Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

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

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

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

CLH-O-0000007034-82-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 September 2021

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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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON LITHIUM CARBONATE [1] LITHIUM CHLORIDE [2] LITHIUM HYDROXIDE [3]

CONTENTS

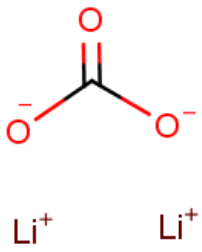
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON LITHIUM CARBONATE [1] LITHIUM CHLORIDE [2] LITHIUM HYDROXIDE [3]

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)

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)
			Repr. 1B (H360, may damage the unborn child) 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).

RAC general comment

Read across

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

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

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

Industry questioned the read across of data in support of classification for reproductive toxicity, especially from lithium carbonate/lithium chloride to lithium hydroxide since there was no data available for the latter on reproductive toxicity, and due to the corrosive nature and high pH of lithium hydroxide. Furthermore, industry commented that while substances used in e.g. pharmaceuticals have greater potential for direct, prolonged exposure to pregnant women and the developing foetuses, pregnant women are unlikely to be exposed to industrial products containing lithium hydroxide. In addition, lithium in industrial products is likely to enter the environment at a quantity or concentration substantially lower than oral administration of lithium carbonate to treat chronic illness. The DS responded that the read across between the different lithium salts

followed the rules described in ECHA's Read-Across Assessment Framework (2017) and that the systemic toxicity of the lithium compounds included in the CLH report was determined by the lithium ion. Further, the DS responded that the CLH proposal was limited to three lithium compounds and that the use of the chemical was not taken into account in the classification.

RAC notes that read across from lithium carbonate to lithium hydroxide was relied on in the REACH registration dossier for lithium hydroxide. Furthermore, a testing proposal for reproductive toxicity of lithium hydroxide using lithium carbonate was submitted. However, the assessment of the testing proposal by ECHA was terminated since testing was already started by industry. RAC notes that corrosivity of the hydroxide anion does not prevent exposure to lithium cation from mixtures, especially if they have a lower pH. According to the REACH Guidance R.7.6.2.3.2 (v6.0), corrosive or highly irritating substances should be tested preferentially via the oral route; however, it must be noted that *in vivo* testing with corrosive substances at concentration/dose levels causing corrosivity must be avoided. However, a vehicle with buffering capacity should be chosen to minimise gastrointestinal irritation, indicating that substances with corrosive properties are not excluded from testing for reproductive toxicity.

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

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

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

Specific concentration limits

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

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-	No data	(ECHA, 2020)	

Property	Value	Reference	Comment (e.g. measured or estimated)
octanol/water			
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
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)	

Property	Value	Reference	Comment (e.g. measured or estimated)
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/cm^3	(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)	
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**	(ECHA, 2020)	

Property	Value	Reference	Comment (e.g. measured or estimated)
	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		
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	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	Negative in experiment 1A and 1C (+/- S9 mix) Experiment 2 : negative with S9 mix	Anonymous, 2000b from (ECHA, 2020) Klimisch

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference** **Reliability**
OECD TG 473 Deviations: no GLP: yes		medium (3 h treatment time, 24 h fixation time); With S9-mix: 0, 100, 333*, 420* and 560* µg/mL culture medium (3 h treatment, 24 h fixation time) Experiment 1B: With and without S9 mix: 0, 300, 350, 400, 450, 500 and 550 µg lithium hydroxide/mL culture medium (3 h treatment, 24 h fixation time); 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	without S9 mix, statistically significant at the lowest (but within the HCD) and the highest concentration. No dose response, very toxic response at the highest dose : not relevant.	score : 2 Supportive 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	Lithium	AA8 CHO cells, up to 10 mM (739 µg/mL;	Positive (- S9 mix):	Pastor et al.,

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference** **Reliability**
anomaly study Guideline: no GLP: no After treatment, cells in anaphase were analysed for any alterations of normal chromosome segregation.	carbonate (purity not provided)	139 µg Li/mL), 3 h treatment - S9 mix Negative control included.	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	2009 Klimisch score : 2 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	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 (- 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
metaphases examined) GLP: no		Negative and positive controls: yes		
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**
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
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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

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

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

Comments received during consultation

Comments were received from one MSCA and three Industry organisations, all supporting

no classification for mutagenicity.

Assessment and comparison with the classification criteria

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

Mutagenicity/genotoxicity studies in vitro

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

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

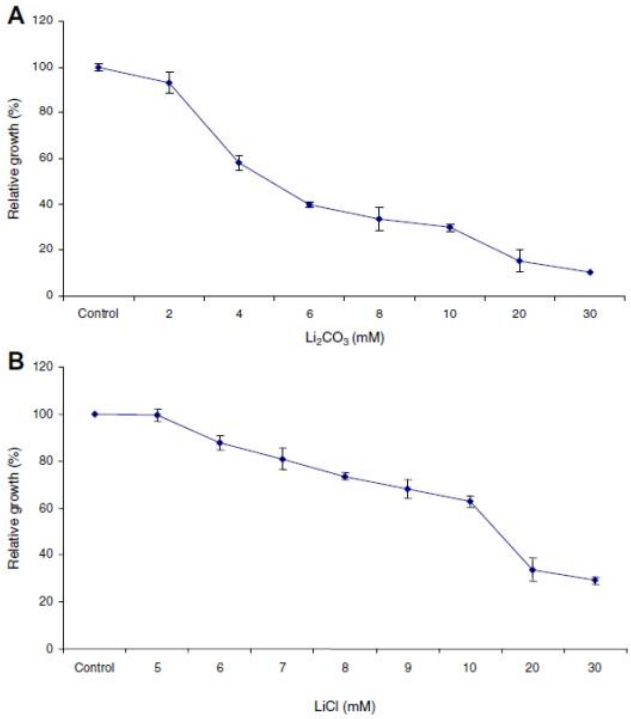


Fig. 1. Effectiveness of different concentrations of lithium salts to inhibit cell growth in the Chinese hamster fibroblast cell line AA8 as shown by the SRB assay. (A) Li_2CO_3. (B) LiCl. While Li_2CO_3 showed as cytotoxic at the lower concentrations (1–5 mM; LD_{50}: 5 mM), the negative effect for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

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

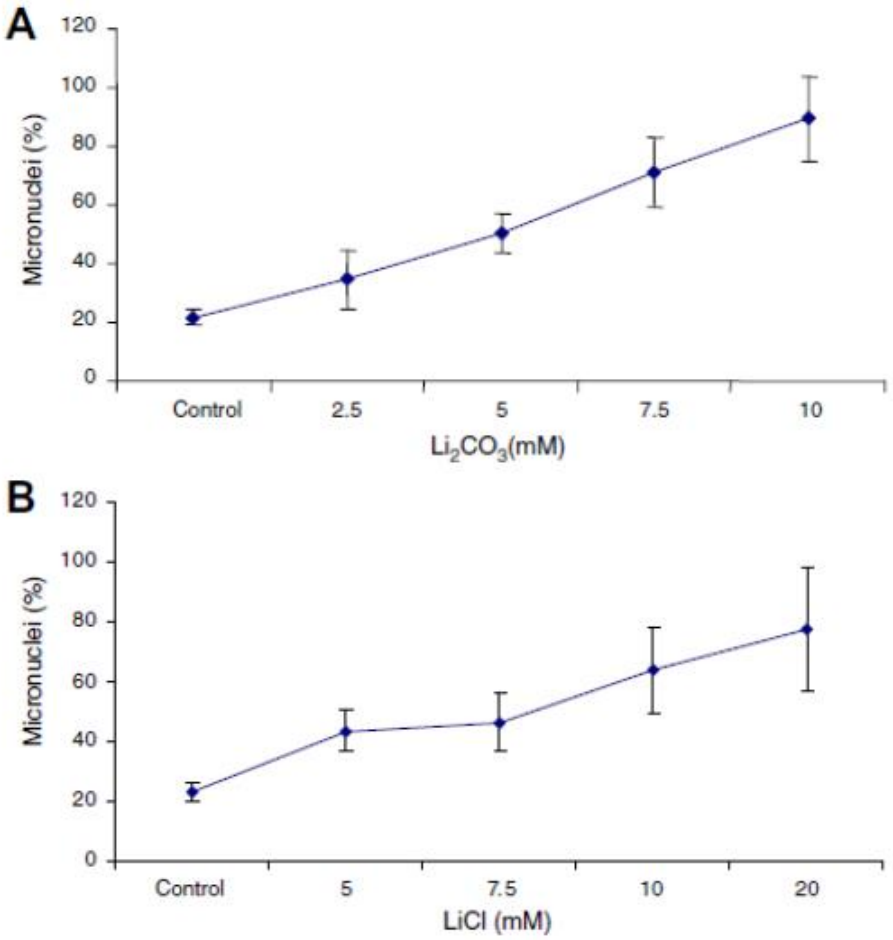


Fig. 2. Dose-dependent induction of micronuclei by both Li_2CO_3 (A), and LiCl (B) in AA8 CHO cells. All the doses tested showed a significant difference in the frequency of micronuclei as compared with control ($p < 0.01$; Student's *t*-test). As can be observed, Li_2CO_3 induced micronuclei with higher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

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

Anonymous (2010a) performed a gene mutation study in mammalian cell according to OECD TG 476 and GLP. In this study, mouse lymphoma L5178Y cells were treated with 0, 12.5, 25, 50, 100 and 200 µg lithium hydroxide/mL for 3 hours with S9-mix, 3 and 24 h

treatment without S9-mix. Cytotoxicity was observed at 200 µg/mL. The results were negative with respect to the mutant frequency in the L5178Y TK +/- mammalian cell mutagenicity test.

Anonymous (2000b) performed a chromosome aberration study in mammalian cells according to OECD TG 473 and GLP. Human lymphocytes from blood samples from one healthy male donor were exposed to lithium hydroxide. In a dose range finding test blood cultures were treated with 0, 10, 33, 100, 333 and 1000 µg lithium hydroxide/mL culture medium with and without S9-mix. Based on the result of the dose range finding, further experiments were performed. Experiment 1A: without S9-mix: 0, 100, 180, 333*, 420* and 560* µg lithium hydroxide/mL culture medium (3 h treatment time, 24 h fixation time) and with S9-mix: 0, 100, 333*, 420* and 560* µg/mL culture medium (3 h treatment, 24 h fixation time). Experiment 1B: with and without S9-mix: 0, 300, 350, 400, 450, 500 and 550 µg lithium hydroxide/mL culture medium (3 h treatment, 24 h fixation time). The experiment was not evaluated due to high cytotoxicity, and a third experiment (Experiment 1C) was performed: with and without S9-mix: 0, 275, 300, 325*, 350*, 375*, 400* (only with S9-mix), 425, 450, 475 and 500 µg lithium hydroxide/mL culture medium (3 h treatment, 24 h fixation time). Based on the results of the dose range finding test and experiments 1A, 1B and 1C the following dose levels were selected to perform an independent repeat (Experiment 2): Without S9-mix: 0, 275*, 300*, 325, 350*, 375, 400, 425 µg lithium hydroxide/mL culture medium (24 h treatment, 24 h fixation time); without S9-mix: 0, 275, 300, 325, 350*, 375*, 400*, 425 µg lithium hydroxide/mL culture medium (48 h treatment, 48 h fixation time);with S9-mix: 350, 375, 400*, 425*, 450*, 475, 500 and 525 µg lithium hydroxide/mL culture medium (3 h treatment time, 48 h fixation time). Test substance concentrations scored for chromosome aberration were selected based on precipitation and cytotoxicity. Lithium hydroxide did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations neither in the absence nor presence of S9-mix in experiments 1A and 1C. Experiment 2 was negative in the presence of S9-mix and lithium hydroxide did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations. In the absence of S9-mix, a statistically significant increase in chromosome aberrations were observed at the lowest (however within the historical control data (HCD)) and the highest concentration. It is noted that no dose response was observed, and the highest dose was a very cytotoxic concentration. Overall, no increase in structural chromosome aberrations in peripheral human blood lymphocytes were seen after a 3-h treatment with lithium hydroxide with concentrations up to 560 µg lithium hydroxide/mL.

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

(1986), reproduced from Annex I to the CLH report.

TABLE 1

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

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

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

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

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

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

In vivo studies in mammalian somatic or germ cells

Two *in vivo* studies in mammalian somatic cells or germ cells were included by the DS. The studies were all disregarded. Sobti et al. (1989) performed one *in vivo* chromosome aberration assay and one *in vivo* sister chromatid assay with lithium carbonate (0, 1.2, 12, 120 mg/kg bw / 0, 0.23, 2.3, 23 mg Li/kg bw) and lithium chloride (0, 0.212, 2.125,

21.25 mg/kg bw / 0, 0.035, 0.35, 3.5 mg Li/kg bw) in mice exposed by single gavage application 72 h before bone marrow preparation. The chromosome aberration assay was positive, however, without a dose response. The frequency of various types of aberrations and the number of cells studied were not provided, no positive control and with negative control values higher than in other published reports (Lagerkvist and Lindell, 2002). The sister chromatid exchange assay was negative. For lithium carbonate there was a slight increase compared to control, however, not statistically significant. There was no information on number of cells studied, no positive control and the negative control values were higher than in other published reports.

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

Human data

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

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

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

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

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

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

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

In a review Aral and Vecchio-Sadus (2008) reported another study (Genest and

Villeneuve, 1971) that found no aberrations in 19 lithium-treated manic-depressive patients compared to 23 controls. However, they reported that the mitotic index was significantly reduced.

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

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

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

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

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	Lithium carbonate (purity not provided) Animals were treated once i.v with N-nitrosourea.	The additional exposure to lithium carbonate for 3 months had no significant effect on the tumour incidence. Animals in the high dose	Ziche et al., 1980 Klimisch

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
GLP: no Female Buffalo/N-rats 24-27 animals per dose group	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	group rapidly lost weight and were killed in week 2. No further information available	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-Dawley 600 animals in total	Lithium carbonate (purity not provided) Animals were treated once with 20 mg 7,12-dimethylbenzanthracene/animal via gavage. Afterwards animals were exposed to 0, 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	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 months. The additional treatment with lithium carbonate did not result in a higher tumour incidence.	Ziche et al., 1980 Klimisch score : 2 Weight of 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 databases; (4) review of the literature on thyroid and renal tumors in patients treated with lithium	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
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	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

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		long term lithium use in cases (0.22%) and controls (0.20%)		
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 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	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
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.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No carcinogenicity or long-term animal studies according to current guidelines were available to the DS. The DS included four tumour promotion studies and one repeated dose toxicity study. Three studies from the same group did not indicate any tumour

promoting activity. In contrast, a fourth study described an increased urinary bladder tumour rate in animals exposed to lithium carbonate and N-butyl-N-(4-hydroxybutyl)-nitrosamine in comparison to animals only treated with N-butyl-N-(4-hydroxybutyl)-nitrosamine. This study could, however, not be adequately assessed due to lack of information. In an insufficiently documented 2-year rat toxicity study with exposure to lithium chloride, no occurrence of tumours was described.

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

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

Comments received during consultation

One commenting MSCA supported no classification for carcinogenicity.

Assessment and comparison with the classification criteria

Animal data

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

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

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

used for the evaluation of a possible tumour promoting activity of lithium carbonate.

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

Human data

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

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

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

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

Martinsson et al. (2016) investigated the cancer incidences in two cohorts of patients with bipolar disorder in a prospective cohort study. One cohort consisted of 3049 patients (age 50-84 years) without lithium treatment and the other consisted of 2393 patients (age 50-84 years) treated with lithium (at least one prescription of lithium per year) between 1 July 2005 and 31 December 2009. Cancer incidences in persons with bipolar disorders were compared to data of the general population (about 2 600 000 men and women). The incidence rate ratios, adjusted for age and gender, of first cancer and site-specific cancer diagnosis between 1 July 2005 and 31 December 2009 were calculated. They concluded that the overall cancer risk was not increased in patients with bipolar disorder. Neither bipolar disorder (IRR = 1.03, 95% CI: 0.89-1.19) nor lithium treatment of bipolar disorder (IRR = 1.04, 95% CI: 0.89-1.23) was associated with increased incidence of unspecified cancers. It is noted that the risk of respiratory, gastrointestinal, and endocrine cancer was increased in patients without lithium treatment, but not in patients with lithium treatment.

Pottegård et al. (2016a) found no association between long term (5 years) lithium use and increased risk of colorectal adenocarcinoma. In this case-control study the cases included patients diagnosed with incident colorectal adenocarcinoma during 2000-2012

(n=36248) while the controls were ten matched cancer free controls per case. Long-term use of lithium was similar among cases (0.22%) and controls (0.20%), yielding an odds ratio of 1.13 (95% confidence interval (CI), 0.89–1.43) for colorectal adenocarcinoma. The odds ratio for colorectal adenocarcinoma in cases were 1.13 (95% CI: 0.89-1.43) while odds ratios for different subsides were for proximal colon: 1.01 (95% CI: 0.66-1.55), distal colon: 1.52 (95% CI: 1.05-2.20) and rectum: 0.80 (95% CI: 0.50-1.30). It is noted that this study lacks data on life-style habits, including smoking, obesity and alcohol consumption in addition to exact data on lithium consumption.

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

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

(i) randomly selected sample of 1,500,000 out of all persons registered in Denmark on 1 January 1995

(ii) all patients having their first psychiatric contact ever in the period from 1994 to 2012 and receiving a main diagnosis of a single manic episode or bipolar disorder

(iii) all persons exposed to either lithium or anticonvulsants.

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

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

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

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

criteria are not meet and the results should be supported by other studies.

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

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

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

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

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

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 1. Experiment: n=20 per dose group, male animals sacrificed after 90 d of exposure 2. Experiment n=20 per dose group, male animals (treated for 90 days with lithium carbonate) caged with untreated female animals to determine fertility index 3. Experiment 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	1. Experiment ≥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. 2. Experiment 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 3. Experiment 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 x 10^8, 1.42 x 10^8, 1.21 x 10^8, 1.12 x 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	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	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,	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**
provided Only results of 425 mg lithium chloride/kg bw/day group documented, no data on general toxicity and number of animals per group	lithium chloride/L, about 0, 85, 170, 250, 425, 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	reduced relative litter weights No toxic effects in the three lowest dose groups (no 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	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,	<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
mM group) n=6 per sex per group in 25 mM group and control group Insufficient reporting	Treatment of 20 mM started 3-7 weeks before mating till end of pregnancy or lactation; 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 6^{th} 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 Female Crl CD (SD) rats (25 animals/dose group)	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 GD 19, examination on GD 20 Exposure via gavage	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, 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)	Anonymous, 2010b Klimisch score : 1 Key study
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-	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.	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	Teixeira et al., 1995 Klimisch score : 2 Supportive study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
water group 13 females in control group	Cross over at parturition of half of animals between lithium group and deprived water group.	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.	
Prenatal and postnatal Developmental Toxicity Study Guideline: no GLP: no Sprague-Dawley rats 11 and 13 female animals per dose group	Lithium carbonate (purity not provided) 0, 1000 ppm lithium carbonate 0, 1000 mg lithium carbonate/kg diet, 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	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 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.	Ibrahim and Canolty, 1990 Klimisch score : 2 Supportive study
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	Fritz, 1988 Klimisch score : 2 Supportive study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		findings) LOAEL: 100 mg lithium carbonate/kg bw/day	
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 Tif:RAIf rats 28 female animals in exposure group, 29 in control group	Lithium carbonate ('purissima') 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	Dams: reduction of body weight gain (12.9%, control: 19%) and feed consumption, polyuria, 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	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,	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	Kelley et al., 1978 Klimisch score : 2 Supportive study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
12 females per dose group	observation until PND 21	offspring during lactation period (6.5 vs. 8.0 in treated and control, respectively)	
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
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	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	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	28 Application via capsule	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 Guideline: no GLP: no HaM/ICR mice 3-4 female animals per dose group, no control group	Lithium carbonate (purity not provided) 200, 300, 465 mg lithium carbonate/kg bw/day, 37.57, 56.36, 87.36 mg Li/kg bw/day from GD 6-15, examination on GD 18 Exposure via gavage	Dams: no information on maternal toxicity Offspring: prenatal mortality in the highest dose group (26%), cleft palate 11/37 (30%) in 3/4 litters 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	Szabo, 1970 Klimisch score : 4 Not assignable 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	Lithium chloride 0, 1 mEq drinking water 0, 10 mg/kg bw/d From mating until end of weaning From delivery until end of	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.	Messiha, 1986 Klimisch score : 3 Disregarded study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
5 females per group Few animals used in each groups and limited details provided by the authors	weaning	Decrease brain weight after postnatal exposure.	
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	Correlation between lithium exposure early in pregnancy and cardiac malformation:	Patorno et al., 2017

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		lithium during the first pregnancy trimester	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).	
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 cohorts, 7 case control studies and 48 cases reports	Evidence that lithium is teratogenic is quite weak. Due partly to heterogeneity in the results, uncertainty remains, the risk cannot be ruled out, and lithium have to be avoid during pregnancy	McKnight et al., 2012
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	Yacobi and Ornoy, 2008

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			general rate of major anomalies.	
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.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on sexual function and fertility

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

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

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

Developmental toxicity

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

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

Medical data were not available in the framework of the dossier, but the DS noted that

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

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

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

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

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

Lactation

The DS considered that there was no doubt that lithium can be transferred to infants via breast milk. However, the existing data did not clearly indicate that severe toxic effects were induced in infants exposed to lithium via breast milk. In experimental animal studies, effects observed could not clearly be distinguished from effects caused by gestational exposure, and there was no evidence that neonates were more sensitive than adults. There was one case report indicating that maternal serum levels in the toxic range could also lead to toxic effects in the infant. However, the DS concluded that the database was not sufficient for a classification for effects on or via lactation.

Comments received during consultation

Effects on sexual function and fertility: Comments from an International NGO and two MSCAs supported the classification as Repr. 1B. One MSCA considered that a classification as Repr. 2 was more appropriate since no adverse effects on sexual function

and fertility up to doses inducing some systemic toxicity was observed in the OECD TG 416 study (according to GLP). Fertility effects were indicated in various other non-guideline studies. However, the MSCA considered that the quality of evidence was less convincing due to deficiencies in the studies, e.g. substance purity information missing, no information on systemic effects/absence of systemic effects.

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

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

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

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

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

Comments from five Industry or Trade Organisations (same comment from one Company-Manufacture) did not support the classification as Repr. 1A for development. They questioned the validity of the studies included in the CLH report since results of a recent study performed under GLP showed no lithium related effects on development. The foetal effects reported in the non-test guideline studies were considered most likely secondary to excessive toxicity or extreme conditions. As regards the human data, epidemiological studies from therapeutic use of lithium showed equivocal and often contradictory results and should not be used for classification for development. Further,

animal studies were carried out on healthy animals and epidemiological studies were carried out on humans with neurological diseases, so how this impacts the interpretation of the data should have been included.

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

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

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

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

2000).

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

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

Animal data

Studies in rats

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

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

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

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

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

P0 generation and no general toxicity in the F1 and F2 generation.

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

In the study by Thakur et al. (2003) male Wistar rats, 20/group, were exposed to 0, 500, 800, 1100 mg/kg lithium carbonate (purity not provided) in the diet for 90 days corresponding to approximately 0, 20, 32, 44 mg lithium carbonate/kg bw/d, (calculated using the ECHA guidance on information requirements and chemical safety assessment R8, 2012, table R8-17), and 0, 3.8, 6.0, 8.3 mg Li/kg bw/d. RAC used this study in the overall weight of evidence assessment for effects on sexual function and fertility. The study included 3 experiments: **Experiment 1:** The animals were exposed to lithium carbonate for 90 days and sacrificed. General toxicity measured as decreased body weight (body weight calculated based on the testis weight per body weight (%), see table below) was in the 0, 500, 800 and 1100 mg/kg bw/d 461 g, 460 g (0% decrease), 387 g (16% decrease) and 408 g (10% decrease), respectively. The reproductive tract was histologically analysed, and sperm parameters and testosterone measured. At doses at and above 800 mg/kg diet a statistically significant reduction in the absolute weight of testes (up to 36%), epididymis (up to 27%) and accessory sex organs (up to 38%) was reported (see table below).

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

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

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

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

indicate an adverse effect". The reduced absolute testes weight is therefore by RAC considered as an effect that should be taken into account in the classification.

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

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

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

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

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

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

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

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

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

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

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

It is noted that no information was provided on systemic toxicity and lithium plasma or serum levels in the study by Thakur et al. (2003). However, the study is considered to cover an important and relevant dose range close to the doses used in the OECD TG 416 study where maternal toxicity at top dose in the P0 generation was very mild and no general toxicity was reported in the F1 and F2 generation investigating endpoints relevant for male fertility in 20 male rats/group with sufficient details. In addition, this is the only study analysing and reporting an impact on fertility of the observations made on sperm and hormonal parameters in several studies. RAC therefore considers the study as relevant for classification noting that the 2-generation study was performed with gavage dosing whereas the Thakur et al. (2003) study administrated lithium carbonate via the diet.

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

In the study by Toghiani et al. (2012) hormonal measurement and histological examination of the testicular tissue was performed in male Wistar rats (6/dose group) following exposure for 48 days via gavage to 0, 10, 20, 30 mg lithium carbonate/kg bw/d corresponding to 0, 1.9, 3.8, 5.6 mg Li/kg bw/d (purity not provided, purchased from Tehran Darou Co, Pharmaceutical company). RAC used this study in the overall weight of evidence assessment for effects on sexual function and fertility. Results: A dose-dependent and statistically significant decrease in the relative testicular tissue weight (0.55 g, 0.43 g, 0.32 g and 0.25 g in 0, 10, 20 and 30 mg/kg bw/d, respectively), germ

and somatic cells in seminiferous epithelium (spermatogonia (up to 42% in high dose group), primary spermatocytes (up to 53% in high dose group), spermatids (up to 57% in high dose group), spermatozoids (up to 70% in high dose group), Sertoli cells (up to 19% in high dose group) and Leydig cells (up to 37% in high dose group)) was reported, see table below:

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

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

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

With a similar study design (seems to be the same study as Toghiani et al., 2012, but with other parameters included) (48 days exposure via gavage to 0, 10, 20, 30 mg lithium carbonate/kg bw/d corresponding to 0, 1.9, 3.8, 5.6 mg Li/kg bw/d) Toghyani et al. (2013) analysed sperm in the epididymis in 6 Wistar male rats/dose group. Results: A dose-dependent reduction in numbers of normal sperm (97%, 88%, 88% and 70% in the 0, 10, 20 and 30 mg/kg bw/d dose group, respectively), sperm motility (96%, 68%, 48% and 39% in the 0, 10, 20 and 30 mg/kg bw/d dose group, respectively), and number of sperm cells in cauda epididymis (2.19×10^8, 1.42×10^8, 1.21×10^8 and 1.12×10^8 in the 0, 10, 20 and 30 mg/kg bw/d dose group, respectively) was reported. No other parameters were assessed, and few details were provided limiting the acceptability of this study. However, the results are in agreement with the effects reported in the studies by Thakur et al. (2003), Zarnescu and Zamfirescu (2006) and Toghiani et al. (2012).

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

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

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

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

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

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

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

Statistical significance of the effects reported were mainly reached when serum lithium levels were in the upper range of therapeutic doses or even exceeded the therapeutic range, i.e. in the low dose group on observation days 21 and 28 and in the high dose group on observation day 14. RAC notes the mortality reported in the high dose group, however, considers the effects observed in the low dose group as related to exposure to lithium and used the study in the overall weight of evidence assessment for effects on sexual function and fertility.

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

Gralla and McIlhenny (1972) investigated effects of lithium carbonate following oral exposure via gavage on fertility and general reproductive performance in Charles River rats (20/sex/group). Females were treated 14 days before cohabitation with approximately 50, 150 and 300 mg lithium carbonate/kg bw/d corresponding to 0, 9.3, 28.1, 56.2 mg Li/kg bw/d. The plasma concentration was 1.4 mmol/L after daily exposure to 300 mg/kg bw/d for 3 days. Males were treated 70 days before cohabitation with approximately 20, 50 and 100 mg lithium carbonate/kg bw/d corresponding to 3.8, 9.3, 18.7 mg Li/kg bw/d. One half of the treated females were sacrificed on GD 13 and the number and distribution of implantation sites were recorded. The remaining females

were allowed to deliver and nurse their offspring to PND 21. Results: Two pregnant rats died unexpectedly for unknown reasons, no further information in which dose group. No effects on reproduction were reported (no further information), and no effects in offspring observed (details not reported).

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

Studies in mice

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

In the study by Mroczka et al. (1983) CFW mice mating pairs were exposed to drinking water containing lithium chloride concentrations of 0, 10, 20, 30, 50, 100 or 200 mM corresponding to approx. 0, 85, 170, 250, 425, 850, 1700 mg lithium chloride/kg bw/d and 0, 14.2, 28.3, 41.7, 70.8, 141.6 and 282.2 mg Li/kg bw/d starting about 2 or 5 weeks before mating. Results: Mice in the highest dose group refused to drink and died within one week. Mice in the 100 mM group survived but did not reproduce (no further information). Mice in the 425 mg/kg bw/d group (with corresponding plasma concentrations in the therapeutic range 0.67 mM), had fewer litters of normal size at birth, prolonged intervals between litters and increased postnatal mortality, including loss of entire litters, whereas no effects were observed in the three lowest dose groups. Due to insufficient reporting (only results of 425 mg lithium chloride/kg bw/d group documented, no data on general toxicity and number of animals per group) this study was considered not reliable and RAC did therefore not include this study in the weight of evidence assessment.

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

Several studies were included by the DS and are shortly described below:

In a study with Swiss male mice (20/group) given a cumulative dose of 23.25 mg lithium carbonate/mice (approx. 22 mg/kg bw/d) i.p.) for 35 days a statistically significant decrease in testes weight (0.16±0.016 g vs 0.24±0.027 g in controls) and body weight (30.45±1.43 g vs 31.06±2.23 g in controls) were reported (Ali, 2008). Further, histopathological changes in the testes included disappearance of spermatogonia, decreased number of spermatocytes, Sertoli 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 considered to be limited since limited information on systemic toxicity was included and the exposure was by i.p. route.

In the study in Swiss mice (10/group) by Mostafa et al. (2010), described in more detail in the developmental toxicity section, an increased diameter of the seminiferous tubules and a decrease in primary spermatocytes count, nuclear diameter of Leydig cells, diameter of epididymis ductulus and testosterone level was reported in 21-d-old offspring after i.p. exposure during gestation and lactation to lithium carbonate (25 mg/kg bw/d from GD 10 to the end of lactation).

Ghosh et al. studied mechanistic aspects of lithium exposure on male fertility in several studies. In the study of Ghosh et al. (1990b) adult male Wistar rats, 8/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. Further, at the two highest concentrations, serum FSH, LH, prolactin (PRL) and testosterone plasma levels were significantly decreased as well as testicular 3-beta-hydroxysteroid dehydrogenase and 17- beta-hydroxysteroid dehydrogenase, two key enzymes in androgen biosynthesis.

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.

Ghosh et al. (1991b) exposed sexually immature male rats (35 days old) subcutaneously to 2 mg lithium chloride/kg bw/d (0.33 mg Li/kg bw/d) for 15, 20, or 25 days. The protocol and results were very similar to Ghosh et al. (1990b). 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/l. Lithium effects were partially reversible by PRL application as shown by the same group in a second comparable experiment where rats were treated with PRL 8 hours after lithium chloride treatment (Ghosh et al., 1991a). Animals revealed a significant restoration of testicular weight in comparison to lithium-treated animals not receiving PRL. Body weights of the lithium-treated animals in all groups did not differ from controls.

Banerji et al. (1983) exposed adult male Sprague-Dawley rats (20/group) by i.p.

injection to 100 mg lithium chloride/kg bw twice daily for 2 or 7 days. The FSH plasma level was not affected. The plasma LH levels were 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 in the 7-d exposure group died on the 6th day, and a number of rats in 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 i.p. injection of 34.7 mg/kg (5 mEq/kg bw corresponding to plasma lithium concentration of 1.66 ±0.29 mEq/L lithium) on the pro-oestrus surge of LH in female C57BL/6 mice (3 injections the same day) (Banerji et al. 1986). Animals were sacrificed in the evening. A statistically significant reduction in plasma LH level was reported in the lithium-exposed mice compared to controls, but as in the previous experiment, no change in pituitary LH. The pro-oestrus surge of LH is considered necessary for normal ovulation. Plasma levels of FSH was statistically significantly increased in lithium-exposed mice compared to control mice. A concomitant statistically significant increase in pituitary FSH was also seen in the lithium-exposed mice. The authors explained that this could be due to that lithium interferes with secretion rather than the synthesis of LH.

Finally, studies were performed by sub-cutaneous or by inter-peritoneal injection on female rats with various duration of exposure. lithium exposure in OVX rats for 3 and 7 days reported a significantly reduction in plasma LH and FSH levels (Sheikha et al., 1989). As in the study of Banerji et al. (1986), it was observed that the duration of the oestrous cycle was increased in lithium-treated rats with longer metoestrous and dieostrous phases (Jana et al., 2001). In addition, it was shown that lithium induced follicular atresia, significant decreases in serum progesterone concentration and ovarian weight (Mirakhori et al., 2013; Khodadadi et al., 2013).

Human data

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

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

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

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

The effect of lithium therapy on human PRL levels were studied in several studies and summarised in HCN, (2000). Four studies did not report any effect of lithium treatment

on plasma PRL levels; however, a fifth study reported an increase in PRL levels during lithium therapy. HCN concluded that due to these contradictory results no final conclusions could be drawn.

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

Summary of the human and animal data

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

Studies investigating effects of lithium carbonate on the male rat reproductive tract consistently showed significant effects on sperm number (decrease up to 70%) or production (decrease up to 70%), sperm function, and/or male reproductive organ structure, as well as on testosterone levels (decrease up to 81%). All five studies on male reproduction were performed with the identical rat strain as used in the 2-generation study (Wistar rats) and four of them used doses in the same range as the 2-generation study (Thakur et al., 2003; Zarnescu and Zamfirescu, 2006; Toghyani et al., 2013; Toghiani et al., 2012). However, it is noted that in the 2-generation study oral administration by gavage was used (Anonymous, 2012, Van Deun et al. 2021) whereas in the Thakur et al. (2003) study lithium carbonate was given in the diet. Further, in the 90-d study (Thakur et al., 2003) with subsequent mating, effects were reported on male fertility, evident as a reduction in male fertility index (from 90% in control group to 40% in the high dose group), confirming the consequence of the effects reported on the reproductive organs. Only the study by Allagui et al. (2006) used higher, partially lethal doses, and the results on sperm parameters and testosterone levels should be interpreted with caution. RAC notes that a complete study report of the 2-generation OECD TG 416 study was not available, but based on information provided on sperm parameters, reasons for the contradictory findings on sperm parameters and male reproductive organs are not known. In a fertility study with lithium chloride in mice, reduced fertility was also observed. However, this study was disregarded due to the high doses used in the study as well as the limited information provided (Mroczka et al., 1983).

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

The reproductive effects were also supported by the results reported on biochemical measurements performed in various studies including a decreased level of testosterone,

FSH, LH and PRL, as well as on key enzymes in androgen biosynthesis.

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

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

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

Developmental toxicity

Animal data

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

Studies in rats

In the key developmental toxicity study female Crl CD (SD) rats (25 rats/dose) were exposed to 0, 10, 30 or 90 mg lithium carbonate/kg bw/d by gavage from GD 6 to 19 corresponding to 0, 1.88, 5.64, 16.91 mg Li/kg bw/d (purity 99.6%) (Anonymous, 2010b, Van Deun et al., 2021). The study was similar to OECD TG 414 (deviation was exposure from GD 6 instead of GD 5) and according to GLP. Serum analysis revealed a clear dose-related systemic exposure to lithium (0.24, 0.52, 1.39 mM Li). Maternal toxicity: In the high dose group a slight but statistically significant reduction in body weight gain (66.9% increase in bw from GD 1 to laparotomy compared to 74.4% in controls), and in food intake (up to 18.3% below controls) and water intake was reported. Further, piloerection was reported in a few dams. Foetal toxicity: No foetal malformations, and no test item related increase in the incidence of external/internal, skeletal or soft tissue variations or skeletal retardations was reported.

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

In the prenatal and postnatal study by Teixeira et al. (1995) pregnant female Wistar rats received either tap water ad libitum or tap water with 10 mM lithium chloride (purity not provided) corresponding to 53 mg lithium chloride/kg bw/d or 8.83 mg Li/kg bw/d from GD 1 to the end of lactation, or were water restricted until weaning of pups. Following

birth, pups were fostered to form five experimental groups: a) control water restricted (21 litters), b) Li+ during prenatal and lactating periods (18 litters), c) Li+ during prenatal period only and then water restricted (22 litters), d) water restricted prenatally and Li+ during lactating period (25 litters), and e) Control, no treatment, not water restricted (13 litters). Maternal toxicity: No information included. Foetal toxicity: No malformations, stillborn or differences between litter size were reported at birth. A reduction in the number of pups with normal righting reflex at birth in both the water restricted (78.5%) and lithium-treated litters (70.5%) compared to the control group (94.2%) was reported; however, the lithium-treated group had also a reduced correct righting reflex compared to the water restricted group. A statistically significant delay in the day of eye opening and in the avoidance of visual cliff was reported following lithium exposure and in the water restricted group compared to the control group. Further, a statistically significant lower body weight was reported at PND 21 in pups exposed to lithium during lactation; however, lithium exposure prenatally did not have any effects on the body weight compared to the water restricted group.

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

In the prenatal developmental toxicity study by Fritz (1988) the effects of lithium carbonate (purity not provided) on the developing kidney were investigated in three experiments. **experiment 1**: Female Tif:RAIf rats (Sprague Dawley-derived) (14-19/dose group) were exposed by gavage to 100 mg lithium carbonate/kg bw/d corresponding to 0 and 18.79 mg Li/kg bw/d from GD 6-10, GD 11-15 or GD 16-20 and examination on GD 21. Maternal toxicity: Reduced body weight gain and feed consumption, polyuria. In GD 16-20 exposed dams, 7 died one day before expected delivery (no gross pathological findings). Foetal toxicity GD 6-10: Embryonic and foetal deaths (3.8% of the implantation sites), dilatation of renal pelvis with obsolete or missing papillae: 0/67 in foetuses. Foetal toxicity GD 11-15: Embryonic and foetal deaths (7.0% of the implantation sites), dilatation of renal pelvis with obsolete or missing papillae

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

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

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

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

maternal body weight in the C/Li group. Foetal toxicity: In the C/Li and Li/Li groups severe structural changes, consisting of up to 3 mm cortical cysts, extensive interstitial fibrosis with cell infiltration, and atrophy of the cortical collecting ducts was reported. The kidneys from C/C and Li/C groups were normal. Functionally, in the C/Li and Li/Li groups growth retardation, polyuria with lowering of renal concentration ability, and uremia associated with as much as 80% lowering of the normal glomerular filtration rate (GFR) was reported. However, in the Li/C group no effects on the concentration ability were reported. The study concluded that postnatal development of the rat kidney was particularly sensitive to nephrotoxic effects of lithium.

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

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

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

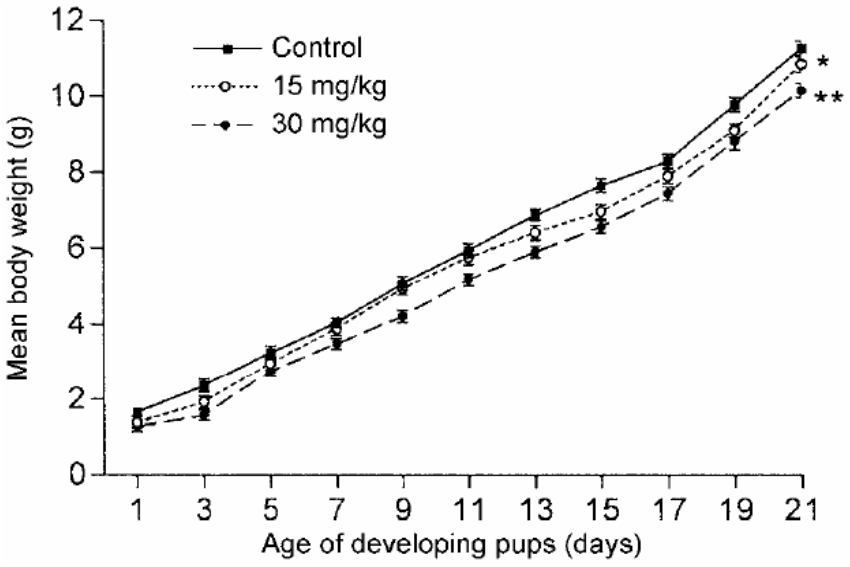
*p<0.01, **p<0.05; [a], number of dams

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

Studies in mice

In a neurodevelopmental study comparable to OECD TG 426, Swiss Webster mice (at least 7 pregnant/dose) were exposed via drinking water to lithium chloride (analytical grade). The doses were 0, 90 and 180 mg lithium chloride/kg bw/d corresponding to 0, 15, 30 mg Li/kg bw/d from GD 1 to PND 15 with neurobehavior examination until PND 21. The lithium doses were calculated based on average total volume of drinking water consumed by the animals in 24 h and, the lithium doses per day dissolved in the water.

However, no analytical verification of the doses was included (Abu-Taweel, 2012). Three pups/litter were examined/test. No information was included with regard to maternal toxicity and with regard to foetal toxicity, a dose-dependent statistically significant decrease in body weight gain from PND 1 to PND 21 was reported, see figure below from Abu-Taweel, 2012:



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

The decrease in brain acetylcholine esterase may have led to disturbances in the behaviour of the animals. The authors of the study included that lithium is considered to affect neuronal communication (Phiel et al., 2001) and affect nerve excitation through the synthesis, activation, and inactivation of various neurotransmitters (Casado et al., 1989; Ghoshdastidar, 1999). Therefore, lithium exposure in the present study, could

have produced developmental abnormalities in the brain of the exposed offspring leading to the observed teratological and behavioural effects reported in the offspring. However, it was noted that assessment of other neurotransmitters like catecholamines (serotonin, norepinephrine, dopamine, etc.) in the brain was not included which would be considered to give a better explanation for effects on behavioural. This study indicates that lithium chloride can induce neurodevelopmental toxicity following exposure during gestation and lactation. However, the study used few animals (7/dose group compared to OECD TG 426 with 20/dose group), included no neuropathology, and with no information on maternal toxicity.

In the prenatal and postnatal developmental toxicity study, albino mice (5 females/group) were exposed to lithium chloride 0 ans 1 mEq in drinking water corresponding to 0 and 10 mg lithium chloride/kg bw/d corresponding to 1.7 mg Li/kg bw/d from mating until end of weaning (Messhia, 1986). Maternal toxicity: no information. Foetal toxicity: 14 days after weaning a significant decrease in brain weight in males and females, kidney weight in females, and testis weight in males was reported. The decrease in brain weight was confirmed after postnatal exposure, suggesting an effect on lactation.

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

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

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

It is noted that induction of cleft palate in mice has also been reported by Loevy and Catchpole, 1973. In this study, CD1 mice received 15.5 mg of lithium chloride/mouse (about 620 mg lithium chloride/kg bw/d corresponding to 100 mg Li/kg bw/d) in sterile water by subcutaneous injection on 2 or 3 days from GD 11 to GD 13. The mice were sacrificed on day 17 of pregnancy. Maternal toxicity: No effects were reported. Foetal

toxicity: An increased incidence of resorptions (11 – 21 vs. 4 in controls) as well as cleft palates were reported in the offspring injected on days 11, 12, and 13 (15.1%); on days 12 and 13 (7.2%); and on days 11 and 12 (3.4%).

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

Studies in mice with i.p. route of exposure

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

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

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

Overall, RAC considers that studies with i.p. exposure to lithium carbonate during

pregnancy leading to high peak exposure, and resulting in developmental toxic effects, including neural tube defects and exencephaly are not regarded as relevant for the assessment of developmental toxicity following exposure to lithium chloride.

Study in New Zealand white rabbits

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

Study in pigs

In a pre- and postnatal toxicity study, pigs (12 females/group) were exposed in the diet to 0 and 3000 mg lithium carbonate/kg diet corresponding to approximately 0 and 40 mg lithium carbonate/kg bw/d and approximately 0, 7.5 mg Li/kg bw/d, from GD 30-114, with observation until PND 21. The serum lithium concentrations on GD 60 were 1.4 mEq/L and on GD 110 2.3 mEq/L (Kelley et al., 1978). Maternal toxicity: Decreased body weight gain, significant at GD 110 (23% reduction, no further information available). Five out of 12 exposed pigs did not complete pregnancy. Offspring toxicity: Prenatal mortality increased (adjusted mean number of live piglets/litters: 9.6 in exposed vs 11.3 in control, adjusted mean number of stillbirths and mummies/litter: 2.1 in exposed vs 0.6 in control), reduced litter birth weight (11.1 vs. 15.4 kg in exposed and control, respectively), reduced survival of offspring during lactation (6.5 vs. 8.0 in exposed and control, respectively). RAC considers that due to the design (only one dose) and limited reporting the study is of limited validity, but is considered to support the finding in Wistar rats including that gestational exposure to lithium carbonate might cause severe developmental effects (e.g. increased number of stillbirths, and reduced postnatal survival reported in the study by Fritz, 1988 and Marathe and Thomas, 1986).

Study in Rhesus monkey

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

Summary of the animal study findings: In the OECD TG 414 study in rats no developmental toxicity was reported but the highest dose tested induced only slight maternal toxicity. However, some of the developmental toxicity studies, although not performed according to OECD TG 414, indicated that lithium may induce developmental toxicity, including reduced pup body weight, decreased litter size, neurobehavioral effects

and nephropathy as well as delayed ossification and malformations, however, often reported at doses which were potentially maternally toxic. Few studies provide sufficient information on maternal toxicity and/or lithium plasma concentrations, which therefore limits the interpretation of these studies. Therefore, in these studies it is not possible to conclude if the effects seen in pups were secondary, non-specific effect of maternal toxicity. Studies in rats indicated an effect on the kidney in the offspring; however, effects on the kidney were reported at doses inducing marked maternal toxicity including mortality. On the other hand, an effect on the kidney could be considered as substance-related since the kidneys is one of the target organs of lithium toxicity. Studies in mice point to a neurotoxic effect following *in utero* exposure to lithium chloride, however, with no information on maternal toxicity and few animals/dose groups. RAC notes that no effects on neurodevelopment were reported in two epidemiological studies following exposure to lithium during pregnancy. However, the epidemiological studies investigating neurodevelopmental effects were limited and of questionable quality.

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

Human data

Cardiac malformations

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

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

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

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

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

A meta-analysis combining data from 6 cohort studies with a total of 727 pregnancies with lithium exposure and 21 397 reference pregnancies in mothers with a mood disorder, but without lithium exposure, was performed by Munk-Olsen et al. (2018). The cohorts included pregnancies resulting in live-born singleton deliveries from 1997 to 2015, where health-related information was available both for the mother and for the infant. The analysis was performed following a shared protocol established a priori to minimise heterogeneity related to selection criteria, exposure, outcome and covariate definitions, and statistical methodology. The odds ratios (OR) were adjusted for maternal age at delivery (in years), primiparity, calendar year of birth, and treatment with any other psychotropic medication during pregnancy. Furthermore, pregnancies in which mothers were prescribed known teratogenic medications in pregnancy were altogether excluded from the analysis. The study showed that lithium treatment during pregnancy was not associated with preeclampsia, foetal distress, or postpartum haemorrhage. Further, no difference between groups were observed for caesarean section, preterm birth, low birth weight, or small for gestational age. Lithium exposure during the first trimester (654 out of 727 children (90%) was exposed during the first trimester) was associated with a statistically significantly increased risk of major malformations such as cardiovascular defects, neural tube defects, hypospadias and epispadias (7.4% vs 4.3%;

pooled adjusted OR = 1.71, 95% CI: 1.07-2.72), but not for major cardiac malformations (2.1% vs 1.6%, pooled adjusted OR = 1.54, 95% CI: 0.64-3.70). No Ebstein's anomaly was reported in the meta-analysis. It is noted that 74.8% in the lithium-treated group and 61.2% in the reference group used other psychotropic drugs, but as explained the ORs were adjusted for the use of such drugs. The authors concluded that their results suggest an association between lithium exposure and major malformation, but that this association was much smaller than previously reported. The study is considered as a good quality study with robust methodology; however, no serum lithium levels were measured, so no dose-response analysis of the effects could be performed.

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

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

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

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

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

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

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

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

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

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

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

During the stakeholder consultation information on a retrospective cohort study by Poels et al. (2020) was submitted which also investigated miscarriage due to lithium exposure during pregnancy. The odds ratio adjusted for the age at conception and the clustering of pregnancies per woman was calculated. In the crude unadjusted analysis, miscarriages

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

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

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

pregnancies with lithium exposure included and that no information regarding the number of lithium-treated women with normal children was provided.

Källen and Tandberg (1983) performed a retrospective cohort study from 1973-1979. The study included 350 mother-child pairs with mothers having a previous history of inpatient ward treatment due to manic depressive disease. Maternity health care records were available for 82% of the pairs and were used to divide them into groups of (1) no indication of psychiatric illness before pregnancy (N=110), (2) psychiatric illness but no indication of drug use (N=80), (3) psychotropic drug use, but not lithium (N=38), (4) lithium use only (N=41) and (5) lithium and other psychotropic drug use (N=18). In women treated only with lithium 4/41 had neonatal deaths, 5/41 had malformed infants, 2/41 had dead and malformed infants and 3/41 had heart defects (no Ebstein's anomaly). The numbers of same outcomes were reported for the other groups mentioned above, but no statistical testing was performed to compare the groups. Heart defects were also observed in the no drug group (2/80, of which one was related to a Down's syndrome) and in the group exposed to both lithium and other drugs (1/18). There were differences in prevalence of smoking between the groups (those with lithium or other drug treatment tended to be more often smokers). However, as there was no statistical testing or risk estimation overall between the groups, also the potential confounding effect of smoking or any other potential confounders was not analysed. It was reported separately that when the entire cohort was compared to all Swedish pregnancies, adjusted for age, parity and hospital district, the frequency of heart defects was increased just reaching statistical significance, but no numerical values were presented. Such a comparison was not reported for the lithium-exposed part of the cohort, while the authors noted that 4 of the 6 cardiac defects occurred among infants exposed to lithium, either alone or together with other drugs. This study is considered to be of limited value due to the lack of proper comparison between lithium-exposed and unexposed groups with relatively small numbers of cases as well as lack of control for potential confounding by other factors.

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

Overall, the epidemiological studies concluded that lithium therapy adds only a small risk for cardiovascular malformations including Ebstein's anomaly and does not increase the

general rate of major anomalies. Furthermore, when reviewing the data accumulated until today the risk is lower than previously anticipated from the Register of Lithium babies. Although the association is weak, RAC still considers the increase in cardiac malformations to be reliable evidence, since these are rare malformations and the population at risk, the pregnant women under lithium therapy will never contribute to a high number of cases. However, the impact of lithium exposure during pregnancy may on the other hand have been underestimated since a higher rate of therapeutic abortions has been reported in lithium-treated pregnant women (10% vs 6% in Jacobson et al., 1992). RAC also notes that no cardiovascular malformations were reported in the experimental developmental toxicity studies in animals.

Neurodevelopmental effects

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

In a cohort study by Van der Lugt et al. (2012) the long-term effect of lithium on children neurodevelopment was studied. Fifteen children born between 1994 and 2007 that were exposed to lithium *in utero*, but not breastfed, were studied at 3-15 years of age. The mother's serum lithium levels were 0.6-0.8mmol/L during pregnancy. Tests on neurological or cognitive development as well as the parent's response on the child's behaviour checklist was included. No adverse effects on growth, neurological, cognitive and behavioural development was reported. However, the group of children studied was small, with no appropriate control group, and other medication besides lithium was used by the pregnant mothers.

Summary epidemiological studies

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

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

In the seventies, retrospective studies, based on the Registry of Lithium Babies, i.e. children from women who had been treated with lithium during the first trimester of

pregnancy, indicated an association between first trimester lithium exposure and cardiac malformations (Schou et al., 1973; Weinstein and Goldfield, 1975; Weinstein, 1976). However, the studies are not considered robust enough to prove this association. The studies had methodological deficiencies and the results are therefore difficult to interpret. They did not assess if cardiovascular anomalies occurred more frequently in infants exposed to lithium during pregnancy compared to non-exposed infants. Further, in several cases malformations were reported in infants exposed *in utero* to other drugs in addition to lithium.

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

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

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

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

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

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

neurodevelopmental effects are limited and of questionable quality.

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

In conclusion, RAC is of the opinion that in a weight of evidence assessment, and taking into account that classification is hazard based, the epidemiological studies showing weak evidence of an increase in rare cardiac malformations in infants exposed to lithium during the first trimester of pregnancy should be considered for the classification of lithium. Especially, the study by Patorno et al. (2017) including appropriate controls, a balanced assessment, and due consideration of bias or confounding factors is considered robust and relevant for classification. The study concluded that maternal use of lithium during the first trimester is associated with an increased risk of the cardiac malformation evident as right ventricular outflow tract obstruction defect, compatible with Ebstein's anomaly, also showing that this association is dose-dependent. This is supported by the reported findings of the other recent and robust epidemiological studies, especially the large study by Munk-Olsen et al. (2018) and also the smaller study by Diav-Citrin et al. (2014). It is noted that the earlier studies described above and included in the analyses by Yacobi et al. (2008) and McKnight et al. 2012 have methodological deficiencies and are difficult to interpret quantitatively, but do not contradict the observations of the more robust studies. Cardiac malformations are considered as serious, although a rare malformation. It is noted that there is a limited number of pregnancies where lithium has been used during the first trimester. Therefore, the finding of cardiac malformations should not be dismissed. RAC notes that a classification for developmental toxicity is supported by experimental animal studies where some concerns for neurodevelopmental effects in rats and mice as well as decreased pup body weight and litter size were reported. RAC is of the opinion that a **classification as Repr. 1A; H360D for the three lithium compounds is warranted** based on the human epidemiological data.

Adverse effects on or via lactation

Experimental animal data:

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

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

In the study by Ibrahim and Canolty (1990), rats were exposed to 50 mg lithium carbonate/kg bw/d corresponding to 9.4 mg Li/kg bw/d, with one group only receiving

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

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

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

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

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

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

Human data

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

In a clinical study with 10 mother-child pairs, the mothers were treated with lithium (600-1200 mg lithium/d during pregnancy and lactation) and the sampling of the children were between 1 and 52 weeks of age (Viguera et al., 2007). In this study, the breast milk concentrations were between 11-56% of serum levels. Maternal serum, breast milk, and infant serum concentrations of lithium averaged 0.76, 0.35, and 0.16 mEq/L, respectively. No serious adverse effects were reported.

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

In a single case study, toxic effects were reported in a breast-fed child (cyanosis,

electrocardiographic changes, floppy muscles). The lithium serum concentrations in the mother were extreme, 16 mM, and serum level in the infant also very high (6 mM). The symptoms resolved after the discontinuation of breastfeeding (HCN, 2000).

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

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

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

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

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

However, information provided during stakeholder consultation described a potential mechanism of toxicity in infants. Due to an immature excretory system in infants, there is an increased possibility of adverse reactions since lithium is eliminated via renal excretion. It is noted that the infants were also exposed to lithium during pregnancy, so the infant serum concentration from *in utero* exposure combined with lithium exposure from breastfeeding adds up due to the immature excretory systems of infants. Further, it is noted that in experimental animal studies, lithium induced severe renal structural changes in the developing rat kidney (Christensen et al., 1982; Fritz, 1988, noting that in

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

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

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

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

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Annex I to the 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 PHYSICAL HAZARDS

Evaluation not performed for this substance

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

3 HEALTH HAZARDS

3.1 Germ cell mutagenicity

3.1.1 In vitro data

3.1.1.1 Anonymous, 2000a

Study reference:

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

See also confidential Annex I for confidential information

Detailed study summary and results:

Test type

- OECD Guideline 471 (Bacterial Reverse Mutation Assay version from July 1997), GLP compliance is given
- Lithium hydroxide was tested in concentrations of 0, 3, 10, 33, 100, 333, 1000, 3330 and 5000 µg/plate with and without S9-mix.
- Positive control substances: sodium azide (TA 1535 without S9), 9-aminoacridine (TA 1537 without S9), daunomycine (TA 98 without S9), methylmethanesulfonate (TA 100 without S9), 4-nitroquinoline-N-oxide (WP2uvrA without S9), 2-aminoanthracene (TA 1537, TA 1535, TA 98, TA 100, E. coli WP2uvrA with S9)
  Solvent control: yes
- Vehicle: Milli-Q-water

- Evaluation criteria:

  A test substance is considered negative (not mutagenic) in the test if:

  a) The total number of revertants in any tester strain at any concentration is not greater than two times the solvent control value, with or without metabolic activation.

  b) The negative response should be reproducible in at least one independently repeated experiment.

  A test substance is considered positive (mutagenic) in the test if:

  a) It induces a number of revertant colonies, dose related, greater than two-times the number of revertants induced by the solvent control in any tester strains, either with or without metabolic activation. However, any mean plate count of less than 20 is considered to be not significant.

  b) The positive response should be reproducible in at least one independently repeated experiment.

Test substance

- The test material is lithium hydroxide
- EC number: 35-287-3
- CAS number: 1310-65-2
- Degree of purity: 92.9%
- Impurities do not affect the classification
- Batch number: [confidential information]

Administration/exposure

- Strains tested: S. typhimurium TA 1535, TA 1537, TA 98 and TA 100, E. coli WP2 uvr A
- Metabolic activation system: S9 mix containing 5 % (v/v) S9 fraction (no further information available)
- Details on test system and conditions:

  The test substance was ground and the stock solution was filter (0.22 µm)-sterilized. Test substance concentrations were prepared directly prior to use.

  Range finding study: Lithium hydroxide was tested in the tester strains TA 100 and WP2uvrA with concentrations of 0, 3, 10, 33, 100, 333, 1000, 3330 and 5000 µg/plate in the absence and in the presence of S9 mix.

  Mutation assay: Based on the results of the dose range finding study, lithium hydroxide was tested up to concentrations of 5000 µg/plate in the absence and in the presence of S9-mix in two mutation experiments. The first mutation experiment was performed with the strains TA 1535, TA 1537 and TA 98 and the second mutation experiment was performed with the strains TA 1535, TA 1537, TA 98 TA 100 and WP2uvrA
- Statistical methods: not performed

Results and discussion

- Tested dose levels are based on results of a dose range finding study
- No precipitation was observed at any dose level
- No cytotoxicity was observed at any dose level (reduction of the bacterial background lawn)
- Genotoxic effects: negative with and without metabolic activation
- Concurrent negative (solvent/vehicle) and positive control data valid: yes

The results of the two experiments can be seen in the following two tables:

Table AI - 1: Mutagenic response of lithium hydroxide in *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (range finding experiment)

Dose (g/plate)	Mean number of revertant colonies/3 replicate plates (±S.D.) with different strains *of Salmonella typhimurium* and one *Escherichia coli* strain				
	TA 1535	TA 1537	TA 98	TA 100	WP_2uvrA
Without S9-mix					
positive control	219±33	435±100	371±39	466±22	172±32
solvent control	12±6	7±3	16±3	65±2	9±2
3				77±13	12±3
10				72±7	11±3
33				79±8	8±2
100	12±1	7±5	18±3	74±8	12±3
333	15±1	7±1	16±4	65±7	7±3
1000	14±3	4±2	19±3	80±8	9±2
3330	11±3	8±3	12±4	77±13	5±2
5000	12±1	6±2	12±5	77±11	4±1
With S9-mix					
positive control	296±23	703±22	1346±230	1199±176	237±37
solvent control	14±6	6±2	26±3	90±10	11±3
3				96±4	12±3
10				99±5	12±4
33				95±9	9±3
100	11±3	6±3	31±4	78±11	12±2
333	15±1	4±1	27±9	101±5	10±1
1000	18±7	9±1	26±6	83±12	11±3
3330	15±6	5±1	25±3	86±14	7±4
5000	14±2	4±1	22±3	65±1	4±1

Table AI - 2: Mutagenic response of lithium hydroxide in *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (mutation assay)

Dose (g/plate)	Mean number of revertant colonies/3 replicate plates (±S.D.) with different strains of Salmonella typhimurium and one Escherichia coli strain				
	TA 1535	TA 1537	TA 98	TA 100	WP_2uvrA
Without S9-mix					
positive control	195±2	287±105	642±125	608±24	696±16
solvent control	10±1	4±2	15±4	69±10	8±1
100	12±4	4±2	13±2	79±13	10±1
333	8±3	4±3	16±7	68±10	8±3
1000	12±5	5±2	15±2	70±7	11±2
3330	11±4	4±2	10±4	60±11	6±1

5000	7±1	5±2	11±3	64±8	7±3
With S9-mix					
positive control	203±14	367±45	551±25	709±131	70±8
solvent control	9±1	3±2	23±3	67±6	11±5
100	10±4	3±1	23±3	84±14	12±3
333	8±2	3±3	25±4	70±8	12±2
1000	9±4	6±3	22±6	68±11	7±2
3330	11±5	3±2	14±4	49±6	7±2
5000	5±3	3±2	13±2	54±8	3±1

3.1.1.2 Haworth et al., 1983

Study reference:

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

Detailed study summary and results:

Test type

Ames test, no GLP compliance given

- Doses: up to 10000 µg Li-chloride/mL.

At least five doses of test chemical, in addition to the concurrent solvent and positive controls, were tested on each strain in the presence of S-9 mix or buffer.

To select the dose range for the mutagenesis assay, the test chemicals were checked for toxicity to TA100 up to a concentration of 10 mg/plate or the limit of solubility, both in the presence and absence of S-9 mix. One or more parameters were used as an indication of toxicity: viability on complete medium (EGG) and reduced numbers of revertant colonies per plate and/or thinning or absence of the bacterial lawn (CWR, EGG, SRI). If toxicity was not apparent in the preliminary toxicity determination, the highest dose tested was 10 mg/plate; otherwise the upper limit of solubility was used. If toxicity was observed, the doses of test chemical were chosen so that the high dose exhibited some degree of toxicity. Occasionally, in the earlier tests, the high dose was greater than 10 mg/plate.

- positive and negative control groups and treatment: 2-Aminoanthracene (2-AA) was tested on all strains in the presence of rat and hamster S-9. 4-Nitro-o-phenylenediamine (NOPD) was tested on TA98 without S-9. Also without S-9, sodium azide (SA) was tested on TA100 and TA1535, and 9-aminoacridine (9-AAD) was tested on TA1537.
- criteria for evaluating results

The data were evaluated in an ad hoc manner by each testing laboratory and by NTP personnel. Prior to statistical analysis no formal rules were used; however, a positive response was indicated by a reproducible, dose-related increase, whether it be twofold over background or not. The matrix of test strains and activation systems used allowed the investigators to detect trends or patterns that might not be as evident if only one strain and activation system were examined. In addition to the standard “positive” and “negative” categories,

there is also “questionable” (or “inconclusive”). This applied to low-level responses that were not reproducible within the laboratory or to results that showed a definite trend but with which the investigator did not feel comfortable in making a “+” or “-” decision. It also included tests in which an elevated revertant colony yield occurred at only a single dose level.

Test substance

- Test material used in the study is lithium chloride
- EC number: 231-212-3
- CAS number: 7447-41-8
- Degree of purity: unknown
- Batch number: 775647

Administration/exposure

- Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100
- Type and composition of metabolic activation system:
  - species and cell type: Liver S-9 fractions from male Sprague-Dawley rats and male Syrian hamsters
  - induced
  - chemicals used for induction: Aroclor 1254

Results and discussion

- Cytotoxic concentrations with and without metabolic activation: no information
- Genotoxic effects: negative

3.1.1.3 Anonymous, 2010a

Study reference:

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

See also confidential Annex I for confidential information

Detailed study summary and results:

Test type

- OECD Guideline 476 (In Vitro Mammalian Cell Gene Mutation Test, version from 1997), GLP compliance is given (including certificate)
- 0, 12.5, 25, 50 100 and 200 µg/mL

- Positive control substances: methylmethanesulfonate (without S9 mix), 3-methylcholanthrene (with S9 mix)
- Historical control data available: yes
- METHOD OF APPLICATION

  In medium assay without metabolic activation:

  The cells for the experiments were obtained from logarithmically growing laboratory stock cultures and were seeded into a series of tubes at 1 x 10^7 cells per tube. The cells were pelleted by centrifugation, the culture medium was removed, and the cells were resuspended in a final volume of 20.0 mL of treatment medium that contained 5 % heat inactivated fetal bovine serum. The dosed tubes were closed, vortexed and placed on a roller drum at approx. 37 degree C at 10 - 15 rpm for an exposure period of 3 hours. The cells were washed and resuspended in growth medium. Cell densities were adjusted to 2 x 10^5/mL and the cells were plated for survival and incubated for the expression period in parallel, i.e. an aliquot of the cells was diluted to 8 cells/mL and 0.2 mL of each culture were placed in two 96 well microtiter plates (192 wells, averaging 1.6 cells/well) and incubated for 1 week at 37 ± 0.4 degree C whereas the rest of the cells was incubated for 2 days at 37 ± 0.4 degree C for the expression period. The cells for the plating of survival were counted after 1 week and the number of viable clones was recorded. The cells in the expression period were maintained below 10^6 cells per mL and a minimum of 4 concentration levels plus positive and negative control was selected for 5-trifluoro-thymidine (TFT) resistance. At the end of the expression period, the selected cultures were diluted to 1 x 10^4 cells/mL and plated for survival and TFT resistance in parallel (plating efficiency step 2). The plating for survival was similar to the above described method. For the plating for TFT resistance, 3 µg/mL TFT (final concentration) were added to the cultures and 0.2 mL of each suspensions placed into four 96-well microtiter plates (384 wells, averaging 2 x 10^3 cells/well). The plates were incubated for 12 days at 37 ± 0.4 degree C and wells containing clones were identified microscopically and counted. In addition, the number of large and small colonies was recorded with an automated colony counter that can detect colony diameters equal or greater than 0.2 to 0.3 mm. Large colonies are defined as >= 1/3 and small colonies < 1/3 of the well diameter of 6 mm.

  Assay with metabolic activation:

  The activation assay is often run concurrently with the non-activation assay; however, it is an independent assay performed with its own set of solvent and positive controls. In this assay, the above-described activation system was added to the cells together with test item.

Test substance

- The test material used in the study is lithium hydroxide monohydrate
- EC number: 215-183-4

- CAS number: 1310-65-2
- Degree of purity: 57.8 wt% LiOH
- Impurities do not affect the classification
- Batch number: [confidential information]

Administration/exposure

- The indicator cell used for this study was the L5178Y mouse lymphoma cell line that is heterozygous at the TK locus (+/-). The particular clone (3.7.2C) used in this assay is isolated by Dr. Donald Clive (Burroughs Wellcome Company, Research Triangle Park, NC).
- Target gene: Thymidine kinase (TK)
- Type and composition of metabolic activation system: liver post-mitochondrial fraction (S9 mix) from Aroclor 1254-induced rats.
- The test was carried out employing 2 exposure times without S9 mix: 3 and 24 hours, and one exposure time with S9 mix: 3 hours; this experiment with S9 mix was carried out twice.

  In the preliminary experiment without and with metabolic activation, concentrations tested were 0, 0.25, 1, 2.5, 10, 25, 100 and 200 µg/mL. Cytotoxicity (decreased survival) was noted at the top concentration of 200 µg/mL. Hence, in the experiments without or with metabolic activation the concentrations of 0, 12.5, 25, 50 100 and 200 µg/mL were used. In the main study, cytotoxicity (decreased survival) was noted immediately after treatment (plating efficiency step 1) and in the following plating for 5-trifluoro-thymidine (TFT) resistance (plating efficiency step 2) in the presence and absence of metabolic activation at the top concentration of 200 µg/mL.
- Vehicle: Aqua ad iniectabilia
- Statistical methods: no data
- Evaluation criteria:

  The minimum criterion considered necessary to demonstrate mutagenesis for any given treatment was a mutant frequency that was >= 2 times the concurrent background mutant frequency. The observation of a mutant frequency that meets the minimum criterion for a single treated culture within a range of assayed concentrations was not sufficient evidence to evaluate a test item as a mutagen. A concentration-related or toxicity-related increase in mutant frequency should be observed. The ratio of small: large colonies will be calculated from the results of the determination of small to large colonies. If the test item is positive, the ratio of small to large colonies for the test item will be compared with the corresponding ratios of the positive and negative controls. Based on this comparison, the type of the mutagenic properties (i.e. basepair substitutions, deletions or large genetic changes frequently visible as chromosomal aberrations) of the test item will be discussed. A test item is evaluated as non-mutagenic in a single assay only if the minimum increase in mutant frequency is not observed for a range of applied concentrations that extends to toxicity causing 10%

to 20% relative growth or a range of applied concentrations extending to at least twice the solubility limit in culture media.

Results and discussion

- INFORMATION ON CYTOTOXICITY:

  In the main study, cytotoxicity (decreased survival) was noted immediately after treatment (plating efficiency step 1) and in the following plating for 5-trifluoro-thymidine (TFT) resistance (plating efficiency step 2) in the presence and absence of metabolic activation at the top concentration of 200 µg/mL. Cytotoxicity is defined as a reduction in the number of colonies by more than 50 % compared with the negative control. Exposure to the test item at the concentration of 200 µg/mL in the absence of metabolic activation resulted in relative survival of 28 % and 34 % (plating efficiency step 1) and 20 % and 33 % (plating efficiency step 2), and in the presence of metabolic activation of 28 % and 26 % (plating efficiency step 1) and 23 % and 17 % (plating efficiency step 2). Therefore, the test item was considered cytotoxic at the top concentration of 200 µg/mL. No relevant change in pH and osmolality was noted.

  The limit of solubility was about 34 mg/mL.

  In the preliminary experiment without and with metabolic activation, concentrations tested were 0, 0.25, 1, 2.5, 10, 25, 100 and 200 µg/mL. Cytotoxicity (decreased survival) was noted at the top concentration of 200 µg/mL. Hence, in the experiments without or with metabolic activation the concentrations of 0, 12.5, 25, 50 100 and 200 µg/mL were used. In the main study, cytotoxicity (decreased survival) was noted immediately after treatment (plating efficiency step 1) and in the following plating for 5-trifluoro-thymidine (TFT) resistance (plating efficiency step 2) in the presence and absence of metabolic activation at the top concentration of 200 µg/mL.

  The mean values of mutation frequencies of the negative controls ranged from 61.61 to 98.34 per 10^6 clonable cells in the experiments without metabolic activation, and from 68.23 to 82.61 per 10^6 clonable cells in the experiments with metabolic activation and, hence, were well within the historical data-range. The mutation frequencies of the cultures treated with Lithium hydroxide monohydrate ranged from 64.74 to 92.63 per 10^6 clonable cells (3 hours exposure) and 50.42 to 92.34 per 10^6 clonable cells (24 hours exposure) in the experiments without metabolic activation and 75.88 to 105.59 per 10^6 clonable cells (3 hours exposure, first assay) and 45.04 to 99.10 per 10^6 clonable cells (3 hours exposure, second assay) in the experiments with metabolic activation. These results were within the range of the negative control values and, hence, no mutagenicity was observed according to the criteria for assay evaluation.

  Under the present test conditions, Lithium hydroxide monohydrate, tested up to a pronounced cytotoxic concentration in the absence and presence of metabolic activation in two independent

experiments, was negative with respect to the mutant frequency in the L5178Y TK +/- mammalian cell mutagenicity test.

3.1.1.4 Pastor et al., 2009

Study reference:

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

Detailed study summary and results:

Test type

1. Micronucleus assay
    - Doses based on the results on cytotoxicity for both lithium salts
        - Li2CO3: 2.2–10 mM
        - LiCl: 5–20 mM
    - Untreated controls were only treated with cytochalasin B for comparison. DMSO was used as negative control.
    - Recovery of the cells from treatment was for 22–24 h, to proceed through mitosis without cytokinesis and show up in the next interphase as binucleated cells. Then cells were harvested by 5 min trypsinization, and then the cell suspensions were centrifuged at 144 g for 6 min at room temperature. Supernatant was discarded and the cell pellet gently resuspended in methanol: acetic acid (3:1) fixative before a new centrifugation. This latter process was repeated, and the fixed cells were dropped on clean glass microscope slides. Finally, dried slides were stained with 3% Giemsa in Sörensen phosphate buffer (pH 6.8) and mounted in DPX (Gurr, England) synthetic resin. Two thousand binucleated cells from both control and lithium-treated preparations were scored blind, and classified as normal or micronucleated cells. All the experiments were carried out in triplicate. A one-tailed Student’s t-test was used to determine if the number of micronuclei observed in cells treated with either Li2CO3 or LiCl was significantly higher than that found in the untreated cells (control cells). For kinetochore labelling in fixed cells, slides were placed for 5 min in PBS, with 0.1% Tween 20, and the excess fluid was drained. The slides were then incubated in a humidified incubator for 1 h at 37°C with CREST sera (Anti-kinetochore serum was purchased from Antibodies Incorporated, Davis, CA, USA) diluted 1:1 in PBS, 0.1% Tween 20 and 1% bovine serum albumin. Upon careful removal of the coverslip, slides were rinsed twice with PBS-0.1% Tween 20 and excess fluid was drained before addition of the

secondary antibody [FITC-labelled goat anti-human IgG from Sigma (diluted 1:100 with PBS with 0.1% Tween 20 and 1% bovine serum albumin)]. Upon incubation for 1 h and another round of rinse and drain, 40 ll of propidium iodide (PI) solution (0.1 lg/ml) in antifade buffer was added onto the slides kept in a dark box at 4°C.

- The coded slides were examined for micronuclei using a fluorescence microscope OLYMPUS Vanox AHBT3 with 600-fold and 1000-fold magnification.

2. Chromosome aberration assay
    - Doses based on the results on cytotoxicity for both lithium salts
        - Li2CO3: 2.2–10 mM
        - LiCl: 5–20 mM
    - Treatment for 3h
    - Cultures that did not receive any treatment served as negative controls. As positive control, cells were treated with the topoisomerase I inhibitor (poison) camptothecin (CPT); 20 µM final concentration.
    - After the treatment period, the cultures were washed and maintained in fresh medium for 12h to allow the cells to recover and reach mitosis. Finally, Colcemid (0.2 mM) was added for 2.5 h to the cell culture to arrest metaphases. The flasks were gently shaken to dislodge the mitotic cells, which were collected by centrifugation as described above, treated with 0.075 M KCl for 1.5 min (hypotonic treatment), fixed in 3:1 methanol:acetic acid, and dropped onto clean glass microscope slides. The slides were stained with 3% Giemsa in Sörensen's buffer (pH 6.8), and mounted in DPX. Two hundred metaphases per concentration were examined.
    - All the experiments were carried out in triplicate.
3. Comet assay
    - Doses based on the results on cytotoxicity for both lithium salts
        - Li2CO3: 2.2–10 mM
        - LiCl: 5–20 mM
    - Treatment for 3h or 24h
    - Comet assay was basically performed according to the original protocols of Singh et al. (1988) with some modifications
    - Positive controls of both cell lines were obtained after irradiation of exponentially growing cells with 5 Gy X-rays using an X-ray machine (Philips MG 103/2.25 system, Germany, 100 KVp, 15 mA, dose rate 1 Gy/min). To determine the initial DNA damage cells were irradiated on ice.

- The slides were examined at 200x magnification using a 20x objective on a fluorescent microscope OLYMPUS Vanox AHBT3, excitation filter at 550 nm and barrier filter at 590 nm.

4. Anaphase anomaly
    - Doses based on the results on cytotoxicity for both lithium salts
        - Li2CO3: 2.2–10 mM
        - LiCl: 5–20 mM
    - Treatment for 3h
    - After the treatment period, the cultures were washed and maintained in fresh medium for 6 h
    - to recover. Cells were subsequently harvested by 5 min trypsinization, and then the cell suspensions were centrifuged at 144 g for 6 min at room temperature. Supernatant was then discarded and the cell pellet directly resuspended with fixation mix (without any hypotonic treatment). Fixed cells were dropped on clean glass microscope slides, Giemsa stained and mounted.
    - The cells in anaphase were finally analysed for any alterations of normal chromosome segregation such as multipolar anaphases or lagging chromosomes.

Test substance

- lithium carbonate (Li2CO3) or lithium chloride (LiCl)
- Degree of purity: no information
- Batch number: no information

Administration/exposure

- Ovary fibroblast Chinese hamster cell line AA8
- No metabolic activation system
- Statistical methods: For the determination of significance of the difference between the means, Student's t-test was used. Statistical treatment and plotting of the results were performed using the Sigma Plot and MS Excel for Windows XP software. The results from three independent experiments are presented as mean ± standard deviation of the mean. Differences were considered to be significant when $p < 0.01$.

Results and discussion

1. Cytotoxicity
    - The AA8 cell line when cells were treated with a range of concentrations (1–30 mM) of either Li2CO3 or LiCl. As can be seen, the Sulforhodamine B (SRB) assay provided results that clearly indicate a dose-dependent cytotoxic effect of both Li2CO3 and LiCl in AA8 cell line, but for any of the concentrations tested Li2CO3 appears to have a higher cytotoxicity than LiCl. While Li2CO3 showed as cytotoxic in the SRB assay at the lower concentrations

(1–5 mM; LD50: 5 mM), the negative effect for LiCl was only observed at higher concentrations (6 mM and higher; LD50: 15 mM).

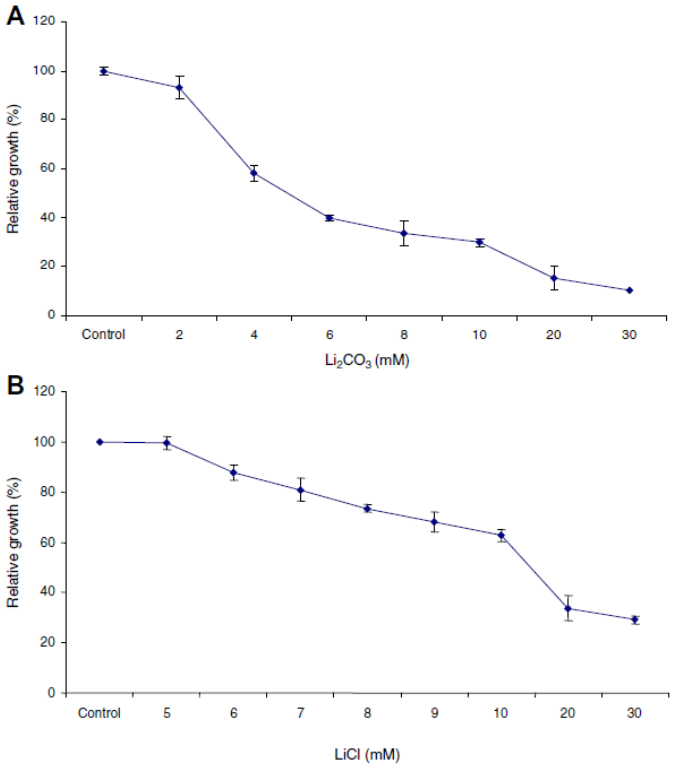


Fig. 1. Effectiveness of different concentrations of lithium salts to inhibit cell growth in the Chinese hamster fibroblast cell line AA8 as shown by the SRB assay. (A) Li_2CO_3. (B) LiCl. While Li_2CO_3 showed as cytotoxic at the lower concentrations (1–5 mM; LD_{50}: 5 mM), the negative effect for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

2. Micronucleus assay

- both lithium salts induced micronuclei in a dose-dependent fashion but again, in agreement with the results on cytotoxicity, Li2CO3 was more efficient than LiCl to induce micronuclei at any of the doses tested (Fig. 2A and B). It has been reported that micronuclei can be caused by osmolarity (Henderson et al., 2000; Fenech et al., 2003; Meintières and Marzin, 2004). In order to rule out that the observed effects of Li solutions as seen by the micronucleus assay might be caused by an in specific osmolarity effect, an assay using equimolar concentrations of NaCl was performed, with negative results (not shown). Most of the micronuclei (over 95 percent) were kinetochore-positive, which seems to point to an aneuploid effect of both Li2CO3 and LiCl rather than a clastogenic (chromosome breakage) effect.

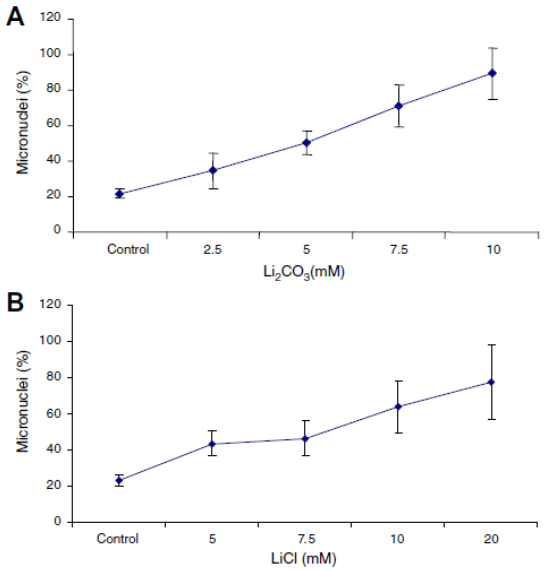


Fig. 2. Dose-dependent induction of micronuclei by both Li_2CO_3 (A), and LiCl (B) in AA8 CHO cells. All the doses tested showed a significant difference in the frequency of micronuclei as compared with control ($p < 0.01$; Student's *t*-test). As can be observed, Li_2CO_3 induced micronuclei with higher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

3. Chromosome aberration assay
    - Chromosome aberration was not increased in lithium treated cells as compared with untreated controls. As positive control, cells treated with the topoisomerase I inhibitor (poison) camptothecin (CPT) showed a high frequency of chromosome aberrations, mainly of the exchange type.
4. Comet assay
    - As to the possible induction of DNA breakage by lithium salts, the alkaline SCGE or ‘‘Comet assay”, which provides a measure of both single- and double-strand breaks in DNA as well as alkali-labile sites, was the method of choice. AA8 cells treated with Li2CO3 or LiCl for either 3, or 24 h showed that the treatment was ineffective in the induction of DNA damage, with no increase in tail moment as compared with controls untreated with lithium salts. H2O2 showed as very effective in the induction of DNA strand breaks in AA8 cells (positive control).
5. Anaphase anomaly
    - An interesting observation was the increased frequency of anomalous anaphases in the cells treated with lithium salts, as compared with controls. Multipolar anaphases (mostly tripolar) and lagging chromosomes, as a result of failure in normal chromosome segregation, were observed as a result of treatment with either Li2CO3 or LiCl. On the other hand, no increase of endoreduplication frequency (metaphases showing diplochromosomes) above the very low rate characteristic of control AA8 CHO fibroblasts was observed for the dose range of lithium salts analysed.

3.1.1.5 Anonymous, 2000b

Study reference:

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

See also confidential Annex I for confidential information

Detailed study summary and results:

Test type

- OECD Guideline 473 (In Vitro Mammalian Chromosome Aberration Test, version from July 1997), GLP compliance is given
- Test concentrations with justification for top dose:
  Dose range finding test: 0, 10, 33, 100, 133, 1000 µg/mL with and without S9-mix
  Chromosome aberrations: Without S9-mix: 0, 275, 300, and 530 µg lithium hydroxide/mL culture medium (24 h treatment, 24 h fixation time); 350, 375 and 400 µg lithium hydroxide/mL culture medium (48 h treatment, 48 h fixation time). With S9-mix: 400, 425 and 450 µg lithium hydroxide/mL culture medium (3 h treatment, 48 h fixation time)
- Positive control substances: mitomycin C (without S9-mix), cyclophosphamide (with S9-mix)
- **Chromosome preparation:** During the last 3 h of the culture period, cell division was arrested by the addition of the spindle inhibitor colchicine (0.5 µg/mL medium). Thereafter the cell cultures were centrifuged for 5 min at 1300 rpm (150 g) and the supernatant was removed. Cells in the remaining cell pellet were swollen by a 5 min treatment with hypotonic 0.56 % (w/v) potassium chloride solution at 37 °C. After hypotonic treatment, cells were fixed with 3 changes of methanol:acetic acid fixative (3:1 v/v).
- **Preparation of slides:** Fixed cells were dropped onto cleaned slides which were immersed for 24 h in a 1:1 mixture of 96 % (v/v) ethanol/ether and cleaned with a tissue. The slides were marked with the study identification number and group number. Two slides were prepared per culture. Slides were allowed to dry and thereafter stained for 10 - 30 min with 5 % (v/v) Giemsa solution in tap water. Thereafter the slides were rinsed in tap-water and allowed to dry. The dry slides were cleared by dipping them in xylene before they were embedded in MicroMount and mounted with a coverslip.
- **Mitotic index/dose selection for scoring the cytogenetic assay:** The mitotic index of each culture was determined by counting the number of metaphases per 1000 cells. At least three analysable concentrations were used. Chromosomes of metaphase spreads were analysed of those cultures with an inhibition of the mitotic index of about 50 % or greater whereas the mitotic index of the lowest

dose level was approximately the same as the mitotic index of the solvent control. Also cultures treated with an intermediate dose were examined for chromosome aberrations.

- **Analysis of slides for chromosome aberrations:** To prevent bias, all slides were randomly coded before examination of chromosome aberrations and scored. An adhesive label with study identification number and code was stuck over the marked slide. At least 100 metaphase chromosome spreads per culture were examined by light microscopy for chromosome aberrations. In case the number of aberrant cells, gaps excluded, was >= 25 in 50 metaphases no more metaphases were examined. Only metaphases containing 46 chromosomes were analysed. The number of cells with aberrations and the number of aberrations were calculated.

Test substance

- The test material used in the study is lithium hydroxide
- EC number: 35-287-3
- CAS number: 1310-65-2
- Degree of purity: 92.9 %
- Impurities do not affect the classification
- Batch number: [confidential information]

Administration/exposure

- Human lymphocytes from blood samples from healthy male donor
- Type and composition of metabolic activation system: S9 mix of Aroclor 1254 induced rat liver
- Details on test system and conditions:

  Cytogenetic assay: Lithium hydroxide was tested in the absence and presence of 1.8 % (v/v) S9-fraction in duplicate in two independent experiments.

  Experiment 1: Lymphocyte cultures (0.4 mL blood of a healthy male donor was added to 5 mL or 4.8 mL culture medium, without and with metabolic activation respectively and 0.1 mL (9 mg/mL) Phytohaemagglutinin) were cultured for 48 h and thereafter exposed in duplicate to selected doses of Lithium Hydroxide for 3 h in the absence and presence of S9-mix. After 3 h treatment, the cells exposed to Lithium Hydroxide were rinsed once with 5 mL of HBSS and incubated in 5 mL of culture medium for another 20-22 h (24 h fixation time).

  Experiment 2: Lymphocyte cultures (0.4 mL blood of a healthy male donor was added to 5 mL or 4.8 mL culture medium, without and with metabolic activation respectively and 0.1 mL (9 mg/mL) Phytohaemagglutinin) were cultured for 48 h and thereafter exposed in duplicate to selected doses of Lithium Hydroxide for 3 h in the absence and presence of S9-mix. After 3 h treatment, the cells exposed to Lithium Hydroxide in the presence of S9-mix were rinsed once with 5 mL of HBSS and incubated in 5 mL of culture medium for another 44-46 h (48 h fixation time). The cells which were

treated for 24 and 48 h in the absence of S9-mix were not rinsed after treatment but were worked up immediately after 24 h and 48 h (24 h and 48 h fixation time).

- Vehicle: DMSO
- Evaluation criteria:

  A test substance was considered positive (clastogenic) in the chromosome aberration test if:

  a) It induced a dose-related statistically significant (Chi-square test, $P < 0.05$) increase in the number of cells with chromosome aberrations.

  b) a statistically significant increase in the frequencies of the number of cells with chromosome aberrations was observed in the absence of a clear dose-response relationship. A test substance was considered negative (not clastogenic) in the chromosome aberration test if none of the tested concentrations induced a statistically significant (Chi-square test, $P < 0.05$) increase in the number of cells with chromosome aberrations. The preceding criteria were not absolute and other modifying factors might enter into the final evaluation decision.
- Statistical methods:

  The incidence of aberrant cells (cells with one or more chromosome aberrations, inclusive or exclusive gaps) for each treatment group was compared to that of the solvent control using Chi-square statistics: X*2 = (N-1)x(ad-bc)*2/(a+b)(c+d)(a+c)(b+d) where b = the total number of aberrant cells in the control cultures, d = the total number of non-aberrant cells in the control cultures, n0 = the total number of cells scored in the control cultures, a = the total number of aberrant cells in treated cultures to be compared with the control, c = the total number of non-aberrant cells in treated cultures to be compared with the control, n1 = the total number of cells scored in the treated cultures, N = sum of n= and n1 If P [X*2 > (N-)x(ad-bc)*2/(a+b)(c+d)(a+c)(b+d)] (two-tailed) is small ($P < 0.05$) the hypothesis that the incidence of cells with chromosome aberrations is the same for both the treated and the solvent group is rejected and the number of aberrant cells in the test group is considered to be significantly different from the control group at the 95 % confidence level.

Results and discussion

- **Dose range finding test:**

  Lithium hydroxide precipitated in the culture medium at a concentration of 1000 µg/mL, therefore a concentration of 1000 µg/mL was used as the highest concentration of lithium hydroxide. In the dose range finding test, blood cultures were treated with 0, 10, 33, 100, 333 and 1000 µg Lithium Hydroxide per mL culture medium with and without S9-mix. The pH of a concentration of 1000 mg lithium hydroxide/mL was 11.83 (compared to 8.15 in the solvent control).

  Cytogenetic assay:

  Based on the results of the dose range finding test the following dose levels were selected for the cytogenetic assay:

Experiment 1A: Without S9-mix: 0, 100, 180, 333, 420 and 560 µg lithium hydroxide/mL culture medium (3 h treatment time, 24 h fixation time) With S9-mix: 0, 100, 333, 420 and 560 µg lithium hydroxide/mL culture medium (3h treatment, 24 h fixation time); Lithium hydroxide precipitated in the culture medium at a concentration of 560 µg/mL, therefore a concentration of 560 µg/mL was used as the highest concentration of lithium hydroxide in the first cytogenetic assay. Since the highest dose level of 560 µg lithium hydroxide/mL was too cytotoxic to the cells (mitotic index of 21 % both in the absence and in the presence of S9-mix) and no dose level resulting in a mitotic index of 50 % could be selected in both the absence and presence of S9-mix, an additional experiment was performed with the following dose levels:

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); Because of the high cytotoxicity in cultures treated with 350 µg/mL lithium hydroxide and upwards in the presence and absence of S9-mix, the test was not used for evaluation but a third experiment was performed.

Experiment 1C: With and without S9-mix: 0, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 µg lithium hydroxide/mL culture medium (3 h treatment, 24 h fixation time). Despite the narrow concentration range used, the mitotic index of cultures treated with 375 and 400 µg/mL lithium hydroxide (without S9-mix) drastically decreased from 128 % to 0 %. In the presence of S9-mix, cytotoxicity was observed at a concentration of 375 µg/mL lithium hydroxide and upwards. The pH of the concentrations 275, 300, 325, 350, 375, 400 and 425 µg/mL was 9.61, 9.69, 9.66, 9.68, 9.66, 9.80 and 10.19, respectively. Possibly these high pH values also play a role in the cytotoxicity of lithium hydroxide. Since it was not possible to determine a concentration which caused the appropriate 50 % inhibition of the mitotic index, the following doses were selected for scoring of chromosome aberrations:

From experiment 1A: With and without S9-mix: 333, 420 and 560 µg lithium hydroxide/mL (3 h treatment, 24 h fixation time); From experiment 1C: Without S9-mix: 325, 350, and 375 µg lithium hydroxide/mL culture medium (3 h treatment time, 24 h fixation time); With S9-mix: 325, 350, 375, and 400 µg lithium hydroxide/mL culture medium (3 h treatment, 24 h fixation time); For cultures with S9-mix four doses were selected, since only one of the duplicate cultures contained scorable metaphases at concentrations of 375 and 400 µg/mL lithium hydroxide. Based on the results of the dose range finding test and experiments 1A, 1B and 1C the following dose levels were selected to perform an independent repeat:

Experiment 2: Without S9-mix: 275, 300, 325, 350, 375, 400 and 425 µg lithium hydroxide/mL culture medium (24 and 48 h treatment time, 24 and 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). Based on these observations the following doses were selected for scoring of chromosome aberrations: Without S9-mix: 275, 300 and 350 µg lithium hydroxide/mL culture medium (24 h treatment time, 24 h fixation time); 350, 375 and 400 µg lithium hydroxide/mL

culture medium (48 h treatment time, 48 h fixation time), with S9-mix: 400, 425 and 450 µg lithium hydroxide/mL culture medium (3 h treatment time, 48 h fixation time).

Evaluation of the results: The ability of lithium hydroxide to introduce chromosome aberrations in human peripheral lymphocytes was investigated. The test was carried out in duplicate in three independent experiments. The number of cells with chromosome aberrations found in the solvent control cultures were within the laboratory historical control data range [min = 0, max = 5 (mean = 0.8, standard deviation = 1.0) aberrant cells per 100 metaphases in the absence of S9-mix; gaps excluded and min = 0 max = 5 (mean = 0.8, standard deviation = 0.9) aberrant cells per 100 metaphases in the presence of S9-mix, gaps excluded]. The positive control chemicals (MMC-C and CP) both produced statistically significant increases in the frequency of aberrant cells. It was therefore concluded that the test conditions were adequate and that the metabolic activation system (S9-mix) functioned properly.

Experiments 1A and 1C: Due to the steepness of the dose response curve for cytotoxicity of lithium hydroxide it was not possible to determine the number of chromosomal aberrations at a mitotic index of 50 %. Therefore, chromosome aberrations were scored from two independent experiments (experiment 1A and 1C) at different concentrations. As a result of extreme cytotoxicity, only 102 and 103 metaphases could be scored in the absence and presence of S9-mix, respectively, in experiment 1A at a concentration of 560 µg/mL lithium hydroxide. At the other concentrations tested, 200 metaphases were scored per concentration. In experiment 1C in the presence of S9-mix at the highest concentrations of 375 and 400 µg/mL only one of the two duplicate cultures could be scored due to extreme cytotoxicity. Both in the absence and presence of S9-mix lithium hydroxide did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations in both experiments 1A and 1C.

Experiment 2: In the absence of S9-mix, at the 24 hours continuous treatment time, lithium hydroxide induced statistically significant increases in the number of cells with chromosome aberrations at the lowest tested concentration of 275 µg/mL (only when gaps were included) and at the highest cytotoxic concentration of 350 µg/mL both when gaps were included and excluded. At the intermediate concentration of 300 µg/mL lithium hydroxide did not induce a statistically significant increase in the number of cells with chromosome aberrations. Since the increase of chromosome aberrations at 275 µg/mL was observed only when gaps were included and furthermore the increase was within the historical control data range the increase was not considered biologically relevant. Scoring of the additional 200 metaphases at the concentration of 350 µg/mL lithium hydroxide verified the statistically significant increase. However, the observed increase within or just on the border of our historical control data range (min = 0, max = 5 aberrant cells per 100 metaphases, gaps excluded), and is observed at a very toxic concentration. In addition, higher concentrations tested at the prolonged treatment time of 48 hours in the absence of metabolic activation did not induce significant increases in the number of cells with chromosome aberrations.

Furthermore, the irregular toxicity profile and the non-physiological test conditions (pH > 9) may be considered cofounding factors. Therefore, the observed increase in the number of aberrant cells at the concentration of 350 µg/mL is considered not biologically relevant. At the continuous treatment time of 48 hours exposure of cells to 350, 375 or 400 µg/mL lithium hydroxide did not induce a significant increase in the number of cells with chromosome aberrations. In the presence of S9-mix, lithium hydroxide did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations.

Conclusion: Finally, it is concluded that this test is to be considered valid and that lithium hydroxide is not clastogenic under the experimental conditions of this test.

3.1.1.6 Slameňová et al., 1986

Study reference:

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

Detailed study summary and results:

Test type

1. Gene mutation assay (HGRPT)
    - The assay for the detection of 6-thioguanine-resistant (6-TG r) mutations was carried out as described previously (Slamenova and Gabelova, 1980; Slamenova et al., 1984).
    - Treatment for 60 mins
2. DNA strand breaks
    - Method described by Kohn (Kohn et al., 1981, Procedure A). In preliminary experiments, a comparison was made between the original method and modified method and the results were very satisfactory
    - Treatment for 60 mins

Test substance

- Lithium carbonate
- Degree of purity: no information

Administration/exposure

- V79 Chinese hamster and heterodiploid human EUE cells were used. Their origin and culture conditions have been described previously
- Type and composition of metabolic activation system: For the preparation of S9 fraction, method described by Kuroki (Kuroki et al., 1979) and Carver (Carver et al., 1980) was employed.
- Test concentrations, and reasoning for selection of doses if applicable
    - HGPRT: 1500, 2000, 2500, 3000 µg/ml

- DNA strand breaks: 150, 250, 500 µg/ml

Results and discussion

1. HGPRT
   - in the absence of S9 fraction, Li carb manifested the highest mutagenic effect at a concentration of 2000 ~g/ml (ratio of induced to spontaneous mutations = 4.4).
   - In the presence of the S9 fraction, the mutagenic response manifested by Li carb was lower in the presence of S9 fraction and cofactors than in their absence. It is obvious to speak about the partial detoxification of the drugs by the S9 fraction.

TABLE 1

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

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

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

2. DNA strand breaks
   - Alkaline elution of DNA in cells treated with Li carb is shown in Fig. 6. If part A and part B are compared, it can be seen that the number of single-strand breaks ascertained immediately after treatment and 60 min after Li carb-treatment was similar. Hence breaks induced by Li carb were not repaired during 60 rain of post-Li carb-treatment.
   - The nature of the single-strand breaks induced by Li carb is not known; however, as with single-strand breaks induced by other kinds of chemical compound, they can be induced chemically (Strauss et al., 1968; Lawley and Brooks, 1973), enzymatically (Strauss and Robbins, 1968, Lawley and Orr, 1970; Lindahl and Anderson, 1972; Kirtikar and Goldthwait, 1974), or as a result of alkali treatment (Strauss et al., 1968; Lindahl and Anderson, 1972; Bronk et al., 1973; Lawley and Brooks, 1973; Peterson et al., 1974).

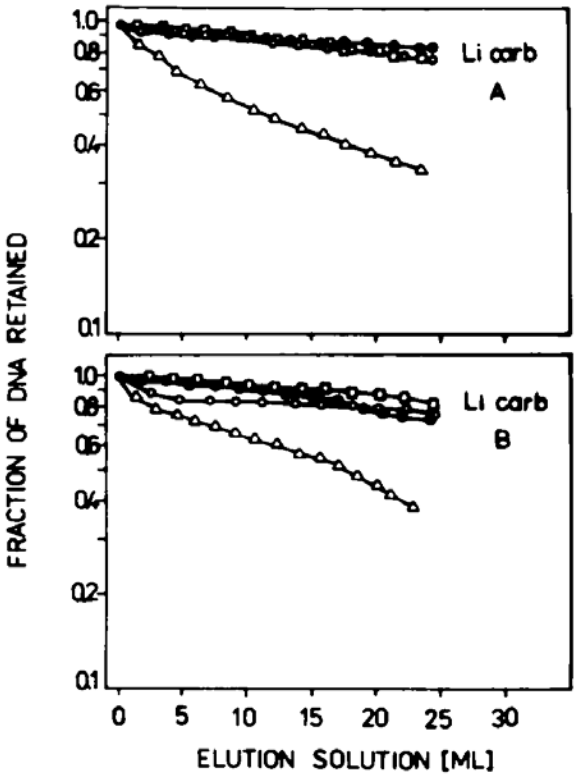


Fig. 6.

3.1.1.7 De La Torre et al., 1976

Study reference:

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

Detailed study summary and results:

Test type/Administration/exposure

- 40 ml of peripheral blood from a healthy donor were divided into 4 cultures, using a modified procedure described by Moorhead et al. ('60): The first culture (group A) was the control; 1 ml of sterile Hank's solution was added to 10 ml of culture media. The second (B), third (C), and fourth (D) cultures contained 10 ml of culture media each, to which 0.5, 1.0, and 1.5 mg of LiCI, dissolved in 0.5, 1.0, and 1.5 ml of sterile Hanks solution, respectively, were added. This procedure was repeated twice for a total of 3 times, using blood from the same donor (sets I, 11, and 111). A total of 866 metaphases were examined in coded slides and scored blind.

Test substance

- Lithium Chloride
- Degree of purity: no information
- Batch number: no information

Results and discussion

- Group A (control, lithium-free) had breaks (1.2%) and gaps (0.8%), and satellite associations that did not exceed 12.6%, or 31 instances (table 1). Chromosomes that appeared to be attached to one another by their satellites were considered to show satellite associations. These associations were double, triple, and quadruple, etc., and each type was scored as 1, 2, and 3 instances of satellite association, respectively. Group B (0.5 mg LiCl) showed an increase in breaks (7.9%) and gaps (14.4%), and satellite associations increased to 25.2%, or 51 instances. Group C (1 mg LiCl) also showed an increase in breaks (4.5%) and gaps (14%), and a low frequency of deletions (2.2%) and translocations (0.6%); satellite associations (31.8%) were consistently increased over the control and group B. Group D showed a definite increase in breaks (10.9%), gaps (20.5%), deletions (4.2%), and translocations (1.7%) ; satellite associations increased to 31%, or 74 instances

3.2 Carcinogenicity

3.2.1 Animal data

3.2.1.1 Ziche et al., 1980

Study reference:

Ziche, M.; Maiorana, A.; Oka, T.; Gullino, P.M. (1980). Influence of lithium on mammary tumor growth in vivo. Cancer Letters, 9, 219-224

Detailed study summary and results:

Test type/ Test substance/ Test animals/Administration/exposure

Six hundred Sprague-Dawley females, 50-days-old, received 20 mg DMBA suspended in 1 ml paraffin oil, by stomach gavage. Mammary tumors appeared in 480 animals within 120 days; these animals were used for other purposes. The remaining 120 rats not developing mammary tumors were randomized into 5 groups as follows: group 1 received deionized water alone as drinking fluid; groups 2 and 3 received deionized water to which lithium carbonate was added at concentrations of 0.01 M and 0.001 M, respectively; and groups 4 and 5 received sodium carbonate alone at concentrations of 0.01 M and 0.001 M, respectively.

One hundred and forty five Buffalo/N females, 50-days-old, received 1 i.v. injection of NMU in 1 ml distilled water at a dose of 10 mg/200 g rat. These animals were randomized into 7 groups as follows: group 1 received deionized water alone; groups 2, 3 and 4 received lithium carbonate dissolved in deionized water at concentrations of 0.01 M, 0.001 M and 0.0005 M, respectively; and groups 5, 6 and 7 received sodium carbonate alone at concentrations of 0.01 M, 0.001 M and 0.0005 M, respectively.

In all animals treated with DMBA or NMU, the appearance of tumors was recorded for 3 months following initiation of lithium treatment per OS. Twenty Buffalo/N females, each bearing 1 mammary tumor induced by 3 successive NMU injections, were selected when the tumors were about 1 g in size. For each tumor, measurements of 2 diameters were made every 3 days for 2 weeks, and the increment of volume over time was plotted. The formula $4/3\pi^3$ was utilized to calculate the change in volume (r was obtained as the mean of 2 perpendicular diameters that were always measured in the same position for each tumor). At the end of 2 weeks, all animals were castrated and randomized into 4 groups of 5 animals each. Two groups received as drinking fluid lithium carbonate dissolved in deionized water at concentrations of 0.01 M and 0.02 M, respectively, and 2 groups received sodium carbonate alone at the same concentrations. The growth curve of each tumor was plotted for 2 months following castration of the animals.

Results and discussion

- First experiment

NMU was injected i.v. at a dose previously known to induce mammary carcinomas in about one-half of the animals, and a comparison of tumor frequency was made between rats given either lithium or sodium dissolved in deionized water or deionized water alone. At lithium doses of 0.01 M, the animals consumed a few ml of solution per day and lost weight rapidly. They were sacrificed during the second week of treatment and discarded. The animals given water with lithium at concentrations of 0.001 M and 0.0005 M consumed fluid and increased in weight as did the controls. There was no indication that lithium enhanced tumor incidence over a period of 3 months after the treatment was initiated.

- Second experiment

Lithium treatment was applied to rats that received DMBA but failed to show tumors at a time when the majority of their counterparts had developed mammary carcinomas. In such animals that are partially refractory to DMBA treatment, mammary tumors appear later in time with a relatively low frequency, a limited growth rate, and the macroscopic and microscopic appearances of fibroadenomas. Again, lithium treatment failed to induce any acceleration in the appearance of these tumors during the interval between the fourth and seventh months following DMBA treatment.

- Third experiment

The possibility that lithium could favor the regrowth of hormone dependent tumors was tested by comparing the regrowth curves of NMU tumors in which ovariectomy had induced regression. The regrowth curves of lithium carbonate and sodium carbonate treated animals failed to reveal any consistent difference and were similar to those previously reported for animals drinking standard tap water. Castrated animals of this group were able to thrive on doses of 0.01 M and 0.02 M lithium carbonate in contrast to the animals of the preceding experiments.

3.2.2 Human data

3.2.2.1 Martinsson et al., 2016

Study reference:

Martinsson, L.; Westman, J.; Hallgren, J.; Osby, U.; Backlund, L. (2016). Lithium treatment and cancer incidence in bipolar disorder. Bipolar Disorders, 18, 33-40

Detailed study summary and results:

Test type

- Material and methods

The Swedish National Patient Register (NPR) contains information on all hospital inpatient treatments in Sweden since 1987. For each hospitalization, the patient's unique national registration number, diagnosis, and dates of admission and discharge are registered. The clinical diagnoses are classified according to the World Health Organization (WHO) International Classification of Diseases (ICD). The Swedish Drug Prescription Register (DPR) automatically registers all drug prescriptions that have been dispensed at Swedish pharmacies since 1 July 2005. The Swedish Cancer Register (SCR) covers the entire Swedish population. The register is based on notification of malignant and certain benign tumors. It is generally considered to be of good quality as approximately 99% of the cases are morphologically verified.

Study population and follow-up

From the NPR, 18,660 subjects who had been admitted to the hospital between 1 January 1987 and 30 June 2005 with a main diagnosis of bipolar disorder (BD) were identified. Of the 18,660 patients, 2,245 had previously been diagnosed with schizophrenia and were consequently excluded. Subjects who had died (n = 4,004), emigrated (n = 623), or been diagnosed with cancer before the start of this study (n = 1,271) were identified by cross-checking the SCR, the migration register and the causes of death register, and were then excluded from the study. Cancer incidence is low in early life and young populations below 50 years of age. Based on clinical experience, it is unusual that lithium treatment is introduced in later life. Since the purpose of the study was to investigate the possible effects of long-term lithium usage, we wanted the exposure to lithium treatment to be as long as possible. In total, we had few cases among patients with BD >84 years of age. Thus, when subdividing the data according to type of cancer and five-year age groups, many strata had no cases for the analyses, providing very limited information for the analyses. Therefore, patients younger than 50 years or older than 84 years (n = 4,459) were excluded. Time since first diagnosis of BD in NPR was a mean of 9.4 years and a median of 8.8 years, and the interquartile range was 4.3–15.0 years. By linking individuals to DPR, lithium use among patients could be determined. Lithium exposure was defined as fulfillment of at least one prescription of prescribed lithium per year in DPR during the period from July 2005 to December 2009. Patients were stratified into two exposure groups based on their lithium purchasing habits: 'patients with BD with lithium treatment' fulfilled prescriptions on an annual basis throughout the study period, and 'patients with BD without lithium treatment' were individuals with zero prescription purchases during the study period. Lithium purchasing habits before the start of the study were not considered. Patients with occasional purchases (n = 616) were excluded from the cohort. Thus, the total population of subjects with BD in the study was comprised of 5,442 patients: 2,393 lithium users and 3,049

who did not fulfil lithium prescriptions during the study period. Each person was followed from 1 July 2005 until 31 December 2009, or until the date of death or the date of diagnosis of cancer, depending on which came first, and thus the follow-up period was 4.5 years. Incidence rate ratios (IRRs), adjusted for age and gender, of first cancer and site-specific cancer diagnosis between 1 July 2005 and 31 December 2009 were calculated in patients with BD compared to the general population (GP). Since lithium is known to affect certain organs specifically, cancer incidences in these organs were compared in detail case by case in order to detect any small difference in tendency between cases in the bipolar cohort with and without lithium and the GP.

- Results

As displayed in Table 1, there were 2,593,011 subjects (10,992,446 p-y) in the GP between the ages of 50 and 84 years. The number of patients with BD with lithium treatment was 2,393 (9,940 p-y), whereas the number of patients with BD without lithium treatment was 3,049 (12,167 p-y).

In patients with BD, there were 327 (6.0%) cancer cases compared to 166,443 (6.4%) cases in the GP. In patients with BD with lithium treatment, there were 142 (5.9%) cancer cases compared to 185 (6.0%) in those without lithium treatment.

Table 1. Description of cohort

	Follow-up, n	Person-years	Mean age (SD), years	Median age (SD), years	Cancer cases, n
Men					
BD − Li	1,220	4,819	63.0 (8.7)	62	73
BD + Li	1,024	4,266	61.4 (8.3)	60	64
GP	1,297,986	5,463,530	62.9 (9.1)	61	92,939
Women					
BD − Li	1,829	7,348	64.8 (9.8)	63	112
BD + Li	1,369	5,673	62.7 (9.0)	61	78
GP	1,295,025	5,528,916	64.4 (9.8)	63	73,504
Total					
BD − Li	3,049	12,167	64.1 (9.4)	63	185
BD + Li	2,393	9,940	62.1 (8.7)	60	142
GP	2,593,011	10,992,446	63.6 (9.5)	62	166,443

BD = bipolar disorder; GP = general population; Li = lithium; SD = standard deviation.

As shown in Table 2, there was no difference in risk of overall cancer, neither in patients with BD with lithium treatment compared to the GP (IRR = 1.04, 95% CI: 0.89–1.23) nor in patients with BD without lithium treatment compared to the GP (IRR = 1.03, 95% CI: 0.89–1.19).

The cancer risk was significantly increased in the digestive organs in patients with BD without lithium treatment (IRR = 1.47, 95% CI: 1.12–1.93), but there was no significant increase in patients with BD with lithium compared to the GP (IRR = 1.34, 95% CI: 0.96–1.87). In the respiratory system and the intrathoracic organs (C30–C39), the cancer risk was significantly increased in patients with BD without lithium treatment (IRR = 1.72, 95% CI: 1.11–2.66), but not in patients with BD with lithium compared to the GP (IRR = 1.23, 95% CI: 0.68–2.22). The cancer risk was significantly increased in endocrine glands and related structures (C73–C75) in patients with BD without lithium (IRR = 2.60, 95% CI: 1.24–5.47), but not in patients with BD with lithium (IRR = 1.41, 95% CI: 0.45–4.36) compared to the GP. No differences in risk were found in site-specific cancers in skin, breast, female genital, male genital, urinary, eye brain and nervous system,

malignant neoplasm secondary and ill-defined, lymphoid haematopoietic and related tissue in patients with BD compared to the GP regardless of lithium exposure (Table 2).

When comparing cancer types from these sites case by case, no specific cancer type was substantially over-represented.

Table 2. Cancer incidence in the general population (GP) and in patients with bipolar disorder with and without lithium

Overall cancer	Rate ratio	Confidence interval	p-value	Cancer cases, n
All				
No lithium versus GP (all)	1.03	0.89–1.19	0.72	185
Lithium versus GP	1.04	0.89–1.23	0.60	142
Lithium versus no lithium	1.02	0.82–1.27	0.88	
Men				
No lithium versus GP (men)	0.89	0.71–1.12	0.32	73
Lithium versus GP	0.96	0.75–1.22	0.73	64
Lithium versus no lithium	1.08	0.77–1.51	0.67	
Women				
No lithium versus GP (women)	1.14	0.95–1.37	0.16	112
Lithium versus GP	1.13	0.90–1.41	0.29	78
Lithium versus no lithium	0.99	0.74–1.32	0.94	
Digestive organs (C15–C26)				
No lithium versus GP	1.47	1.12–1.93	0.01	52
Lithium versus GP	1.34	0.96–1.87	0.08	35
Lithium versus no lithium	0.91	0.59–1.40	0.67	
Respiratory system and intrathoracic organs (C30–C39)				
No lithium versus GP	1.72	1.11–2.66	0.02	20
Lithium versus GP	1.23	0.68–2.22	0.50	11
Lithium versus no lithium	0.72	0.34–1.49	0.37	
Skin (C43–C44)				
No lithium versus GP	0.92	0.63–1.35	0.68	26
Lithium versus GP	1.04	0.68–1.59	0.86	21
Lithium versus no lithium	1.13	0.64–2.01	0.68	
Breast (C50)				
No lithium versus GP	1.07	0.73–1.57	0.75	26
Lithium versus GP	1.28	0.86–1.92	0.22	24
Lithium versus no lithium	1.21	0.69–2.10	0.51	
Female genital organs (C51–C58)				
No lithium versus GP	1.07	0.59–1.93	0.82	11
Lithium versus GP	1.39	0.77–2.52	0.27	14
Lithium versus no lithium	1.30	0.56–3.00	0.54	
Urinary organs (C64–C68)				
No lithium versus GP	0.76	0.38–1.52	0.44	8
Lithium versus GP	0.75	0.34–1.68	0.49	6
Lithium versus no lithium	0.99	0.34–2.85	0.98	
Eye, brain and central nervous system (C69–C72)				
No lithium versus GP	1.06	0.40–2.83	0.90	4
Lithium versus GP	0.33	0.05–2.33	0.26	1
Lithium versus no lithium	0.31	0.03–2.76	0.29	
Endocrine glands and related structures (C73–C75)				
No lithium versus GP	2.60	1.24–5.47	0.01	7
Lithium versus GP	1.41	0.45–4.36	0.55	3
Lithium versus no lithium	0.54	0.14–2.09	0.37	
Malignant neoplasms, secondary and ill-defined (C76–C80)				
No lithium versus GP	1.69	0.84–3.38	0.14	8
Lithium versus GP	0.59	0.15–2.35	0.45	2
Lithium versus no lithium	0.35	0.07–1.64	0.18	
Lymphoid, haematopoietic, and related tissue (C81–C96)				
No lithium versus GP	0.48	0.20–1.16	0.10	5
Lithium versus GP	0.51	0.19–1.37	0.18	4
Lithium versus no lithium	1.07	0.29–3.97	0.92	

3.2.2.2 Pottegard et al., 2016b

Study reference:

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

Detailed study summary and results:

Test type

- Methods

The study was conducted within a nationwide case-control population. The use of lithium among persons diagnosed with upper urinary tract cancers (UUTCs) (cases) to that of cancer-free persons (controls) were compared to estimate the OR for UUTCs associated with long-term use of lithium defined as at least 5 years of cumulative exposure.

- Data sources

Five Danish nationwide registries were used: the Danish Cancer Registry, the National Prescription Registry, the National Patient Register, Registers in Statistics Denmark on educational level and income, and the Civil Registration System.

Virtually all medical care in Denmark is furnished by the national health authorities, allowing true population-based register linkage studies covering all inhabitants of Denmark. Data were linked by the personal identification number, a unique identifier assigned to all Danish residents since 1968. All linkages were performed within Statistics Denmark, a governmental institution that collects and processes information for a variety of statistical and scientific purposes.

- Cases and controls

From the Danish Cancer Registry, we identified all individuals in Denmark with a first-time diagnosis of UUTCs, defined as invasive cancer of the kidney, renal pelvis or ureter, between January 1, 2000, and December 31, 2012, using the date of cancer diagnosis as the index date. To ensure the validity of our case material, cases were restricted to histologically verified UUTCs. Exclusion criteria were age outside the range 18–85 years at index date and any residency outside Denmark within 10 years prior to index date, thus ensuring at least 10 years of follow-up for all study subjects. Individuals with a previous history of cancer (except non-melanoma skin cancer), von-Hippel Lindau syndrome, and cystic kidney disease were excluded.

Controls were selected by the use of risk set sampling. For each case, 40 controls were selected among all Danish citizens fulfilling the exclusion criteria for cases and of the same gender and birth year. Controls were assigned an index date identical to that of the corresponding case.

Subjects were eligible for sampling as controls before they became cases. Thereby, the calculated ORs are unbiased estimates of the incidence rate ratios that would have emerged from a cohort study in the source population.

- Exposure definition

The primary exposure was use of lithium. Ever use of lithium was defined as having filled two or more prescriptions of lithium prior to the index date. Long-term use of lithium was defined as ≥5 years of treatment prior to the index date.

The duration of each prescription required for estimation of cumulative exposure duration is not recorded in the Prescription Registry. To overcome this limitation, a method based on the waiting time distribution was

used, providing an estimate of the average duration of each lithium prescription of 64 days. Thus, was considered an individual exposed from the date of filling a prescription for lithium and 64 days onward.
In all exposure calculations, prescriptions redeemed within 12 months prior to the index date was disregarded. Such recent exposure is unlikely to be associated with cancer development, and moreover, drug use has been shown to increase up to 12 months prior to a cancer diagnosis, raising the possibility of reverse causation bias.

- Main analysis

The analysis followed a conventional matched case-control approach using conditional logistic regression. In the main analysis, UUTCs (i.e., cancers of the renal parenchyma, renal pelvis or ureter) were considered as a composite end-point and estimated ORs for UUTCs associated with long-term use of lithium. In all analyses, use of lithium was compared with non-use (<2 prescriptions) of lithium.
Any confounding effect from age, gender and calendar time was handled via the matching procedure. Further, the following potential confounders were identified and incorporated in the logistic regression: (1) Use of drugs known or suspected to modify renal function or risk of UUTC, including low-dose aspirin and non-aspirin NSAIDs, paracetamol, statins, thiazides, beta-blockers, vascular calcium channel blockers, inhibitors of the renin-angiotensin system, and loop diuretics. (2) Prior diagnoses of diseases known or suspected to modify renal function or risk of renal or other cancers, including hypertension, type 1 or type 2 diabetes, chronic obstructive pulmonary disease, alcohol-related disease, and moderate to severe renal disease. (3) Highest achieved education (as a crude measure of socioeconomic status). As in the assessment of drug exposure, the period 12 months prior to the index date in the identification of confounder status was disregarded. A number of pre-planned sub-analyses and sensitivity analyses was performed.

- First, as an explorative analysis of a potential dose–response effect, analyses stratified according to cumulative duration of lithium use were performed.
- Second, associations for UUTCs with lithium use was examined within subgroups defined by gender, age, or history of renal disease, diabetes or hypertension.
- Third, ORs were computed for associations between long-term use of lithium and two subtypes of UUTCs, i.e., renal cancers (of which the majority are renal cell carcinomas) and cancers of the renal pelvis or ureter (which comprise almost exclusively urothelial carcinomas) as two separate outcomes.
- Fourth, the analyses were stratified by clinical stage, i.e., localized or non-localized disease.
- Fifth, all analyses were repeated using lamotrigine or valproate as primary exposure instead of lithium. The rationale behind these analyses was that if important unmeasured or unknown confounders were associated with use of lithium, these confounders would also be associated with other drug therapy used for the same indication. Lamotrigine and valproate are both used as ‘mood stabilizers’ in bipolar affective disorder.
- Sixth, as drug use before 1995 (start of Prescription Registry) was unavailable, some long-term use of lithium was misclassified in this study. To evaluate the true exposure length among patients with a

minimum of 5 years of lithium use, an explorative analysis within the OPED database was performed (initiated in 1990).

- Finally, as a sensitivity analysis, the 1 year lag time was changed to 0 or 2 years, respectively.

- Results

Were identified 9444 incident UUTCs (cancers of the kidney, renal pelvis or ureter) between January 1, 2000, and December 31, 2012. After exclusions, the study population comprised 6477 cancer cases that were matched to 259,080 controls.

The use of lithium in the Danish population was stable during the study period. Among cases and controls, 0.37% and 0.43%, respectively, had used lithium (≥2 prescriptions), and 0.22% and 0.17% had used lithium for more than 5 years (Table 1).

Table 1. Characteristics of upper urinary tract cancer cases and their age- and sex-matched controls

	Cases (*n*=6477)	Controls (*n*=259,080)
Age, median (IQR, years)	65 (57–73)	65 (57–73)
Male gender	4184 (64.6%)	167,360 (64.6%)
Renal cancer	5648 (87.2%)	NA
Pelvis or ureter cancer	829 (12.8%)	NA
Use of lithium prior to index date		
Non-use	6453 (99.6%)	257,978 (99.6%)
Ever use	24 (0.37%)	1102 (0.43%)
Long-term use (≥5 years)	14 (0.22%)	447 (0.17%)
Drugs		
Non-aspirin NSAID	3542 (54.7%)	127,821 (49.3%)
Aspirin	1639 (25.3%)	54,488 (21.0%)
Paracetamol	1243 (19.2%)	41,774 (16.1%)
Statins	1394 (21.5%)	47,111 (18.2%)
Loop diuretics	858 (13.2%)	26,513 (10.2%)
Thiazides	1637 (25.3%)	50,153 (19.4%)
β-Blockers	1619 (25.0%)	51,228 (19.8%)
Vascular calcium-channel blockers	1424 (22.0%)	39,955 (15.4%)
Inhibitors of RAS	2108 (32.5%)	61,302 (23.7%)
Diagnoses		
Hypertension	1022 (15.8%)	27,275 (10.5%)
Diabetes, type 1	156 (2.4%)	4297 (1.7%)
Diabetes, type 2	688 (10.6%)	21,073 (8.1%)
COPD	471 (7.3%)	16,624 (6.4%)
Alcohol-related disease	530 (8.2%)	18,739 (7.2%)
Moderate/severe renal disease	145 (2.2%)	2603 (1.0%)
Highest achieved education		
Short (7–10 years)	2444 (37.7%)	89,942 (34.7%)
Medium (11–13 years)	2301 (35.5%)	92,518 (35.7%)
Long (>13 years)	974 (15.0%)	46,928 (18.1%)

IQR, interquartile range; RAS, renin-angiotensin system; COPD, chronic obstructive pulmonary disease.

These exposure prevalences yielded overall adjusted odds ratios (ORs) for UUTCs associated with ever or long-term use of lithium, respectively, of 0.9 (95% CI, 0.6–1.3) and 1.3 (95% CI, 0.8–2.2) (Table 2). Adjustment for potential confounders had generally minimal impact on the ORs. The 14 cases associated with long-term lithium use comprised ten cases of renal cell carcinomas, three urethelial carcinomas and one case of uncertain histology.

Table 2. Association between exposure to lithium and risk of upper urinary tract cancer, specified by exposure pattern within the entire follow-up period, excluding the last year prior to the index date

Exposure group	Cases	Controls	Adjusted OR[a]	Adjusted OR[b]
Non-use	6453	257,978	1.0 (ref.)	1.0 (ref.)
Ever use	24	1102	0.9 (0.6–1.3)	0.9 (0.6–1.3)
Long-term use (≥5 years)	14	447	1.3 (0.7–2.1)	1.3 (0.8–2.2)
Duration of use:				
<1 year	3	213	0.6 (0.2–1.8)	0.5 (0.2–1.7)
1–4.99 years	7	442	0.6 (0.3–1.3)	0.6 (0.3–1.4)
5–9.99 years	10	332	1.2 (0.6–2.3)	1.2 (0.7–2.3)
≥10 years	4	115	1.4 (0.5–3.8)	1.5 (0.5–4.0)

[a]Adjusted for age and gender (by design; risk-set matching).
[b]Fully adjusted model, adjusted for *(1)* low-dose aspirin and non-aspirin NSAIDs, paracetamol, statins, thiazides, beta-blockers, vascular calcium-channel blockers, inhibitors of the renin-angiotensin system, and loop diuretics; *(2)* hypertension, type 1 or type 2 diabetes, chronic obstructive pulmonary disease, alcohol-related disease, and moderate to severe renal disease; and *(3)* highest achieved education.

No material variation in the association of lithium use and UUTC risk was seen in analyses defined according to duration of lithium use (Table 2). Individuals aged ≥70 years experienced a slightly higher OR (OR, 1.9; 95% CI, 0.9–3.9) than those of younger ages (Table 3). A similar difference in ORs was seen in separate analyses of females (OR, 1.6; 95% CI, 0.8–3.3) and males (OR, 1.0; 95%CI, 0.5–2.3) (Table 3). Long-term use of lithium was associated with slightly higher ORs for cancers of the pelvis/ureter (OR, 1.7; 95% CI, 0.5–5.4) compared with renal cancers (OR, 1.2; 95% CI, 0.7–2.2). Similarly, a slightly increased OR was observed for localized UUTCs (OR, 1.6; 95% CI, 0.8–3.0), whereas the OR was close to unity for non localized UUTCs (OR, 0.8; 95%CI, 0.3–2.6) (Table 3). However, the statistical precision of the stratified analyses was limited and none of the associations was statistically significant.

Table 3. Associations between long-term exposure to lithium (≥5 years) and risk of upper urinary tract cancer, specified by patient subgroups, type of cancer and stage

Subgroup	Cases exposed/unexposed	Controls exposed/unexposed	Adjusted OR[a]	Adjusted OR[b]
All	14/6477	447/259,080	1.3 (0.7–2.1)	1.3 (0.8–2.2)
Males	6/4184	240/167,360	1.0 (0.4–2.2)	1.0 (0.5–2.3)
Females	8/2293	207/91,720	1.5 (0.8–3.1)	1.6 (0.8–3.3)
Age <50 years	0/624	11/24,960	—	—
Age 50–69 years	6/3515	259/140,600	0.9 (0.4–2.1)	1.0 (0.4–2.2)
Age 70+ years	8/2338	177/93,520	1.8 (0.9–3.7)	1.9 (0.9–3.9)
No history of renal disease	14/6332	428/256,477	1.3 (0.8–2.2)	1.4 (0.8–2.4)
No history of hypertension	13/5455	399/231,805	1.4 (0.8–2.4)	1.5 (0.8–2.5)
No history of diabetes	12/5770	404/237,180	1.2 (0.7–2.2)	1.2 (0.7–2.2)
Subtype				
Renal cancers	11/5648	377/225,920	1.2 (0.6–2.1)	1.2 (0.7–2.2)
Renal pelvis and ureter cancers	3/829	70/33,160	1.7 (0.5–5.5)	1.7 (0.5–5.4)
Stage				
Localized	10/3561	254/142,440	1.6 (0.8–3.0)	1.6 (0.8–3.0)
Non-localized	3/2254	148/90,160	0.8 (0.3–2.5)	0.8 (0.3–2.6)
Unknown	1/662	45/26,480	0.9 (0.1–6.5)	0.9 (0.1–6.8)

[a]Adjusted for age and gender (by design).
[b]Fully adjusted model, adjusted for *(1)* low-dose aspirin and non-aspirin NSAIDs, paracetamol, statins, thiazides, beta-blockers, vascular calcium-channel blockers, inhibitors of the renin-angiotensin system, and loop diuretics; *(2)* hypertension, type 1 or type 2 diabetes, chronic obstructive pulmonary disease, alcohol-related disease, and moderate to severe renal disease; and *(3)* highest achieved education.

Analyses of lamotrigine or valproate as primary exposure yielded results similar to those of the main analyses, with ORs for UUTCs of 0.7 (95%CI, 0.4–1.3) and 0.9 (95%CI, 0.6–1.5) associated with long-term use of lamotrigine and valproate, respectively. Increasing the lag time to 24 months yielded a slightly

increased OR for UUTCs associated with long-term use of lithium (OR, 1.5; 95% CI, 0.9–2.6), whereas the OR was close to unity in the analysis with no lag time (OR, 1.2; 95% CI, 0.7–2.0).

In the evaluation of misclassification of long-term exposure due to left truncation of prescription history in 1995, using the OPED database, 1683 ever users (≥2 prescriptions) and 877 long-term users (≥5 years) of lithium during 1995–2012 were identified. Among the long-term users, 66.2% (n=581) had also used lithium during 1990–1994 (median 744 days; interquartile range, 318–1257 days). Among subjects with <5 years use of lithium within the study exposure period, the corresponding prevalence was 19.4% (n=326, median 705 days; interquartile range, 366–1130 days).

Finally, assuming a true OR of 1.3, it was estimated that 12,364 person-years of long-term lithium use would be required to elicit one additional case of UUTC. The upper limit of the confidence interval for the OR of 1.3 (i.e., 2.2) would correspond to one additional UUTC for every 3091 person-years of lithium exposure.

3.2.2.3 Kessing et al., 2015

Study reference:

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

Detailed study summary and results:

Test type

- Methods
    - The registers

Data were obtained by linking Danish population based registers using the unique personal identification number that is assigned to all 5.4 million persons living in Denmark. In this way, the Medicinal Product Statistics register was linked with the Danish Medical Register on Vital Statistics, the Danish National Hospital Register, and the Danish Psychiatric Central Register.

The Medicinal Product Statistics register contains data on all prescribed medication purchased at pharmacies since 1 January 1995. In Denmark, all medications such as lithium, which are prescribed by doctors, are purchased at pharmacies only. Information on indications for the drug is not available in the register.

The Danish Medical Register on Vital Statistics contains dates of death. The Danish National Hospital Register contains data on all patients treated at all general hopsitals as inpatients or outpatients in Denmark from 1 January 1977 onwards as a part of the official Danish Health Survey. Likewise, all psychiatric admissions have been registered in a nationwide register, The Danish Psychiatric Central Register, from 1 April 1970 onwards. Since 1 January 1994, the International Classification of Diseases,

Tenth Revision (ICD-10) has been in use in both registers.

The study was approved by the Danish Data Protection Agency (Nr.: 2013-41-2281).

- The study cohorts

The study included two cohorts. Cohort I consisted of (i) a randomly selected sample of 1,500,000 out of all persons registered in Denmark on 1 January 1995; (ii) all patients having their first psychiatric contact ever (as inpatients or outpatients) in the period from 1994 to 2012 and receiving a main diagnosis of a single manic episode or bipolar disorder (ICD-10 code: DF30-31.9 + 38.00) on that occasion; and (iii) all persons exposed to either lithium or anticonvulsants identified on the date of their first prescription of either drug between 1995 and 2012. Cohort II was the sub-cohort of patients with a diagnosis of bipolar disorder. Thus, the analyses of Cohort I investigated the effect of drugs, regardless of the indication.

Patients were excluded if they had had a hospital contact, as an inpatient or outpatient, with a diagnosis of renal or upper urinary tract tumors, malignant or benign (ICD-8: 237.39 to 337.99 inkl. + ICD-10: D30, D30.0 til D30.9 inkl + C64 til C68.9, inclusive) prior to inclusion into the study – that is, 1 January 1995 for the random sample of the population and prior to inclusion in the study.

- Outcomes

We included three separate outcomes:

- Renal or upper urinary tract tumors, malignant or benign: ICD-10: C64-C68.9 + D30-30.9
- Renal or upper urinary tract tumors, malignant: ICD-10: C64-C68.9
- Renal or upper urinary tract tumors, benign: ICD-10: D30-30.9

For sensitivity analyses, we included two additional outcomes:

- Renal malignant tumors: ICD-10: C64 + C64.9
- Renal benign tumors: ICD-10: D30 + D30.0

- Results
  - Cohort I

Cohort I comprised data from 1,712,040 persons (835,964 men and 876,076 women) aged between 0 years and 110 years [median 40 years, interquartile range (IQR): 22–57 years]. Within the limitations of the study period (start: 1 January 1995; end: renal or upper urinary tract tumor, death or 31 December 2012), 24,272 patients started treatment with lithium (age at first prescription: median =48 years, IQR: 22–57 years) and 386,255 patients started treatment with anticonvulsants (age at first prescription: median = 50 years, IQR: 22–57 years). The main analysis included 22,379,270 person-years at risk, of which 1% were exposed to lithium and 10.8% were exposed to anticonvulsants.

Table 1 presents hazard rate ratios (HR) for renal or upper urinary tract tumors, malignant and benign, malignant, and benign, respectively, related to the number of prescriptions for lithium, anticonvulsants, antipsychotic agents, and antidepressants and adjusted for age, gender, employment status, calendar year, a diagnosis of bipolar disorder, and use of all other types of medication. Patients with a main diagnosis of a single manic episode/bipolar disorder were found to have a decreased rates of renal or upper urinary tract tumors compared to individuals without a diagnosis, and regardless of outcome measure.

Table 1 shows that rates of all three outcome measures did not increase with the number of prescriptions for lithium or anticonvulsants. The rate of renal or upper urinary tract tumors decreased slightly with the number

of prescriptions for antipsychotic agents, regardless of outcome measure, and increased slightly with the number of prescriptions for antidepressants in relation to malignant and benign tumors as well as benign tumors.

Prescription of all other types of medication was associated with a moderately increased rates of all three outcome measures (non-significantly in relation to benign tumors), but a decreased rates in women and individuals in work. Effect modification analyses using likelihood ratio tests showed no significant changes in the association between lithium and renal or upper urinary tract tumors with respect to simultaneous use of anticonvulsants, regardless of the outcome measures (all $p > 0.5$).

In sensitivity analyses with malignant renal tumors or benign renal tumors as outcomes, respectively, no statistically significant associations were found in the trend test in relation to the number of lithium prescriptions (malignant: HR = 1.01, 95% CI: 0.95–1.08, p = 0.7; benign: HR = 1.09, 95% CI: 0.86–1.40, p = 0.5).

Table 1. Cohort I: rates of renal and upper urinary tract tumors (RUT) (malignant and benign, malignant, benign) relative to the number of prescriptions with lithium, anticonvulsants, antipsychotic agents, and antidepressants

		RUT: malignant and benign (n = 12,961)	Trend test	RUT: malignant (n = 8,631)	Trend test	RUT: benign (n = 6,086)	Trend test
Variables	Units	HR (95% CI)	HR (95% CI) p-value[a]	HR (95% CI)	HR (95% CI) p-value[a]	HR (95% CI)	HR (95% CI) p-value[a]
Lithium	0	1	1.01 (0.97–1.05)	1	1.00 (0.96–1.06)	1	0.99 (0.93–1.05)
	1–2	0.67 (0.43–1.06)	0.70	0.61 (0.34–1.11)	0.90	0.74 (0.40–1.39)	0.70
	3–9	1.29 (0.93–1.78)		1.26 (0.83–1.90)		1.18 (0.73–1.91)	
	10–19	0.80 (0.52–1.23)		0.61 (0.32–1.13)		1.08 (0.64–1.84)	
	20–29	1.18 (0.76–1.81)		1.34 (0.80–2.24)		0.80 (0.38–1.69)	
	30–39	0.94 (0.55–1.64)		1.16 (0.62–2.17)		0.59 (0.22–1.59)	
	40–59	0.91 (0.56–1.48)		0.86 (0.46–1.61)		0.88 (0.44–1.79)	
	>60	1.17 (0.80–1.72)		1.12 (0.68–1.83)		1.07 (0.59–1.92)	
Anticonvulsants	0	1	1.01 (1.00–1.03)	1	1.00 (0.98–1.02)	1	1.01 (0.99–1.04)
	1–2	1.18 (1.10–1.27)	0.10	1.15 (1.05–1.25)	0.80	1.14 (1.03–1.28)	0.20
	3–9	1.08 (0.98–1.19)		1.09 (0.97–1.22)		0.97 (0.83–1.12)	
	10–19	1.07 (0.94–1.22)		1.04 (0.89–1.21)		1.02 (0.85–1.23)	
	20–29	1.14 (0.97–1.33)		0.98 (0.79–1.21)		1.36 (1.10–1.68)	
	30–39	0.97 (0.79–1.20)		0.91 (0.70–1.18)		1.06 (0.79–1.42)	
	40–59	0.99 (0.82–1.19)		0.82 (0.64–1.05)		1.19 (0.93–1.52)	
	>60	1.05 (0.90–1.22)		1.02 (0.85–1.23)		0.94 (0.75–1.19)	
Antipsychotic agents	0	1	0.96 (0.95–0.98)	1	0.96 (0.93–0.98)	1	0.97 (0.94–1.00)
	1–2	1.08 (0.99–1.19)	0.0005	1.10 (0.99–1.24)	0.0006	1.05 (0.91–1.21)	0.05
	3–9	0.88 (0.77–1.00)		0.77 (0.65–0.91)		1.02 (0.85–1.21)	
	10–19	0.79 (0.66–0.95)		0.78 (0.62–0.98)		0.77 (0.59–1.02)	
	20–29	0.80 (0.63–1.02)		0.78 (0.58–1.06)		0.88 (0.63–1.24)	
	30–39	0.76 (0.56–1.03)		0.75 (0.51–1.09)		0.71 (0.44–1.13)	
	40–59	0.67 (0.50–0.90)		0.64 (0.44–0.93)		0.59 (0.37–0.93)	
	>60	0.93 (0.77–1.12)		0.90 (0.71–1.14)		0.98 (0.74–1.29)	
Antidepressants	0	1	1.01 (1.00–1.03)	1	1.00 (0.99–1.02)	1	1.03 (1.01–1.04)
	1–2	1.17 (1.08–1.26)	0.03	1.15 (1.05–1.26)	0.80	1.18 (1.05–1.31)	0.007
	3–9	1.12 (1.04–1.21)		1.13 (1.03–1.24)		1.13 (1.01–1.26)	
	10–19	1.05 (0.95–1.15)		0.98 (0.87–1.11)		1.09 (0.95–1.25)	
	20–29	1.02 (0.90–1.16)		0.95 (0.80–1.11)		1.04 (0.86–1.26)	
	30–39	1.13 (0.98–1.32)		1.00 (0.83–1.21)		1.21 (0.97–1.50)	
	40–59	1.18 (1.03–1.35)		1.10 (0.93–1.30)		1.30 (1.07–1.58)	
	>60	0.97 (0.85–1.11)		0.94 (0.80–1.11)		1.05 (0.86–1.28)	
Bipolar disorder	No	1		1		1	
	Yes	0.61 (0.43–0.86)		0.59 (0.38–0.91)		0.61 (0.37–1.01)	
All other medications	No	1		1		1	
	Yes	2.77 (2.39–3.21)		2.88 (2.40–3.47)		2.78 (2.26–3.43)	
Gender	Male	1		1		1	
	Female	0.33 (0.32–0.35)		0.34 (0.32–0.35)		0.31 (0.29–0.33)	
Employment status	Work/ student	1		1		1	
	Disabled	1.48 (1.37–1.59)		1.57 (1.42–1.72)		1.38 (1.24–1.55)	
	Retired	1.14 (1.06–1.22)		1.08 (0.99–1.17)		1.28 (1.16–1.43)	
	Unemployed	1.42 (1.23–1.65)		1.30 (1.06–1.58)		1.57 (1.28–1.92)	
	Other	1.60 (1.43–1.78)		2.08 (1.83–2.36)		1.04 (0.86–1.27)	

Total N = 1,712,040 individuals. Adjusted for age, gender, employment status, calendar year, diagnosis of bipolar disorder, and use of all other types of psychotropic agents, as well as somatic medications.
CI = confidence interval; HR = hazard ratio.
[a]Trend tests among treated patients.

- Cohort II

Cohort II included 9,651 patients with a main diagnosis of a single manic episode/bipolar disorder at first psychiatric contact (as inpatients or outpatients) as recorded in the Danish Psychiatric Central Register (4,089 men and 5,562 women), aged between eight and 100 years (median 52 years, IQR: 39–65 years). Within the limitations of the study period (start: 1 January 1995, end: renal or upper urinary tract tumor, death or 31 December 2012), 5,513 patients started treatment with lithium (age at first prescription: median = 53 years, IQR: 39–65 years) and 5,616 patients started treatment with anticonvulsants (age at first prescription: median = 50 years, IQR: 39–65 years). The main analysis included a total of 74,306 patient-years at risk, of which 61.4% were exposed to lithium and 50.0% were exposed to anticonvulsants. The number of patients not receiving 'other types of psychotropic as well as somatic medication' was too small to include this variable in the analyses.

As can be seen from Table 2, no trend tests showed statistically significant associations for any of the three outcome measures (except for antipsychotic agents in relation to malignant and benign renal or upper urinary tract tumor). As in Cohort I, women and individuals in work systematically had a decreased rates (adjusted for other variables in Table 2).

Figures 1 and 2 show the cumulative incidences of renal or upper urinary tract tumor (malignant or benign) starting at the 30th, 45th, 60th, and 75th birthday, according to ever treatment with lithium and anticonvulsants, respectively. Cumulative ten- and 15-year risks of renal or upper urinary tract tumors were not increased compared to patients unexposed to lithium and anticonvulsants, respectively.

Table 2. Cohort II: rates of renal and upper urinary tract tumors (RUT) (malignant and benign, malignant, benign) relative to the number of prescriptions with lithium, anticonvulsants, antipsychotic agents, and antidepressants

		RUT: malignant and benign (n = 37)	Trend test	RUT: malignant (n = 22)	Trend test	RUT: benign (n = 17)	Trend test
Variables	Units	HR (95% CI)	HR (95% CI) p-value[a]	HR (95% CI)	HR (95% CI) p-value[a]	HR (95% CI)	HR (95% CI) p-value[a]
Lithium	0	1	1.03 (0.73–1.46)	1	0.85 (0.54–1.32)	1	1.32 (0.78–2.26)
	1–9	0.66 (0.19–2.32)	0.90	0.63 (0.135–2.91)	0.50	0.65 (0.075–5.64)	0.30
	≥10	1.07 (0.54–2.12)		0.72 (0.295–1.74)		1.75 (0.604–5.09)	
Anticonvulsants	0	1	0.84 (0.55–1.29)	1	0.63 (0.33–1.21)	1	1.05 (0.59–1.87)
	1–9	0.78 (0.31–1.94)	0.40	1.05 (0.371–2.98)	0.20	0.30 (0.038–2.33)	0.90
	≥10	0.70 (0.30–1.67)		0.40 (0.108–1.47)		1.11 (0.351–3.50)	
Antipsychotic agents	0	1	0.58 (0.37–0.90)	1	0.75 (0.42–1.33)	1	0.36 (0.18–0.73)
	1–9	0.70 (0.33–1.49)	0.02	0.95 (0.349–2.58)	0.30	0.37 (0.123–1.14)	0.05
	≥10	0.34 (0.14–0.82)		0.56 (0.180–1.76)		0.13 (0.033–0.54)	
Antidepressants	0	1	1.24 (0.81–1.91)	1	1.02 (0.62–1.68) 0.90	1	2.36 (0.85–6.58)
	1–9	0.93 (0.31–2.77)	0.30	0.53 (0.13–2.14)		3.49 (0.36–33.65)	0.10
	≥10	1.55 (0.65–3.66)		1.04 (0.39–2.82)		5.57 (0.72–43.26)	
Gender	Male	1		1		1	
	Female	0.23 (0.11–0.49)		0.14 (0.05–0.43)		0.32 (0.12–0.89)	

Total N = 9,651 patients with a main diagnosis of a single manic episode/bipolar disorder. Adjusted for age, gender, and calendar year.
CI = confidence interval; HR = hazard ratio.
[a]Trend tests among all patients.

3.2.2.4 Zaidan et al., 2014

Study reference:

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

Detailed study summary and results:

Test type

- Methods
    - Study design, setting, and participants

A retrospective cohort study was conducted and considered for inclusion of all patients under long-term lithium therapy referred, between 1996 and 2011, to the Nephrology Departments at Necker and Tenon University Hospitals (Paris, France). The detection of potential renal tumors was based on the analysis of renal ultrasound investigations. Thus, only patients with available renal imaging results were included in the study. Patient care and the conduct of the study complied with good clinical practice and the Declaration of Helsinki guidelines.

    - Data sources

For each patient, demographic, clinical, and biological characteristics at the time of renal imaging were obtained from medical records, and included sex, age at lithium initiation, age at renal imaging, medical history, weight, height, and cigarette smoking. Glomerular filtration rate was estimated according to the Modification of Diet in Renal Disease Study equation. The result of renal ultrasound performed in the routine management of patients with chronic kidney disease (CKD) was reviewed for all patients. Further radiological investigations, including magnetic resonance imaging or computed tomography scan, were also recorded when performed, mostly in case of a suspect lesion found by renal ultrasound. The results of renal imaging were classified as follows: normal, presence of typical renal cyst(s), or atypical renal lesions. Only patients with histologically proven tumors were considered as having solid renal tumors, except for patients with typical images of renal angiomyolipoma. When a biopsy or a nephrectomy was performed, histological specimens were reviewed centrally by a senior pathologist specialized in renal tumoral pathology

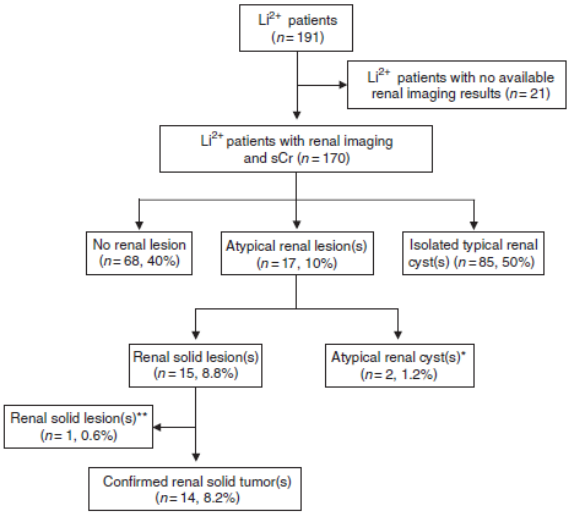


Figure 1 | Study design and renal ultrasound findings. *Renal cancer was ruled out by further imaging investigation and no histological confirmation was required. **The patient declined further investigations and was lost to follow-up.

- Results
    - Baseline characteristics of lithium-treated patients and renal imaging findings

Between 1996 and 2011, 191 patients who were under lithium therapy and followed at two nephrology departments (Paris, France) were eligible for the study. Renal imaging results were available for 170 patients, including 108 women (63.5%) and 62 men (36.5%), who were included in the study. Baseline characteristics are detailed in Table 1. The mean age at the time of renal imaging was 65.1±11.5 years, and the mean age at lithium therapy initiation was 41.1±11.9 years.

The mean duration of lithium exposure was 21.3±10.4 years and the mean estimated glomerular filtration rate (eGFR) at renal imaging was 40.0±17.1 ml/min per 1.73m^2. Renal ultrasound findings are shown in Figure 1. Eighty-five patients (50%) had isolated typical renal cysts. Seventeen patients (10%) had atypical renal lesions on renal ultrasound including two with atypical renal cysts and one with two renal solid lesions. This patient declined further investigation and was lost to follow-up. Fourteen lithium-treated patients (8.2%) were diagnosed with renal solid tumors.

    - Renal solid tumors in lithium-treated patients

The renal solid tumors included seven renal malignant tumors (4.1%) and seven benign tumors (4.1%). Pathological analysis was performed in all but two patients who had imaging findings typical of angiomyolipoma. The renal cancers included three clear-cell renal cell carcinoma (RCC), two papillary RCC, one clear cell carcinoma with leiomyomatous stroma, and one hybrid tumor characterized by the association of an oncocytoma and a chromophobe RCC. Benign renal solid tumors included four other cases of oncocytoma, one of which presented with six oncocytomas that were associated with numerous papillary adenomas, one mixed epithelial and stromal tumor, and two angiomyolipomas. The mean age at diagnosis was 64.4±8.6 years. The mean duration of lithium exposure at diagnosis of renal cancer and benign tumor was 23.9±11.7 and 20.2±9.9 years, respectively. The diagnosis of renal tumor was established after lithium withdrawal in four patients, three of whom had renal cancer with a delay between lithium withdrawal and cancer diagnosis of 3, 5, and 18 years. Considering the limited number of cases, no association could be observed between age, gender, duration of lithium exposure, eGFR, obesity and ever smoking, and an increased risk of renal tumor in lithium-treated patients.

    - Comparison of the frequency of renal solid tumors between lithium-treated and lithium-free patients

As CKD has been identified as a potential risk factor for renal carcinoma, we investigated whether the frequency of renal solid tumors in lithium-treated CKD patients was increased as compared with lithium-free CKD patients. Lithium-free patients were identified from the active files of the same two nephrology departments using the criteria detailed in the Methods section. Two sex-, age-, and eGFR-matched lithium free patients, followed during the same period, were randomly selected for each lithium-treated patient blindly to the result of their renal imaging. Of the 340 matched patients, only one had a renal cancer, none had oncocytoma, and four had angiomyolipoma (Table 3). Three patients had atypical renal cysts that did not require further pathological investigation. The frequency of renal cancer and oncocytoma was significantly

higher among lithium-treated patients than among their sex-, age-, and eGFR-matched lithium-free patients (4.1% vs 0.3%, P=0.002 and 2.4% vs 0%, P=0.01, respectively). In contrast, the frequency of angiomyolipoma did not differ between the two groups (1.2% in both groups, P=0.60).

Table 3 | Comparison between lithium-treated and lithium-free patients

	Li^{2+}-treated patients ($n = 170$)	Li^{2+}-untreated patients ($n = 340$)	*P*
Age (yr)	65.1 ± 11.5	65.0 ± 11.6	0.93
Women/men	108/62	216/124	0.92
eGFR (ml/min per 1.73 m^2)	40.0 ± 17.1	39.6 ± 16.9	0.82
Renal solid tumors			
Renal cancer	7 (4.1)	1 (0.3)	0.004
Oncocytoma	4 (2.4)[a]	0 (0)	0.02
Angiomyolipoma	2 (1.2)	4 (1.2)	0.66

Abbreviations: eGFR, estimated glomerular filtration rate; Li^{2+}, lithium; *n*, number; RCC, renal cell carcinoma; yr, years.
Continuous variables are shown as means ± s.d. and categorical variables are shown as number (percentage).
[a]The oncocytoma associated with chromophobe RCC is not included among the four cases.

- Comparison of the incidence of renal cancers between lithium-treated patients and the French general population

Using the French National estimates of renal cancer incidence, the incidence of renal cancer in lithium-treated patients was compared to the general population. The incidence was 10.12 times higher among lithium-treated patients overall than in the French general population (95% confidence interval (CI) (4.06–20.86)) (Table 4). The Standardized Incidence Ratios (SIRs) were highly significant for both men and women (SIR 7.51, 95% CI (1.51–21.95) and 13.69, 95% CI (3.68–35.06), respectively).

3.3 Reproductive toxicity

3.3.1 Animal data

3.3.1.1 Toghyani et al., 2013

Study reference:

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

Detailed study summary and results:

Test type

Effect of lithium on the sperm concentration and motility and forms of abnormal cells has been examined.

Test substance

- Lithium carbonate
- Degree of purity: no information

- Batch number: Tehran Darou Co.: serial no. 8808

Test animals

- Wistar males
- 3 rats/group
- Adult, 230g

Administration/exposure

- Route of administration: oral (gavage)
- duration and frequency of test/exposure period: 48 day of spermatogenesis process
- doses/concentration levels: 0, 20, and 30 mg/kg bw/day
- vehicle: distilled water

Description of test design:

- Total sperm count obtained from the cauda epididymis

The sperm motility was inhibited by adding 50 µL of 10% formalin to the sperm solution, and then, total number of sperm cells was estimated in 1 mL of the sperm solution prepared on a neubauer slide with a magnification of ×10.

- Determining the percentage of motile sperms

After several times of sampling, the solution containing epididymal pieces was placed in the laboratory environment and shaken gently for 20 minutes in order to extract the sperms. One drop of the sperm solution was placed on the slide, and the percentage of sperm motility with progressive movement was estimated using a light microscope with a magnification of ×10, then with ×40 in 10 fields, and the percentage of sperms with normal and abnormal morphology in the fields was recorded.

- Papanicolaou staining for morphological analysis of sperm cells

In this method, the sperm fluid was fixated by adding 100 µL of 10% formalin, and then, a smear was made on the slide and Papanicolaou staining was carried out sequentially. The slide was immersed in 70% methanol for 15 min and rinsed by running tap water and then dipped in acid alcohol for 1 s and again rinsed in running tap water. After staining with OG-6 (orange) for 2-4 min, the slide was dipped in distilled water for 1 s and immersed in alcohol 1 for 1 s and then put in alcohol 2 for 2 s. Then, it was stained with EA-50 for 2-4 min. Once again, the slide was dipped in distilled water for 1 s and immersed in alcohol 1 for 1 s and then put in alcohol 2 for 2 s and air dried. Finally, the slide was immersed in xylene for 10 min and again air dried. Morphology of the sperms in terms of normal and abnormal shape of the head and tail was analyzed in the prepared slides and mean data were recorded.

Results and discussion

The analysis by one-way ANOVA test showed that mean percentage of motility, number, and percentage of normal and abnormal sperm cells were not equal in different groups. The status of experimental groups and the control group to each other was determined using the Duncan post hoc test.

Table 1: Comparison of mean percentage of motility, number, and percentage of normal and abnormal sperm cells in cauda epididymis

Group	Percentage of sperm motility±SD	Percentage of normal sperms±SD	Total number of sperms in cauda epididymis±SD
Control	96.00±0.89	97.33±1.21	2.19×10^{8}±9715966.24
Lithium carbonate 10 mg/kg BW	67.66±1.21	87.50±0.55	1.42×10^{8}±3881580.43
Lithium carbonate 20 mg/kg BW	48.17±3.43	88.00±0.89	1.21×10^{8}±5750362.31
Lithium carbonate 30 mg/kg BW	38.50±2.59	70.83±3.43	1.12×10^{8}±3488074.92

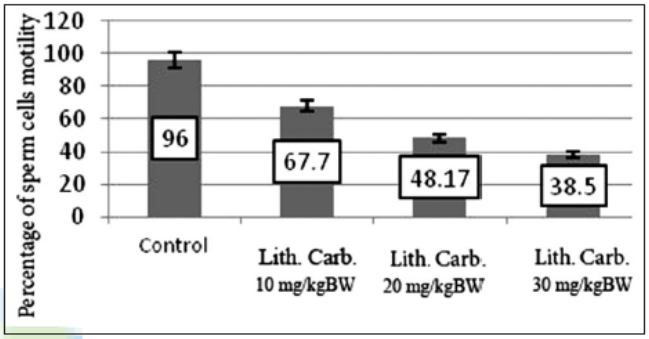


Figure 1: Percentage of sperm cell motility in groups receiving lithium shows a dose-dependent reduction

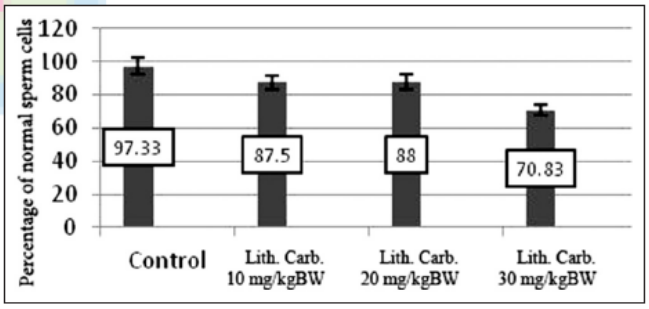


Figure 2: Percentage of normal sperm cells obtained from cauda epididymis is reduced in treatment with lithium

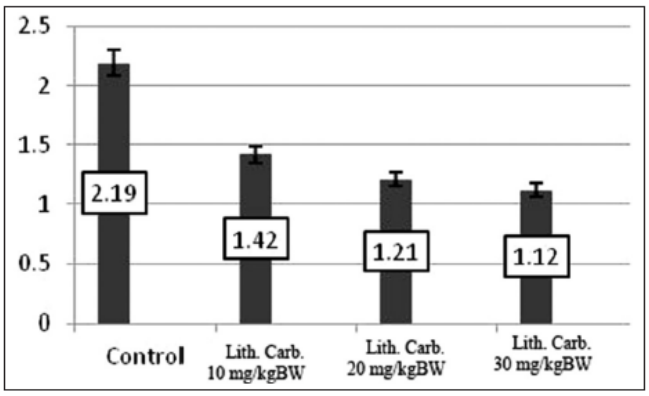


Figure 3: Number of sperm cells in the cauda epididymis is reduced in experimental groups treated with lithium

3.3.1.2 Toghiani et al., 2012

Study reference:

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

Detailed study summary and results:

Test type

Effect of three doses of 10, 20 and 30 mg/kg Bw/day of lithium carbonate at histological and cellular modifications of testes and also on the level of LH, FSH and testosterone hormones was examined.

Test substance

- Lithium carbonate
- Degree of purity: no information
- Batch number: Tehran Darou Co., serial no. 8808

Test animals

- Species/strain/sex: Wistar male rats
- No. of animals per sex per dose: 6
- Age and weight at the study initiation: adult rats with mean weight of 200-250 g and 7-8 weeks old

Administration/exposure

- Route of administration – oral (gavage)
- duration and frequency of test/exposure period: treated for 48 days.
- doses/concentration levels, rationale for dose level selection: 10, 20 and 30 mg/kg BW/day
- historical control data if available
- vehicle: sterile distilled water
- test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation: 0.5 ml of lithium carbonate solution which was prepared by dissolving the specific amount of white powder of lithium carbonate for each group with sterile distilled water.

Description of test design:

- Hormonal measurement:

Blood sample of 4-5 ml was drawn from each rat and collected in glass tubes specific for centrifuges. The blood samples were maintained in the laboratory setting for 1 hour to form clots and then centrifuged for 2 min at 500 rpm. In this way, serum was separated from the blood and surfaced the clot. The serum was removed using a 1000 sampler and transmitted to smaller tubes. These tubes were kept in a freezer at -20°C in order to be used in ELISA kits for measurement of LH, FSH and testosterone hormones (Ghosh et al., 1991a; Ghosh et al., 1990b). Finally, optical absorption of the prepared solutions was recorded according to the kit guidelines at wavelength of 450 nm and concentration of each hormone was recorded in ng/ml.

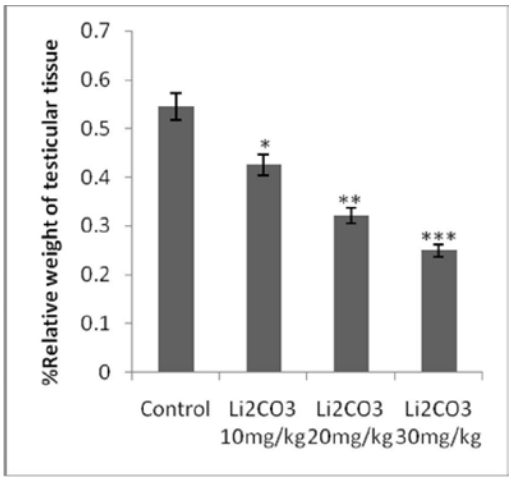
- Preparation of tissue sections:

The removed testicular tissue was placed in Bouin's fixative solution for 24 hours after being weighed. Then, paraffin blocks and serial sections of the tissue were prepared and the slides were stained with hematoxylin and eosin. Histological examination: The testicular tissue sections were observed and examined using an ordinary optical microscope (Hm-Lux3, Germany) with 10X, 40X and 100X magnification and the spermatogonia, primary spermatocytes, spermatozoa, Sertoli and Leydig cells were counted in each microscopic field in 10 seminiferous tubules which had a round shape and an appropriate cross-section. All sections selected for cell counting and histological analysis are located in the similar Stage. Morphological changes of the seminiferous tubules and germinal epithelium of the groups were compared with each other. In this study, besides counting cells and comparing mean germinal cell and testicular somatic cell count, the score count method or Johnson's model was used for the level of spermatogenesis which is the common method for histopathological measurements (Johnson et al., 1980).

Results and discussion

- Relative weight of the testicular tissue

This index was reduced in groups receiving lithium carbonate in that it reached 0.24936 ± 0.021927 g in the group receiving 30 mg/kg Bw/day of lithium carbonate while it reached 0.54428 ± 0.02791 g in the control group. This difference is Significant ($p<0.001$). This index reached 0.32121 ± 0.13154 g in the group receiving 20 mg/kg Bw/day of lithium carbonate, which is significantly different from that of the control group ($p<0.01$). Likewise, this index reached 0.42535± 0.04978 g in the group receiving 10 mg/kg Bw/day lithium carbonate, which has a Significant difference with that of the control group ($p<0.05$)



- Johnson’s score count

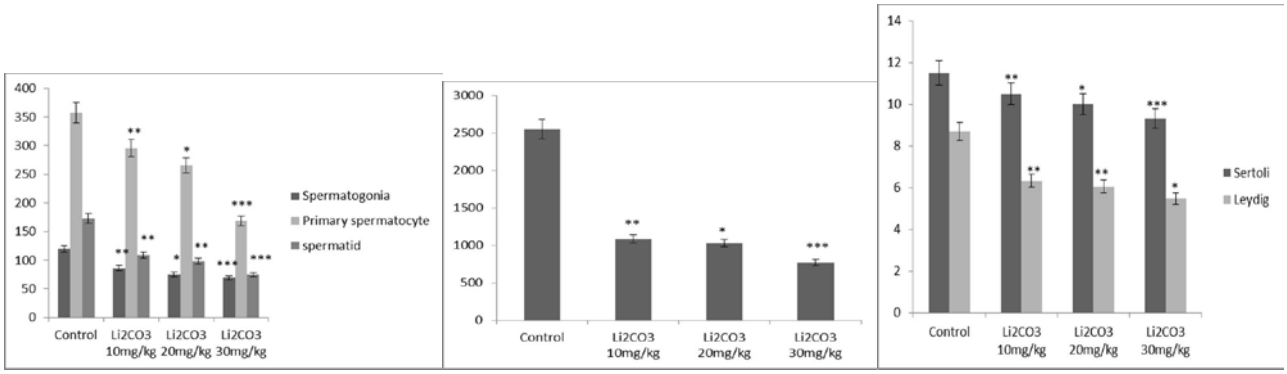
According to the score count, density of germinal and somatic cells in seminiferous tubules was based for the scoring and is shown in Table below. On the basis of the results, the control group scored 10, and the groups receiving 10, 20, and 30 mg/kg Bw/day of lithium carbonate scored 8, 4.9 and 3.7, respectively.

Table 1: Johnson's scoring system for the level of spermatogenesis

Groups	Score
Control	**10**
10 mg/kgBW Lithium carbonate	**8**
20 mg/kgBW Lithium carbonate	**4.9**
30 mg/kgBW Lithium carbonate	**3.7**

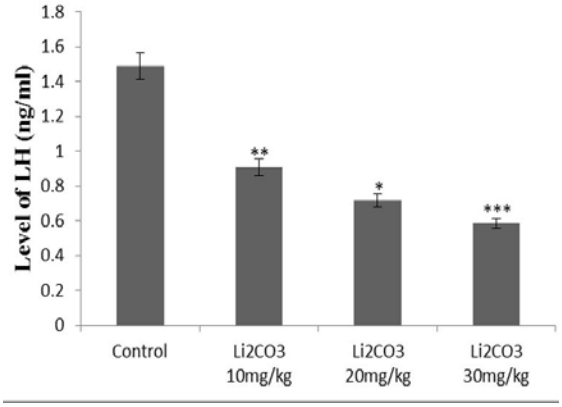
Cell count and comparisons among control and experimental groups: Through considerable histological changes in testicles, lithium caused a decrease in the number of germ and somatic cells in seminiferous epithelium, an increase in interstitial space, and an increase in the number of cells being destroyed. The decreased number of cell count is shown in the table below.

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

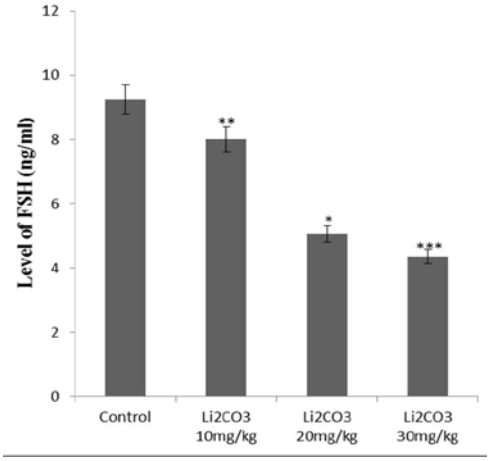


Results of the hormonal measurement:

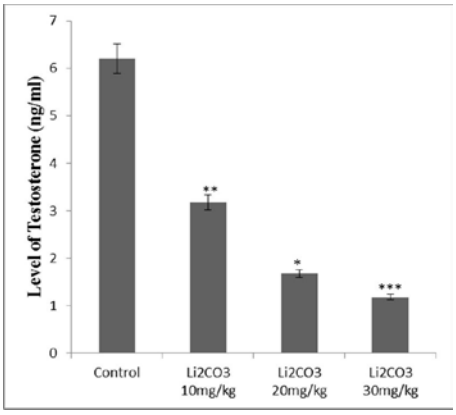
The lowest level of LH belongs to the group receiving 30 mg/kg BW lithium carbonate (0.584±0.03184 ng/mg, p<0.001).Mean ± SD values of LH hormone (ng/ml) in the groups receiving 20 and 10 mg/kg BW of lithium carbonate and the control groups were 0.716±0.07806 (p<0.05), 0.908±0.07851 (p<0.01) and 1.49±0.07137 ng/ml, respectively.



With regards to FSH, it had such regular decrease in the same order as for LH. So, the lowest rate was recorded for the group receiving 30 mg/kg BW of lithium carbonate as 4.355±0.01655ng/ml (p<0.001). Mean ± SD values of FSH hormone (ng/ml) in the groups receiving 20 and 10 mg/kg BW of lithium carbonate were 5.063±0.06188 (p<0.05), 8.009±0.33806 (p<0.01). This reduction was significant as compared with the control group with 9.246±0.16106 ng/ml of FSH.



Testosterone level (ng/ml) was reduced significantly in the experimental groups as compared with that in the control group. It was 1.183±0.16553 ($p<0.001$), 1.67±0.0618 ($p<0.05$) and 3.17±0.33806 ($p<0.01$) ng/ml in the groups receiving 30, 20, and 10 mg/kg BW of lithium carbonate, and 6.2±0.16106 ng/ml in the control group.



3.3.1.3 Anonymous, 2012

Study reference:

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

See also confidential Annex I for confidential information

Detailed study summary and results:

Test type

Test performed according to OECD Guideline 416 (Two-Generation Reproduction Toxicity Study, version from January 22nd, 2001), GLP compliance is given (including certificate)

Test substance

- Test material used in the study is lithium carbonate
- Degree of purity: [confidential]

- Impurities do not affect the classification
- Lot/batch No.: [confidential]

Test animals

- Male and female Wistar rats
- 25 animals per sex and dose for the parental and F1 generation.
- Age at study initiation: 8 - 10 weeks
- Weight at study initiation (P): males 211 - 254 g, females 158 - 190 g

Administration/exposure

- Route of administration – oral (gavage)
- Duration of treatment: (P)-Treatment commenced from the age of 9 weeks and continued throughout the treatment period until F1 litters were weaned. Parents and pups not selected for F1 generation were sacrificed. (F1)-Treatment commenced for F1 generation from the time of weaning and continued until F2 were weaned and sacrificed.
- Frequency of treatment: once daily
- 0, 5, 15 and 45 mg/kg bw/day,

  Dose selection was based on different studies regarding repeated oral exposure as well as oral exposure of pregnant rats with lithium carbonate (Ibrahim at al., 1990; Fritz et al., 1988; Marathe & Thomas et al., 1986; Hansen et al., 2010). Further a dose range finding study was conducted treating male and female rats for 28 consecutive days orally by gavage. Based on the available literature and experimental data provided, the dose levels of 5, 15 and 45 mg/kg bw/ day were selected for this two generation toxicity study in consultation with the Sponsor. Vehicle control group (G1) animals were administered Milli-Q water throughout the study.
- Historical control data: yes available
- Vehicle: Milli-Q water, Amount of vehicle: 10 mL/kg bw
- PREPARATION OF DOSING SOLUTIONS: Required quantity of the test item was weighed and mixed in Milli-Q water to attain desired concentrations. Dose formulations were prepared once every 8 days as stock solution for each dose. The prepared stock solution was mixed by inversion before taking for daily use. Homogenity of the dose formulation was maintained by constant stirring using magnetic stirrer. The prepared stock solution was stored in the experimental room. Following procedure was adopted when 1000 mL of dose solution was prepared. The quantities of 0.5, 1.5 and 4.5 g of test item was weighed and mixed in Milli-Q water to attain desired concentrations of 0.5, 1.5 and 4.5 mg/mL for the low, mid and high dose groups respectively. The weight of the test item and the volume of the test item prepared varied depending upon the requirement.

For active ingredient concentration analysis, samples of test formulation were taken from all doses including vehicle control, prepared on Day 1 and once in 3 months intervals thereafter during the treatment period. The collected samples were sent to Analytical R&D Department of Advinus Therapeutics Limited, Bangalore for concentration analysis. The method of analysis was the same as the validated method done under Advinus Study No. G7467. The test item concentrations in gavage prepared for dosing on 18.01.2011, 12.04.2011, 12.07.2011 and 20.09.2011 with nominal concentrations of the test item in gavage samples 0 mg/mL, 0.5 mg/mL, 1.5 mg/mL and 4.5 mg/mL indicated that the test item concentrations in solution were within the permissible limits of ± 10 % from the nominal concentrations.

Description of test design:

- Details on mating procedure:
  - M/F ratio per cage: 1:1
  - Length of cohabitation: 2 weeks
  - Proof of pregnancy: vaginal plug / sperm in vaginal smear referred to as day 0 of pregnancy- After 14 days of unsuccessful pairing replacement of first male by another male with proven fertility.
  - Further matings after two unsuccessful attempts: no data
  - After successful mating each pregnant female was caged (how): The females were housed individually in polysulfone cages (size: L 425 x B 266 x H 175 mm) during gestation and lactation period until weaning and sacrifice. The sterilized nesting material (paper shreds) was provided on GD 20 or near term. The steam sterilized corn cob was used as bedding material throughout the experiment. The cage along with bedding material was changed at least once a week.
- Male rats: exposure for at least 10 weeks prior to the mating period Treatment was continued during mating and up to and including the day before sacrifice which was done after the completion of the mating process.
- Female rats: exposure for at least 10 weeks prior to mating. Treatment was continued through mating, pregnancy and up to the weaning of F1 offspring, after which, parental females were sacrificed. F1-generation offspring were treated from weaning till they were sacrificed after obtaining F2 weanlings.
- STANDARDISATION OF LITTERS- Performed on day 4 postpartum, a maximum of 8 pups/litter (4/sex/litter as nearly as possible); excess pups were killed and discarded.
- Parameters assessed for P:
  - CAGE SIDE OBSERVATIONS: Yes- Time schedule: daily
  - DETAILED CLINICAL OBSERVATIONS: Yes- Time schedule: daily

- BODY WEIGHT: Yes- Time schedule for examinations: Individual body weights of parental rats were recorded initially and at weekly intervals thereafter in males and in the pre mating period in females for both generations. All dams were weighed on presumed gestation days 0, 7, 14 and 20 and on lactation days 1, 4, 7, 14 and 21 and weights were recorded.
- FOOD CONSUMPTION: Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes. After day 0 of pregnancy the food intake was recorded on gestation days (GD) 7, 14, 20 and on lactation days (LD) 4, 7, 14, 21.
- WATER CONSUMPTION: Yes- Time schedule for examinations: thwice a week for males and females. After Day 0 pregnancy, the water intake was recorded on presumed GDs 4, 7, 10, 14, 18 and 20 and on LDs 4, 7, 11,14, 16, 18 and 21. Food and water consumption for both sexes was not measured during the cohabitation period.
- Estrous cyclicity: The oestrous cycle length and pattern were evaluated by vaginal smears examination for all females during a minimum of 2 weeks prior to mating and during mating. The oestrous cycle length was calculated for all females as the period between two successive diestrus stages.
- Sperm parameters: For all the P and F1 males at termination, sperm from the right vas deferens was collected for evaluation of sperm motility. The sperm smears for the sperm morphology were prepared for all animals but evaluation was performed for the randomly selected 10 animals per group only. Likewise the right testis and corresponding epididymis were collected from all males for enumeration of homogenisation detergent resistant testicular spermatids and cauda epididymal sperm reserves, respectively. The sperm count was restricted to the selected animals. As there were no treatment-related effects observed in the sperm morphology, testicular spermatid count and epididymal sperm count, the examination was not extended to the remaining animals in control and high dose as well as all the animals in the lower dose groups. The frozen testes and epididymides samples were discarded.
- Postmortem examinations:
    - SACRIFICE- Male animals: All surviving animals were sacrificed after completion of the mating process. Maternal animals: All surviving animals were sacrificed after the last litter of each generation was weaned.
    - GROSS NECROPSY- Gross necropsy consisted of the following tissues and organs for P and F1 parental animals: Adrenal glands, Brain, Epididymides, Gross lesions, Kidneys, Testes, Liver, Ovaries, Pituitary, Prostate, Sciatic nerves, Seminal vesicles and coagulating glands and their fluid, Spinal cord (cervical, thoracic and lumbar), Spleen, Thyroid with parathyroids, Uterus (with oviducts and cervix), Vagina. The

following tissues were collected from one randomly selected pup per sex per litter (F1 and F2 offspring): Brain, Coagulating glands, Epididymides, Gross lesions, Kidneys, Ovaries, Prostate, Seminal vesicles, Spleen, Testes, Thymus, Uterus and vagina

- HISTOPATHOLOGY / ORGAN WEIGHTS - The tissues indicated above were prepared for microscopic examination and weighed, respectively.

- Parameters assessed for F1 and F2:
  - Number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, weight gain, physical or behavioural abnormalities
  - GROSS EXAMINATION OF DEAD PUPS: no
  - Postmortem examinations:
    - SACRIFICE- The F1 offspring not selected as parental animals and all F2 offspring were sacrificed after weaning. These animals were subjected to postmortem examinations (macroscopic and/or microscopic examination) as follows:
    - GROSS NECROPSY- Gross necropsy consisted of the following tissues that were collected from one randomly selected pup per sex per litter (F1 and F2 offspring): Brain, Coagulating glands, Epididymides, Gross lesions, Kidneys, Ovaries, Prostate, Seminal vesicles, Spleen, Testes, Thymus, Uterus and vagina
    - HISTOPATHOLOGY / ORGAN WEIGTHS: The tissues indicated above were prepared for microscopic examination and weighed, respectively.
- Reproductive indices: Male mating index, female mating index, male fertility index, female fertility index and the fecundity index. Offspring viability indices: The following indices were determined: live birth index, 24-hour survival index, 4th day survival index, 7th day survival index, 14th day survival index and 21st day survival index.

Results and discussion

- Statistical treatment of results:

  The statistical analysis of the experimental data was carried out using the validated package in Excel and using licensed copies of SYSTAT Statistical package ver.12.0. All quantitative variables like body weight, feed intake, sperm parameters, organ weights and organ weight ratios data were tested for homogeneity of variances (Levene's test) within the group before performing One-way analysis of variance (ANOVA). When the data are found to be non-optimal (non-normal or heteroschedastic), ANOVA was done using suitable transformation. Comparison of means between treatment groups and vehicle control group was done using Dunnett's test when the overall treatment 'F' test is found to be significant. For the characters namely pre-implantation loss (%), post implantation loss (%), number of corpora lutea, implantations and pre-coital interval (days) was analysed after suitable transformation (Arc sine, $\sqrt{x + ½}$) of the data. One-way analysis of variance (ANOVA) was carried

out for the transformed data. Dunnett's pair-wise comparison of the treated means with the control mean was done when the group differences are found significant. Z test was performed for testing the differences in proportions for the characters namely mating and fertility indices. Since the parametric tests proposed above are expected to be applicable and more efficient, the non-parametric test (Kruskal Wallis followed by Mann Whitney U test) was used only for the non-normal data measured in the nominal and ordinal scales wherever necessary. Statistically significant differences ($p \leq 0.05$), indicated by the aforementioned tests are designated by the superscripts throughout the report as stated below: +/-: Significantly higher (+)/lower (-) than the vehicle control group

- PARENTAL ANIMALS AND F1

  CLINICAL SIGNS AND MORTALITY (Parental animals)

  P-Generation: There were no clinical signs and mortalities observed in control, 5 and 15 and 45 mg/kg bw/day doses. However, incidences of hair thinning with hair re-growth were randomly observed in all the groups. These were considered incidental as these are common findings in rodents.

  F1-Generation: There were no clinical signs and mortalities observed in control, 5 and 15 and 45 mg/kg bw/day doses. However, incidences of hair thinning with hair re-growth were randomly observed in all the groups. These were considered incidental as these are common findings in rodents.One male rat (Rk5986) died during blood collection in the 45 mg/kg bw/day dose group prior to sacrifice because of overdose of anaesthesia. One female rat died on GD 24 (Rk6042) due to dystocia in the 15 mg/kg bw/day dose group. The cause of death could not be ascertained as there were no gross and microscopic changes observed in this animal.

  BODY WEIGHT AND FOOD CONSUMPTION (Parental animals)

  P-Generation Males: The mean body weights were comparable with the control group during weeks 1 to 10 of treatment at 45 mg/kg bw/day dose group. However, the treatment significantly increased the mean body weights (5.9 to 7.1 %) during weeks 11 to 14 and net weight gains (16.6 %) at the end of 14 weeks of treatment when compared to vehicle control group. The weekly mean body weights and net weight gains were unaffected by the treatment at 5 and 15 mg/kg bw/day doses when compared to the vehicle control group. Terminal body weights observed at necropsy were significantly increased (8 %) at the highest test dose group. Significantly higher food intake was observed during weeks 2 to 10 (8.2 to 12.7 %) at 45 mg/kg bw/day dose when compared to the vehicle control. The food intake was unaffected by the treatment at 5 and 15 mg/kg bw/day doses when compared to the vehicle control group.

  P-Generation Females: The mean body weights were unaffected by the treatment at 45 mg/kg bw/day dose. However, the net weight gains were apparently higher (9.7 %) when compared to the vehicle control but statistically not significant at the end of 10 weeks of treatment. The weekly mean body weights and net weight gains were unaffected by treatment at 5 and 15 mg/kg bw/day doses

when compared to the vehicle control group. Terminal body weights observed at necropsy were not altered due to treatment with the test substance. Significantly higher food intake was observed during weeks 4 – 6 (7.9 to 9.3 %) at 45 mg/kg bw/day when compared to the vehicle control. The food intake was not altered by the treatment at 5 and 15 mg/kg bw/day doses.

F1-Generation Males: The weekly mean body weights and net weight gains as well as terminal body weights at necropsy were unaffected by treatment at all tested doses compared to the vehicle control group. The food intake was unaffected by the treatment at all tested doses compared to the vehicle control group. However, an incidence of significantly lower food intake during week 4 at 15 mg/kg bw/day dose observed was considered to be incidental because of its isolated occurrence.

F1-Generation Females: The mean body weights were unaffected by the treatment at 45 mg/kg bw/day dose. However, the net weight gains were apparently higher (8.5 %) when compared to the vehicle control but statistically not significant at the end of 10 weeks of treatment. The weekly mean body weights and net weight gains were unaffected by treatment at 5 and 15 mg/kg bw/day doses when compared to the vehicle control group. Terminal body weights observed at necropsy were not affected by the test substance. The food intake was not altered by the treatment at all the tested doses when compared to the vehicle control group.

WATER INTAKE

P-Generation Males: The water intake was significantly higher (22.1 to 40.0 %) during weeks 1 to 10 at 45 mg/kg bw/day dose when compared to the vehicle control. At 15 mg/kg bw/day, the water intake was significantly higher (12.5 to 23.4 %) during weeks 3 to 5 and 7 to 10 when compared to vehicle control. On weeks 1, 2 and 6, the water intake was apparently higher (7.6 to 10.5 %) when compared to vehicle control but statistically not significant. The water intake was unaffected by the treatment at 5 mg/kg bw/day dose when compared to the vehicle control group.

P-Generation Females: The water intake was not altered by the treatment at all the tested doses.

F1-Generation Males: Significantly higher water intake (10.1 to 31.7 %) was observed during weeks 2, 3 and 5-12 at 45 mg/kg bw/day when compared to the vehicle control. On weeks 1 and 4, the water intake was apparently higher (8 to 9.7 %) when compared to vehicle control but statistically not significant. At 15 mg/kg bw/day, the water intake was significantly higher (13.2 to 30.4 %) during weeks 6, 8, 10 and 12 when compared to vehicle control during premating period. During weeks 7, 9 and 11, the water intake was apparently higher (6.3 to 14.6 %) when compared to vehicle control but statistically not significant. The water intake was unaffected by treatment at 5 mg/kg bw/day when compared to the vehicle control group except for incidental finding of significantly higher water intake observed during week 10.

F1-Generation Females: The water intake was not altered by the treatment at all the tested doses.

REPRODUCTIVE FUNCTION: ESTROUS CYCLE (PARENTAL ANIMALS)

P-Generation: The calculated mean oestrous cycle length was 4.07, 3.90, 3.85 and 3.95 days in vehicle control, 5, 15 and 45 mg/kg bw/day doses, respectively. The mean oestrous cycle length in the treated groups was not significantly different from the vehicle control group.

F1-Generation: The calculated mean oestrous cycle length was 3.89, 4.43, 4.0 and 4.05 days in vehicle control, 5, 15 and 45 mg/kg bw/day doses, respectively. The oestrous cycle length was significantly higher at 5 mg/kg bw/day dose. This was considered as incidental as there was no dose dependency observed.

REPRODUCTIVE FUNCTION: SPERM MEASURES (PARENTAL ANIMALS)

There were no intergroup differences in the percentage of total motility, percentage of progressive sperm motility and sperm morphology evaluated. Cauda epididymal sperm counts and testicular spermatid counts data were comparable between control and 45 mg/kg bw/day dose group. The minimal weight decrease observed in the cauda epididymides weight at 45 mg/kg bw/day did not show any changes in the sperm counts and hence considered as not toxicologically relevant. The percentage of progressive motile sperms in 15 and 45 mg/kg bw/day dose groups were higher. These minimal changes however were considered as not toxicologically relevant.

REPRODUCTIVE PERFORMANCE (PARENTAL ANIMALS)

P-Generation:

Pre-coital time: The mean pre-coital interval was apparently higher but statistically not significant in all the doses tested. This was considered incidental, as the normal biological range is 1-4. Further this was within the historical range (HD for mean precoital time is 2.57 days with a range of lowest 1 to highest 18 days).

Gestation Length: There were no treatment-related effects on the gestation length (average days to litter) at 5 and 15 mg/kg bw/day doses. At 45 mg/kg bw/day dose, the gestation length (average days to litter) was significantly longer when compared to concurrent vehicle control. This was however considered incidental as the increase was within the historical range.

Fertility Indices: No treatment-related changes were observed in the fertility indices of sires and dams. The incidences of higher male and female fertility indices observed at 45 mg/kg bw/day were considered toxicologically insignificant as the significance was due to the slightly lower control value. No treatment-related changes were observed in the uterine/implantation data except slightly higher post-implantation loss at 45 mg/kg bw/day dose, which subsequently led to lower mean litter size. The post-implantation loss observed was due to the lower number of pups in four dams (Rk5876, Fk5879, Rk5881, and Rk5889) only.

F1-Generation:

Pre-coital time: The mean pre-coital time was apparently higher but statistically not significant in all the doses tested. This was considered incidental, as the normal biological range is 1-4 as per the

available literature. Further this was within the historical range (HD for mean precoital time is 2.53 days with a range of lowest 1 to highest 12 days).

Gestation Length: There were no treatment-related effects on the gestation length (average days to litter) at all the tested doses.

Fertility Indices: No treatment-related changes were observed in the fertility indices of sires and dams at 5 and 15 mg/kg bw/day dose. At 45 mg/kg bw/day dose, the treatment significantly reduced the male and female fertility indices and was less than the Advinus historical control data (HD range: Male fertility index: 88 to 100 %, Female fertility index: 80 to 100%). However, literature showed historical ranges for male fertility index of 70 to 100% and for female fertility index of 72 to 100%, (Hood, 2nd Ed, Developmental and Reproductive Toxicology). In addition, there were no significant changes in the oestrous cyclicity, sperm parameters and reproductive organ weights and histology (including follicle count) in these animals. Hence, as it was only observed in F1 generation and in absence of other interrelated reproductive findings it was not considered a direct test article related reproductive effect but rather a possible indirect (stress related) effect.

ORGAN WEIGHTS (PARENTAL ANIMALS)

P-Generation:

Males: In liver, a significant increase in the absolute and relative weights was observed at 45 mg/kg bw/day. This change corresponded to the microscopic finding of higher incidences of increased hepatocyte cytoplasmic rarefaction in the livers at 45 mg/kg bw/day. In adrenals, a significant increase in the absolute and relative weights (combined and individual weights) was observed at 45 mg/kg bw/day in males. This increase corresponded to the microscopic finding of increased cortical cell vacuolation observed at 45 mg/kg bw/day in males. The increase in the absolute but not relative thyroid weight in 45 mg/kg bw/day dose group males was considered test item related but was not associated with any microscopic change in thyroid tissue. Some variations in other organ weights were noted but none were considered to be test item related.

Females: There were no statistically significant inter group differences in the organ weights of females.

F1-Generation:

Males: In males test item related increase in the liver weight (absolute and relative) was observed at 45 mg/kg bw/day. This change corresponded to the microscopic finding of higher incidences of increased hepatocyte cytoplasmic rarefaction in the livers at 45 mg/kg bw/day.

Females: There were no statistically significant inter group differences in the organ weights of females.

GROSS PATHOLOGY (PARENTAL ANIMALS)

There were no test item-related gross findings in males or females of the P and F1 generation.

HISTOPATHOLOGY (PARENTAL ANIMALS)

P-Generation: Microscopically, higher incidence of increased cytoplasmic rarefaction was observed in the liver at 45 mg/kg bw/day dose group in males. In females, higher incidences of focal basophilic hepatocytes and hepatocellular hypertrophy were observed at 45 mg/kg bw/day. Hepatocellular hypertrophy was of minimal severity and not observed in the lower dose groups. The basophilic hepatocytes involved approximately 10 to 15 hepatocytes with focal distribution. The relation of this lesion to test item administration is not clear as only low incidences were observed with minimal severity and focal distribution. In kidneys, higher incidences with minimal severity of dilated tubules were observed in 45 mg/kg dose groups of both males and females (11/25 and 3/25, respectively). In addition, a slightly more severe (mild) dilatation was observed also in males and females (10/25 and 13/25, respectively). Increased incidences were also observed at 15 mg/kg bw/day in males (11/25) and females (3/25). Adrenals showed higher incidences of cortical vacuolation in males at 45 mg/kg bw/day. In thyroid glands, increased colloid was observed in the follicular lumen at 45 mg/kg bw/day in females. Microscopic examination of reproductive organs did not reveal any test item related changes.

F1-Generation: Microscopically, higher incidences of increased cytoplasmic rarefaction were observed in the liver at 45 mg/kg bw/day in males. In females, incidences of basophilic hepatocytes were observed at 45 mg/kg bw/day. The basophilic hepatocytes involved approximately 10 to 15 hepatocytes with focal distribution. The relation of this lesion to test item administration is not clear as only low incidences were observed with minimal severity and focal distribution. In kidneys, higher incidences with minimal severity of dilated tubules were observed in males and females at 15 mg/kg (6/25 and 8/25 respectively) and 45 mg/kg (19/25 and 16/25 respectively) and were considered as test item related. In addition, slightly more severe (mild) dilation was only seen in 2/25 males of 45 mg/kg group. 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. Microscopic examination of reproductive organs did not reveal any test item related changes.

- Details on results (F1and F2 pups)

  VIABILITY (OFFSPRING)

  F1 pups: At the doses tested there were no treatment-related effects on the number of pregnancies of the parental generation, number littered and number of live litters. The mean litter and mean viable litter size was not altered by the treatment at 5 and 15 mg/kg bw/day doses. At the high dose, the mean litter size and mean viable litter size were significantly lower when compared to vehicle control, however the findings fell well within historical range (HD range for mean litter size is 8.3 to 12.3), whereas the control values (mean values of 12.2 and 12.0, respectively) were at the higher boundaries of the historical control range. Therefore this finding was not considered to be

toxicologically relevant. There were no external abnormalities in live or dead pups in any of the groups tested. No treatment-related changes were observed in the survival data of pups up to lactation day 21 at all the doses tested. An incidence of higher number of dead pups up to Day 4 was observed at 45 mg/kg bw/day dose. This variation was due to the fact that 8 pups were dead in one dam (Rk5879) of no good health. With this one dam the overall rate was within the historical control range of 2-16.

F2 pups: Test item at the doses tested had no treatment-related effects on the number of pregnancies of the F1 generation, number littered and number of live litters. The mean litter size and mean viable litter size were not altered by treatment. There were no external abnormalities in live or dead pups in any of the groups tested. No treatment-related changes were observed in the survival data of pups up to lactation day 21 at all doses tested.

CLINICAL SIGNS (OFFSPRING)

There were no clinical signs observed in control, 5 and 15 and 45 mg/kg bw/day doses of the F1 and F2 pups.

BODY WEIGHT (OFFSPRING)

There were no test item-related changes in terminal body weights in male and female pups of F1 and F2 litters.

SEXUAL MATURATION (OFFSPRING)

The average age at acquisition of balano-preputial separation in the control 5, 15 and 45 mg/kg bw/day exposure dose groups were 43.64, 43.20, 43.92 and 44.16 days, respectively. The mean age and body weights at acquisition of balano preputial separation were not affected by treatment when compared to vehicle control group. The average duration post weaning of vaginal opening (patency) in the control 5, 15 and 45 mg/kg bw/day exposure dose groups were 31.56, 31.72, 31.68 and 32.20 days, respectively. The mean age and body weights at acquisition of vaginal patency were not affected by treatment when compared to vehicle control group.

ORGAN WEIGHTS (OFFSPRING)

There were no test item-related changes in organ weights in male and female pups of F1 and F2 litters.

GROSS PATHOLOGY (OFFSPRING)

No gross internal lesions or external abnormalities were observed in male and female pups of F1 and F2 litters.

HISTOPATHOLOGY (OFFSPRING)

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

OTHER FINDINGS (OFFSPRING)

Pinna detachment:

F1 litters: The observation for pinna detachment was started on postnatal day (PND) 1. The first sign of detachment began on PND 2 in all groups and the process was completed on PND 4 in all groups. The pinna detachment was seen in a significantly higher percentage of pups on PND 2 and 3 at 45 mg/kg bw/day dose. This earlier onset was considered incidental.

F2 litters: The observation for pinna detachment was started on postnatal day (PND) 1. The first sign of detachment began on PND 2 in all groups and the process was completed on PND 4 in all groups. The pinna detachment was seen in a significantly higher percentage of pups on PND 3 at 5 mg/kg bw/day and PND 2 and 3 at 45 mg/kg bw/day doses. This earlier onset was considered not toxicologically relevant.

Incisor Eruption:

F1 litters: The observation for incisor eruption was started on PND 7. The first sign of eruption of incisors was noticed on PND 9 in control, 5 and 15 mg.kg bw/day doses and on PND 8 in the 45 mg/kg bw/day dose. The eruption was completed in all pups on PND 12 at 5 and 15 mg/kg bw/day doses and was delayed by one day (PND 13) in control and 45 mg/kg bw/day dose. The incisor eruption was seen in a significantly higher percentage of pups on PND 11 and 12 at 5 mg/kg bw/day dose, PND 9, 11 and 12 at 15 mg/kg bw/day and on PND 9 at 45 mg/kg bw/day doses. This change was considered not toxicologically relevant as there was no dose dependency observed.

F2 litters: The observation for incisor eruption was started on PND 7. The first sign of eruption of incisors was noticed on PND 8 in the 5 mg/kg bw/day dose and was delayed by one day in other groups. The eruption was completed in all pups on PND 13 in all the groups. The incisor eruption was seen in a significantly lower percentage of pups on PND 10 and 12 at 15 mg/kg bw/day dose and at a significantly higher percentage of pups on PND 9 to 11 at 45 mg/kg bw/day doses. This earlier onset was considered not toxicologically relevant as there was no dose dependency observed.

Ear Opening:

F1 litters: The observation for ear opening was started on PND 10 and continued until the criterion was met in all pups. The ear canal of both ears began to open on PND 12 in all the treated groups and was delayed by one day in the control group. The ear opening was completed on PND 15 in 5, 15 and 45 mg/kg/bw/day doses and was delayed by 1 Day in the control group. No significant changes were observed between the control and treated groups.

F2 litters: The observation for ear opening was started on PND 10 and continued until the criterion was met in all pups. The ear canal of both ears began to open on PND 12 in all the groups. The ear opening was completed on PND 15 in all the groups. The ear opening was seen in a significantly higher percentage of pups on PND 12 at 45 mg/kg bw/day dose group. This earlier onset was considered not toxicologically relevant due to lack of dose correlation.

Eye Opening:

F1 litters: The observation for the total separation of the upper and lower eye lids and the complete opening of both eyes was started on PND 13 and the first sign of eye opening began on PND 14 in all groups. The eye opening was completed in all pups on PND 16 in all the treated groups and was delayed by one day in the control group. The eye opening was seen in a significantly higher percentage of pups on PND 14 and 15 at 45 mg/kg bw/day dose. This earlier onset was considered not toxicologically relevant.

F2 litters: The observation for the total separation of the upper and lower eye lids and the complete opening of both eyes was started on PND 13 and the first sign of eye opening began on PND 14 in all groups. The eye opening was completed in all pups on PND 17 in all groups. The eye opening was seen in a significantly higher percentage of pups on PND 15 and 16 at 5 mg/kg bw/day, PND 15 at 15 mg/kg bw/day and on PND 14 and 15 at 45 mg/kg bw/day doses. This earlier onset was considered not toxicologically relevant.

- Effect levels for P, F1 and F2:
  NOAEL (P-generation): 15 mg/kg bw/d (male/female, basis for effect level: systemic toxicity)
  NOAEL (F1-generation): 45 mg/kg bw/d (male/female, basis for effect level: reproductive and foetal toxicity)
  NOAEL (F2-generation): 45 mg/kg bw/d (male/female, basis for effect level: reproductive and foetal toxicity)

3.3.1.4 Zarnescu and Zamfirescu, 2006

Study reference:

Zarnescu, O.; Zamfirescu, G., Effects of lithium carbonate on rat seminiferous tubules: an ultrastructural study, International Journal of Andrology, 29, 576-582, 2006 (Zarnescu und Zamfirescu, 2006)

Detailed study summary and results:

Test type

Male fertility screening study. Albino Wistar rats were exposed to lithium carbonate dissolved in physiological saline for 21 days. No GLP compliance is given.

Test substance

- Test material used in the study is lithium carbonate
- Degree of purity: no information available
- Impurities: no information available
- Batch number: no information available

Test animals

- Male Wistar rats
- No. of animals per sex per dose: Control group 4 males, test group 10 males
- Age at study initiation: 4-6 month
- Weight at study initiation: 100-120 g

Administration/exposure

- Route of administration: oral (gavage)
- Duration of treatment: 21 days
- Frequency of treatment: Once a day
- Doses: 0, 35 mg/kg bw/day, animals received once a day 0.5 mL oral gavages of 0.7 % lithium carbonate aqueous solution.
- Control group treated as exposed animals but with physiological saline
- No historical control data available
- Vehicle: physiological saline
- Test substance formulation: no further information available

Description of test design:

- Animals were not mated
- At the end of the experiment, all animals were killed, and testes were removed. Testes fragments were fixed in 2.5 % glutaraldehyde, buffered at pH in 0.1 M sodium cacodylate, then post-fixed in 1 % ismium tetraoxide, in the same cacodylate buffer, dehydrated and embedded in Epon 812. Ultra-thin sections were stained with uranyl acetate and lead citrate, and then studied with Philipps 208S electron microscope operating at 80 KV (208S Phillips/FEI, Brno, Czech Republic).

Results and discussion

- In the seminiferous epithelium loss of germ cell attachment and appearance of expanded intercellular spaces between spermatogenic cells were observed. Early stages of spermatogenic cells showed nuclear protrusions or swellings because of an extensive enlargement of the outer nuclear membrane. Round spermatids exhibited abnormally shaped acrosomes and dilation of the subacrosomal space. Many abnormal, degenerated late spermatids with random orientation were seen towards the basal and adluminal compartments of the seminiferous epithelium. In addition, spermatids exhibited alteration in F-actin bundle ectoplasmic specialization and contained many mitochondria-associated granular bodies.
- No quantitative analysis of results was done, only microscopic pictures are shown in the results part of the publication.
- Under the conditions of the present study, ultrastructural findings in rat seminiferous tubules were observed (e.g., loss of germ cell attachment, tunica propria effects, damage of spermatogenic cells, spermatids). The deduction of a NOAEL was not possible (single dose).

3.3.1.5 Allagui et al., 2006

Study reference:

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

Detailed study summary and results:

Test type

Influence of 28 day lithium exposure on thyroid and sex hormone levels

Test substance

- Lithium carbonate
- Degree of purity: no information
- Batch number: no information

Test animals

- Species/strain/sex: male and female wistar rats
- No. of animals per sex per dose
- Age and weight at the study initiation: “mature” about 100g.

Administration/exposure

- Route of administration – oral (feed)
- duration and frequency of test/exposure period: 28 days
- doses/concentration levels, rationale for dose level selection: 0, 2000, 4000 mg/kg diet/day
- control group and treatment: normal food

- vehicle: normal food

Description of test design:

The animals weight was measured daily for establishing growth curves. Food consumption per cage was measured daily in order to determine lithium uptake.

On the seventh, fourteenth, twenty-first and twenty-eight day, animals were rapidly sacrificed in the early morning with minimum stress handling. Collected blood samples were immediately spun down and serum was maintained at -20°C until use. The testes, prostate, epididymis, ovaries and uterus were rapidly taken,-cleared of fat, weighed and frozen at - 200C. On day 28, the testes were fixed in Bouin's fixative (48 hours), embedded in paraffin and colored with hematoxyline-eosine for histological study.

- RIA analysis of serum hormones

Calibration (standard curves) and measures were performed following the manufacturer's recommendations (immunoassay kits, Immunotech®, Marseille, France). 125I-labelled antibody raised against thyroxine (T4) was incubated together with serum samples in the presence of a biotinylated thyroxine-like ligand, in avidine-coated tubes. I251-labelled antibody to tri-iodothyronine (FT3) was incubated together with serum samples in FT3 analog-coated tubes. 251-labelled progesterone or estradiol was incubated together with serum and in tubes previously coated with specific anti-testosterone or anti-estradiol antibody. In all cases, after incubation, the free fraction was removed by aspiration and washing. Fixed radioactivity was measured with a gamma-counter and serum hormone concentrations, in inverse proportion to radioactivity, were interpolated from standard curves. All measures were carried out in duplicate.

Results and discussion

- Effect of lithium poisoning on body growth and food consumption

Adjunction of 2 g and 4 g LiCO3/kg of food resulted in growth arrest and a dose-dependent decrease of body weight in both male and female animals. In the Li2 group, the treatment was stopped on day 14 because of a mortality rate of 50-60%. Throughout the first 2 weeks of the experiment, food consumption was found to decrease in a dose and time-dependent manner in treated animals compared to controls (C). As calculated from the ingested food quantity, the respective amounts of absorbed lithium carbonate in the Li1 and Li2 groups were 0.17 and 0.20 g/male rat during the first week, then 0.12 and 0.17 g/male rat during the second week. Similar changes were found in female rats. In the Li1 group, serum lithium concentrations during the first 2 weeks (0.44-0.62 mmol/L) were found to be close to that used for the treatment of bipolar disorders in human (0.4-0.8 mM). However, during the third and fourth week, Li concentrations (1.02-1.34 mmol/L) exceeded the toxicity threshold. In the Li2 group, on day 14 the amount of ingested lithium carbonate resulted in toxic serum lithium concentrations, in male and female rats (1.45 and 1.29 mmol/L, respectively).

- Effect of lithium poisoning on circulating thyroid hormones

The serum levels of FT4 and FT3 decreased in a dose-dependent manner in both male (Figure 2A, C) and female (Figure 2B, D) lithium-treated rats. Table 3 shows that the FT4/FT3 ratio was almost similar in the male and female control animals. This ratio was significantly increased in the Li2 group on days 7 and 14, for serum lithium concentrations ranging from 0.66 to 1.45 mmol/L. In the Li1 group, this ratio was only

increased in males on days 21 and 28 (serum lithium concentrations < 1.27 mmol/L). The increase of the FT4/FT3 ratio was mainly due to the more important decrease of the FT3 concentrations. Compared to controls, FT3 levels were decreased by 61% on day 28 in males of the Li1 group or on day 7 in males of the Li2 group, whereas the corresponding FT4 levels were decreased by 30 and 38% only. In Li2 females, the FT3 level was decreased by 73% on day 14, whereas the FT4 level was down by 44% only.

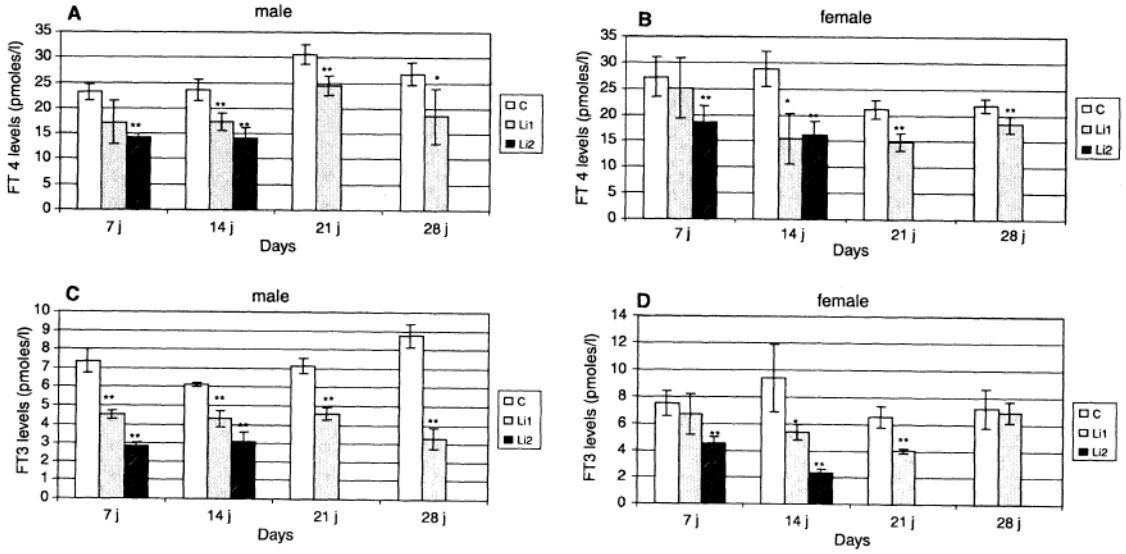


Table 3 Free thyroxine (FT4) over free tri-iodothyronine (FT3) ratio in serum of rats given either normal food pellets (C = controls) or food pellets containing either 2 g (Li1) or 4 g (Li2) lithium carbonate/kg pellets after 7, 14, 21 and 28 days

	Male			*Female*		
	C	*Li1*	*Li2*	*C*	*Li1*	*Li2*
Day 7	3.15 ± 0.21	3.75 ± 0.76	4.97 ± 0.47**	3.65 ± 0.46	3.76 ± 0.42	4.14 ± 1.00*
Day 14	3.90 ± 0.40	4.06 ± 0.22	4.56 ± 0.24**	3.43 ± 0.83	3.00 ± 1.10	6.90 ± 1.43**
Day 21	4.31 ± 0.50	5.52 ± 0.51**		3.26 ± 0.51	3.68 ± 0.49	
Day 28	3.08 ± 0.39	5.25 ± 1.37**		3.12 ± 0.49	2.67 ± 0.27	

*$P < 0.05$.
**$P < 0.01$.
Values correspond to the mean ± SD established from four animals.

- Effect of lithium poisoning on circulating sex hormones

Compared to controls, testosterone level was decreased by > 50% on day 7 (serum Li concentration: 0.75 mM) and day 14 in Li2 male rats, whereas in Li1 animals, such a decrease was observed on days 21 and 28. In the females of the Li1 group, estradiol levels were significantly increased by 54% on day 21 and by 91% on day 28. In the Li2 animals, a 97% increase was obtained on day 14. The increase of estradiol level appeared for Li concentration >1 mM. Interestingly, testosterone and estradiol levels were shifted in opposite directions, but both in dose- and time-dependant manners.

Table 4 Serum concentrations of testosterone (ng/mL) in male rats given either normal food pellets (C) or food pellets containing 2 g (Li1) or 4 g (Li2) Li carbonate/kg pellets

Testosterone (ng/mL)	*Day 7*	*Day 14*	*Day 21*	*Day 28*
C	1.23±0.33	1.40±0.37	3.07±0.68	1.96±0.32
Li1	0.89±0.36 (−28%)	1.14±0.40 (−19%)	1.53±0.54* (−50%)	0.84±0.32** (−57%)
Li2	0.55±0.10* (−55%)	0.53±0.09* (−62%)		

*$P < 0.05$, Student's t-test.
**$P < 0.01$, Student's t-test.
Values correspond to the mean±SD established from four animals.

Table 5 Serum concentrations of estradiol (pg/mL) in female rats given either normal food pellets (control, C) or food pellets containing 2 g (Li1) or 4 g (Li2) Li carbonate/kg pellets

Estradiol (pg/mL)	*Day 14*	*Day 21*	*Day 28*
C	19.53±0.49	27.69±1.02	18.82±1.23
Li1	25.85±2.35 (+32%)	42.63±6.61* (+54%)	35.92±4.74** (+91%)
Li2	38.51±1.65** (+97%)		

*$P < 0.05$, Student's t-test.
**$P < 0.01$, Student's t-test.
Values correspond to the mean±SD established from four animals.

- Effect of lithium on spermatogenesis

Compared to controls, strong abnormalities appeared in the testes of Li1-treated animals, on day 28. On day 28, 73±2% of azoospermia was found and 70±5% of spermatozoa were deprived of flagella. Similar observations were found on day 14 in Li2-treated rats.

- Effect of lithium poisoning on the estrous cycle

Cytological examination of the vaginal epithelium in rats allows the determination of the estrous cycle phases. As reported in Table 6, most of control animals were in estrus or post-estrus phases, while Li1-treated animals were mostly in post-estrus on days 7 and 14 and in diestrus on days 21 and 28. Similarly, on day 14, Li2-treated animals were mostly in post-estrus or diestrus.

Table 6 Cytological examination of the vaginal epithelium in control rats (C) or in rats given food pellets containing 2 g (Li1) or 4 g (Li2) lithium carbonate/kg pellets

	Day 7				*Day 14*				*Day 21*				*Day 28*			
	Pr	*E*	*Po*	*Di*	*Pr*	*E*	*Po*	*Di*	*Pr*	*E*	*Pe*	*Di*	*Pr*	*E*	*Po*	*Di*
C	1	5	3			6	2			4	3			5	3	
Li1		1	8		2	1	5	1			2	7			2	8
Li2	1	4	2	3			6	7								

Values correspond to the number of rats in pro-estrus (Pr), estrus (E), post-estrus (Po) and di-estrus (Di).

3.3.1.6 Thakur et al., 2003

Study reference:

Thakur, S.C.; Thakur, S.S.; Chaube, S.K.; Singh, S.P., Subchronic supplementation of lithium carbonate induces reproductive system toxicity in male rat, Reproductive Toxicology, 17, 683-690, 2003 (Thakur et al., 2003)

Detailed study summary and results:

Test type

Effect of subchronic exposure (90 days) on reproductive organs of male Wistar rats. No GLP compliance is given.

Test substance

- Test material used in the study is lithium carbonate
- Degree of purity: no information available
- Impurities: no information available
- Batch number: no information available

Test animals

- Male Wistar rats
- No. of animals per sex per dose: 20 male animals per dose group
- Age at study initiation: sexually mature animals (no further information available)
- Weight at study initiation: 210 ± 10 g

Administration/exposure

- Route of administration: oral (in feed)
- Duration of treatment: 90 days
- Frequency of treatment: daily via feed
- Doses levels: 500, 800, 1100 mg/kg of diet for 90 days
- Control group: control animals received vehicle control feed
- Historical control data: no information available
- Vehicle: no information available
- Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation: no information available
- Actual doses (mg/kg bw/day): no information available

Description of test design:

- Three experiments were performed:
    - Experiment 1: Male animals treated as described above and sacrificed after 90 d of exposure
    - Experiment 2: Male animals treated as described above. After 90 d of exposure animals were caged with unexposed females to determine fertility index
    - Experiment 3: Male animals treated as described above. After 90 d of exposure and a 30 d recovery period animals were caged with unexposed females to determine fertility

- Details on mating procedure:
  - M/F ratios per cage: 1 :2
  - The day of sperm detection in vaginal smear was considered as day 0 of pregnancy
- The dams were killed on day 21 post-conception and fertility index was analysed
- Pups were not analysed
- In experiment 1: organ weights of testis, epididymis, seminal vesicle and prostate were determined and organs were analysed histologically. Sperm parameters and serum testosterone were measured.
- In experiments 2 and 3 fertility indices were analysed (mating index, male fertility index)
  Mating index (%) = (number of females showing evidence of mating/number of females placed with males) x 100
  Male fertility index (%) = (number of males that became sire/number of males placed with females) x 100

Results and discussion

- Statistical analysis:
  Fertility data and proportions were analysed by using the Chi-square-test. All other data were analysed by ANOVA followed by two-tailed t-testor Mann-Whitney when ANOVA test yielded statistical differences ($p < 0.05$ or 0.01)
- Starting at 800 mg/kg diet reduced absolute weight of testes, epididymis and accessory sex organs was observed, relative organ weights were not affected. See Table AI - 3 for details.

Table AI - 3: Weight of genital organs. Values presented are mean ± SD

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

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

- Dose dependent effects (reduced sperm number from cauda epididymis and the daily sperm production, reduced serum testosterone and testicular interstitial fluid volume) were observed, being significant at 800 mg/kg diet. Number of abnormal spermatozoa was already significant increased at the lowest dose. For details see Table AI - 4 and Table AI - 5.

  Histological analysis showed in the highest dose severe degenerative changes in the testes and accessory reproductive organs. These effects were also observed at 800 mg/kg diet to a milder degree.

Table AI - 4: Effect of lithium carbonate exposure on serum testosterone level and interstitial fluid volume (IFV). Values presented are mean ± SD

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

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

Table AI - 5: Effect of lithium carbonate exposure on sperm parameters. Values presented are mean ± SD

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

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

- Experiment 2:

  Significantly decreased male fertility index at 800 mg/kg diet and above, mating index was not affected (see Table AI - 6).

- Experiment 3:

  Significantly decreased male fertility index at 800 mg/kg diet and above, mating index was not affected (see Table AI - 6).

Table AI - 6: Effect of lithium carbonate on fertility parameters. Values presented are mean ± SD

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

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

3.3.1.7 Banerji et al., 1986

Study reference:

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

Detailed study summary and results:

Test type

Mouse, female fertility study

Test substance

- Lithium chloride
- Degree of purity: no information
- Batch number: Ralston Purina Company, Richmond, IN

Test animals

- Species/strain/sex: virgin C57BL/6 mice
- No. of animals per sex per dose: 20
- Age and weight at the study initiation: adult

Administration/exposure

- Route of administration – oral (feed)
- duration and frequency of test/exposure period: 15 days
- doses/concentration levels, rationale for dose level selection: 0.4% in diet
- control group and treatment: standard laboratory chow

- vehicle: standard laboratory chow

Description of test design:

Vaginal smears, obtained from both the treated and control groups, were examined each morning for the next 15 days. The aims of this experiment were to evaluate whether repeated lithium treatment (a) had an adverse effect on the estrous cycle, and (b) affected the proestrous LH surge in the event lithium did not interfere with the regularity of the estrous cycle.

Results and discussion

Results of the effects of chronic lithium treatment on the estrous cycle in mice are presented in Fig. 2. None of the mice showed any irregularity in their estrous cycle for the first 3 days following the introduction of food containing lithium. On day 4, however, 30 percent of the mice on lithium showed an irregularity in their estrous cycle since they had entered diestrus unexpectedly. The percentage of experimental mice showing an abnormal estrous cycle gradually increased on days 5, 6 and 7 (60, 80 and 96%, respectively) until 100% of the lithium-treated mice displayed a constant diestrous vaginal smear on day 8. Thus, once an abnormality in the estrous cycle was initiated, as evident from diestrous vaginal smear, the animals remained in persistent diestrus for the remainder of the experimental period (Fig. 2).

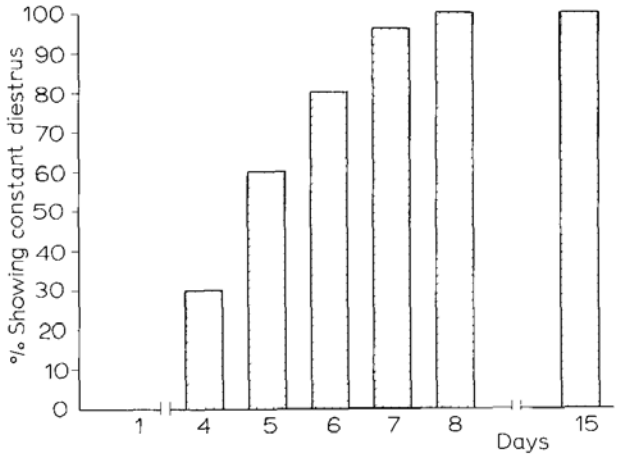


Fig. 2. Effects of long-term lithium treatment on the estrous cycle in mice.

3.3.1.8 Abu-Taweel et al, 2012

Study reference:

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

Detailed study summary and results:

Test type

Neurodevelopmental Toxicity Study in Swiss-Webster mice, no GLP compliance is given.

Test substance

- The test material used in the study is lithium chloride
- EC number: 231-212-3
- CAS number: 7447-41-8
- Degree of purity: analytical grade (no further information available)
- Impurities: no information available
- Batch number: no information available

Test animals

- Male and female Swiss Webster mice
- At least 7 pregnant females per dose group; 3 pups per litter investigated per test per PND 1-21
- Age at study initiation: 8-9 weeks, dams were not weighted

Administration/exposure

- Route of administration – oral (drinking water)
- Duration: from GD 1 to PND 15 of pups
- Frequency of exposure: over the whole exposure duration via drinking water, from PND 16 -21 dams received deionized distilled water without lithium chloride
- Doses: 0, 15, 30 mg lithium chloride/kg bw/day
- Rationale for dose selection: on the basis of literature reported for rodents and pilot studies in the laboratory of the authors (no further information available)
- Control animals received deionized distilled water only.
- No historical control data available
- Vehicle: deionized distilled water
- Test substance formulation: Lithium chloride was dissolved in deionized distilled water. The Lithium doses were calculated on the basis of the average total volume of drinking water consumed by the animals in 24 h.

Description of test design:

- Details on mating procedure (M/F ratios per cage: 1:3, length of cohabitation: until appearance of vaginal plug)
- Premating exposure period: none
- Standardization of litters: yes on PND 0, 8 pups per dams
- Parameters assessed for P:

No assessment of maternal toxicity

- Parameters assessed for F1:
  - Body weight: Pups were weighted every second day
  - Observation of day of eye opening and hair appearance
  - Behavioural observations: Three pups of each litter were colour-marked and subjected to various behavioural tests every second day.
    - Righting reflex
    - Cliff avoidance
    - Rotating reflex
    - Locomotor activity (tested only on PND 22)
  - Biochemical studies

    On PND 22 one male animal from every litter was sacrificed, brains and liver were removed. Acetylcholinesterase (AChE) activity was determined in brain tissue and total acid phosphatase (AcP) and alkaline Phosphatase (AIP) in liver homogenate.

Results and discussion

Note: Since dams were exposed during gestation until PND 15, pups were also exposed to lithium via lactation. Due to this fact, only data obtained on PND 0 or 1 can be used to determine developmental toxicity of lithium during pregnancy, because later in their life it cannot be excluded that effects are caused by lithium transmitted via milk from their exposed mothers.

- Statistical analysis:

  Data on body weight, dates of morphological developments, sensory motor reflexes and biochemical analyses were compared within the experimental groups by analysis of variance (ANOVA) and subsequently by Student-Newman-Keuls multiple comparison test. Significance levels were defined by $P \leq 0.5$, 0.01 and 0.001. Data on locomotor activity were compared within the experimental groups by ANOVA and subsequently were analysed using Mann-Whitney U tests.

- Body weight:

  Dose-dependent reduction in body weight on PND 1. Significantly reduced body weight in the high dose exposure group on PND 1 ($P < 0.001$). No information on significance in low dose available (no further information available)

- Observation of day of eye opening and hair appearance:

  Eye opening and body hair appearance were significantly delayed by treatment. However these effects cannot be evaluated any further in this context since exposure of dams continued during lactation.

- Behavioural observations:
    - Righting reflex, cliff avoidance and rotating reflex
      On PND 1 righting reflex time, cliff avoidance time and rotating reflex time were dose dependently and significantly increased ($P < 0.001$).
    - Locomotor activity (tested on PND 22)
      Inhibition of locomotor activity of male, weaned pups (females not investigated). These effects cannot be evaluated any further in this context since exposure of dams continued during lactation.

- Biochemical studies (on PND 22)
  Acetylcholinesterase (AChE) activity in brain tissue was found to be inhibited in a dose dependent and significant way.
  Total acid phosphatase (AcP) and alkaline Phosphatase (AIP) in liver homogenate were also found to be inhibited in a dose dependent and significant way.
  These effects cannot be evaluated any further in this context since exposure of dams continued during lactation.

3.3.1.9 Anonymous, 2010b

Study reference:

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

Detailed study summary and results:

Test type

Test performed according to OECD Guideline 414 (Prenatal Developmental Toxicity Study, version from 2001), GLP compliance is given (including certificate)

Test substance

- Test material used in the study is lithium carbonate
- Degree of purity: 99.6 % (not confidential)
- Impurities do not affect the classification
- Batch number: 0000001125 (not confidential)

Test animals

- Male and female Crl: CD(SD) rats
- No. of animals per sex per dose: 25
- Age at study initiation: 8-9 weeks
- Weight at study initiation: 185 - 234 g

Administration/exposure

- Route of administration – oral, gavage
- Duration and frequency of exposure period: daily from GD 6 - 19
- Dose groups: 0, 10, 30 or 90 mg Lithium Carbonate/kg b.w./day
- Control group received the vehicle (methocel) at a constant volume of 5 mL/kg b.w. orally once daily in the same way as exposed animals.
- Historical control data: no information available
- Vehicle: 0.5% aqueous hydroxypropyl methyl cellulose gel (Methocel)
- PREPARATION OF DOSING SOLUTIONS: The test item was suspended in the vehicle to the appropriate concentrations and was administered orally at a constant volume of 5 mL/kg b.w. once daily from the 6th to the 19th day of pregnancy. The dose of the test item was adjusted to the animal's body weight daily. The test item mixtures were freshly prepared every day approx. 1 h before use. Applied volume: 5 mL/kg bw/day
- Details on analytical verification of doses or concentrations:
  For the analysis of the test item formulations, samples of approx. 10 mL were taken at the following times: At the beginning of the administration period: Analysis of concentration/homogeneity. At start of administration, during (middle) administration and before administration to the last animal of each group (3 samples/dose level group). Total number of samples: 9. At termination of the administration period data time point when the majority of animals was dosed: Analysis of concentration/homogeneity. At start of administration, during (middle) administration and before administration to the last animal of each dose level group (3 sample/dose level group). Total number of samples: 9. Thus, the sum of all samples is 18. The samples were labelled with the study number, species, type of sample, test item, concentration, sampling time and date and were stored immediately after withdrawal at -20 degree C or colder until dispatch. The formulation samples were analysed for Lithium levels according to GLP by the Test Site AllessaChemie GmbH. The Phase Plan “Bestimmung des Lithiumgehaltes in Trägergemisch mittels ICP-OES (Teil-Prüfplan VP-Nummer 005/2010)” and any amendments to this Phase Plan are part of the LPT Study Plan 24635. The analysis of the test item-carrier mixtures for Lithium levels revealed that the formulations used for the administrations in groups 2 to 4 were correctly prepared. The measured actual concentrations ranged from 96.45 % to 103.64 % of the nominal values. The results were within the expected range of the theoretical concentrations.

Description of test design:

- Details on mating procedure:

  Sexually mature ('proved') male rats of the same breed served as partners. The female breeding partners were randomly chosen. Mating was monogamous: 1 male and 1 female animal were placed together in one cage during the dark period. Each morning a vaginal smear was taken to check for the presence of sperm. If findings were negative, mating was repeated with the same partner. The day on which sperm was found was considered as the day of conception (day 0 of pregnancy). This procedure was repeated until enough pregnant dams were available for all groups. Rats which did not become pregnant were excluded from the analysis of the results and replaced by other animals. A post-mortem negative staining according to SALEWSKI was carried out in the replaced animals in order to confirm the non-pregnancy status.
- Duration of treatment / exposure: From the 6th to the 19th day of pregnancy, no premating exposure period
- Frequency of treatment: Once daily from the 6th to the 19th day of pregnancy.
- Standardization of litters: no
- **Maternal examinations:**

  CAGE SIDE OBSERVATIONS: No data

  DETAILED CLINICAL OBSERVATIONS: Yes - Time schedule: Immediately after administration, any signs of illness or reaction to treatment were recorded. In case of changes, the animals were observed until the symptoms disappeared. In addition, animals were checked regularly throughout the working day from 7.00 a.m. to 3.45 p.m. On Saturdays and Sundays, the animals were checked regularly starting from 7.00 a.m. to 11.00 a.m. with a final check performed at approximately 3.30 p.m.

  BODY WEIGHT: Yes - Time schedule for examinations: The weight of each rat was recorded on day 0 of gestation (the day of detection of a positive mating sign), followed by daily weightings - always at the same time of the day. The body weight gain was also calculated in intervals (i.e. day 0-3, 3- 6, 6-9, 9-12, 12-15, 15-18 and 18-20).

  FOOD CONSUMPTION: Yes - Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes

  OVARIES AND UTERINE CONTENT: The ovaries and uterine content were examined after termination: Yes

  Examinations included: Gravid uterus weight, number of corpora lutea, number of implantations, number of early resorptions, number of late resorptions
- **Fetal examinations:**

Weights of foetuses and weights of the placentae were determined (foetuses were considered as runts if their weight was less than 70 % of the mean litter weight). Foetuses were inspected externally for damages, especially for malformations. The foetuses were sacrificed by an ether atmosphere.

Results and discussion

- Statistics:
  For all numerical values, homogeneity of variances was tested using the BARTLETT chi-square test. When the variances were homogeneous, the DUNNETT test ($p <= 0.01$) was used to compare the experimental groups with the control group. In case of heterogeneity of variances, the STUDENT's t-test was carried out, limit of significance was $p <= 0.01$. For the comparison of classification measurements (for example malformation-, resorption-, retardation- and variation rate) the FISHER's exact test ($n < 100$) or chi2-test with YATES' correction for continuity ($n >= 100$) ($p <= 0.05$ and $p <= 0.01$) was employed.

- **Details on maternal toxic effects:**
  Slight but significant reductions were noted for the net weight change and the food intake.

  Mortality: None of the dams treated with 10, 30 or 90 mg Lithium Carbonate/kg b.w./day died prematurely during the course of the study.

  Clinical signs: Pilo-erection was noted in four high-dosed dams treated with 90 mg Lithium Carbonate/kg b.w./day on two to four days, starting on gestation day 17 or 19 and lasting until laparotomy on gestation day 20. The drinking water intake of all high-dosed dams treated with 90 mg Lithium Carbonate/kg b.w./day was increased starting on gestation day 17, 18 or 19 and lasting until laparotomy on gestation day 20.

  Body weight and body weight gain: Marginal reductions were noted for the mean body weights of the high-dosed dams (90 mg Lithium Carbonate/kg b.w./day) during the last gestation days. The increase in the mean body weight from the start value (day 0 of pregnancy) was 66.9 % at the time point of laparotomy (control: 74.4%). Significant reductions (at $p \leq 0.01$) were noted for the net weight change of the high-dosed dams from day 6 of gestation to laparotomy on gestation day 20 (carcass weight minus day 6 body weight).

  Food consumption: Slight but statistically significant reductions (at $p <= 0.01$ or $p <= 0.05$) were determined for the relative food consumption of the high-dosed dams (90 mg Lithium Carbonate/kg b.w./day) on gestation days 7, 9, 11 to 13 and 19 (up to 18.3% below the control value).

Drinking water consumption: Increased intake of drinking water was noted in all high dosed females treated with 90 mg Lithium Carbonate/kg b.w./day on one to four days, starting on gestation day 17 (qualitative observation by visual appraisal).

Necropsy findings: No test item-related pathological findings were noted.

Uterus and carcass weights: The gravid uterus weight and the carcass weight were not influenced by the exposure to the test item.

Toxicokinetics: The toxicokinetic analysis based on Lithium plasma levels revealed a clear dose related systemic exposure to Lithium. Mean peak plasma levels of 1.66, 3.59 and 9.65 mg Li/L plasma, respectively, were observed at 10, 30 or 90 mg Lithium Carbonate/ kg b.w./day on gestation day 19. The plasma concentrations declined with a mean elimination half-life for Lithium between 8.4 to 12.0 hours. Toxicokinetics demonstrated dose proportional increases of Lithium plasma concentrations between 10 and 90 mg Lithium Carbonate/kg b.w./day. Peak time and half-life and increased with dose levels.

- **Details on fetal toxic effects:**
  No test item-related influence was noted on the prenatal fetal development at 10, 30 or 90 mg Lithium Carbonate/kg b.w./day with respect to the number of corpora lutea, implantation sites, resorptions, sex distribution, fetal and placental weights, number of live foetuses at birth and the values calculated for the pre- and post implantation loss when compared to the control. No dead foetuses or runts were noted at laparotomy.

  Malformations: No malformations were noted in the foetuses during external/ internal examination, skeletal examination (according to DAWSON) or soft tissue evaluation (according to WILSON).

  Variations: No test item-related variations were noted in the foetuses during external / internal examination, skeletal examination (according to DAWSON) or soft tissue evaluation (according to WILSON).

  Retardations: No test item-related influence was noted for the incidence of skeletal retardations.

- Effect levels for P and F1:
  NOEL (dams): 30 mg/kg bw/d (basis for effect level: systemic toxicity)

NOEL (F1-generation): 90 mg/kg bw/d (male/female, highest dose tested, basis for effect level: embryotoxicity)

3.3.1.10 Teixeira et al., 1995

Study reference:

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

Detailed study summary and results:

Test type

Prenatal and postnatal Developmental Toxicity Study

Test substance

- Lithium Chloride
- Degree of purity: no information
- Batch number: no information

Test animals

- Species/strain/sex: female wistar rats
- No. of animals per sex per dose: 44 females in lithium group, 46 females in deprived-water group, 13 females in control group
- Age and weight at the study initiation: adult, about 200g

Administration/exposure

- Route of administration – oral (drinking water)
- duration and frequency of test/exposure period: from GD1 to end of lactation. Upon birth, part of the litter from lithium and deprived-water groups were cross-fostered to form the following experimental groups: a) 22 litters that were given Li during the prenatal period and were submitted to water restriction during the lactating period (LiP group), b) 25 litter that were water restricted prenatally and given Li during the lactating period only (LiL group). Pups that received the same treatment postnatally as during the prenatal period formed the following groups: c) 13 litters that were neither given Li nor were water-restricted (control-NS group), d) 21 litter that were not given Li but were water restricted (Control-S group) and e) 18 litter that were given Li during the prenatal and lactating period (LiPL group).
- doses/concentration levels, rationale for dose level selection: 10 mM in drinking water
- vehicle: tap water

Description of test design:

The following tests were employed to evaluate offspring:

- Physical landmark of development
- Surface righting reflex
- Pup retrieval
- Motor coordination
- Sensory-motor performance

Results and discussion

No malformation was observed in any of the pups. The stillborn incidence was 2/144 (1.3%) in the control-NS group, 4/439 (1%) in the control-S group and 7/467 (1.5%) in the Li group.

Water deprivation or Li treatment did not affect the number of female pups born. In contrast, the mean number of male pups born was affected by treatments ($F(2,100) = 6.81$; $P \leq 0.01$), so that water deprivation or Li treatment reduced the overall number of male born ($P \leq 0.01$ in each comparison) compared to untreated litters.

There was a reduction in the proportion of pups with a normal righting reflex at birth ($x^2=26.48$). This reduction was particularly marked in both the water-deprived and Li treated litters ($P \leq 0.001$). The latter group had a reduced correct righting reflex even when compared to the water-deprived group ($P=0.01$). The latency to perform this task, however, was not affected by the treatments.

In the pup retrieval test, there was no difference in the average latency to pup rescue between the two control (control-NS= 13.1±5.6s, N=13; Control-S=21.0±10.4s N=46) and the treated group (15.0±9.4s, N=40) or in the proportion of females which did not recover their pups (control-NS=3%, control-S=5% and Li group=6%) within the stipulated time.

Lithium treatment or water deprivation did not affect birth weight. ANOVA revealed a body weight decreased in in water-deprived rats on day 7 ($F(1.94)=5.67$, $P \leq 0.001$), day 14 ($F(4.94)=6.57$, $P \leq 0.001$) and day 21 ($F(4.94)=8.85$ $P \leq 0.001$), which attained statistical significance when comparing the control-NS group to any of the experimental groups ($P \leq 0.01$ in each comparison).

Pups from postpartum Li treatment (i.e. LiL and LiPL litters) had lower body weights by day 21 when compared to the water-deprived group ($P \leq 0.05$ and $P \leq 0.01$, respectively). In contrast, Li treatment during the prenatal period did not influence body weight compared to the water-deprived group.

All treatment affected the critical day of maturation with the exception of pinna detachment or motor coordination, so that there was a reduction in the proportion of rats with a positive index on the critical day of eye opening ($x^2=98.28$, $P \leq 0.001$), and in the cliff avoidance test ($x^2=18.08$, $P \leq 0.001$). The Fisher exact test, applied to the eye opening date, gave a P value of 0.001 in all comparisons between the untreated and water-restricted or Li groups. The ability to avoid cliff was reduced in all groups when compared to the untreated group (P $P \leq 0.001$ in all cases), but was not different when Li treated litters were compared to water deprived ones.

3.3.1.11 Ibrahim and Canolty, 1990

Study reference:

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

Detailed study summary and results:

Test type

Prenatal and postnatal Developmental Toxicity Study

Test substance

- Lithium carbonate
- Degree of purity: no information
- Batch number: No. L-3876 Sigma Chemical Company, St. Louis, MO

Test animals

- Species/strain/sex: Female Sprague-Dawley rats
- No. of animals per sex per dose: 11 in control group and 13 in Li treated group
- Age and weight at the study initiation: 210 ±15g

Administration/exposure

- Route of administration – oral (feed)
- duration and frequency of test/exposure period: from GD1 to end of lactation
- doses/concentration levels, rationale for dose level selection: 1g/kg diet
- control group and treatment: AIN purified rat-mouse diet
- vehicle: AIN purified rat-mouse diet

Description of test design:

On day one of gestation, as determined by the presence of a copulatory plug, the females were assigned to either the control or lithium group. These groups were designated as C and L, respectively. Throughout gestation, one group was fed the control diet and the other fed the lithium-containing diet. Body weights and feed intakes were recorded daily. At parturition, after determining the total litter weight and the number of live and dead pups, the number of pups in each litter was reduced to six, standardizing by selecting males when possible. Dams in each of the two groups were assigned to one of four experimental groups during lactation. Half of the dams fed the control diet and half fed the lithium diet during gestation were continued on the same diet throughout lactation. These groups were designated as CC and LL, respectively. The diet for the other half of each group was switched; that is, the group fed the control diet during gestation was fed the lithium diet during lactation and vice versa (group CL and group LC, respectively). Body weights and feed intakes of dams were recorded daily. At the end of the 21-day lactation period, blood and organs (brain, heart, liver, kidney and spleen) were obtained from each dam and one of her randomly selected pups. The remaining pups were used in another experiment.

Results and discussion

Body weight changes and feed intakes of dams during gestation are shown in Table I. Pregnant dams ingesting lithium had lower body weight gains, feed intake and feed efficiencies during gestation than those consuming the control diet.

TABLE 1

Body Weight and Feed Intake of Dams Fed During Gestation Diets Supplemented With 0 (C) and 1,000 (L) ppm Lithium Carbonate

Variables	C	L	%[1]
Number of rats	11	13	
Body weight gain, g	156 ± 5[2]	97 ± 4*	62
Feed intake, g	398 ± 10	320 ± 8*	80
Feed efficiency	0.39 ± 0.01	0.30 ± 0.01*	77

As shown in Table 2, ingesting lithium during gestation significantly decreased litter size, total litter weight and mean weight of pups. When the lithium group was compared to the control group, litter size was decreased 25%, total litter weight was decreased 30%, and mean pup weight was decreased 10%.

TABLE 2

Size and Weight of Litter and Mean Pup Weight When Dams Were Fed Diets Supplemented With 0 (C) and 1,000 (L) ppm Lithium Carbonate

Variables[1]	C	L	%[2]
Number of dams	11	13	
Litter size	12.5 ± 0.8[3]	9.4 ± 0.6*	75
Total litter weight, g	76.0 ± 5	53.0 ± 3*	70
Mean pup weight, g[4]	6.3 ± 0.1	5.7 ± 0.2*	90

Survival of pups in the standardized litters at the end of a 21-day lactation period, was affected by dietary treatment (Table 3). The highest mortality was observed in group LL, the group ingesting lithium during both gestation and lactation. Dietary treatment also significantly affected total litter weight and mean weight of pups. Total weight of the standardized litters was decreased in both groups ingesting lithium during lactation, but not in the group ingesting lithium only during gestation. Group CL had the lowest mean pup weight, however, the mean pup weight was not reduced in the other two groups ingesting lithium.

TABLE 3
Weight and Size of Standardized Litters and Mean Pup Weights at the End of Gestation

Variables	Dietary Treatment[1]				P[2]
	CC	CL	LC	LL	
Number of Dams	5	6	7	6	
Litter Size[3]	6.0 ± 0.1[4,a]	6.0 ± 0.0[a]	5.7 ± 0.2[a]	4.8 ± 0.5[b]	0.0166
Litter Wt, g[5]	351 ± 6[a]	266 ± 19[b]	327 ± 14[a]	256 ± 27[b]	0.0050
Pup Weight, g[6]	58 ± 1[a]	44 ± 3[b]	57 ± 2[a]	54 ± 4[a]	0.0119

Body weights and feed intakes of dams at the end of the lactation period are shown in Table 4. Dams fed the lithium-containing diet during lactation (groups CL and LL) had significantly lower body weights than the group fed the control diet during both gestation and lactation (group CC). Body weights of dams in the group fed the lithium-containing diet during gestation and the control diet during lactation (group LC) were not significantly different from those of dams in the groups fed during both gestation and lactation the control diet (group CC) or the lithium-containing diet (group LL). Lithium ingestion had no effect on the feed intake.

TABLE 4
Body Weight and Feed Intake of Dams Fed Diets Supplemented With 0 or 1000 ppm Lithium Carbonate During Lactation

Variable	Dietary Treatment[1]				P[2]
	CC	CL	LC	LL	
Number of Animals	5	6	7	6	
Body weight, g	269 ± 8[3,a]	238 ± 10[c]	259 ± 4[a,b]	242 ± 2[b,c]	0.0087
Feed intake, g	929 ± 29[a]	861 ± 110[a]	937 ± 61[a]	844 ± 48[a]	0.7169

[1]See Table 3 for dietary treatment.
[2]Significance of dietary treatment as determined by one-way analysis of variance.

Organ weights of dams in each of the four groups at the end of the lactation period are given in Table 5. Lithium ingestion had no effect on the weight of brain, heart or spleen. However, lithium significantly affected both liver and kidney weights. Dams fed the control diet throughout gestation and lactation (group CC) had larger kidneys than the other three groups ingesting lithium during gestation or lactation, or both. When dams were fed the control diet during gestation (group CC and CL), liver and kidney weights were reduced when the lithium diet was fed during lactation. When lithium was fed during gestation (group LC and LL), dietary lithium during lactation decreased kidney weight but did not affect liver weight.

TABLE 5
Organ Weights of Dams Fed Diets Supplemented With 0 or 1,000 ppm Lithium Carbonate During Gestation and Lactation

Organ	Dietary Treatment[1] CC (n=5)	CL (n=6)	LC (n=7)	LL (n=6)	P[2]
Brain	1.30 ± 0.07[3,a]	1.32 ± 0.03[a]	1.23 ± 0.01[a]	1.24 ± 0.03[a]	0.2992
Heart	0.98 ± 0.05[a]	0.88 ± 0.06[a]	0.99 ± 0.03[a]	0.88 ± 0.03[a]	0.1210
Liver	18.97 ± 0.61[a]	13.85 ± 1.77[b]	17.85 ± 0.38[a]	16.18 ± 0.63[a,b]	0.0116
Kidney	2.05 ± 0.04[a]	1.79 ± 0.07[b,c]	1.90 ± 0.04[b]	1.73 ± 0.03[c]	0.0020
Spleen	0.67 ± 0.05[a]	0.58 ± 0.07[a]	0.68 ± 0.08[a]	0.65 ± 0.04[a]	0.7221

g

When organ weights were expressed relative to brain weight, there was a significant effect of lithium carbonate on liver and kidney, but not heart or spleen (Table 6). As with absolute organ weights, liver and kidney weights relative to brain weights were decreased when lithium was ingested only during lactation (groups CC vs. CL). Furthermore, when lithium was ingested during gestation, diet during lactation did not affect organ weights.

TABLE 6
Organ Weights Expressed Relative to Brain Weight[1] for Dams Fed Diets Supplemented With 0 and 1,000 ppm Lithium Carbonate

Organ	Dietary Treatment[2] CC (n=5)	CL (n=6)	LC (n=7)	LL (n=6)	P[3]
	g/g brain wt				
Heart	0.77 ± 0.05[4,ab]	0.67 ± 0.05[b]	0.81 ± 0.02[a]	0.71 ± 0.02[ab]	0.0652
Liver	14.74 ± 0.76[a]	10.61 ± 1.52[b]	14.58 ± 0.45[a]	13.12 ± 0.82[ab]	0.0225
Kidney	1.59 ± 0.08[a]	1.37 ± 0.07[b]	1.55 ± 0.05[ab]	1.40 ± 0.05[b]	0.0467
Spleen	0.52 ± 0.04[a]	0.45 ± 0.05[a]	0.56 ± 0.07[a]	0.52 ± 0.02[a]	0.4901

Lithium ingestion significantly affected the weights of all organs except spleen when weights were expressed relative to body weight (Table 7). Controlling for dietary treatment during gestation, dams fed the lithium rather than the control diet during lactation (groups CC vs CL and LC vs LL) had smaller relative weights for kidney and heart (Table 7). Liver weight relative to body weight was reduced by lithium ingestion during lactation when the control diet (group CC vs CL) but not the lithium diet (group LC vs LL) was fed during gestation.

TABLE 7
Organ Weights Expressed Relative to Body Weight for Dams Fed Diets Supplemented With 0 and 1,000 ppm Lithium Carbonate

Organ	Dietary Treatment[1] CC	CL	LC	LL	P[2]
	g/100 g body weight				
Brain	0.37 ± 0.03[3,ab]	0.35 ± 0.01[b]	0.41 ± 0.01[a]	0.41 ± 0.01[a]	0.0127
Heart	0.28 ± 0.01[b]	0.24 ± 0.02[c]	0.33 ± 0.01[a]	0.29 ± 0.01[b]	0.0001
Liver	5.51 ± 0.08[a]	3.69 ± 0.48[b]	5.95 ± 0.13[a]	5.32 ± 0.25[a]	0.0001
Kidney	0.59 ± 0.02[ab]	0.48 ± 0.02[c]	0.63 ± 0.01[a]	0.56 ± 0.01[b]	0.0001
Spleen	0.19 ± 0.01[ab]	0.16 ± 0.02[b]	0.23 ± 0.03[a]	0.21 ± 0.01[ab]	0.0943

Organ weights of pups at the end of the lactation period are given in Table 8. There were no significant effects of lithium ingestion on brain, heart, liver and kidney. However, spleen weight was significantly affected by dietary lithium carbonate. When lithium was fed only during lactation (group CL), spleen weight was significantly decreased compared to the other three groups.

TABLE 8
The Effect of Maternal Dietary Treatment on Organ Weights of Pups at the End of Lactation Period

Organ	Dietary Treatment[1] CC (n=5)	CL (n=6)	LC (n=7)	LL (n=6)	P[2]
	g				
Brain	1.06 ± 0.03[3,a]	1.13 ± 0.07[a]	1.06 ± 0.03[a]	1.07 ± 0.03[a]	0.5978
Heart	0.31 ± 0.03[a]	0.24 ± 0.02[b]	0.29 ± 0.02[ab]	0.31 ± 0.01[a]	0.0877
Liver	2.79 ± 0.21[a]	2.22 ± 0.22[a]	2.62 ± 0.16[a]	2.56 ± 0.18[a]	0.2434
Kidney	0.61 ± 0.03[a]	0.52 ± 0.04[a]	0.60 ± 0.03[a]	0.57 ± 0.04[a]	0.3497
Spleen	0.23 ± 0.01[a]	0.17 ± 0.02[b]	0.23 ± 0.01[a]	0.22 ± 0.02[a]	0.0356

3.3.1.12 Fritz et al., 1988

Study reference:

Fritz, H., Lithium and the developing rat kidney in transplacental target organ toxicity, Arzneimittel-Forschung, 38, 50-54, 1988 (Fritz, 1988)

Detailed study summary and results:

Test type

Prenatal developmental toxicity study in rats, no GLP compliance given

Test substance

- Test material used in the study is lithium carbonate
- Degree of purity: 'purissima' (no further information available)
- Impurities: no information available
- Batch number: no information available

Test animals

- Male and female Tif:RAIf rats
  Experiment I: 14 – 19 females
  Experiment II: 14 dams in exposure group, 17 dams in control group
  Experiment III: 29 dams in exposure group, 28 dams in control group
- Age and weight at the study initiation: no information available

Administration/exposure

- Route of administration – oral (gavage)
- Duration and frequency of exposure:
  Experiment I: daily from GD 6 to 10, GD 11-15 or GD 16-20, examination on GD 21
  Experiment II: once daily from GD16-20, examination on GD 21 or PND 11-19
  Experiment III: once daily from GD 16-20, examination on PND 35-40, 10 dams were allowed to nurse their own pups, the remaining pups were crossfostered, and offspring were separated from dams on PND 28
- Doses:
  Experiment I: 100 mg lithium carbonate/kg bw/d
  Experiment II: 0, 100 mg lithium carbonate/kg bw/d
  Experiment III: 0, 60 mg lithium carbonate/kg bw/d
- Rationale for dose level selection: daily doses of 100 mg/kg bw/d administered to rats is within the range of human therapeutic plasma concentration of 0.6 – 1.6 meq/L. To obtain enough pups surviving to weaning age it was decided also to set up an experimental group receiving 60 mg/kg bw/d presuming low toxicity in dams as well as in foetuses or new-borns.

- Control group and treatment: Control animals received distilled water only
- Historical control data: no results reported in form of numbers, however, for experiment I results were compared with "standard-range derived from series of an untreated control population". This can be interpreted as historical control data from the laboratory.
- Vehicle: distilled water

Description of test design:

- Details on mating procedure (M/F ratios per cage: 1 : 1-3, proof of pregnancy: spermatozoa in vaginal smear or a vaginal plug)
- Premating exposure period: none
- Standardization of litters: no
- Parameters assessed for P and F1:
    - Experiment I: foetuses examined macroscopically, weighted individually, skeletal or visceral staining with particular consideration of urogenital system, dams macroscopically assessed for pathological changes of kidney or other organs, body weight changes and feed consumption
    - Experiment II:

      Examination on GD 21: visceral evaluation; from foetuses of 3 control litters and 4 medicated litters slices of the lumbar area were embedded in paraplast, cut at 5 µm and stained with haematoxylin-eosin for histological examination.

      Examination on PND 11/12 and 18/19: interim sections carried out at 2 males and 3 females per litter on PNDs 11/12 and 18/19
    - Experiment III:

      Dams: body weight, food and water consumption examined days 16-20 p.c. and a water/food index was calculated.

      Offspring: clinical signs and mortality checked daily, weighted on PNDs 21, 28 and 35, litter weights recorded on PNDs 4,7 and 14 and average individual weights derived from these data. On PNDs 29-32 negative geotaxis and exploratory locomotion (EL) were examined to detect gross behavioural abnormalities. An EL-index was determined. EL was determined by observing the behaviour of the pup being placed in a cylindrical cage

Results and discussion

- Experiment I (for some details see Table AI - 10):

  Dams: reduction of body weight gain and feed consumption, polyuria

  Offspring: Reduced implantations, reduced body weight

  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)

Table AI - 7: Results in foetuses from dams treated with 100 mg/kg bw/d

Group	Treatment days p.c.	Number of dams	Embryonic and fetal deaths[a]	Live foetuses		Enlarged renal pelvis	
				Number	Weight [g]	Litters	Foetuses
1	6 – 10	16	3.8	203	4.91 ± 0.41[c]	0/16	0/67
2	11 – 15	19	7.0	225	4.82 ± 0.81[c]	1/19	3/75 (4.0 %)
3	16 - 20	14	38.5[b]	123	4.48 ± 0.53[c]	4/14	7/41 (17.1 %)

[a] in % implantations sites, [b] largely above normal range (3 – 6 %), c slightly to largely below normal range (5.1 – 5.5 g). Results taken from Fritz (1988)

- Experiment II (for some details see Table AI - 11):
  Dams: reduced body weight gain in treated animals (11.5%, control: 21.5%), mortality (2/4), 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

Table AI - 8: Results in foetuses from dams treated with 100 mg/kg bw/d or distilled water only

Group	Number of dams	Number of dams sacrificed near term	Enlarged renal pelvis		Number of dams raising the offspring	
			Litters	Foetuses	Day 1 p.p.[a]	Day 4 p.p.
Control	17	9/17	0/9	0/133	8/17	7/8
Li_2CO_3	14	7/14	4/7	20/93 (21.5 %)	7/14	4/7

[a] Number of dams selected for delivery and offspring observed day 1 postpartum. Results taken from Fritz (1988)

- Experiment III:
  Dams: reduction of body weight gain and feed consumption, polyuria, increased water consumption, macroscopically normal kidneys (for details see Table AI - 12).

  Offspring: reduced litter size (PND1: 10.9±5.8, control: 16.0±2.1), no effects on the kidneys ((for details see Table AI - 13).

Note: Lithium exposed offspring crossfostered by control dams showed a statistically significant increase in the mortality rate. However, according to the author this is due to one instance with death of 10/12 pups until day 4 p.p. and any experimental importance of this finding is doubtful.
General behaviours of the pups were similar in all groups (see Table AI - 14).

Table AI - 9: Results in dams treated with 60 mg/kg bw/d or distilled water only

Group	Number of dams	Bodyweight gain (%)	Food consumption per day [g]	Water consumption per day [mL = g]	Index water /food
Control	28	+ 19.0	23.1	46.6	2.02
Li_2CO_3	29	+ 12.9*	18.4*	60.0*	3.26

* $p < 0.01$ (t-test, one-tailed), results taken from Fritz (1988)

Table AI - 10: Postnatal development of progeny: litter sizes, mortality

Group	Number of litters	Number of pups on day 1 p.p.	Litter size on day 1 p.p.	Mortality rates					
				Litters affected[a]	% Offspring affected				
					Days postpartum				
					4	7	14	21	35
A (control)	9	144	16.0 ± 2.1	3/9	1.4	1.4	1.4	2.8	4.2
B (Li_2CO_3)	8	87	10.9 ± 5.8*	1/8	2.3	2.3	2.3	2.3	2.3
A → B[b]	10	146		3/10	1.4	2.1	2.7	3.4	3.4
B → A[b]	10	149		5/10	14.1	14.1	14.1	14.8	14.8

[a] at least one pup noted; number of litters with dead pups/ number of total litters, [b] A-litters fostered by B-dams, B-litters fostered by A-dams, * $p < 0.05$ (t-test, one-tailed). Results taken from Fritz (1988)

Table AI - 11: Postnatal development of progeny: bodyweight gain and exploratory locomotion (EL)

Group	Average bodyweight [g]		Body weight index[a]					EL
	Day 4 p.p.	Day 21 p.p.	Pre-weaning (days p.p.)			Post-weaning (days p.p.)		
			4 →7	4→14	4→21	21→35		
						Males	Females	
A (control)	8.0	37.7	0.48	1.81	3.69	2.01	1.92	1.52
B (Li_2CO_3)	8.6	40.9	0.47	1.94	3.76	1.94	1.72	1.48
A → B[b]	8.7	41.6	0.46	1.88	3.77	2.03	1.90	1.51
B → A_b	8.1	40.5	0.50	2.08	4.01	1.95	1.76	1.49

[a] Calculated as follows: (bw day n p.p. – bw day 4 (21) p.p.) / (bw day 4 821) p.p. [b] A-litters fostered by B-dams, B-litters fostered by A-dams. Results taken from Fritz (1988)

3.3.1.13 Marathe and Thomas, 1986

Study reference:

Marathe, M.R.; Thomas, G.P., Embryotoxicity and teratogenicity of lithium carbonate in Wistar rat Toxicology Letters, 34, 115-120, 1986 (Marathe und Thomas, 1986)

Detailed study summary and results:

Test type

Prenatal Developmental Toxicity Study, no GLP compliance given

Test substance

- Test material used in the study is lithium carbonate
- Degree of purity: no information available
- Impurities: no information available
- Batch number: no information available

Test animals

- Female Wistar rats
- No. of animals per sex per dose: n=20 in control, n=11 or 13 in high and low dose group
- Age at the study initiation: no information available
- Weight at the study initiation: 200 – 250 g

Administration/exposure

- Route of administration – oral (gavage)
- Duration and frequency of exposure period: Daily application from GD 6-15
- Doses: 0, 50 or 100 mg/kg bw/d
- Rationale for dose level selection: The doses selected were comparable to the human therapeutic doses
- Control group: Same treatment as exposed animals, exposure to the vehicle only (agar)
- Historical control data: no information available
- Vehicle: agar
- Test substance formulation: Lithium carbonate was homogenized and suspended in 0.2 % sterile agar at 50 mg/mL

Description of test design:

- Details on mating procedure (M/F ratios per cage: no information available, length of cohabitation: no information available, proof of pregnancy: observation of spermatozoa in vaginal smear)
- On GD 20 the dams were killed and foetuses were recovered by caesarean section
- Parameters assessed for P:

- Body weights were taken on days 0, 6, 13 and 20. Doses were computed based on the body weights taken on day 6 and day 13. Observations were made every day for onset and duration of symptoms. On day 20 of gestation, the dams were killed by an overdose of ether anaesthesia.
- Uterine content was examined after termination,
- Number of implantations
- Number of early resorptions
- Number of late resorptions:
- The uteri of dams which did not appear to be pregnant were stained with a 10 % aqueous solution of sodium sulphide and were examined for evidence of early resorption sites.

- Parameters assessed for F1:
  - Observation of gross abnormalities
  - Number of live and dead foetuses
  - Body weight and sex
  - One third of the total number of foetuses/sex/dam were fixed in Bouin's fluid and the remaining 2/3 in 95 % ethanol. The free-hand razor-blade technique of Wilson was employed to study visceral malformations in the foetuses fixed in Bouin's fluid. The foetuses fixed in ethanol were eviscerated, cleared in 2 % KOH and stained with Alizarin Red S stain to study skeletal malformations.

Results and discussion

- 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). For details see Table AI - 15.
- No foetal malformations were found in foetuses from the dams treated with 50 mg/kg. Wide bone separation in the skull and incomplete ossification of sternebrae were found in control and 50 mg/kg-treated rat foetuses, but their incidence was significantly higher in the 100 mg/kg-treated rat foetuses (see Table AI - 16).
- No visceral malformations were seen in foetuses of 50 and 100 mg/kg bw/d treated dams.

Table AI - 12: Effect of exposure on Wistar rat foetuses, values are expressed as mean ±SD

Dose [mg/kg bw]	Implantations	Live pups	Resorptions		Average weight of pups [g]	
			Early	Late	Male	Female
Control	10.25 ± 0.68	9.00 ± 0.68	1.00 ± 0.24	0.25 ± 0.10	3.65 ± 0.07	3.32 ± 0.10

						n = 10
50	8.69 ± 0.78	8.23 ± 0.78	0.31 ± 0.17	0.15 ± 0.10	3.33 ± 0.10	3.28 ± 0.08
100	7.91** ± 0.71	4.73* ± 1.29	1.91 ± 0.97	1.18 ± 0.67	2.42* ± 0.40 n = 8	2.24* ± 0.37 n = 9

* p < 0.01, ** p < 0.05

Table AI - 13: Skeletal malformations observed in foetuses from Wistar rats

Findings	Dose [mg(kg bw/d]		
	0 (n = 95)	50 (n = 107)	100 (n= 54)
(1) Wide bone separation in skull	5 (5.26 %)	4 (3.74 %)	10 (18.54 %)
(2) Incomplete ossification of sternebrae	10 (10.53 %)	8 (7.48)	21 (38.89 %)
(3) Wavy ribs	0	0	28 (51.85 %)
(4) Shorthening of bones			
(a) Radius and ulna	0	0	20 (37.04 %)
(b) Humerus	0	0	20 (37.04 %)
(c) Tibia and fibula	0	0	18 (33.33 %)
(d) Femur	0	0	22 (40.74 %)
(5) Deformity in			
(a) Scapula	0	0	20 (37.04 %)
(b) Pelvic bones	0	0	18 (33.33 %)
(6) 14th rib	11 (11.58 %)	16 (14.95 %)	7 (12.96 %)

3.3.2 Human data

3.3.2.1 McKnight et al., 2012

Study reference:

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

Detailed study summary and results:

Test type

Systematic review and meta-analysis of studies investigating the association between lithium and all reported major adverse effects.

- Search strategy and selection criteria

Searches in Medline (1966–2010), Medline In-Process and other non-indexed citations (from 1966 to October, 2010), Embase (1980–2010), the Cumulative Index to Nursing and Allied Health Literature (1982–2010), PsycINFO (1806–2010), the Cochrane Library database (inception–2010), Biosis Previews (1926–2010), TOXNET database (inception–2010; webappendix), and archives of the journals Lithium, Lithium Therapy Monographs, and Teratology (search terms listed in webappendix). All relevant references were

checked for additional and unpublished citations. Major textbooks of mood disorders and conference abstracts were hand-searched. Pharmaceutical companies that market lithium, relevant clinicians, and authors of trials with incompletely reported data were contacted. All studies were assessed for meeting inclusion criteria, and those used for analysis were reviewed by a second researcher.

Studies were included in the review if they investigated one or more of the adverse events of interest. Randomised controlled trials (RCTs) comparing lithium with placebo, no treatment, or other drug therapies in patients with depression or bipolar disorder were considered most reliable if they included safety data for adverse effects, followed by prospective cohort studies comparing patients given lithium with those not given lithium, and then case-control studies. In the absence of controlled studies, were included uncontrolled prospective studies following up patients with depression or bipolar disorder given lithium and, finally, individual case reports. For each outcome, all studies meeting inclusion criteria were assessed and tabulated, but only the highest available form of evidence was included in the formal analysis. When only poor quality data from a higher level of evidence were available, the next level down were routinely included. For adverse events that often occur after months or even years of treatment, observational studies are often more informative than are RCTs.

- Outcomes

The main outcomes investigated were: renal function (glomerular filtration rate [GFR, normal >90 mL/min], renal concentrating ability [maximum urinary concentrating ability, normal 800–1200 mOsm/kg]); thyroid function (thyroid stimulating hormone [TSH, normal 0·5–5·7 IU/mL], subclinical hypothyroidism [raised TSH with normal thyroxine] or clinical hypothyroidism [raised TSH and low thyroxine], or hyperthyroidism [depressed TSH and high thyroxine]); parathyroid function (total calcium [normal 2·1–2·8 mmol/L] and parathyroid hormone [PTH, normal 10–70 pg/mL]); bodyweight (clinically significant change in bodyweight [>7% total weight in kg]); hair disorders; skin disorders; and teratogenicity (risk of major congenital and cardiac malformations in infants exposed to lithium in utero).

Study quality was judged by assessing design aspects likely to introduce biases, method of randomisation and concealment of treatment allocation, blinding, length of follow-up, reporting withdrawals and dropouts, and method of analysis for RCTs; and likelihood of measurement bias, handling of confounding, and loss to follow-up for observational studies. Authors were contacted when published reports did not contain adequate details.

- Results

62 studies of the teratogenic potential of lithium were identified: seven cohort studies, seven case-control studies, and 48 case reports. Six case-control studies (n=264) measured the association between Ebstein's anomaly and lithium. The odds of exposure to lithium in cases of Ebstein's anomaly did not differ significantly from controls; however, estimates are unstable because of the low number of events (Peto OR 0.27, 95% CI 0.004–18.17, p=0·54; heterogeneity χ^2=0.00 [df 1], p=0.96; Mantel-Haenszel OR 2.0, 95% CI 0.20–20.6, p=0.54; heterogeneity χ^2=1.98 [df 1], p=0.16). A case-control study of 10 698 infants born with any major congenital abnormality and 21 546 healthy controls showed no significant association between

lithium and congenital abnormalities (Peto OR 2.62, 95% CI 0.74–9.20, p=0.132). The number of infants exposed to lithium was low in cases (six of 10 698) and controls (five of 21 546).

The evidence that exposure to lithium is teratogenic is quite weak, and these findings accord with the notion that the risk has been overestimated. Thus, the risk estimates were not significant, although the upper confidence limit is consistent with a clinically significant increase in risk of congenital malformations. Therefore, uncertainty about the risk of harm remains. The present clinical recommendation is to avoid lithium in pregnancy.

This review suggests a sounder approach would be to explain the uncertainty of risk to women of childbearing age, considering the balance of risks between harm to the baby and maternal mood instability before continuation or stopping of lithium therapy.

3.3.2.2 Yacobi and Ornoy, 2008

Study reference:

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

Detailed study summary and results:

Test type

- Methodology

All studies that discussed the teratogenic and embryotoxic effect of Li intake during pregnancy on infants born to mothers with Bipolar Disorder (BD) were reviewed. For this purpose data were obtained from all published studies and case reports in English, referenced in Medline between the years 1969 and 2005 that included the key words: lithium and pregnancy, with related phrases such as lithium and embryotoxicity, lithium and teratogenicity, lithium and Ebstein's anomaly, lithium, pregnancy and Ebstein's anomaly and lithium and cardiac anomalies. This review surveys case report studies (including 24 separate cases) between the years 1969–2005; retrospective studies; a follow-up study and controlled prospective studies.

- Results
    - Case reports

The 24 case report studies found by the authors are not detailed here. In this 24 case report, 15 women were also treated with antidepressants and/or anti-psychotics or other drugs. Moreover, although in the reported cases most anomalies were of the cardiovascular system, the number of the unreported lithium-treated women with normal children were not known, a fact that it have been to consider when evaluating case reports.

    - Retrospective studies

Lithium babies registry

In a retrospective study published in its final form in 1980, including all 225 cases of the registry, Weinstein reported 25 malformed infants from a total of 225 reported lithium babies (11.1%). This rate consisted of 18 (8%) cardiovascular defects — six of which were Ebstein's anomaly. The other seven defects involved

different systems. Ten of the 25 malformed infants died during the first postnatal week (Table 2). In addition to the 25 malformed infants, there were seven stillborn infants, two children had Down syndrome and one had congenital toxoplasmosis. This registry was published partly in several other studies, by Weinstein and Goldfield (1975), Weinstein (1976, 1979), and by Schou et al. (1973). Troyer et al. (1993) reviewed the records of these 225 infants from the International Registry of Lithium Babies and found 84 complete maternal-infant data that included gestational age at delivery and birth weight. They found that lithium therapy during pregnancy increased the incidence of premature birth, as over one-third (35.7%) were prematurely born. They also demonstrated more than two-fold increase in large for gestational age infants (LGA) among prematures compared to the term born infants (37% vs 15.0%, Table 3).

Retrospective cohort study

From a total of 350 infants born to manic-depressive women, information was obtained by Kallen and Tandberg (1983) for 287 cases (82%). In this group, a sub-group of 59 infants exposed to either lithium alone or lithium in combination with other psychotropic agents was compared to 190 untreated women and to 38 women treated with antidepressants other than lithium. In the 59 Li-exposed group, a 10.2% neonatal death rate was reported, as well as a 11.9% malformation rate (seven infants) and 6.8% (four infants) rate of heart defect. Although the rate of anomalies among the lithium-treated newborns was higher as compared to control untreated manic-depressive women, the difference was not statistically significant and, due to the small sample size, could still be random. Ebstein's anomaly was not demonstrated.

Nested case-control study

Kallen (1991) evaluated all infants with cardiac defects born to 716 women hospitalized for BD and compared two matched controls for each malformed infant. Fourteen cardiac defects were identified, more than twice the expected rate. One infant had a chromosomal anomaly and was therefore excluded. Among the 13 left, seven had Ventricular Septal Defect and two had a systolic murmur — all considered to be relatively mild cardiac anomalies. The others had other cardiac anomalies, including one case of Ebstein's anomaly that was not exposed in utero to lithium or other antidepressants. There was no significant difference in the rate of exposure to lithium between the malformed (3/13) and the control infants (4/20). Thus, lithium could not be associated with the increase in the rate of cardiac anomalies. One possible explanation for the lack of this association, given by the author, was the fact that most women stopped lithium once pregnancy was diagnosed.

Retrospective case control study

Czeizel and Racz (1990) studied 10,698 children with congenital anomalies between the years 1980–1987. Their study included all malformed still-born and live-born cases diagnosed from birth till the age of one year, as well as prenatally diagnosed and electively terminated malformed fetuses. They observed no association with maternal lithium use during pregnancy; however, only six infants were exposed to lithium.

- Studies on cardiac anomalies, especially Ebstein's anomaly

In a joint case-control study Kallen collected 25 cases of Ebstein's anomaly and 44 cases of tricuspid atresia. In addition, he added 15 cases of Ebstein's anomaly that were reported in France. None of these infants with

Ebstein's anomaly or with tricuspid atresia were exposed in-utero to lithium (Table 5). Sipek reported 89 cases of infants with Ebstein's anomaly in a Czech study, conducted between 1960–1985. None of their mothers were treated with lithium during pregnancy. The author, however, could not rule out possible occupational exposure to lithium. No statistical differences were found in exposure to lithium between these mothers and a control group of women with children without anomalies (7.1% vs. 11.4%).

Zalzstein et al. analyzed data from 59 patients diagnosed as having Ebstein's anomaly, using 168 children with neuroblastoma as a control group. They found that none of the mothers of the 59 children had lithium therapy during pregnancy. These results led them to the conclusion that lithium does not increase the rate of cardiac anomalies, especially of Ebstein's anomaly.

In a case-control study, Edmonds and Oakley compared 76 infants who were "possibly" born with Ebstein's anomaly with the same number of control infants. Of the 76 infants, 34 were confirmed as Ebstein's anomaly. However, none of them or of the control group were born to women with BD or were exposed to Li during pregnancy.

- Prospective studies

Only one record linkage study and two published prospective studies regarding lithium use in pregnancy were found, and they were all negative regarding major anomalies.

In a record linkage study of Michigan Medicaid recipients (Rosa, personal communication cited by Briggs et al. 2005), only two (3.2 %) of 62 infants of women treated with lithium during the first trimester of pregnancy were reported to have major congenital anomalies, and none of them was cardiac.

In an uncontrolled prospective study, Cunniff et al. (1989) identified 72 lithium-treated women with BD. Of these, six had pregnancy termination, four resulted in first trimester spontaneous abortions and 12 were lost to follow-up. Only 50 were known as live-born infants, two of them reported as having major malformations: one lumbar myelomeningocele and the other unilateral inguinal hernia. The rate of anomalies was not different from that generally observed in controls. There were no cases of cardiac anomalies.

In another controlled prospective study, Jacobson et al. (1992) studied the pregnancy outcome of 138 Li-exposed pregnant women (who gave birth to 105 liveborn infants), and 148 controls. They observed one case of Ebstein's anomaly, but failed to show any differences with respect to major congenital anomalies and number of livebirths between controls and lithium exposed group (Table 6).

In the lithium-exposed group, four malformations were observed: two neural tube defects, one of which was exposed also to carbamazepine. The third infant had meromelia and died because of prematurity, and one fetus was diagnosed as severe Ebstein's anomaly at 16 week gestation and pregnancy was terminated. In the control group the three anomalies observed were: ventricular septal defect (VSD); congenital hip dislocation

(CDH) and cerebral palsy (CP) with torticolis. Birth weight was higher in the lithium group (Table 6).

Table 6. *The effect of lithium therapy given to women with B-D on pregnancy outcome and malformation rate among their infants — a controlled prospective study (50)*

Treatment group / no. of cases	Lithium intake 1st trimester mean / range	normal livebirth / stillbirth no. (%)	term delivery / premat. no. (%)	birth weight* / range gestational age* / range	spontan. abortions / therapeut. no. (%)	maternal age* / range	congenital malformat.	Types of malformations
Li-exposed / 138	927mg/d 50-2400mg/d	105 (76%) / 1	99 (72%) / 6 (4%)	3475±660** / 539-5024 39.1±3.0 / 23-42.5	13 (9%) / 15 (10%)	30.0±5.3 15-40	3 (3%)	1. meningomyelocele, hydrocephalus (treated: Li + carbamazepine 2.spina bifida + tethered cord 3. meromelia,born& died 23wk,1/twins 1 severe form of Ebstein's anomaly diagnosed wk.16, pregnan. terminated (treated:fluoxetine,trazodone+L-thyoxin)
control / 148	drugs other than Lithium	123 (83%) / 0	116 (78%) / 7 (5%)	3383±566 / 950-4896 39.2±2.5 / 26-43	12 (8%) / 9 (6%)	29.8±5.3 16-40	3 (2%)	1. Ventricular septal defect 2 Congenital hip dislocation 3. Cerebral palsy & torticolis

Four sets of twins were born in the Li group: one pair died from complications of prematurity at 23 weeks.
One set of twins was born in the control group.
* Mean ± SD
** significantly higher than controls

In a controlled prospective study from the Israeli Teratogen Information Service, yet unpublished, Diav-Citrin et al. observed, among 105 pregnant women exposed in pregnancy to lithium (86% were exposed from the first trimester), 79 liveborn infants. Two infants were malformed (2.9%). One infant had cryptorchidism that was operated and the other had Ebstein's anomaly. This was compared to 1,234 control infants with 39 cases of major malformations (3.2%), none of which had Ebstein's anomaly. In addition, there was an 8.7% rate of pregnancy interruptions (vs. 2.9 in the controls) and 14.3% of spontaneous abortions (vs. 5.9% in the controls).

In summary: Of a total of at least 377 lithium treated pregnancies (in one study the number of pregnancies was unknown) with 296 liveborn infants, there were eight malformed (2.7%), not different from controls. However, two of the malformed infants had Ebstein's anomaly while none of the 43 malformed infants among 1,354 controls (3.2%) had this cardiac anomaly. A sample size of 296 liveborn infants exposed to lithium with a ratio of 1:4.6 (lithium/control) and a power of 80%, assuming a baseline risk of 3% for major anomalies, enables detection of a 2.27-fold increase in the overall rate of major anomalies (with 95% CI).

- Developmental Follow-up studies

Several human case reports have demonstrated transient neurodevelopmental deficits in infants born to lithium exposed mothers. However, long-term developmental studies on lithium-exposed children are generally lacking. In a single prospective follow-up study, Schou (1976) compared the motor and mental development of 60 non-malformed lithium babies to their own 57 siblings, who were not exposed to lithium in-utero. The data were calculated based on the information obtained from questionnaires and letters sent to doctors (psychiatrists/general practitioners) who primarily reported the children. Out of 60 lithium-exposed children, 10 were abnormally developed, in six developmental delay was transitory and in four it was permanent. In this group, three children were exposed to lithium only in the first trimester and the seven others throughout pregnancy. In the 57 control siblings, six children were identified with persistent abnormal development. The data shown by Schou indicates that in-utero exposure to lithium does not increase the risk of developmental (both motor and mental) disorders.

3.3.2.3 Munk-Olsen et al., 2018

Study reference:

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.

Detailed study summary and results:

Test type

- Methods
    - Participating cohorts

This study combined primary data from 6 cohorts using meta-analysis: three population-level register-based cohorts in Denmark, Sweden and Ontario, Canada, and three clinical cohorts (i.e., women under psychiatric secondary care) from the Netherlands, the United Kingdom, and the United States. A joint study protocol was created prior to dataset creation and analysis, including specific definitions for selection criteria, each included variable, and statistical analysis. Each study site obtained local ethical approval. All cohorts comprised pregnancies resulting in live-born singleton deliveries from 1997 to 2015, where health-related information was available both for the mother and for the infant. Pregnancies in which mothers were prescribed known teratogenic medications in pregnancy (thalidomide, valproate, retinoids, antineoplastic drugs, misoprostol, and methotrexate) were excluded from the analysis.

    - Lithium exposure

The lithium-exposed group comprised pregnancies with lithium exposure during the index pregnancy. For register-based cohorts, lithium exposure during pregnancy was defined as at least two dispensations of lithium during pregnancy that were dispensed any time from one month prior to conception until the delivery, or a single lithium dispensation during pregnancy when there was at least one other lithium dispensation within six months before or after this date. Dispensations of lithium were identified using the Anatomical Therapeutic Chemical (ATC) Classification System code N05AN01 in Denmark and Sweden and the corresponding Drug Identification Numbers in Ontario, Canada. For clinical cohorts, medical records were used to define lithium use during pregnancy. For the lithium-exposed group, we did not require a documented psychiatric diagnosis, as non-psychiatric indications for lithium are rare.

For analyses with specific focus on congenital malformations, the authors were interested in lithium exposure during early pregnancy, and further defined lithium exposure in the first trimester as follows: For register-based cohorts: 1) At least two dispensations of lithium in the first trimester (from one month before the date of conception to 90 days of gestation); or 2) One dispensation in the first trimester with at least one other dispensation within 6 months before or after this date. For clinical cohorts: Medical records were used to define lithium use in the first trimester.

- Mood disorder reference group

The reference group comprised women with a known history of mood disorder (bipolar disorder or major depressive disorder) without exposure to lithium from 90 days before pregnancy until the delivery. For register-based cohorts, maternal mood disorder was defined as at least one inpatient and/or at least two outpatient contacts for bipolar disorder (equivalent to the International Classification of Diseases, 10th revision (ICD-10) codes F30–F31) or major depressive disorder (ICD-10 codes F32–F33) from 2 years prior to the date of pregnancy to the delivery date. For clinical cohorts, maternal mood disorder was defined as any medical history of bipolar disorder or major depressive disorder before delivery.

- Outcomes of interest

Outcomes of interest were selected based on theoretical risks for general medication exposure in pregnancy and prior research on lithium use specifically. Outcomes were divided into four subcategories: 1) Pregnancy complications, identified in pregnancy or within 42 days after delivery, using hospital-based diagnoses for preeclampsia (ICD-10 code O14), diabetes during pregnancy (ICD-10 code O24), fetal distress (ICD-10 code O68), and postpartum hemorrhage (ICD-10 code O72); 2) Labour and delivery outcomes, identified in hospital, including caesarean section (ICD-10 codes O82 and P03·4; surgical code KMCA), preterm birth (<37 weeks gestation), low birth weight (<2500g), and small for gestational age (i.e. a birth weight below the 10th percentile of birth weight by gestational age and sex); 3) Neonatal hospital admission to a special care baby unit in the first 28 days of life; 4) Congenital malformations excluding chromosomal abnormalities in the child diagnosed by age 1 year, including all singular and combined structural defects, syndromes, sequences, and associations, such as cardiovascular defects, neural tube defects hypospadias, and epispadias (ICD-10 codes Q00–Q89, excluding minor malformations according to the EUROCAT Guide 1·4). Major cardiac malformations were defined as atrial and atrioventricular septal defects and Ebstein's anomaly (ICD-10 codes Q20–Q26), but excluding atrial septal defect (ICD-10 code Q21·1) and patent ductus arteriosus (ICD-10 code Q25·0) in infants born prior to 37 weeks gestation.

- Results

A total of 727 lithium-exposed pregnancies were identified (n=557, or 76.6% from register-based cohorts). Women in the lithium exposure group were more likely to be older, nulliparous, and to have filled a prescription for a psychotropic medication other than lithium during pregnancy, compared to the reference group (N=21,397).

Lithium use during pregnancy was not associated with preeclampsia (pooled prevalence of 1.8% in lithium-exposed vs. 2.1% in reference group, pooled OR=0.97, 95% CI: 0.52–1.80), diabetes in pregnancy (6.4% vs. 5.4%, pooled OR=1.20, 95% CI: 0.81–1.78), fetal distress (14.1% vs. 13.2%, pooled OR=1.00, 95% CI: 0.76–1.32), or postpartum hemorrhage (7.4% vs. 7.1%, pooled OR=1.28, 95% CI: 0.64–2.57). No differences between the lithium-exposed group and the reference group were observed for caesarean section (26.5% vs. 25.8%, pooled OR=0.94, 95% CI: 0.66–1.33), preterm birth (13.1% vs. 10.0%, pooled OR=1.24, 95% CI: 0.83–1.84), low birth weight (6.4% vs. 7.2%, pooled OR=0.98, 95% CI: 0.72–1.35), or small for gestational age (7.5% vs. 9.3%, pooled OR=0.90, 95% CI: 0.67–1.21). In-utero lithium exposure was

associated with an increased risk of neonatal admission to a special care baby unit prior to 28 days of age (27.5% vs. 14.3%, pooled OR=1.62, 95% CI: 1.12–2.33).

There were 51 lithium-exposed children (7.2%) and 856 children from reference group (4.3%) with major malformations diagnosed by one year of age. Lithium exposure was not statistically significantly associated with increased odds of major malformation (pooled OR=1.58; 95% CI: 0.90–2.79), nor major cardiac malformations (2.0% vs. 1.6%, pooled OR=1.31, 95% CI: 0.50–3.47), but statistical heterogeneity was high. For example, in Denmark, lithium exposure was associated with both major malformation and major cardiac malformation risk, but this association was not observed in data from the other 4 sites. Of 727 lithium exposed children, 654 (90.0%) were exposed to lithium in the first trimester. In total, 47 children from the lithium exposure in the first trimester group were diagnosed with major malformations and 16 with major cardiac malformations. Lithium exposure was associated with an increased risk of major malformations (7.4% vs. 4.3%, pooled OR=1.71, 95% CI: 1.07–2.72), but not major cardiac malformations (2.1% vs. 1.6%, pooled OR=1.54, 95% CI: 0.64–3.70) (Figure 1c–1d), in comparison to the reference group of mood disorders. Note, that no Ebstein's anomaly cases were observed in any of the participating study sites.

The "leave-one-out approach" analyses demonstrated an overall stability of the main findings, except for the association between lithium exposure in the first trimester and major malformations. This latter relation became non-significant when each of Denmark, Sweden, and the USA were left out. Pooled ORs from the register-based cohorts substantially overlapped those of the clinical cohorts, except for postpartum hemorrhage, where a strong relation was observed in clinical cohorts (pooled OR=2.58, 95% CI: 1.21–5.52) but not in register-based cohorts (pooled OR=0.79, 95% CI: 0.41–1.51).

Results from additional analyses in a subgroup that included only the Danish and Swedish data were generally consistent with those of the main analysis. Adjustments for education status, marriage status, antiepileptic and psychotropic medication use during pregnancy did not differ from the main results. When lithium exposed pregnancies were compared to pregnancies where women were using lithium before and after pregnancy but not during pregnancy, results were also generally consistent with those of the main analysis. However, the odds ratios of major malformations was elevated among children exposed to lithium in pregnancy (pooled OR=2.09, 95% CI: 1.10–3.96), although this was not the case specifically for major cardiac malformations (pooled OR=1.28, 95% CI: 0.13–12.39, Table 3). The relative risk of adverse outcomes in lithium exposed children were similar when comparing to the reference group with maternal diagnosis of mood disorder or to the reference group with maternal diagnosis of bipolar disorder, although the relative risk of neonatal readmission was attenuated to null when comparing to the reference group with maternal diagnosis of bipolar disorder.

Table 3. Pooled prevalence rate and odds ratio of health outcomes in lithium use *during* pregnancy group compared to lithium use *around* pregnancy group in sub-analyses based on data from Sweden and Denmark

Health outcomes	Lithium exposure during pregnancy			Lithium exposure around pregnancy			Pooled adjusted OR (95% CI) in lithium exposure during pregnancy versus around pregnancy [a]	I-squared (%)
	Pooled N	N with outcome	Pooled prevalence with 95% CI (%)	Pooled N	N with outcome	Pooled prevalence with 95% CI (%)		
Pregnancy complications [b]								
Fetal distress	356	15	0·6 (0·0, 1·5)	597	16	1·7 (0·6, 2·7)	0·91 (0·35, 2·37)	3·5
Labour and delivery outcomes								
Caesarean section	356	97	26·4 (21·9, 31·0)	597	131	21·9 (18·6, 25·2)	1·02 (0·45, 2·34)	74·7
Preterm birth	356	46	12·9 (9·4, 16·4)	597	54	8·9 (6·6, 11·2)	1·44 (0·92, 2·26)	0·0
Low birth weight	356	26	7·2 (4·5, 9·9)	597	31	5·0 (3·2, 6·7)	1·22 (0·68, 2·17)	0·0
Small for gestational age	356	18	3·4 (1·6, 5·3)	597	27	4·3 (2·7, 6·0)	0·85 (0·32, 2·22)	43·8
Neonatal readmission <28 days	356	77	20·9 (16·7, 25·1)	597	83	13·8 (11·0, 16·6)	1·65 (1·14, 2·41)	0·0
Congenital malformations								
Major malformations	356	31	7·1 (4·4, 9·7)	597	20	3·1 (1·7, 4·5)	2·09 (1·10, 3·96)	0·0
Major cardiac malformations	356	8	1·2 (0·1, 2·3)	597	9	1·5 (0·5, 2·4)	1·28 (0·13, 12.39)	52·1

3.3.2.4 Patorno et al., 2017

Study reference:

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.

Detailed study summary and results:

Test type

- Method
    - Data Source and Study Cohort

Data were collected from the U.S. Medicaid Analytic eXtract (MAX) for 46 U.S. states and the District of Columbia for the years 2000 through 2010. The cohort included all pregnancies in women 12 to 55 years of age that resulted in live births for which Medicaid covered the health care expenses. The authors excluded women who had private insurance or restricted medical benefits and those who did not have an appropriate enrolment type (i.e., capitated managed care, fee-for-service primary care case management, or no managed care, depending on the state). The approach that was used for the development of the study cohort has been described previously (Palmsten et al., 2013; Margulis et al., 2013).

- Study Conduct

The study was approved by the institutional review board at Brigham and Women's Hospital, and the need for informed consent was waived. The study funder (the National Institute of Mental Health) had no role in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; or the preparation, review, or approval of the manuscript.

- Definition of Exposure

Exposure was defined as at least one filled prescription for lithium during the first trimester (first 90 days after the date of the last menstrual period). The primary reference group included women with no lithium or lamotrigine dispensings during the 3 months before the start of pregnancy or during the first trimester. The criterion of no dispensing during the 3 months before the start of pregnancy was imposed to avoid misclassifying as unexposed women who still had medications from an earlier filling available at the start of

pregnancy. We also identified a secondary reference group of women who had at least one filled prescription for lamotrigine during the first trimester. Patients who were exposed to both lithium and lamotrigine during the first trimester (67 patients) were excluded. Lamotrigine was chosen for comparison because it is an effective treatment for bipolar disorder, often in combination with other psychotropic medications, and has not been associated with an increased risk of congenital malformations. Women who were exposed to lamotrigine were compared with the reference group of unexposed women and were used as the active reference group for women who were exposed to lithium. The former contrast (exposure to lamotrigine vs. no exposure) allows an indirect and stable comparison of lithium and lamotrigine by using a common reference. The latter contrast can further limit residual confounding by indication.

- Outcomes

The primary outcome was the presence of a cardiac malformation in the infant. The positive predictive value for this outcome definition has been previously estimated at 77.6%. Secondary outcomes were major congenital malformations overall, defined as the presence of any major malformation, and noncardiac congenital malformations, defined as the presence of a major malformation in the absence of a cardiac defect. Because the use of lithium in early pregnancy has been previously associated with an increased risk of Ebstein's anomaly, the authors assessed right ventricular outflow tract obstruction defects as a secondary outcome. They focused on overall right ventricular outflow tract obstruction defects rather than on Ebstein's anomaly for two reasons: first, Ebstein's anomaly has a specific code in the International Classification of Diseases, 9th Revision (ICD-9), but it may be classified under the general group of right ventricular outflow tract obstruction malformations; second, clinicians may be more likely to label a right ventricular outflow tract obstruction defect as Ebstein's anomaly in infants known to have been exposed to lithium than in unexposed infants, and thus Ebstein's anomaly may be more prone to differential misclassification than right ventricular outflow tract obstruction defects more generally.

- Covariates

The authors considered the following covariates as potential confounders: maternal age at delivery, race or ethnic group, year of delivery, smoking status, maternal psychiatric disorders and medical conditions, concomitant medication use, and general markers of the burden of disease, including the Obstetric Comorbidity Index and measures of the intensity of health care use. Maternal coexisting conditions and medication use were measured during the 3 months before pregnancy through the end of the first trimester. Measures of health care use (e.g., number of physician visits) were captured only during the 3 months before pregnancy to avoid their being influenced by early awareness of possible pregnancy complications.

- Results
  - Study Cohort and Patient Characteristics

The study cohort included 1,325,563 pregnancies that met the inclusion criteria. Of these, 663 (0.05%) were in women who were exposed to lithium and 1945 (0.15%) were in women who were exposed to lamotrigine during the first trimester. As compared with unexposed women, women who were exposed to lithium were older, more likely to be white, and had a higher prevalence of psychiatric disorders (particularly bipolar

disorder and depression) and pain conditions. They were more likely to use psychotropic and pain medications and had a higher overall burden of disease. After propensity-score adjustment, baseline characteristics were well balanced between the two groups.

- Absolute and Relative Risks of Cardiac Malformations

The prevalence of cardiac malformations was 2.41 per 100 live births among infants exposed to lithium, 1.15 per 100 among unexposed infants, and 1.39 per 100 among infants exposed to lamotrigine (Table 2). The adjusted risk ratio for cardiac malformations among infants exposed to lithium was 1.65 (95% confidence interval [CI], 1.02 to 2.68) as compared with nonexposed infants and 2.25 (95% CI, 1.17 to 4.34) as compared with lamotrigine-exposed infants. The adjusted differences were 0.95 cases per 100 births (95% CI, −0.22 to 2.12) and 1.43 cases per 100 births (95% CI, 0.09 to 2.77), respectively. The adjusted risk ratio for cardiac malformations among infants exposed to lamotrigine as compared with unexposed infants was 0.89 (95% CI, 0.61 to 1.30) (Table 2). The adjusted risk ratio for noncardiac defects among infants exposed to lithium as compared with unexposed infants was 1.22 (95% CI, 0.81 to 1.84).

Table 2. Absolute and Relative Risk of Cardiac, Noncardiac, and Overall Malformations among Infants Exposed to Lithium during the First Trimester as Compared with Lamotrigine-Exposed or Unexposed Infants.*

Variable	No Exposure to Lithium or Lamotrigine	Exposure to Lamotrigine	Exposure to Lithium
No. of pregnancies	1,322,955	1945	663
Cardiac malformations			
Events	15,251	27	16
Prevalence per 100 births	1.15	1.39	2.41
Unadjusted risk ratio (95% CI)	Reference	1.20 (0.83–1.75)	2.09 (1.29–3.40)
Propensity-score–adjusted risk ratio (95% CI)	Reference	0.89 (0.61–1.30)	1.65 (1.02–2.68)
Unadjusted risk ratio (95% CI)	—	Reference	1.74 (0.94–3.21)
Propensity-score–adjusted risk ratio (95% CI)	—	Reference	2.25 (1.17–4.34)
Noncardiac malformations			
Events	27,816	49	22
Prevalence per 100 births	2.10	2.52	3.32
Unadjusted risk ratio (95% CI)	Reference	1.20 (0.91–1.58)	1.58 (1.05–2.38)
Propensity-score–adjusted risk ratio (95% CI)	Reference	0.90 (0.68–1.18)	1.22 (0.81–1.84)
Unadjusted risk ratio (95% CI)	—	Reference	1.32 (0.80–2.16)
Propensity-score–adjusted risk ratio (95% CI)	—	Reference	1.63 (0.96–2.78)
Overall malformations			
Events	43,067	76	38
Prevalence per 100 births	3.26	3.91	5.73
Unadjusted risk ratio (95% CI)	Reference	1.20 (0.96–1.50)	1.76 (1.29–2.40)
Propensity-score–adjusted risk ratio (95% CI)	Reference	0.90 (0.72–1.12)	1.37 (1.01–1.87)
Unadjusted risk ratio (95% CI)	—	Reference	1.47 (1.00–2.14)
Propensity-score–adjusted risk ratio (95% CI)	—	Reference	1.85 (1.23–2.78)

* CI denotes confidence interval.

- Secondary and Sensitivity Analyses

The prevalence of right ventricular outflow tract obstruction defects was 0.60 per 100 live births among infants exposed to lithium and 0.18 per 100 among unexposed infants. The adjusted risk ratio for specific cardiac malformations that were associated with lithium was 2.66 (95% CI, 1.00 to 7.06) for right ventricular outflow tract obstruction defects and 1.46 (95% CI, 0.84 to 2.57) for other cardiac defects. Although none of

the identified right ventricular outflow tract obstruction defects in the lithium-exposed infants were specifically coded in claims as Ebstein's anomaly, most were consistent with cardiac defects that frequently co-occur with Ebstein's anomaly (e.g., pulmonary atresia and stenosis). The observed prevalence of Ebstein's anomaly among unexposed infants was approximately 7 cases per 100,000 births. In dose–response analyses based on the first lithium prescription in pregnancy, after propensity-score adjustment, the risk ratio was 1.11 (95% CI, 0.46 to 2.64) for a daily dose of 600 mg or less, 1.60 (95% CI, 0.67 to 3.80) for 601 to 900 mg, and 3.22 (95% CI, 1.47 to 7.02) for more than 900 mg. Results were consistent in analyses that used thirds of the highest prescribed dose during the first trimester. All right ventricular outflow tract obstruction defects that were identified in lithium-exposed infants occurred with a daily dose of more than 600 mg. There was no evidence of a dose–response relationship for lamotrigine. The risk of cardiac malformations among infants exposed to lithium as compared with unexposed infants remained consistently elevated in sensitivity analyses. After authors accounted for potential differences in the probability of termination of malformed fetuses among exposed and unexposed women, the range of plausible adjusted risk ratios for cardiac malformations among lithium-exposed infants was estimated to be 1.67 to 1.80.

3.4 Specific target organ toxicity – single exposure

Evaluation not performed for this substance

3.5 Specific target organ toxicity – repeated exposure

3.6 Aspiration hazard

Evaluation not performed for this substance

4 ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance

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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Version number: 02 **Date: June 2020**

CONTENTS

1 HYPOTHESIS FOR THE ANALOGUE APPROACH

According to the REACH Regulation (Regulation (EC) No 1907/2006) Annex XI 'Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or 'category' of substances. The similarities may be based on: … the common precursors and/or the likelihood of common breakdown products via physical and biological processes, which result in structurally similar chemicals'.

The inorganic lithium compounds

- **lithium carbonate,**
- **lithium chloride, and**
- **lithium hydroxide**

dissociate to the lithium cation (Li+) and the corresponding anion (carbonate – CO_3^{2-}, chloride – Cl-, or hydroxide – OH-) in aqueous solutions, i.e. in body fluids as well as in in vitro systems. The lithium cation remains unchanged in the body. Due to its similar charge and size to the sodium or potassium cation, it can use sodium ion channels to reach the target organs where it exerts its toxicological or pharmacological activity.

The anions are physiological anions, which are naturally present in the body. Carbonate is part of the extracellular bicarbonate buffer system, i.e. the interstitial and blood buffer. Carbonate is an endogenously generated substance. In the acid pH of the stomach, exogenous added carbonate is protonated and finally transformed to carbon dioxide and water. After absorption, carbon dioxide diffuses rapidly into red blood cells, where it is hydrated to form carbonic acid, a reaction accelerated by carbonic anhydrase, which is present in high concentrations in red blood cells. Carbonic acid dissociates into bicarbonate and hydrogen ions, which then become part of the endogenous bicarbonate buffer

Chloride is an electrolyte with a relevant role in physiology. It is an essential compound of the acid produced in the stomach, hydrochloric acid (HCl), and e.g. of sweat. Together with sodium, it controls the water balance of the body and it is relevant for the electrical excitability of the nerves. The hydroxide anion is e.g. generated when carbonate and water react to hydrogen carbonate (bicarbonate) and hydroxide anion. Due to its reactivity, it rapidly reacts with free protons to water. After oral exposure, it is rapidly neutralised by stomach acid.

Read-across hypothesis

Systemic toxicity of inorganic lithium compounds (Li-carbonate, -chloride, -hydroxide) are determined by the lithium ion.

2 SOURCE CHEMICAL(S)

Overview on the chemicals is provided below (Table AII - 1). Data were obtained from ECHA (ECHA, 2017a) (Date of access: 02-February-2017).

Table AII - 1: Overview of the chemicals considered in the category

Chemical name	**Lithium carbonate**	**Lithium hydroxide**	**Lithium chloride**
CASRN	**554-13-2**	**1310-65-2**	**7447-41-8**
Molecular formula	$(Li)_2CO_3$	Li(OH)	Li Cl
MW [g/mol]	73.89 [b]	23.9479 [b] (anhydrous) 41.9627 [b] (monohydrate)	42.394 [b]
Classification according to Regulation (EC) No 1272/2008[1] [#a]	Acute Tox. 4 (H302)[#] Eye Irrit. 2 (H319)[#]	Acute Tox. 4 (H302)[#] Skin Corr. 1B (H314)[#] Eye Dam. 1 (H318)[#]	Acute Tox. 4 (H302)[#] Skin Irrit. 2 (H315)[#] Eye Irrit. 2 (H319)[#]
PBT assessment[2#]	No PBT assessment available (anorganic substance)	No PBT assessment available (anorganic substance)	No PBT assessment available (anorganic substance)

a: only harmonised classification and joint entries reported; #: joint entry;

b: information provided by https://chem.nlm.nih.gov/chemidplus/, date of access: 02-February-2017

3 PURITY / IMPURITIES

Although the purity of the test substance is not always available, especially literature data very often do not provide exact data on substance purity, there is no knowledge about impurities which might mimic the

[1] ECHA, European Chemicals Agency (2017a)

Information on Chemicals - Classification & Labelling Inventory

Online: http://echa.europa.eu/information-on-chemicals/cl-inventory, Disclaimer: http://echa.europa.eu/web/guest/legal-notice , date of access: 02-February-2017

[2] ECHA, European Chemicals Agency (2017b)

Information on Chemicals - Registered Substances

Online: http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances , date of access: 16-NOV-2016

lithium effects. Therefore, missing information on purity of the test substance for some studies are not regarded as relevant for the overall evaluation.

4 ANALOGUE APPROACH JUSTIFICATION

Category justification

The anions influence the physico-chemical properties of the inorganic lithium compounds like water solubility, acidity of the substance (characterised by the (logarithmic) acid dissociation constant pKa), but not the toxicological/physiological function of the lithium cation. Due to the fact that the anions are naturally present in the body they are rapidly integrated into the physiological pool of anions or neutralised in the body so that they do not influence the systemic toxicity of the lithium compounds. Lithium hydroxide, due to its basic properties, causes skin and eye irritancy. However, this is not relevant for the evaluation of the systemic toxicity.

5 DATA MATRIX

Comparative data on physico-chemical parameters as well as human health endpoints are relevant and thus provided in the following table.

Table AII - 2: Comparative data from registration dossier on physico-chemical parameters and human health endpoints

Chemical name	**Lithium carbonate**	**Lithium hydroxide**	**Lithium chloride**
CASRN	**554-13-2**	**1310-65-2**	**7447-41-8**
Physico-chemical data			
Melting Point [°C] at 1013 hPa	722	422.83	608-614
Boiling Point [°C]	Data not available	Data not available	1360-1383
Flash Point [°C]	Data not available	Data not available	Data not available
Density [g/cm³]	2.1	1.5	1.06-2.1 at 20°C
Vapour pressure [hPa]	Data not available	Data not available	4.2E-30 Pa (calculated)
Partition coefficient (log P_{OW})	Data not available	Data not available	-0.46 (calculated)
Water solubility [g/L] at 20 °C	8.4	71-125	569
Mammalian toxicity			
Acute oral [mg/kg]	LD50 (rat) = 525-723	LD50 (rat, female/male) = 210/280* (corresponding to 368/491* for the monohydrate)	LD50 (rat) = 526
Acute inhalation [mg/L]	LC50 (rat, 4 h) > 2	LC50 (rat, 4 h) > 3.4	LC50 (rat, 4 h) > 5.57
Acute dermal [mg/kg]	LD50 (rabbit) > 2000 or > 3000	LD50 (rabbit) > 2000 ; RA from lithium carbonate	LD50 (rabbit) > 2000
Skin irritation/corrosion	Not irritating	Corrosive **	Not irritating

Chemical name	**Lithium carbonate**	**Lithium hydroxide**	**Lithium chloride**
Eye irritation	Eye irritant	No data available	Eye irritant
Skin sensitisation	Not sensitising	No key study available, RA to lithium chloride	Not sensitising
Repeated dose	**Oral** (WoE, rat): NOAEL = 6.43 mg Li/kg bw/day (calculated, based on therapeutic human dose) **Inhalation**: No data available **Dermal**: No data available	No valid **oral** study available, NOAEL = 4.13Li mg/kg bw/day (calculated, based on therapeutic human dose) **Inhalation**: No data available **Dermal**: No data available	**Oral (WoE, rat)**: NOAEL = 85-102 mg/kg bw/d (rat) **Inhalation**: No data available **Dermal**: No data available
Genetic toxicity *in vitro* - Gene mutation (bacteria) - Chromosomal aberration - Gene mutation (mammalian cells)	No data available (Ames) No data available (CA) No data available (GM)	Negative (Ames) Negative (CA) Negative (GM)	No data available (Ames) No data available (CA) No data available (GM)
Genetic toxicity *in vivo*	No data available	No data available	No data available
Reproductive Toxicity **- Fertility**			
	(OECD 416, rat, gavage) NOAEL $_{\text{fertility}}$ = 45 mg/kg bw/day (highest dose tested); LOAEL $_{\text{systemic toxicity}}$ = 45 mg/kg bw/day WoE : effect on male reproduction from several publications	No key study available, RA to lithium carbonate	No key study available WoE : effect on male reproduction from some publications
Reproductive Toxicity **- Developmental toxicity**			
	(OECD 414, rat, gavage) NOAEL $_{\text{maternal toxicity}}$ = 30 mg/kg bw/day; NOAEL $_{\text{embryotoxicity}}$ = 90 mg/kg bw/day (highest dose tested)	No key study available, RA to lithium carbonate	No key study available, WoE : teratogenic effects from some publications
	Teratogenic effects reported in human treated by lithium as a medicine		

* Probably due to corrosive properties; ** based on in vitro assay

RA: read-across

WOE: weight of evidence

CA: chromosomal aberration

GM: gene mutation

6 CONCLUSIONS FOR READ ACROSS APPROACH

The chemicals lithium carbonate, lithium chloride and lithium hydroxide have no current harmonized classification. They are all self-classified for Acute toxicity after oral exposure (Cat. 4).

According to self-notified classifications, lithium carbonate and lithium chloride are classified as eye irritants (Cat. 2), whereas lithium hydroxide is classified as causing severe eye damage (Cat. 1), indicating that lithium hydroxide is locally more reactive. This is also obvious from the classification of lithium hydroxide as skin corrosive (Cat. 1B) whereas lithium carbonate is not classified for skin local effects and lithium chloride is only classified as skin irritant (Cat. 2). As discussed, these differences in the local reactivity are mainly due to the physico-chemical properties of the lithium compounds determined by the anion, but do not influence the systemic toxicity.

The acute oral toxicity values (see Table AII – 2), in rats (LD50 values) for lithium salts are similar. A slightly lower value was however obtained for lithium hydroxide, which was obviously due to severe local effects observed in the stomach of the treated animals.

Existing data on repeated dose toxicity, genetic toxicity or reproductive toxicity are limited for a comparison of the toxicity of the three substances of interest, because in the registration dossiers data requirements under REACH were covered by read-across, so no direct comparison is possible. However, published data on genetic and reproductive toxicity, mainly disregarded in the registration dossier, indicate that the genetic and reproductive toxicity of the substances is comparable.

Due to the reasons outlined above (Li-anion as common breakdown product), the three substances are regarded as similar and therefore suitable for a category approach. This interpretation is supported by the common risk assessment for lithium compounds as performed by several organisations (Hartwig, 2014; HCN, 2000; Lagerkvist and Lindell, 2002; Montelius, 2003; Moore, 1995).

7 REFERENCES TO ANNEX II

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Information on Chemicals - Classification & Labelling Inventory

Online: http://echa.europa.eu/information-on-chemicals/cl-inventory, Disclaimer: http://echa.europa.eu/web/guest/legal-notice

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