, Toghiani et al. 2012, Thakur et al. 2003, Banerji et al., 1986) suggesting that lithium interferes with the sexual hormonal system and causes disturbances of the regulatory circuit. Although some species specific differences in the relevance of the single pathways (FSH or LH mediated) are known between rats and humans (Schlatt and Ehmcke, 2014), the regulatory circuits are comparable to a large extent. Therefore, in principle the observations on sperm parameters observed in rats can be regarded as relevant for the human situation. As a matter of fact, in the Levin *et al.* study, a significant decrease in the percentage of sperm viability is observed. However, the reasons for the contradictions between these findings and the two-generation study remain unclear. Particularly, from study by oral route of exposure, similar doses, same strain of rats and route of exposure (gavage) were used.

Therefore, various studies of adequate quality (Klismish scores 1 and 2) demonstrate clear and consistent effects on male reproductive system that can *in fine* induce a decrease of fertility.

Some less clear effects were noted in **female reproductive system**, such as irregularity in oestrous cycle (Banerji et al. (1986)). These data are not sufficient by itself for classification proposal.

10.10.3 Comparison with the CLP criteria

For potential classification on sexual function and fertility, criteria from CLP guidance (ECHA, 2017) were applied.

- Adverse effects on sexual function and fertility are described as "*Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems*." *(ECHA, 2017).*
- *Known human reproductive toxicant. "The classification of a substance in this Category 1A is largely based on evidence from humans."*

Human data are restricted to few case reports, which are not sufficient by themselves to serve as basis for a classification discussion.

- *Presumed human reproductive toxicant. "The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic*

information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate" (ECHA, 2017).

Ten experimental studies have investigated effects of lithium salts in rodent on reproductive function.

No toxicologically significant effects on fertility were observed in a recent two-generation guideline study and in further insufficiently reported and so disregarded rat fertility studies (Anonymous, 2012; **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 71%), sperm function, and/or male reproductive organ structure, but also on testosterone levels (decrease up to 81%). All five studies on male reproduction were performed with the identical rat strain as used in the two-generation study (Wistar rats) and four of them used doses in the same range as the two-generation study (Thakur et al., 2003; Zarnescu and Zamfirescu, 2006; Toghyani et al., 2013, Toghiani et al., 2012). Moreover, the 90-day study (Thakur et al., 2003) with subsequent mating shows consequences on male fertility, with reduced male fertility index (from 90% in control group to 40% in the high dose group), confirming the consequence of the previous mentioned effects. Only the fifth study used higher, partially lethal doses, and is therefore less informative (Allagui et al., 2006). Complete study report of the two-generation guideline study is not available, but based on information available, reasons for these 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 dose range used by the authors and the few information provided (Mroczka et al. 1983).

It has to be noted that these findings are confirmed by mechanistic studies performed with less realistic route 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 as a weight of evidence for supporting the reproductive effects reported by oral route.

The reproductive effects are also supported by the results of biochemical measurements performed in various studies. Indeed, decreased levels of testosterone, FSH, LH and prolactin were reported, and also on key enzymes in androgen biosynthesis.

In conclusion, despite the overall negative findings in the two-generation study, the high consistency of the findings in the 90-day/mating study and the studies on male reproduction, which are recent and robust studies, led to a clear evidence of effects on fertility. A classification in category 1B for the three lithium compounds is therefore warranted.

10.10.4 Adverse effects on development

Table 22: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal Developmental Toxicity Study Similar to OECD TG 414 Deviations: Exposure from GD6 instead of GD5 at the latest GLP: yes	Lithium carbonate (purity 99.6%) 0, 10, 30 and 90 mg lithium carbonate/kg bw/ day 0, 1.88, 5.64, 16.91 mg Li/kg bw/day, Once daily from GD 6 to	Dams: NOAEL: 30 mg/kg bw/day based on maternal toxicity at LOAEL of 90 mg/kg bw/day: pilo-erection, reduced drinking water consumption, reduced feed consumption, reduced body weight gain Offspring: NOEL: 90 mg/kg bw/day No effects on number of corpora lutea, implantation sites, resorptions, sex distribution,	Anonymous, 2010b Klimisch score : 1 Key study

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Female Crl CD (SD) rats (25 animals/dose group)	GD 19, examination on GD 20 Exposure via gavage	fetal and placental weights, number of live foetuses at birth. No dead foetuses or runts were noted at laparotomy. No malformations or variations were noted in the foetuses during external/ internal examination, skeletal examination or soft tissue evaluation. Mean peak plasma levels of 1.66, 3.59 and 9.65 mg Li/L plasma (0.24, 0.52, 1.39 mM Li)	
Neurodevelopmental Toxicity Study Similar to OECD TG 426 Deviations: yes (longer duration of exposure, low number of animals/group, only two doses) GLP: not mentioned Swiss-Webster Strain mice At least 7 pregnant females per dose group; 3 pups per litter investigated per test per GD 1 - PND 21	Lithium chloride (analytical grade) 0, 15, 30 mg Li/kg bw/day 0, 90, 180 mg lithium chloride/kg bw/day Via drinking water from GD 1 to PND 15, neuro-behaviour examination until PND 21	Dams: no information on maternal toxicity provided NOAEL unknown Offspring: significant dose dependent decrease in body weight, delayed eye opening, appearance of body hair, sensory motor reflexes (righting, rotating, cliff avoidance) affected in both sexes, inhibition of locomotor activity of male, weaned pups (females not investigated), effects already significant on PND1 LOAEL 90 mg lithium chloride/kg bw/day	Abu-Taweel, 2012 Klimisch score : 2 Supportive study
Prenatal and postnatal Developmental Toxicity Study Guideline: no GLP: no Wistar rats 44 females in lithium group 46 females in deprived-water group 13 females in control group	Lithium chloride (purity not provided) 0, 10 mM in drinking water + 2 control groups (1 deprived-water group) 0, 53 mg lithium chloride/kg bw/d Daily from GD1 to end of lactation. Cross over at parturition of half of animals between lithium group and deprived water group.	No malformation, stillborn or litter size difference observed at birth. Reduction in the proportion of pups with a normal righting reflex at birth in both the water deprived (78.5%) and lithium treated litters (70.5%) compared to the control group (94.2%), but this group had also a reduced correct righting reflex compared also to the water deprived group. Statistically significant delay in the critical day of maturation compared to control in all groups (day of eye opening and avoidance of visual cliff). Statistically significant lower body weight at day 21 in pups exposed to lithium during lactation.	Teixeira et al., 1995 Klimisch score : 2 Supportive study
Prenatal and postnatal Developmental Toxicity Study Guideline: no GLP: no Sprague-Dawley rats	Lithium carbonate (purity not provided) 0, 1000 ppm lithium carbonate 0, 1000 mg lithium carbonate/kg diet,	Dams: decreased body weight gain and feed intake during gestation. After lactation, decrease in body weight only in groups exposed to lithium during the entire study and during lactation. The group exposed to lithium during gestation showed no difference. Only the absolute liver weight was decreased in group exposed during lactation. Concerning	Ibrahim and Canolty, 1990 Klimisch score : 2 Supportive study

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
11 and 13 female animals per dose group	About 0, 50 mg lithium carbonate/kg bw/day Daily from GD 1 to end of gestation, then switch of diet for half of both groups, and exposure until LD21 Exposure in diet	relative organ weight, heart, kidney and liver were decreased in the same group. Offspring: no gross malformation in newborn animals. At birth, mean pup weight was significantly lower in exposed group (6.3 g and 5.7 g 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 (0.31 g and 0.24 g in control and lithium groups respectively) and spleen weight (0.23 g and 0.17 g in control and lithium groups respectively) were decreased in group exposed during lactation only.	
Prenatal Developmental Toxicity Screening Study. maternal and foetal examinations, examinations of ovaries and uterine content Guideline: no GLP: no Tif:RAIf rats (Sprague Dawley derived) 14-19 female animals per dose group	Lithium carbonate (‘purissima’) 100 mg lithium carbonate/kg bw/d, 0,18.79 mg Li/kg bw/day, once daily from GD 6 to 10, GD 11-15 or GD 16-20, examination on GD 21 Exposure via gavage Comparison with historical control	Dams: reduction of body weight gain and feed consumption, polyuria GD 6-10: Offspring: embryonic and fetal deaths (3.8 % of implantation sites), dilatation of renal pelvis with obsolete or missing papillae: 0/67 (0%) GD 11-15: Offspring: embryonic and fetal deaths (7.0% of implantation sites), dilatation of renal pelvis with obsolete or missing papillae: 3/75 (4%) GD 16-20: Offspring: increased prenatal mortality, embryonic and fetal deaths (38.5% of implantation sites), dilatation of renal pelvis with obsolete or missing papillae: 7/41 (17%) foetuses/4/14 litters GD 16-20: Dams: 7 died one day before expected delivery (no gross pathological findings) LOAEL: 100 mg lithium carbonate/kg bw/day	Fritz, 1988 Klimisch score : 2 Supportive study
Prenatal Developmental Toxicity Study Tif:RAIf rats 20 female animals per dose group	Lithium carbonate (‘purissima’) 0, 100 mg lithium carbonate/kg bw/day 0, 18.79 mg Li/kg bw/day, once daily from GD16-20, examination on GD 21 or PND 11-19 Exposure via gavage	Dams: reduced body weight gain in treated animals (11.5%, control: 21.5%), mortality (2/20), polyuria, increased water consumption Offspring: dilatation of renal pelvis with obsolete or missing papillae: (20/93 foetuses, 22%, control: 0/133 foetuses, 0%, GD21), mortality (half of the animals died PND 1-4 with dilatation of renal pelvis), surviving animals without nephrotoxicity LOAEL: 100 mg lithium carbonate/kg bw/day	
Prenatal Developmental Toxicity Study	Lithium carbonate (‘purissima’)	Dams: reduction of body weight gain (12.9%, control: 19%) and feed consumption, polyuria,	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Tif:RAIf rats 28 female animals in exposure group, 29 in control group	0, 60 mg lithium carbonate/kg bw/day, 0, 11.3 mg Li/kg bw/day, once daily from GD 16-20, examination on PND 35-40, crossfostering Exposure via gavage	increased water consumption, macroscopically normal kidneys Offspring: reduced litter size (PND1: 10.9±5.8, control: 16.0±2.1), no effects on the kidneys LOAEL: 60 mg lithium carbonate/kg bw/day	
Prenatal Developmental Toxicity Screening Study, maternal and foetal examinations, examinations of ovaries and uterine content Guideline: no GLP: no Wistar rats n=20 control, n=11 or 13 females in low or high dose group	Lithium carbonate (purity not provided) 0, 50, 100 mg lithium carbonate/kg bw/day 0, 9.4, 18.79 mg Li/kg bw/day, once daily from GD 6 to GD 15, examination on GD 20 Exposure via gavage	Dams: no information on toxicity Offspring: in the highest dose group: reduced body weight, reduced implantations, increase in number of resorptions, reduced number of pups alive, incomplete ossification of sternebrae (39% vs 0% in control), shortening of several bones (radius, ulna, humerus, tibia, fibula, femur), malformations of scapula (37% vs 0% in control) and pelvic bone (33% vs 0% in control) NOAEL: 50 mg lithium carbonate/kg bw/day	Marathe and Thomas, 1986 Klimisch score : 2 Supportive study
Prenatal and post natal developmental Toxicity Study Guideline: no GLP: no Pig 12 females per dose group	Lithium carbonate 0, 3000 mg/kg diet, about 0, 40 mg lithium carbonate/kg bw/day, about 0, 7.5 mg Li/kg bw/day, from GD 30-114, observation until PND 21	Dams: decrease of body weight gain, significant at GD 110 (about 23% reduction) Offspring: prenatal mortality increased (adjusted mean number of live piglets per litter 9.6 in treated and 11.3 in control, adjusted mean number of stillbirth and mummies per litter 2.1 in treated and 0.6 in control), reduced litter birth weight (11.1 vs. 15.4 kg in treated and control, respectively), reduced survival of offspring during lactation period (6.5 vs. 8.0 in treated and control, respectively)	Kelley et al., 1978 Klimisch score : 2 Supportive study
Prenatal Developmental Toxicity Study Guideline: no GLP: no Albino rats 20 female animals per dose group	Lithium carbonate (purity not provided) 0, 0.675, 2.025, 4.05 mmol lithium carbonate/kg bw/day 0, 49.51, 149.63, 299.25 mg lithium carbonate/kg bw/day, 0, 9.30, 28.11, 56.22 mg Li/kg bw/day, once daily from GD 5 to 15, examination on GD 20 Exposure via gavage	Dams: up to the highest dose no adverse effects on fertility, number of implantation sites, litter size, body weight gain, but 2 females 'died unexpectedly' (no further information) NOAEL: 149.63 mg lithium carbonate/kg bw/day Offspring: no internal and skeletal abnormalities NOAEL: 299.25 mg lithium carbonate/kg bw/day Plasma concentration 1.4 mmol Li/L after daily treatment with 4.05 mmol lithium carbonate/kg bw/day for 3 days.	Gralla and McIlhenny, 1972 Klimisch score : 4 Not assignable study

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal Developmental Toxicity Study Guideline: no GLP: no Albino rats 10 female animals per dose group	Lithium carbonate (purity not provided) 0, 0.675, 2.025, 4.05 mmol lithium carbonate/kg bw/day 0, 49.51, 149.63, 299.25 mg lithium carbonate/kg bw/day, 0, 9.30, 28.11, 56.22 mg Li/kg bw/day, once daily from GD 14 to PND21, examination on PND 21 Exposure via gavage	Dams: up to the highest dose no adverse effects on fertility, number of implantation sites, litter size, body weight gain, but 2 females 'died unexpectedly' (no further information) NOAEL: 149.63 mg lithium carbonate/kg bw/day Offspring: reduced body weight in the highest dose group, no internal and skeletal abnormalities NOAEL: 149.63 mg lithium carbonate/kg bw/day Plasma concentration 1.4 mmol Li/L after daily treatment with 4.05 mmol lithium carbonate/kg bw/day for 3 days.	
Prenatal Developmental Toxicity Study Guideline: no GLP: no New Zealand rabbits 10 female animals per dose group	Lithium carbonate (purity not provided) 0, 0.675, 1.08 mmol lithium carbonate/kg bw/day, 0, 49.51, 79.8 mg lithium carbonate/kg bw/day, 0, 9.30, 14.99, mg Li/kg bw/day, once daily from GD 5 to 18, examination on GD 28 Application via capsule	Dams: mortality in the highest dose group (3/10), no effects observed on number of implantation sites, mean litter size and body weight; one dam of low dose group died for unknown reasons (relevance unclear) NOAEL: 49.51 mg lithium carbonate/kg bw/day Offspring: no grossly visible internal or skeletal defects NOAEL: 79.8 mg lithium carbonate/kg bw/day Plasma concentration in the highest dose group: 1.5-2.4 mM	
Prenatal Developmental Toxicity Study Guideline: no GLP: no Rhesus monkeys 6 females animals in exposure group, 5 animals in control group	Lithium carbonate (purity not provided) 0, 0.67 mmol lithium carbonate/kg bw/day, 0, 49.51 mg lithium carbonate/kg bw/day, 0, 9.30 mg Li/kg bw/d, once daily from GD 14 to 35. Observation until PND 30/12-15 month of age Application via capsule	Dams: no adverse effects described Offspring: no adverse effects observed, no visible malformations, no signs of functional neurologic defects, normal growth, no physical defects clinically at 12-15 month of age NOAEL: 49.51 mg lithium carbonate/kg bw/day Plasma concentration in the highest dose group: 0.2-1.4 mM	
Prenatal Developmental Toxicity Study Dose range finding study	Lithium carbonate (purity not provided) 200, 300, 465 mg lithium carbonate/kg bw/day,	Dams: no information on maternal toxicity Offspring: prenatal mortality in the highest dose group (26%), cleft palate 11/37 (30%) in 3/4 litters	Szabo, 1970 Klimisch score : 4 Not assignable

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Guideline: no GLP: no HaM/ICR mice 3-4 female animals per dose group, no control group	37.57, 56.36, 87.36 mg Li/kg bw/day from GD 6-15, examination on GD 18 Exposure via gavage	In the middle dose group cleft palates were observed in 3/50 (6%) animals in 1/4 litters. In the lowest dose group no adverse effects were observed. NOAEL: 200 mg lithium carbonate/kg bw/day No control group	study
Prenatal Developmental Toxicity Study Guideline: no GLP: no HaM/ICR mice 15-20 female animals per dose group, 16 animals in control group	Lithium carbonate (purity not provided) 0, 200, 465 mg lithium carbonate/kg bw/day, 0, 37.57, 87.36 mg Li/kg bw/day from GD 6-15, examination on GD 18 Exposure via gavage	Dams: mortality in the highest dose group (37%) Offspring: in the highest dose group: dead foetuses and resorption: 32% (control; 12.3%), cleft palate 12/121 in 7/15 litters (control: 0/181, historical control: 6/2881 (0.2%)) In the lowest dose group cleft palate in 1/243 foetuses in 1/20 litters, effect not significant NOAEL: 200 mg lithium carbonate/kg bw/day	
Prenatal and postnatal Developmental Toxicity Study Guideline: no GLP: no Albino mice 5 females per group Few animals used in each groups and limited details provided by the authors	Lithium chloride 0, 1 mEq drinking water 0, 10 mg/kg bw/d From mating until end of weaning From delivery until end of weaning	Dams: no information on maternal toxicity Offspring: significant decreased in brain weight in males and females, kidney weight in females, and testis weight of offspring. Pre and postnatal exposure also induced L-ADH in developing males and females. Decrease brain weight after postnatal exposure.	Messiha, 1986 Klimisch score : 3 Disregarded study
Prenatal Developmental Toxicity Screening Study Guideline: no GLP: no 129 Sv/SL mice 16 female animals per dose group, no control animals No control animals, visceral malformations not examined, maternal effects not reported and only one dose group tested, together with a limited documentation	Lithium carbonate (analytical grade) 2 mg lithium carbonate/mL drinking water, ca. 400 mg lithium carbonate/kg bw/day, ca. 75 mg Li/kg bw/day Exposure on GD 1-18, examination on GD 17 or 18	Dams: reduced number of litters (only 2/16 of pregnant rats), with 60 % resorptions, no further information on maternal effects Offspring: no external or skeletal malformations Serum levels 0.5-1.0 mmol/L	Smithberg and Dixit, 1982 Klimisch score : 3 Disregarded study

Table 23: Summary table of human data on adverse effects on development

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Systematic review and meta-analysis focusing on neurodevelopmental effects	Lithium	7 preclinical studies, 3 cohort studies, and 5 case studies	No effect on neurodevelopment. Many confounding factors	Poels et al., 2018
Meta-analysis from 6 cohort studies	Lithium	727 pregnancies	No difference for caesarean section, preterm birth, low birth weight, or small for gestational age. Exposure during the first trimester associated with increased risk of major malformations (OR = 1.71, 95% CI : 1.07-2.72), but not cardiac malformations (OR = 1.54, 95% CI : 0.64-3.70)	Munk-Olsen, 2018
Cohort study	Lithium	1,325,563 pregnancies between 2000 and 2010, 663 women exposed to lithium during the first pregnancy trimester	Correlation between lithium exposure early in pregnancy and cardiac malformation: RR: 600 mg or less: 1.11 (95% CI = 0.46-2.64) 601 to 900 mg: 1.60 (95% CI = 0.67-3.80), > 900 mg: 3.22 (95% CI = 1.47-7.02).	Patorno et al., 2017
Cohort study	Lithium	183 lithium-exposed pregnancies compared to 72 disease-matched and 748 nonteratogenic-exposed	Rate of total congenital anomalies not different between the 3 groups. Increased number of cardiovascular anomalies in lithium treated group vs. nonteratogenic group (4.1% vs. 0.6%), higher rate of preterm deliveries (13.7% vs. 6.0%), and one case of Ebstein's anomaly. Lack of statistical power. Only pregnancies of women who contacted the Israeli Teratology Information Service	Diav-Citrin et al., 2014
Review and meta-analysis	Lithium	Studies on lithium toxicity published between 1966 and 2010 62 publications assessing teratogenicity of lithium: 7	Evidence that lithium is teratogenic is quite weak. Due partly to heterogeneity in the results, uncertainty remains, the risk cannot be ruled out,	McKnight et al., 2012

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		cohorts, 7 case control studies and 48 cases reports	and lithium have to be avoid during pregnancy	
Cohort study	Lithium	15 children born between 1994 and 2007 and exposed to lithium in utero, but not breastfed Investigated at 3-15 years of age	No adverse effects on growth, neurological, cognitive and behavioural development Small group of children investigated, no appropriate control group, and other medication besides lithium used	Van der Lugt et al., 2012
Review	Lithium	Review of human studies dealing with teratogenic and embryotoxic effects of lithium published between 1969 and 2005 24 case reports 9 of these born to mothers treated with lithium only.	Most anomalies reported for the cases were of the cardiovascular system. All case control and prospective studies were negative. Lithium therapy adds only a small risk for cardiovascular defect, and does not increased general rate of major anomalies.	Yacobi and Ornoy, 2008
Retrospective cohort study	Lithium	years 1973-1979 350 mother-child pairs, medical data available only for 82% of them Lithium therapy only documented for 59 of the manic-depressive patients, 18 of them received lithium together with other psychotropic drugs	Women treated only with lithium: 4/41 neonatal deaths, 5/41 malformed infants, 2/41 dead and malformed infants and 3/41 heart defects (no Ebstein's anomaly) recorded. Small sample size. No statistical method used. Influence of confounding factors not assessed properly	Källén and Tandberg, 1983

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Experimental data in animals:

Except a minor deviation with an exposure starting later than recommended in the guideline, a prenatal developmental toxicity study was performed in SD rats with lithium carbonate with a protocol very similar to OECD guideline 414. This is considered as key study for this endpoint.

Female rats were administered 0, 10, 30 or 90 mg lithium carbonate/kg bw/day by gavage from GD 6 to 19. The NOAEL was 30 mg lithium carbonate/kg bw/day for the dams (maternal NOAEL). The following effects were observed in the highest dose group (90 mg lithium carbonate/kg bw/day): pilo-erection in a few dams, slight but significant reductions for the net weight change and the food intake. The NOAEL for the foetuses was 90 mg lithium carbonate/kg bw/day. There was no foetal malformations, and no test item-related increase in the incidence of external/internal, skeletal or soft tissue variations or skeletal retardations.

Serum analysis revealed a clear dose-related systemic exposure to lithium (0.24, 0.52, 1.39 mM Li). Anonymous, 2010b).

Several other studies are available, scored with a Klimisch score of 2. They are considered as **supportive studies**.

In the guideline two-generation study documented in section 10.10.1, the only developmental effect observed was the mean litter size and mean viable litter size significantly lower at the high dose compared to vehicle control.

Offspring (3 per litter, 7 litters, male and female) of Swiss-Webster mice (at least 7 per dose group), treated from GD 1 to PND 15 to lithium chloride via drinking water (0, 90, 180 mg lithium chloride/kg bw/day) were examined for neuro-developmental effects on PND 1-21. There was a significant dose-dependent effect on postnatal body weight gain which was decreased, age of hair appearance and eye opening which were delayed, and sensory motor reflexes (righting reflex, rotating reflex, cliff avoidance) which were decreased. Locomotor activity of male pups at weaning (PND 21, females not investigated) was reduced (Number of squares crossed, 69%, Wall rears, 30%, Rears, 78%, Locomotion duration 52% and Immobility duration increased by 200%). Further, a dose-dependent decrease in liver acid phosphatase, alkaline phosphatase and brain acetylcholine esterase was reported. The developmental LOAEL is 90 mg lithium chloride/kg bw/day. No details on maternal toxicity nor plasma lithium concentrations were reported, therefore no maternal NOAEL or LOAEL can be determined (Abu-Taweel, 2012). This study points to a neuro-developmental toxicity of lithium chloride in mice due to gestational and/or lactational exposure.

In the first place, the validity of this study is impaired due to limited reporting (e.g. no information on total number of dams, maternal toxicity, drinking water consumption and body weight of dams). However, in comparison to the other oral mice studies the dose is about tenfold lower and therefore no relevant maternal toxicity is expected. This study is regarded relevant in the way that a careful examination of neurotoxic effects has been performed, which have been influenced by lithium treatment.

Nulliparous females Wistar rats were exposed from GD1 to parturition to 53 mg/kg bw/d (10 mM) lithium chloride in drinking water *at libitum*. As pilot studies demonstrated a 35% lower liquid intake in these animals, in addition to control group, a group with tap water with the same average liquid volume as the exposed group was set up. Moreover, at birth, part of exposed litter and water deprived litter were crossed over. No malformation, stillborn or litter size difference was observed at birth. There was a reduction in the proportion of pups with a normal righting reflex at birth. This reduction was marked in both the water deprived and lithium treated litters, but this group had also a reduced correct righting reflex compared to the water deprived group. Both groups showed a delay in the critical day of maturation compared to control. However, concerning the day of eye opening, authors did not discuss if the difference between lithium treated group and the water-deprived group was significant. Concerning the ability to avoid visual cliff, they mentioned that this difference was not significant. Therefore, a doubt remains if these latter effects observed are the consequence of the lithium treatment or the water-deprivation (Teixeira et al., 1995).

Concerning neuro-developmental disorders, Poels *et al.* (2018), in their review, concluded: "*Overall, findings from preclinical studies suggest a deleterious effect of lithium on locomotor activity and delayed development of eye opening and righting reflexes. Additionally, brain weight was found to be lower in lithium-exposed offspring*".

In a developmental study on Sprague-Dawley rats, 11 and 13 females were fed during gestation a casual diet or with lithium carbonate corresponding to an exposure of 50 mg lithium carbonate/kg bw/day. At parturition, half rats of both groups were switch to the other diet to assess the potential effect of exposure during lactation. No gross malformation were observed in newborn animals. At birth, mean pup weight was significantly lower in exposed group. Moreover, decreased body weight gain and feed intake was also observed in dams during gestation (Ibrahim and Canolty, 1990).

This study investigated few parameters and few details are given by the authors but is still informative in a weight of evidence approach.

Fritz (1988) investigated the transplacental effects of lithium carbonate on the developing rat kidney. They exposed Tif:RAIf female rats during several periods of gestation (GD 6-10 or GD 11-15 or GD 16-20) towards 100 mg lithium carbonate/kg bw/d by gavage. Controls received distilled water. On GD 21 dams were sacrificed by carbon-dioxide and uterine contents were examined. Foetuses were removed, submitted to macroscopic pathological examination, weighed and examined for skeletal (about two thirds per litter) or visceral (about one third per litter) effects with particular consideration of the urogenital system. Lithium carbonate treatment caused moderate maternal toxicity including polyuria, except in group exposed from GD16-20 where seven maternal deaths were observed. In offspring increased prenatal and postnatal mortality was observed. Visceral examination of the foetuses revealed an enlargement of the renal pelves associated with rudimentary or missing papillae. The authors of the study interpreted these findings as developmental retardation due to specific lithium activity. After termination of the exposure, i.e. after birth, slight to moderate structural changes of the kidney were apparently compensated.

A dose of 60 mg lithium carbonate/kg bw/d during GD 16-20 still caused moderate maternal toxicity including polyuria. No renal toxicity was observed in the offspring, however reduced litter size was recorded. Overall, the LOAEL for maternal and developmental toxicity, which can be derived from these studies, is 60 mg lithium carbonate/kg bw/d.

The observation that both doses (60 and 100 mg/kg bw/day) caused maternal toxicity but only the highest dose caused kidney effects in offspring supports the interpretation that kidney effects are induced by lithium and are not secondary to maternal toxicity. The observation that no such effects were described in other developmental toxicity studies might be due to the fact that this endpoint was not explicitly addressed in the other studies. Therefore, these findings are regarded relevant for the evaluation. These studies are limited due to the missing information on serum levels in dams and the fact that only one dose per experiment was tested, but are relevant for classification purpose.

Another developmental toxicity study in rats similar to guideline was reported by Marathe and Thomas (1986). Lithium carbonate was administered orally (gavage) to pregnant Wistar rats from GD 6-15 at doses of 0, 50 and 100 mg/kg bw/day. Animals were sacrificed for caesarean section on GD 20. Information on maternal toxicity was not provided. No adverse effects were observed in offspring of the low dose group. At the dose of 100 mg/kg bw/day there occurred reduction in number and weight of litter, increase in the number of resorptions, wavy ribs, short and deformed bones of the limbs, or an increased incidence of incomplete ossification of sternebrae and wide bone separation in the skull. Based on the results of this study, a NOAEL for prenatal developmental toxicity of 50 mg lithium carbonate/kg bw/day was determined.

There remains some uncertainties as to whether maternal toxicity occurred, as no information on this endpoint was provided. However, in the developmental guideline study (Anonymous, 2010b) only slight maternal toxicity was observed at 90 mg/kg bw/day, a dose similar to the highest dose of this study.

In a developmental toxicity study, 12 pregnant pigs were orally exposed to 3000 mg/kg of lithium carbonate in the diet (about 40 mg/kg bw/day) during the last 80 days of gestation (GD 30 - 114). A control group of 11 animals was included in the study. Feed consumption and body weights were measured at 60, 90 and 110 days of gestation. Body weights were also recorded after 21 days of lactation. Number of live and stillborn piglets and body weights were recorded at birth and the piglets were weighted at 21 days of age. The body weights of treated dams were reduced, this effect was only significant on GD 110 (23%). Five out of 12 treated animals did not complete pregnancy. Average offspring born per litter and birth weight of the piglets did not differ. The number of piglets born alive was decreased and number of stillbirth and mummies was increased in treated animals. Litter birth weight and number of piglets alive on PND 21 were reduced in the treatment group, but growth rates did not differ from control piglets. No abnormalities were reported (Kelley et al., 1978).

Due to the design of the study (only one dose) and the limited reporting the study is of limited validity, but it supports the finding in Wistar rats that gestational exposure to lithium carbonate might cause severe developmental effects (e.g. increased number of stillbirth, reduced postnatal survival).

Following studies are scored with Klimich scores of 3 or 4 due to insufficient level of details and/or methodological deviations. They **cannot considered as relevant for classification purpose**.

Gralla and McIlhenny (1972) reported the outcome of developmental toxicity studies in rats, rabbits and monkeys treated with lithium carbonate. All these studies provide only limited information on maternal toxicity.

Twenty pregnant Charles River female albino rats per group were dosed by gavage from gestation day 5 -15 with lithium carbonate solutions at 49.88, 149.63 and 299.25 mg/kg bw/day. A control group received tap water. Animals were sacrificed for caesarean section on GD 20. The fetal number, appearance and uterine distribution were observed. Gross internal malformations were examined in one third after decalcification in Bouin's solution, remainder were examined for skeletal examinations. Maternal mortality occurred at the highest dose level. Two pregnant female rats died unexpectedly for unknown reasons (not clear in which of the three rat studies reported by the authors mortality of the two rats occurred). Except these mortalities, no maternal or developmental toxicity up to the highest dose level were reported ("*Maternal parameters such as fertility, average number of implantation sites, average litter size, body weight gain and offspring body weight at 20 days gestation, offspring mortality and gross appearance after transverse sectioning or skeletal staining revealed no differences between treated and control groups*" no further details provided). Therefore, the maternal NOAEL is 149.63 mg lithium carbonate/kg bw/day, the NOAEL for developmental toxicity is 299.25 mg/kg bw/day.

In a further study reported by Gralla and McIlhenny (1972) Charles River albino rats (n=10 dams per dose group) received lithium carbonate doses of 0, 49.88, 149.63 or 299.25 mg/kg bw/day by gastric intubation from GD 14 through 21 days of lactation. Dams and their offspring were observed for mortality, normal body weight gain and general symptomatology on PND 1, 4 and 21. Offspring were further examined for gross external and internal findings at the end of the study. Two pregnant female rats died unexpectedly for unknown reasons (not clear in which of the three rat studies reported by the authors mortality of the two rats occurred). No other maternal effects are documented. Body weight gain of newborn rats was decreased when nursing females were given 299.25 mg/kg bw/day, but not at lower doses. The maternal NOAEL and the NOAEL for developmental toxicity consist in 149.63 mg lithium carbonate/kg bw/day.

Pregnant female New Zealand albino rabbits (n=10 per group) were dosed per oral with lithium carbonate in capsules from GD 5 -18 at doses of 49.51 or 79.80 mg/kg bw/day. Control animals received empty capsules. On day 28 all dams were sacrificed and autopsied. The uteri were examined for implantation sites, resorption and fetal number and distribution. After caesarean delivery, the offspring were weighed and examined for gross external defects. All rabbit pups were then sacrificed, autopsied and examined grossly for internal defects; skeletons were stained by the alizarin technique and examined. Three dams, which received 79.80 mg/kg bw/day, died late in pregnancy after prolonged anorexia and occasional tremors. One non-pregnant female of the low dose group died unexpectedly overnight (no further information). No other effects were described. The maternal NOAEL is 49.51 mg lithium carbonate/kg bw/day, the NOAEL for developmental toxicity is 79.8 mg/kg bw/day (Gralla and McIlhenny, 1972).

Additionally, a developmental toxicity study was performed in rhesus monkeys by Gralla and McIlhenny (1972). Pregnant female rhesus monkeys (n=6) were dosed with lithium carbonate at 49.51 mg/kg bw/day by capsule from GD 14 to 35. Five additional female monkeys, which received empty capsules, served as controls. The offspring were either taken by caesarean section or the females were allowed to deliver naturally on GD 160 +/- 2. Immediately the offspring were radiographed (full body) and weighed and cranial and limb measurements taken. At 7 and 30 days post-partum, body weight, hematocrit, hemoglobin, RBC, WBC, BUN and blood glucose were determined. "The offspring were closely observed during development, especially for signs of functional neurologic defects" up to 12-15 month of age (no further information). Reproduction was not affected by lithium treatment: seven (2 females, 5 males) or 4 (3 females, 1 male) progeny were delivered from treated or control females, respectively. A set of twins (male) from a treated female was "inadvertently" destroyed. All other parameters investigated were in the normal range (no details

provided). The dose of 49.51 mg/kg bw/day was the NOAEL for maternal and prenatal developmental toxicity.

The validity of these studies is very limited due to the insufficient reporting of methodological details and results. Moreover, the investigation depth remains unclear, and there is probably no in depth visceral examination (“*gross appearance after transverse sectioning”).*

Smithberg and Dixit (1982) exposed different strains of mice to lithium carbonate concentrations of 2 mg/mL drinking water (about 400 mg/kg bw/day). Serum measurements revealed that 2 mg/mL drinking water resulted in serum concentrations similar to the therapeutic range (0.5-1.0 mmol/L). Exposure to lithium carbonate resulted in a high number of resorptions (about 60%).

However, as no control group was present in this study, visceral malformations were not examined, maternal effects were not reported and only one dose group was tested together with a limited documentation, the results are regarded as not valid for evaluation.

Szabo (1970) exposed HaM/ICR mice orally by gavage to lithium carbonate at doses between 200 and 465 mg lithium carbonate/kg bw/day (37.57 to 87.36 mg Li/kg bw/day) from GD 6-15, which is in the range of therapeutic doses (mean lithium plasma level in the range of 0.45 to 1.25 mM). Caesarean section was performed on GD 18 and followed by examinations for visceral and skeletal malformations. The lowest dose caused neither maternal nor foetal deaths and no relevant increase in cleft palate in the foetuses, but the highest dose caused fetal and maternal lethality as well as an increased number of foetuses with cleft palate.

Overall, the study is limited due to its insufficient reporting. Especially, the influence of the massive maternal toxicity on the observed effects is unclear.

Induction of cleft palate in mice has also been reported by Loevy and Catchpole, 1973. CD1 mice received 15.5 mg of lithium chloride/mouse (about 620 mg lithium chloride/kg bw/day or 100 mg Li/kg bw/day) in sterile water by subcutaneous injection daily for 2 or 3 days on days 11 through 13 of pregnancy. The mice were sacrificed on day 17 of pregnancy. The uteri were examined for resorption sites and the foetuses for malformations. Treated animals revealed an increased incidence resorptions, not further specified (11 – 21 vs. 4 in controls). Cleft palates were detected in the offspring injected on days 11, 12, and 13 of 15.1 %; on days 12 and 13 of 7.2 %; and on days 11 and 12 of 3.4 %. The authors reported no maternal toxicity.

Investigations with two different strains of mice and different route of exposure (oral and subcutaneous) point to the induction of cleft palate after lithium carbonate or chloride treatment (Loevy and Catchpole, 1973; Szabo, 1970), a finding not confirmed by investigations performed with A/J mice (Smithberg and Dixit, 1982), a strain especially sensitive for this endpoint. Cleft palates were observed in the Szabo study in the same dose group in which mortality of the dams was seen, but also in a lower dose group, for which no mortality was described. In the other study reporting cleft palates (Loevy and Catchpole, 1973), an increase in resorptions was observed, which also might indicate maternal toxicity/stress. The latter is known to lead also to an increase in malformations, e.g. in cleft palates. No signs of maternal toxicity or stress were reported in the study with A/J mice. Whether induction of cleft palates were secondary to stress or whether the inconsistent findings in the different strains are due to different dose levels remains unclear. Therefore, these data do not provide clear evidence for lithium carbonate developmental toxicity but have to be taken in a weight of evidence approach.

Messiha (1986) exposed 5 albino mice per group to 10 mg/kg bw/d lithium chloride solution or water beginning on mating until end of weaning. Fourteen days after weaning, authors observed a significant decreased in brain weight in males and females, in kidney weight in females, and in testis weight of offspring. The decrease brain weight was confirmed after a postnatal exposure, suggesting effect via lactation. Pre and postnatal exposure also induced alcohol dehydrogenase (L-ADH) in developing males and females.

This study gives however only little evidence, due to the few animals used in each groups and the limited details provided by the authors.

Some **other studies exposed rodents via other route with less relevance**, and are detailed below.

In a developmental study, 10 swiss albino mice per group were exposed intraperitoneally to 25 mg lithium carbonate/kg bw/day from GD10 to the end of lactation (Mostafa et al. 2010). At the end of exposure, authors observed on offspring increased body weight and diameter of seminiferous tubules and also decrease of primary spermatocytes count, nuclear diameter of Leydig cells, diameter of epididymis ductules and testosterone level.

However, this study do not report information on dams.

Smithberg and Dixit (1982) exposed different strains of mice to lithium carbonate concentrations on single or repeated days of gestation intraperitoneally to 0, 0.8, 1.6, 3.2, 5.0 mg lithium carbonate per animal (0, 32, 64, 128, 200 mg lithium carbonate/kg bw/day). Serum measurements revealed that 0.8 mg lithium carbonate per animal i.p. resulted in serum concentrations similar to the therapeutic range (0.5-1.0 mmol/L). Application of lithium carbonate to 129 SV mice in the therapeutic range, or the two-fold or four-fold dose did not cause any adverse effects in the offspring. Only in the highest dose group, which is about six-fold higher than the dose resulting in therapeutic serum concentrations increased incidences of malformations (fused ribs, and/or vertebral defects and exencephaly) were observed, especially after application on GD 9 (19.3, 41.6, 17.1 % malformation after application on GD 8, 9, 10, respectively). Results obtained in A/J mice were similar to the effects seen in 129 SV mice. Lithium treatment did not increase the incidence of cleft palates in A/J mice.

Overall, the study reveals some shortcomings, for example, control animals for single exposure were only exposed on day 9, visceral malformations were not examined and maternal effects were not reported.

Giles and Bannigan (1997) reported that single intraperitoneal treatment of CD-1 mice with 300 mg lithium carbonate/kg bw did not cause maternal toxicity but increased incidence of resorptions (19%) and a 2% incidence of open cranial neural tube defects. Substantial apoptosis in the neuroepithelium of the cranial neural folds beginning 3 hours post-treatment was observed. According to Jurand (1988) similar findings were obtained in JBT/JD mice after single intraperitoneal treatment with concentrations above 330 mg lithium carbonate/kg bw on GD 9. Exencephaly and spinal kinking were observed in offspring. However, these data are not regarded as relevant for the classification, because according to the authors peak serum levels of 9.8 mM were reached one hour after i.p. treatment, indicating that the observed effects might possibly be due to high concentrations.

Studies with intraperitoneal application during pregnancy, especially at doses clearly exceeding the therapeutic range, resulted in developmental toxic effects, including neural tube defects and exencephaly. As this application route may result in peak concentrations which might directly injure maternal and fetal tissues, these studies are not regarded as relevant for the assessment of developmental toxicity.

Altogether, some rat developmental toxicity studies indicate that lithium induces developmental toxicity, including malformations, at doses which are potentially maternally toxic. However, few studies provide sufficient information on maternal toxicity and/or lithium plasma concentrations, which impedes the interpretation of these studies. Investigations in rats point to kidney effects in the offspring at maternal toxic doses, an effect regarded as substance-related, as the kidneys is one of the target organs of lithium toxicity. Additionally, investigations in mice point to neurotoxic effects and induction of cleft palate of gestational lithium exposure.

The limited experimental database, limited with respect to the quality but not the number of studies, does not provide clear evidence of developmental toxicity due to gestational lithium exposure. Especially, the guideline study did not observe developmental toxicity, probably due to the fact, that the highest dose tested was only slightly toxic. However, the data indicate that exceedance of the therapeutic range, which is already toxic to dams, might cause severe developmental effects.

Human data:

A number of studies have been published which examined the developmental toxicity (including teratogenicity) of lithium in humans. They are mostly case reports. Some cohort and case control studies are available, most of them are retrospective studies, only few of them are prospective studies. The summary below focused on two recent reviews (Yacobi and Ornoy, 2008 and McKnight et al., 2012), which were completed with a bibliographic search.

Yacobi and Ornoy (2008) performed a review of human studies published between 1969 and 2005 dealing with teratogenic and embryotoxic effects of lithium. They analysed in total 24 case reports. Nine of these infants were born to mothers with bipolar disorders, who were treated with lithium only. The remaining 15 women received also other drugs. Most anomalies reported for the cases were of the cardiovascular system. But, as discussed by the authors, the number of unreported lithium-treated women with normal children was unknown. They further reported typical effects of perinatal toxicity observed in children like higher rate of prematurities, higher birth weight, goiter, respiratory distress, cyanosis, hyporeflexia, diabetes insipidus. These effects were most often observed when serum lithium levels in the newborns exceeded 1 mM. All case control and prospective studies were negative. The authors also assumed that the high rate of cardiac anomalies from lithium registry seems to be due to the fact that some cases were reported in several publications. All things considered, the authors concluded that "*Reviewing the data accumulated until today regarding lithium exposure and cardiovascular anomalies, including Ebstein's anomaly, it is to be concluded that the risk is much lower than previously thought*". This conclusion is also tempered by the fact that some publications point out the fact that the impact of lithium can also be under-estimated since many pregnant women treated with lithium prefer to abort the malformed foetuses, which is confirmed by a higher rate of therapeutic abortions (10% vs 6% in Jacobson et al., 1992, and 8.6 vs 2.9 in Diav-Citrin 2006). Finaly, Yacobi and Ornoy (2008) considered that lithium therapy adds only a small risk for cardiovascular defect, and does not increased general rate of major anomalies.

McKnight et al. (2012) performed a review and meta-analysis of studies on lithium toxicity profile published between 1966 and 2010. They found 62 publications assessing teratogenicity of lithium. Many of them were also discussed in Yacobi et al. (2008), 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 avoid during pregnancy according to the authors.

It has to be noted that these authors speak about risk. However, classification is based on hazard and not on risk. The authors, in these publication do not question the association between lithium and developmental effects.

A cohort study was recently conducted, involving 1,325,563 pregnancies between 2000 and 2010, among which 663 women were exposed to lithium during the first pregnancy trimester (Patorno et al., 2017). The exposure was defined based on prescription for lithium during the first trimester. The outcome investigated were cardiac malformation, major congenital malformation overall, 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, and take them into account in the statistical analysis. A correlation between lithium exposure early in pregnancy and cardiac malformation was found: the risk ratio 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 601 to 900 mg, and 3.22 (95% CI = 1.47-7.02) for more than 900 mg. Even if the magnitude of this association was smaller than what have been reported in previous studies (in line with review described previously), the authors confirm this association, and also show that this association is dose-dependent.

This is a good quality study based on a substantial cohort.

A meta-analysis performed in 2018 using data from 6 cohort studies is available. A total of 727 pregnancies were identified. Lithium used in pregnancy was not associated with preeclampsia, foetal distress, or postpartum haemorrhage. No difference between groups were observed for caesarean section, preterm birth, low birth weight, or small for gestational age. There were 7.2% of lithium exposed baby and 4.3% children from reference group with major malformations diagnosed by one year of age, difference which was not statistically significant. However, lithium exposure during the first trimester was associated with an increased risk of major malformations (7.4% vs 4.3%; OR = 1.71, 95% CI : 1.07-2.72), but not cardiac malformations (2.1% vs 1.6%, OR = 1.54, 95% CI : 0.64-3.70). Again, authors concluded that their results suggest an association between lithium exposure and major malformation, but that this association was much smaller than previously reported (Munk-Olsen, 2018).

This is a good quality study with a robust methodology.

A Swedish retrospective cohort study investigated the birth outcome in manic-depressive women (n=350 mother-child pairs, but medical data available only for 82% of them) in the years 1973-1979. The number of perinatal deaths and the incidence of heart defects were higher, gestational length shorter and the birth weight lower than expected in the control population, which comprised all births in Sweden in the years 1973-1979. Lithium therapy was only documented for 59 of the manic-depressive patients, 18 of them received lithium together with other psychotropic drugs. In the group of women treated only with lithium during pregnancy 4/41 neonatal deaths, 5/41 malformed infants, 2/41 dead and malformed infants and 3/41 heart defects (no Ebstein's anomaly) were recorded. Due to the small sample size the differences were not statistically significant (Källén and Tandberg, 1983). The validity of these data is limited as the influence of confounding factors was not assessed properly.

Van der Lugt et al. (2012) reported the long-term outcome of 15 children who were born between 1994 and 2007 and exposed to lithium in utero, but were not breastfed. Children were investigated at 3-15 years of age. Tests on neurological or cognitive development and the parents response on the child behaviour checklist did not point to adverse effects on growth, neurological, cognitive and behavioural development of exposed children. However, the group of children investigated was small, no appropriate control group was included, and other medication besides lithium was used. This study is therefore of limited interest for classification.

A study was reported from Israel (Diav-Citrin et al., 2014). In this prospective, comparative, observational study 183 lithium-exposed pregnancies of women who contacted the Israeli Teratology Information Service were followed and compared to 72 disease-matched and 748 nonteratogenic-exposed (i.e. pregnant women counselled for nonteratogenic exposure) pregnancies. The rate of total congenital anomalies without chromosomal or genetic conditions did not differ between the three groups (6.5% lithium-exposed group, 3.3% bipolar disorders, 2.7% nonteratogenic exposures). About 58% of the lithium exposed group took the medication (mean 906 mg) throughout pregnancy and not only during first trimester. In the lithium treated group an increased number of cardiovascular anomalies vs. the nonteratogenic group (5/123 vs. 4/711; 4.1% vs. 0.6%), higher rate of preterm deliveries (18/131 vs., 41/683; 13.7% vs. 6.0%), and one case of Ebstein's anomaly was described. The adjusted odds ratio was 4.75 (95% CI = 1.11-20.36). The increase in cardiovascular anomalies in the lithium group was only significant if both, persistent and spontaneously resolving cardiovascular anomalies, were considered. One of the major shortcomings of the study is that it is primarily based on pregnancies of women who contacted the Israeli Teratology Information Service, which may not represent the general population. Further, the study relies on maternal interview and lacks medical records in most cases.

A systematic review and meta-analysis performed in 2018 focused on neurodevelopmental effects of intrauterine exposure to lithium (Poels et al., 2018). Authors identified 7 preclinical studies, 3 cohort studies, and 5 case studies investigating lithium neurodevelopmental effects. 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, studies investigated neuro-development are sparse, and of questionable quality. Therefore the conclusions have to be taken with care.

10.10.6 Comparison with the CLP criteria

For potential classification on development, criteria from CLP Regulation/guidance (ECHA, 2017) were applied.

"Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency."

- *Known human reproductive toxicant "The classification of a substance in Category 1A is largely based on evidence from humans" (ECHA, 2017).*

Existing epidemiological studies are rather contradictory, of various quality, and can be summarized chronologically:

- ➔ In the seventies, a retrospective study, based on the lithium babies registry, 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), point to an increased risk of malformations in babies exposed during gestation to lithium.
- ➔ Later, valid 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). Cohort studies provided contradictory results, and case reports point to perinatal complications in the context with gestational exposure.
- ➔ In recent publications, a more precise pattern of the effects of lithium on development seems to emerge: authors from reviews (Yacobi *et al.*, 2008), meta-analysis (McKnight *et al.*, 2012) or cohort study (Patorno *et al.*, 2017) lead to very similar conclusions, i.e., the evidence between lithium exposure during pregnancy and cardiac malformation is quite weak, but there is an association, with a magnitude lower than previously reported. In particular, Patorno *et al.* point out to a risk of cardiac malformation particularly at high doses, with a clear dose-response relationship. The relatively weak association has also to be tempered by the higher rate of spontaneous or therapeutic abortion of woman under lithium, which was not taken into consideration by authors of these publications and could lead to a underestimation of developmental effects of lithium.

Data on animals are inconclusive, due to the heterogeneity of results and the overall quality of the dataset. Moreover, the observations on some studies are not in line with the findings from human studies (no increase of cardiac malformation seen in animals studies), which can be explained by a difference in mechanism of action between rodents and human. However, human data, and particularly the homogeneity of recent robust human studies are considered sufficient by themselves to give evidence of developmental effect of lithium.

Also, medical data were not available in the framework of the dossier, but it have to be noted that in 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.

Lithium should therefore be classified as Category 1A substance for development.

10.10.7 Adverse effects on or via lactation

Table 24: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Studies described in the text below already detailed in previous sections			

Table 25: Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Clinical investigation	Lithium carbonate therapy	10 mother-child pairs, investigation of maternal and child lithium serum levels and breast milk levels Maternal dose levels: 400-1200 mg lithium carbonate/day throughout pregnancy and lactation Infant age at sampling: between 1 and 52 weeks of age	Breast milk concentrations 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/liter, respectively, no serious adverse effects observed	Viguera et al., 2007
Clinical investigation	Lithium therapy (no further information)	3 mother-child pairs, determination of serum lithium levels Maternal dose levels: 600-1350 mg lithium/day during pregnancy and lactation Infant age at sampling: 1 month	Maternal serum levels: 0.12-0.97 mM Li Infant serum levels: 0.08-0.11 mM (corresponding to 10-17% of maternal levels)	Bogen et al., 2012

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

In rats, Ahmed *et al.* (2018) observed the presence of lithium in the breast milk.

Very few studies investigating effects of lithium exposure exclusively via breast milk have been identified.

In a study described above (see 10.10.4), Teixeira *et al.* (1995) observed a lower body weight at day 21 in pups exposed to lithium during lactation (in both groups: only after parturition and before and after parturition). Authors also observed a delay in the critical day of maturation compared to control in groups exposed during lactation (day of eye opening and avoidance of visual cliff). However, as this effect is also

observed in group exposed only during gestation, and in water-deprived group, conclusion is difficult to draw.

In a developmental study on Sprague-Dawley rats described in section 10.10.4, 11 and 13 females were fed during gestation a casual diet or containing 1000 mg/kg diet of lithium carbonate. At parturition, half rats of both groups were switched to the other diet and the other half were kept in the same diet as during gestation to assess the potential effect of exposure during lactation. At the end of lactation, the mean pup weight was significantly decreased in group exposed during lactation only. Heart and spleen absolute weight were decreased in group exposed during lactation only. In dams: After lactation, decrease in body weight was observed only in groups exposed to lithium during the entire study and during lactation. The group exposed to lithium during gestation showed no difference with control. Only the absolute liver weight was decreased in group exposed during lactation. Concerning relative (to body weight) organs weight, heart, kidney and liver were decreased in the same group (Ibrahim and Canolty, 1990). Despite the very interesting protocol for assessing effects via lactation, this study remains poorly informative because of the few parameters investigated, the few details given by the authors, and the limited number of rats in each group (5 to 7 in the second part of the study).

In another study described above (see 10.10.4), Messiha (1986) observed a significant decreased in brain weight in males and females, in kidney weight in females, and in testis weight of offspring. The decrease brain weight was confirmed after a postnatal exposure, suggesting effect via lactation. Postnatal exposure also induced L-ADH in developing males and females. This study was however disregarded, due to the few animals used in each groups and the limited details provided by the authors.

One study was mentioned in several reviews, but not available in the framework of this evaluation (Hsu *et al.*, 1978). Authors exposed 13 pregnant McCollum strain rats to 20 mM in drinking water. Corresponding plasma levels are assumed to have been 1.5 to 2.0 mM, based on Trautner *et al.* (1958) who used the same species, dose, and 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. Authors observed that postnatal lithium exposure delayed development, measured by age at eye opening and weaning weight. Mothers exposed to lithium postpartum had a decreased rate of 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 and postnatal treatment.

There are no other animal studies with exposure only during lactation. Animal studies with exposure during gestation and lactation (see section 10.10.4) do not allow to draw final conclusions. No effects on the offspring were reported in a 2-generation rat study (Anonymous, 2012). In a rat developmental toxicity study dams were exposed from GD 14 till PND 21. Offspring in the highest dose group, which caused mortality in 2 dams, showed reduced body weights on PND 21 (Gralla and McIlhenny, 1972). Offspring of mice exposed from GD 1 till PND 15 revealed a dose dependent decrease in body weight (gain), delayed eye opening, appearance of body hair. Sensory motor reflexes (righting, rotating, cliff avoidance) were dose dependently affected on days 1 to 15 of the postnatal phase. On PND 21, i.e. 6 days after termination of exposure, there was no significant difference between treated and controls (Abu-Taweel, 2012). Whether the effects were only due to gestational exposure (significant differences already on PND 1) or additionally influenced by lactational exposure cannot be differentiated due to studies design. Further studies describing postnatal development of monkeys (Gralla and McIlhenny, 1972) or pigs (Kelley *et al.*, 1978) did not include lithium exposure during lactation. Trautner *et al.* (1958) indicated that the growth of offspring exposed during gestation and lactation to lithium chloride was slower than the growth of offspring only exposed during gestation. The relevance of these findings cannot be assessed as no details were reported.

Investigations with mother-infant pairs clearly confirmed that lithium is transferred to breast milk and via breast milk to infant’s serum. 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 (Viguera *et al.*, 2007, Bogen *et al.*, 2012). A single case study reported toxic effects (cyanosis, electrocardiographic changes, floppy muscles) in a breast fed child. 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).

10.10.9 Comparison with the CLP criteria

The two criteria suggested by ECHA (2017) were checked for classification for adverse effects on or via lactation:

1. *"Substances which are absorbed by women and have been shown to interfere with lactation. This relates to effects in the mother that impact adversely on the breast milk, either in terms of the quantity produced or the quality of the milk produced (i.e. the composition). Any effect on the quantity or quality of the breast milk is likely to be due to systemic effects in the mother. However, overt maternal toxicity may not be seen (e.g. the substance may just affect the transfer of a nutrient into the milk with no consequence for the mother)."*

There is no study investigating the quantity and quality of the milk produces, or any suggestion in studies available that lithium compounds can have an impact on breast milk production.

2. *"Substances which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child. This relates to the ability of the substance (including metabolites), to enter the breast milk in amounts sufficient to cause a concern. When the effect on the offspring is caused by the substance (or metabolite) after transport through the milk then the maternal toxicity has no relevance for classification."*

- *"Ideally, studies will be available which inform directly on whether the substance causes adverse effects in the offspring due to an adverse effect on lactation. One generation or multi-generation reproductive toxicity studies, which involve direct exposure or exposure via the milk of the offspring postnatally, usually provide information on this." (ECHA, 2017).*

Only one study investigated postnatal effect of lithium. Authors observed a delayed development, measured by age at eye opening and weaning weight. Mothers exposed to lithium postpartum had a decreased rate of water consumption and weight gain. Two tests of learning and memory, performed after lithium treatment ended, showed a decrease in performance in rat pups. This study is however not available to allow a robust assessment and therefore be used by itself for classification.

No adverse effects have been observed in the offspring (F1 and F2) of the two generation guideline study. Rat offspring in the highest dose group, which caused mortality in 2 dams, in a rat developmental toxicity study with exposure from GD 14 till PND 21 revealed reduced body weight on PND 21. Whether this effect is due to gestational or lactational exposure is unclear. Offspring of mice exposed from GD 1 till PND 15 revealed a dose dependent decrease in body weight (significant differences already on PND 1), delayed eye opening, appearance of body hair. Sensory motor reflexes were dose dependently affected during the postnatal phase. On PND 21, i.e. 6 days after termination of exposure, there was no significant difference between treated and controls. Whether the effects observed in rats and mice were only due to gestational exposure or additionally influenced by lactational exposure cannot be differentiated due to the study design.

- *"In general, positive data should usually be available to show that a substance leads to an adverse effect in offspring due to effects on lactation to support classification. However, in exceptional circumstances, if there are substantiated grounds for concern that the substance may have an adverse effect via lactation then it may be classified as such in the absence of direct evidence. This should be based on a quantitative comparison of the estimated transfer via the milk and the threshold for toxicity in the pups. This might apply in cases where the substance has the capacity to bioaccumulate which would lead to a potentially higher burden in the offspring, or where there is evidence that the offspring may be more sensitive to the substance's toxicity than adult. The mere presence of the substance in the milk alone, without a strong justification for a concern to offspring, would normally not support classification for effects on or via lactation.*

  Overall, classification for effects on or via lactation can be assigned on the basis of toxicokinetic data or a well substantiated estimate of the exposure through the milk alone provided that it is

supported by an argument clearly justifying that the level present in the breast milk would be likely to harm developing offspring (ECHA, 2017).”

Although there is no doubt that lithium can be transferred to infants via breast milk, existing data do not clearly indicate that infants reveal severe toxic effects if exposed via breast milk. In most cases, effects observed could not clearly be distinguished from effects caused by gestational exposure, and there is no evidence that neonates are more sensitive than adults. One case report indicates that in case of maternal serum levels in the toxic range toxic effects could also occur in the baby. But the database is not sufficient for a classification for effects on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classification for reproductive toxicity addresses adverse effects on sexual function and fertility, developmental effects and adverse effects on or via lactation.

Adverse effects on sexual function and fertility

There are no qualified epidemiological studies investigating influence of lithium on fertility available. Findings in animal studies on the effects of lithium on reproduction seem contradictory at first sight. Whereas the 2-generation guideline study with rats did not identify any effects of lithium carbonate on fertility up to slightly toxic doses, impairment of male fertility (decrease fertility index) was described in many other recent fertility studies. Further, several *in vivo* studies reported that lithium carbonate or chloride affected sperm function and production and caused structural changes of the testes. Additionally, mechanistic reports on the interaction of lithium chloride with the sexual hormonal system suggest an influence of lithium on male fertility. Why such effects on sperm parameters and reproductive organ structure were not observed in the 2-generation guideline study, although the same rat strain (Wistar), comparable doses and route of administration were used, is not known. Despite these unresolved issue, results of robust recent experimental studies are very consistent, so a classification as presumed reproductive toxicant (category 1B) is recommended.

Adverse effects on development

Altogether, available epidemiological studies are contradictory, and most of them do not fulfil todays requirements (insufficient number of patients, deficiencies in exposure estimate). In the seventies, the lithium babies registry, point to an increased risk of congenital malformations (mainly on cardiovascular system) in babies exposed during gestation to lithium. Later, valid case-control studies did not identify an association between congenital, and cohort studies provided contradictory results. However, recent robust studies (review, meta-analysis and cohort) drawn similar conclusion on a developmental effect: a correlation between lithium exposure and developmental toxicity (particularly cardiac malformation) exists.

Considering also drug labels recommended discontinuation of treatment until the 9th week of amenorrhea, evidence is considered sufficient to recommend a classification in category 1A.

In conclusion, because evidence for adverse effects of lithium on male fertility and on developmental toxicity, classification to

Repr. 1A, H 360FD;

May damage fertility,

May damage the unborn child

is warranted.

Adverse effects on or via lactation

Lithium carbonate is transferred via mother milk to the babies with infant serum levels about one fourth of the maternal serum levels. There is no robust human or animal studies providing clear evidence for effects in offspring caused by lactational exposure or for impairment of breastmilk production. Therefore, no classification for effects on or via lactation is recommended.

10.11 Specific target organ toxicity-single exposure

Evaluation not performed for this substance

10.12 Specific target organ toxicity-repeated exposure

Evaluation not performed for this substance

10.13 Aspiration hazard

Evaluation not performed for this substance

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance

12 EVALUATION OF ADDITIONAL HAZARDS

Evaluation not performed for this substance

13 ADDITIONAL LABELLING

14 ANNEXES

Annex I: Confidential or non-confidential annex documenting the key studies for assessment

Annex II: Non-confidential annex: READ ACROSS JUSTIFICATION DOCUMENT

15 REFERENCES

Abu-Taweel, G.M. (2012)
Effects of perinatal exposure of lithium on neuro-behaviour of developing mice offspring
Indian Journal of Experimental Biology, 50, 696-701

Ahmed, I.; Manno, F.A.M.; Manno, S.H.C.; Liu, Y.; Zhang, Y.; Lau C. (2018)
Detection of lithium in breast milk and in situ elemental analysis of the mammary gland
Biomedical Optics Express Vol. 9, Issue 9, pp. 4184-4195

Aizenberg, D.; Sigler, M.; Zemishlany, Z.; Weizman, A. (1996)
Lithium and male sexual function in affective patients
Clinical Neuropharmacology, 19, 515-519

Ali, A.M. (2008)
Reproductive system toxicity in male Swiss mice under supplementation of Camcolit
International Journal of Zoological Research, 4, 85-95

Allagui, M.S.; Hfaiedh, N.; Vincent, C.; Guermazi, F.; Murat, J.C.; Croute, F.; El Feki, A. (2006)

Changes in growth rate and thyroid- and sex-hormones blood levels in rats under sub-chronic lithium treatment

Human and Experimental Toxicology, 25, 243-250

Anonymous (2000a)

Study report [details confidential], (2000, unpublished), Bacterial reverse mutation assay with Lithium Hydroxide

Anonymous (2000b)

Study report [details confidential], (2000, unpublished), In vitro mammalian chromosome aberration test with Lithium hydroxide

Anonymous (2010a)

Study report [details confidential], (2010, unpublished), In vitro gene mutation study in mammalian cells with Lithium hydroxide

Anonymous (2010b)

Study report [details confidential], (2010, unpublished), Prenatal Developmental Toxicity Study

Anonymous (2012)

Study report [details confidential], (2012, unpublished), Two-generation reproductive toxicity

Ambrosiani L., Pisanu C., Deidda A., Chillotti C., Stochino M. E., Bocchetta A. (2018)

Thyroid and renal tumors in patients treated with long-term lithium: case series from a lithium clinic, review of the literature and international pharmacovigilance reports

Int J Bipolar Disord; 6:17

Aral, H.; Vecchio-Sadus, A. (2008)

Toxicity of lithium to humans and the environment - a literature review

Ecotoxicology and Environmental Safety, 70, 349-356

Banerji, T.K.; Parkening, T.A.; Collins, T.J.; Rassoli, A. (1983)

Lithium-induced changes in the plasma and pituitary levels of luteinizing hormone, follicle stimulating hormone and prolactin in rats

Life Sciences, 33, 1621-1627

Banerji T.K.; Parkening T.A.; Collins T.J.; Rassoli A.H.; Legate L.S. (1986)

Acute lithium treatment suppresses the proestrous LH surge in mice: chronic lithium leads to constant diestrus

Brain Research, 380 176-180

Bille, P.E.; Jensen, M.K.; Kaalund Jensen, J.P.; Poulsen, J.C. (1975)

Studies on the haematologic and cytogenetic effect of lithium

Acta Medica Scandinavica, 198, 281-286

Blay, S.L.; Ferraz, M.P.; Calil, H.M. (1982)

Lithium-induced male sexual impairment: two case reports

Journal of Clinical Psychiatry, 43, 497-498

Bodén, R.; Lundgren, M.; Brandt, L.; Reutfors, J.; Andersen, M.; Kieler, H. (2012)

Risks of adverse pregnancy and birth outcomes in women treated or not treated with mood stabilisers for bipolar disorder: population based cohort study

BMJ, 345, e7085

Bogen, D.L.; Sit, D.; Genovese, A.; Wisner, K.L. (2012)

Three cases of lithium exposure and exclusive breastfeeding

Arch Womens Ment Health, 15, 69-72

Correa-Villaseñor, A.; Ferencz, C.; Neill, C.A.; Wilson, P.D.; Boughman, J.A. (1994)

Ebstein's malformation of the tricuspid valve: Genetic and environmental factors

Teratology, 50, 137-147

De La Torre, R.; Krompotic, E.; Kowlessar, L. (1976)

The in vivo and in vitro effects of lithium on human chromosomes and cell replication

Teratology, 13, 131-138

Diav-Citrin, O.; Shechtman, S.; Tahover, E.; Finkel-Pekarsky, V.; Arnon, J.; Kennedy, D.; Erebara, A.; Einarson, A.; Ornoy, A. (2014)

Pregnancy outcome following in utero exposure to lithium: a prospective, comparative, observational study

American Journal of Psychiatry, 171, 785-794

ECHA, European Chemicals Agency (2012)

Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health. Version 2.1, November 2012

http://echa.europa.eu/documents/10162/17224/information_requirements_r8_en.pdf

ECHA, European Chemicals Agency (2014)

Guidance on the preparation of dossiers for harmonised classification and labelling. Version 2.0. August 2014

http://publications.europa.eu/en/publication-detail/-/publication/3102d5d7-e68b-11e5-8a50-01aa75ed71a1/language-en

ECHA, European Chemicals Agency (2017)

Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0 - July 2017

Helsinki, Finland. https://echa.europa.eu/documents/10162/13562/clp_en.pdf

ECHA, European Chemicals Agency (2020)

Information on Chemicals - Registered Substances

Online: http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

Edmonds L.D.; Oakley G.P.; (1990)

Ebstein's anomaly and maternal lithium exposure during pregnancy.

Teratology, 41:551-2.

Friedrich, U.; Nielsen, J. (1969)

Lithium and chromosome abnormalities

The Lancet, 2, 435-436

Fritz, H. (1988)

Lithium and the developing rat kidney in transplacental target organ toxicity

Arzneimittel-Forschung, 38, 50-54

Frolov, A.G.; Pliss, G.B. (1991)

Lithium carbonate as a promoter of bladder carcinogenesis in rats

Eksperimentalnaya Onkologiya, 13, 18-20

Frolov, A.G.; Pliss, G.B. (1992)

Lithium carbonate-mediated promotion of N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urothelial tumors in rats

Voprosy Onkologii (St. Petersburg), 38, 1309-1313, quoted from Hartwig, 2014

Ghosh, D.; Biswas, N.M.; Chaudhuri, A.; Ghosh, P.K. (1990a)

Direct effects of lithium chloride on testicular delta^{5}-3 beta and 17 beta-hydroxysteroid dehydrogenase activities in the rat--in vitro study

Acta Physiologica Hungarica, 76, 287-290

Ghosh D.; Chaudhuri A.; Biswas N.M.; Ghosh P.K. (1990b)

Effects Of Lithium Chloride On Testicular Steroidogenic And Gametogenic Functions In Mature Male Albino Rats

Life Sciences, Vol. 46, pp. 127-137

Ghosh, D.; Biswas, N.M.; Ghosh, P.K. (1991a)

Studies on the effect of prolactin treatment on testicular steroidogenesis and gametogenesis in lithium-treated rats

Acta Endocrinologica, 125, 313-318

Ghosh, P.K.; Biswas, N.M.; Ghosh, D. (1991b)

Effect of lithium chloride on testicular steroidogenesis and gametogenesis in immature male rats

Acta Endocrinologica, 124, 76-82

Giles, J.J.; Bannigan, J.G. (1997)

The effects of lithium on neurulation stage mouse embryos

Archives of Toxicology, 71, 519-528

Giles, J.J.; Bannigan, J.G. (2006)

Teratogenic and developmental effects of lithium

Current Pharmaceutical Design, 12, 1531-1541

Gralla, E.J.; McIlhenny, H.M. (1972)

Studies in pregnant rats, rabbits and monkeys with lithium carbonate

Toxicology and Applied Pharmacology, 21, 428-433

Hartwig, A. (2014)

Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, Loseblattsammlung, 56. Lfg

DFG Deutsche Forschungsgemeinschaft, WILEY-VCH Verlag Weinheim

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983)

Salmonella mutagenicity test results for 250 chemicals.

Environ Mutagen 5, Suppl 1: 3–142

HCN, Health Council of the Netherlands: Committee for Compounds toxic to reproduction (2000)

Evaluation of the Effects on Reproduction, Recommendation for Classification. Lithiumcarbonate and Lithiumchloride. Publ. No. 2000/06OSH

Health Council of the Netherlands, The Hague. https://www.gezondheidsraad.nl/sites/default/files/0006osh.pdf

Hoberman, A.M.; Deprospo, J.R.; Lochry, E.A.; Christian, M.S. (1990)

Developmental toxicity study of orally administered lithium hypochlorite in rats

International Journal of Toxicology, 9, 367-379

Hsu J.M.; Rider A.A.; (1978)

Effect of maternal Li ingestion on biochemical and behavioral characteristics of rat pups

FM Johnson, S Johnson (Eds.), Lithium in Medical Practice, University Park Press, Baltimore, pp. 279-287

Ibrahim H.S.; Canolty N.L. (1990)

Effects of Dietary Lithium on Pregnant and Lactating Rats and Their Progeny.

Nutrition Research, Vol. 10, pp. 315-324

Jana D., Nandi D., Maiti R. K., Ghosh D. (2001)

Effect of human chorionic gonadotrophin coadministration on theactivities of ovarianD5-3b-hydroxysteroid dehydrogenase, and 17b-hydroxysteroid dehydrogenase, and ovarian and uterine histology inlithium chloride-treated albino rats

Reproductive Toxicology 15 (2001) 215–219

Jacobson, S.J.; Jones, K.; Johnson, K.; Ceolin, L.; Kaur, P.; Sahn, D.; Donnenfeld, A.E.; Rieder, M.; Santelli, R.; Smythe, J.; Pastuszak, A.; Einarson, T.; Koren, G. (1992)

Prospective multicentre study of pregnancy outcome after lithium exposure during first trimester

The Lancet, 339, 530-533

Jarvik, L.F.; Bishun, N.P.; Bleiweiss, H.; Kato, T.; Moralishvili, E. (1971)

Chromosome examinations in patients on lithium carbonate

Archives of General Psychiatry, 24, 166-168

Källén, B.; Tandberg, A. (1983)

Lithium and pregnancy. A cohort study on manic-depressive women

Acta Psychiatrica Scandinavica, 68, 134-139

Kallen B. (1988)

Comments on teratogen update: lithium.

Teratology, 38: 598.

Kelley, K.W.; McGlone, J.J.; Froseth, J.A. (1978)

Lithium toxicity in pregnant swine

Proceedings of the Society for Experimental Biology and Medicine, 158, 123-127

Kessing, L.V.; Gerds, T.A.; Feldt-Rasmussen, B.; Andersen, P.K.; Licht, R.W. (2015)

Lithium and renal and upper urinary tract tumors - results from a nationwide population-based study

Bipolar Disorders, 17, 805-813

Khodadadi M., Pirsaraei Z.A. (2013)

Disrupting effects of lithium chloride in the rat ovary: Involves impaired formation and function of corpus luteum

Middle East Fertility Society Journal (2013) 18, 18–23

King, M.T.; Beikirch, H.; Eckhardt, K.; Gocke, E.; Wild, D. (1979)

Mutagenicity studies with X-ray-contrast media, analgesics, antipyretics, antirheumatics and some other pharmaceutical drugs in bacterial, Drosophila and mammalian test systems

Mutation Research - Genetic Toxicology, 66, 33-43

Lagerkvist, B.J.; Lindell, B. (2002)

131. Lithium and lithium compounds

The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. Arbete och Hälsa 2002:16. http://hdl.handle.net/2077/4277

Levin R.M.; Amsterdam, J.D.; Winokur, A.; Wein A.J. (1981)

Effects Of Psychotropic Drugs On Human Sperm Motility

Fertil Steril 36:503

Li, L.; Song, H.; Zhong, L.; Yang, R.; Yang, X.Q.; Jiang, K.L.; Liu, B.Z. (2015)

Lithium chloride promotes apoptosis in human leukemia NB4 cells by inhibiting glycogen synthase kinase-3 beta

Int J Med Sci, 12, 805-810

Licht, R.W.; Grabenhenrich, L.B.; Nielsen, R.E.; Berghofer, A. (2014)

Lithium and renal tumors: a critical comment to the report by Zaidan et al

Kidney International, 86, 857

Loevy, H.T.; Catchpole, H.R. (1973)

Lithium ion in cleft palate teratogenesis in CD_1 mice

Proceedings of the Society for Experimental Biology and Medicine, 144, 644-646

Marathe, M.R.; Thomas, G.P. (1986)

Embryotoxicity and teratogenicity of lithium carbonate in Wistar rat

Toxicology Letters, 34, 115-120

Martinsson, L.; Westman, J.; Hallgren, J.; Osby, U.; Backlund, L. (2016)

Lithium treatment and cancer incidence in bipolar disorder

Bipolar Disorders, 18, 33-40

Matsushima, Y.; Hazama, H.; Kishimoto, A. (1986)

Chromosome examination of patients under lithium therapy

Japanese Journal of Psychiatry and Neurology, 40, 625-630

McKnight, R.F.; Adida, M.; Budge, K.; Stockton, S.; Goodwin, G.M.; Geddes, J.R. (2012)

Lithium toxicity profile: a systematic review and meta-analysis

The Lancet, 379, 721-728

Messiha F. S. (1986)

Lithium and the Neonate: Developmental and Metabolic Aspects

Alcohol, Vol. 3, pp. 107-112

Mirakhori F., Zeynali B., Tafreshi A.P., Ameneh Shirmohammadian A. (2013)

Lithium Induces Follicular Atresia in Rat Ovary Through a GSK-3b/b-Catenin Dependent Mechanism

Molecular Reproduction & Development 80:286–296

Montelius, J. (2003)

Consensus Report for Lithium and Lithium Compounds

In: Montelius, J., Scientific Basis for Swedish Occupational Standards XXIV, Arbete och Hälsa. Arbetsmiljöinstitutet Solna, Sweden. www.inchem.org/documents/kemi/kemi/ah2003_16.pdf, 55-65

Moore, J.A. (1995)

An assessment of lithium using the IEHR Evaluative Process for Assessing Human Developmental and Reproductive Toxicity of Agents. IEHR Expert Scientific Committee

Reproductive Toxicology, 9, 175-210

Mota de Freitas, D.; Leverson, B.D.; Goossens, J.L. (2016)

Lithium in Medicine: Mechanisms of Action

Metal Ions in Life Sciences, 16, 557-584

Mroczka, D.L.; Hoff, K.M.; Goodrich, C.A.; Baker, P.C. (1983)

Effect of lithium on reproduction and postnatal growth of mice

Biology of the Neonate, 43, 287-296

Munk-Olsen, T., Liu, X., Viktorin, A., Brown, H. K., Di Florio, A., D'Onofrio, B. M., ... Bergink, V. (2018).

Maternal and infant outcomes associated with lithium use in pregnancy: an international collaboration combining data from 6 cohort studies using meta-analysis covering 727 lithium exposed pregnancies and 21,397 bipolar or major depressive disorder reference pregnancies.

The Lancet Psychiatry, 5(8), 644-652.

Nciri, R.; Allagui, M.; Vincent, C.; Murat, J.C.; Croute, F.; El Feki, A. (2009)

The effects of subchronic lithium administration in male Wistar mice on some biochemical parameters

Human and Experimental Toxicology, 28, 641-646

Newport, D.J.; Viguera, A.C.; Beach, A.J.; Ritchie, J.C.; Cohen, L.S.; Stowe, Z.N. (2005)

Lithium placental passage and obstetrical outcome: implications for clinical management during late pregnancy

American Journal of Psychiatry, 162, 2162-2170

Pastor, N.; Kaplan, C.; Domínguez, I.; Mateos, S.; Cortés, F. (2009)

Cytotoxicity and mitotic alterations induced by non-genotoxic lithium salts in CHO cells in vitro

Toxicology In Vitro, 23, 432-438

Patorno, E.; Huybrechts, K.F.; Bateman, B.T.; Cohen, J.M.; Desai, R.J.; Mogun, H.; Cohen, L.S.; Hernandez-Diaz S. (2017)

Lithium Use in Pregnancy and the Risk of Cardiac Malformations

N Engl J Med 2017;376:2245-54.

Poels E.M.P.; Schrijver L.; Kamperman A.M.; Hillegers M.H.J.; Hoogendijk W.J.G.; Kushner S.A.; Roza S.J. (2018)

Long-term neurodevelopmental consequences of intrauterine exposure to lithium and antipsychotics: a systematic review and meta-analysis

European Child & Adolescent Psychiatry (2018) 27:1209–1230

Pottegård, A.; Ennis, Z.N.; Hallas, J.; Jensen, B.L.; Madsen, K.; Friis, S. (2016a)

Long-term use of lithium and risk of colorectal adenocarcinoma: a nationwide case-control study

British Journal of Cancer, 114, 571-575

Pottegard, A.; Hallas, J.; Jensen, B.L.; Madsen, K.; Friis, S. (2016b)

Long-term lithium use and risk of renal and upper urinary tract cancers

Journal of the American Society of Nephrology, 27, 249-255

Schlatt, S.; Ehmcke, J. (2014)

Regulation of spermatogenesis: an evolutionary biologist's perspective

Seminars in Cell and Developmental Biology, 29, 2-16

Schou, M.; Goldfield, M.D.; Weinstein, M.R.; Villeneuve, A. (1973)

Lithium and pregnancy. I. Report from the Register of Lithium Babies

British Medical Journal, 2, 135-136

Schou, M. (1976)

What happened later to the lithium babies? A follow-up study of children born without malformations

Acta Psychiatrica Scandinavica, 54, 193-197

Schrauzer, G.N.; Klippel, K.-F. (1991)

Lithium in Biology and Medicine

Wiley-VCH Verlag GmbH

Sheikha S.H., LeGate L.S., Banerji T.K. (1989)

Lithium Suppresses Ovariectomy-Induced Surges In Plasma Gonadotropins In Rats

Life Sciences, Vol. 44, pp. 1363-1369

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L. (1988)

A simple technique for quantification of low levels of DNA damage in individual cells.

Exp. Cell Res., 175, 184–191.

Sípek A. (1989)

Lithium and Ebstein's anomaly.

Cor Vasa, 31: 149-56.

Slameňová, D.; Budayová, E.; Gábelová, A.; Morávková, A.; Pániková, L. (1986)

Results of genotoxicity testing of mazindol (degonan), lithium carbonicum (contemnol) and dropropizine (ditustat) in Chinese hamster V79 and human EUE cells

Mutation Research - Genetic Toxicology, 169, 171-177

Smithberg, M.; Dixit, P.K. (1982)

Teratogenic effects of lithium in mice

Teratology, 26, 239-246

Sobti, R.C.; Sharma, M.; Gill, R.K. (1989)

Frequency of sister chromatid exchanges (sces) and chromosome aberrations (cas) caused by three salts of lithium (in vivo)

Cytologia, 54, 245-248

Šrám, R.J.; Binková, B.; Topinka, J.; Fojtíková, I. (1990)

Inhibition of DNA repair synthesis in the rat by in vivo exposure to psychotropic drugs and reversal of the effect by co-administration with α-tocopherol

Mutation Research - Letters, 244, 331-335

Szabo, K.T. (1970)

Teratogenic effect of lithium carbonate in the foetal mouse

Nature, 225, 73-75

Teixeira, N.A.; Lopes, R.C.M.; Secoli, S.R. (1995)

Developmental toxicity of lithium treatment at prophylactic levels

Brazilian Journal of Medical and Biological Research Volume 28, Issue 2, pp 230-239

Thakur, S.C.; Thakur, S.S.; Chaube, S.K.; Singh, S.P. (2003)

Subchronic supplementation of lithium carbonate induces reproductive system toxicity in male rat

Reproductive Toxicology, 17, 683-690

Timson, J.; Price, D.J. (1971)

Lithium and mitosis

The Lancet, 2, 93

Toghiani T.; Gholami M.; Zendedel A.; Assadollahi V. (2012)

The Effects of Low-Dose Lithium Carbonate on the Spermatogenic Parameter in the adults Male Wistar Rats.

Life Sci J;9(4):4360-4367

Toghyani, S.; Dashti, G.R.; Roudbari, N.H.; Rouzbehani, S.; Monajemi, R. (2013)

Lithium carbonate inducing disorders in three parameters of rat sperm

Adv Biomed Res, 2, 55

Trautner, E.M.; Pennycuik, P.R.; Morris, R.J.H.; Gershon, S.; Shankly, K.H. (1958)

The effects of prolonged sub-toxic lithium ingestion on pregnancy in rats

Australian Journal of Experimental Biology and Medical Science, 36, 305-321

Turecki, G.; Smith, M.C.; Mari, J.D.J. (1994)

Lithium mutagenicity

British Journal of Psychiatry, 165, 552-553

Vacaflor, L.; Lehmann, H.E.; Ban, T.A. (1970)

Side effects and teratogenicity of lithium carbonate treatment

Journal of Clinical Pharmacology and the Journal of New Drugs, 10, 387-389

van der Lugt, N.M.; van de Maat, J.S.; van Kamp, I.L.; Knoppert-van der Klein, E.A.; Hovens, J.G.; Walther, F.J. (2012)

Fetal, neonatal and developmental outcomes of lithium-exposed pregnancies

Early Human Development, 88, 375-378

Viguera, A.C.; Newport, D.J.; Ritchie, J.; Stowe, Z.; Whitfield, T.; Mogielnicki, J.; Baldessarini, R.J.; Zurick, A.; Cohen, L.S. (2007)

Lithium in breast milk and nursing infants: clinical implications

American Journal of Psychiatry, 164, 342-345

Warkany, J. (1988)

Teratogen update: lithium

Teratology, 38, 593-597

Weinstein, M.R.; Goldfield, M.D. (1975)

Cardiovascular malformations with lithium use during pregnancy

American Journal of Psychiatry, 132, 529-531

Weinstein, M.R. (1976)

The international register of lithium babies

Drug Information Journal, 10, 94-100

Yacobi, S.; Ornoy, A. (2008)

Is lithium a real teratogen? What can we conclude from the prospective versus retrospective studies? A review

The Israel Journal of Psychiatry and Related Sciences, 45, 95-106

Zaidan, M.; Stucker, F.; Stengel, B.; Vasiliu, V.; Hummel, A.; Landais, P.; Boffa, J.J.; Ronco, P.; Grünfeld, J.P.; Servais, A. (2014)

Increased risk of solid renal tumors in lithium-treated patients

Kidney International, 86, 184-190

Zalzstein, E.; Koren, G.; Einarson, T.; Freedom, R.M. (1990)

A case-control study on the association between first trimester exposure to lithium and Ebstein's anomaly

American Journal of Cardiology, 65, 817-818

Zarnescu, O.; Zamfirescu, G. (2006)

Effects of lithium carbonate on rat seminiferous tubules: an ultrastructural study

International Journal of Andrology, 29, 576-582

Zassadowski, F.; Pokorna, K.; Ferre, N.; Guidez, F.; Llopis, L.; Chourbagi, O.; Chopin, M.; Poupon, J.; Fenaux, P.; Ann Padua, R.; Pla, M.; Chomienne, C.; Cassinat, B. (2015)

Lithium chloride antileukemic activity in acute promyelocytic leukemia is GSK-3 and MEK/ERK dependent

Leukemia, 29, 2277-2284

Ziche, M.; Maiorana, A.; Oka, T.; Gullino, P.M. (1980)

Influence of lithium on mammary tumor growth in vivo

Cancer Letters, 9, 219-224