Regulatory Management Option Analysis (RMOA)

Draft

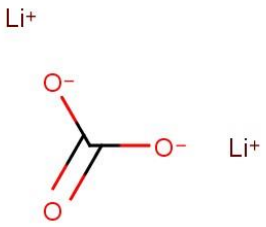
Authority: France

Date: July 2024

Substance name:

Lithium and 3 salts: lithium carbonate, lithium hydroxide, lithium chloride

General structure: Li, Li_2CO_3, LiOH, LiCl

Revision history

Version	*Date*	*Description*
1	July 2024	

Comments and additional relevant information are invited on this RMOA by XXX.

Additional specific questions to MSCAs:

The eMSCA would appreciate to receive any information that you are aware of.

Substances within this group:

EC/List number	CAS number	Substance name [and Substance name acronyms (*)]	Chemical structure(s)	Registration type (full, OSII or TII, NONS, cease manufacture), highest tonnage band among all the registrations (t/y)
231-102-5	7439-93-2	Lithium	Li	Full, OSII or TII, 100-1,000
209-062-5	554-13-2	Lithium carbonate		Full, > 1,000
215-183-4	1310-65-2	Lithium hydroxide	LiOH	Full, > 1,000
231-212-3	7447-41-8	Lithium chloride	LiCl	Full, > 1,000

Contents

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Foreword

The purpose of the assessment of regulatory needs of a group of substances is to help authorities conclude on the most appropriate way to address the identified concerns for a group of substances or a single substance, i.e. the combination of the regulatory risk management instruments to be used and any intermediate steps, such as data generation, needed to initiate and introduce these regulatory measures.

An assessment of regulatory needs can conclude that regulatory risk management at EU level is required for a (group of) substance(s) (e.g. harmonised classification and labelling, Candidate List inclusion, restriction, other EU legislation) or that no regulatory action is required at EU level. While the assessment is done for a group of substances, the (no) need for regulatory action can be identified for the whole group, a subgroup or for single substance(s).

The assessment of regulatory needs is an important step under ECHA's Integrated Regulatory Strategy. However, it is voluntary, i.e., it is not part of the processes defined in the legislation but aims to support them.

The assessment of regulatory needs can be applied to any group of substances or single substance, i.e., any type of hazards or uses and regardless of the previous regulatory history or lack of such. It can be done based on different level of information. A Member State or ECHA can carry out this case-by-case analysis. The starting point is available information in the REACH registrations and any other REACH and CLP information. However, more extensive set of information can be available, e.g. assessment done under REACH/CLP or other EU legislation, or can be generated in some cases (e.g. further hazard information under dossier evaluation). Uncertainties associated to the level of information used should be reflected in the documentation. It will be revisited when necessary. For example, after further information is generated and the hazard has been clarified or when new insights on uses are available. It can be revisited by the same or another authority.

The responsibility for the content of this assessment rests with the authority that developed it. It is possible that other authorities do not have the same view and may develop further assessment of regulatory needs. The assessment of regulatory needs does not yet initiate any regulatory process but any authority can consequently do so and should indicate this by appropriate means, such as the Registry of Intentions.

For more information on Assessment of regulatory needs please consult ECHA website[1].

[1] https://echa.europa.eu/understanding-assessment-regulatory-needs

Glossary

ADEME	*Agence de la transition écologique* [French Agency for Ecological Transition]
ADHD	Attention-Deficit/Hyperactivity Disorder
AGS	Ausschuss für Gefahrstoffe
Anses	*Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'environnement et du travail* [French Agency for Food, Environmental and Occupational Health & Safety]
ARN	Assessment of Regulatory Needs
ASD	Autism Spectrum disorders
BAT	Best available techniques
BE	Biomonitoring equivalent
BL	Binding limit value
BOEL	binding occupational exposure limit value
BRGM	*Bureau de Recherches Géologiques et Minières* (French geological survey]
CAD	Chemical Agent Directive
CARACAL	Competent Authorities for Registration, Evaluation, Authorisation and restriction of Chemicals (REACH) and Classification, Labelling and Packaging (CLP)
CCH	Compliance Check
CLH	Harmonised classification and labelling
CLP	Regulation (EC) No 1272/2008 of 16/12/08 on classification, labelling and packaging (CLP) on substances and mixtures
CMR	Carcinogenic, mutagenic and/or toxic to reproduction
CMRD	Carcinogens, Mutagens or Reprotoxic substances Directive
CRM	Critical raw materials
CSR	Chemical safety report
DEv	Dossier evaluation
DFG	Deutsche Forschungsgemeinschaft
DNEL	Derived no effect level
DS	Dossier submitter
DWD	Drinking water directive

EC50	Effect concentration 50
ECHA	European Chemical Agency
ED	Endocrine disruptor
EEE	Electrical and electronic equipment
EFSA	European Food Safety Authority
ELV	Emission limit value
eMSCA	Evaluating Member State competent authority
EQS	Environmental quality standards
EU	European Union
HBSL	Health Based Screening Level
IED	Industrial Emissions Directive
ILIA	International Lithium Association
INRS	*Institut national de recherche et de sécurité*
IOEL	indicative occupational exposure limit value
JRC	Joint Research Centre
LC50	Lethal concentration 50
Li	Lithium
LiCl	Lithium chloride
Li2CO3	Lithium carbonate
LiOH	Lithium hydroxide
LMP	Lithium Metal Polymer
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
L(O)Q	Limit (of) quantification
MAK	Maximale Arbeitsplatzkonzentrationen
MELCC	*Ministère de l'Environnement et de la Lutte contre les changements climatiques* (Québec) [Ministry of the Environment from Quebec]
MS	Member State
MSCA	Member State competent authority
NO(A)EL/NO(A)EC	No observed (adverse) effect level/concentration
NONS	Notified new substances

NORMAN	Network of Reference Laboratories for the Monitoring of Emerging Environmental Substances
OEL	Occupational exposure limit
OSH	Occupational Safety and Health
OSII or TII	On-site isolated intermediate or transported isolated intermediate
PACT	Public activities coordination tool
PBT/vPvB	Persistent, bioaccumulative and toxic/very persistent and very bioaccumulative
PC	Product category
PDE	Permissible daily exposure
PNEC	Predited no effect concentration
PoD	Point of Departure
PPE	Personal protective equipment
PPRTV	Provisional peer reviewed toxicity value
p-RfD	Provisional Reference Dose
RAC	Risk Assessment Committee
RBSP	River Basin-Specific Pollutants
RCR	Risk characterisation ratio
RDA	Recommended dietary allowance
REACH	Regulation (EC) No 1907/206 of 18/12/06 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
RMM	Risk management measure
RMOA	Regulatory management options analysis
RoHS	Restriction of Hazardous Substances
SEv	Substance evaluation
STOT RE	Specific target organ toxicity, repeated exposure
SVHC	Substance of very high concern
TDI	Tolerable daily intake
TDS	Total diet studie
TPE	Testing proposal examination
TRV	Toxicological reference value
UF	Uncertainty factor

USGS	United States Geological Survey
US EPA	U.S. Environmental Protection Agency
WFD	Water Framework Directive

1 Overview of the substance

1.1 Context and scope of the RMOA

In 2017, a CLH report on lithium carbonate was initiated by a consultant on request of the European Commission. France MSCA proposed to be MS rapporteur of this dossier and extended its scope to three lithium salts: lithium carbonate, lithium chloride and lithium hydroxide. In 2019, France MSCA submitted the dossier to ECHA with a CLH proposal as Repr. 1A for the development and 1B for the fertility (H360FD) based on adverse effects on development and sexual function and fertility[2]. This proposal was approved by RAC with an added classification as Lact..(H362)[3].

This dossier is for now pending as, on request of the European Commission, ECHA requested the RAC to review an additional publication (Boyle et al. (2017)), which was provided by industry stakeholders at CARACAL and, if necessary, to amend its opinion in relation to the classification for reproductive toxicity of the three lithium salts.

During this RMOA, France MSCA evaluated lithium and its salts with respect to the endocrine disruption hazard properties. In addition, France MSCA wanted to ensure the adequacy of worker protections regarding a known reprotoxicant. Indeed, lithium, naturally found as salts, is widely used in various sectors. Besides, France MSCA wanted to check available data regarding potential risks for human through environmental exposure and environmental exposure leading to risks for environmental species. Indeed, it is important to identify if risks are expected while considering the growing amount of lithium used in Europe and the new projects on lithium mining appearing in Europe.

France, through ANSES, decided to pursue its investigation on assessing lithium safe use by building a RMOA to determine if further regulatory risk management actions are needed.

1.2 Substance identifiers

Table 1: Substance identity

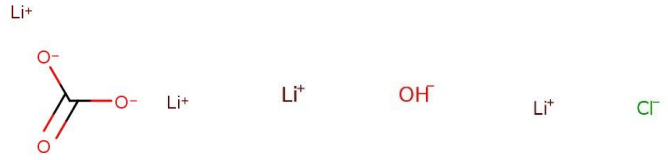
SUBSTANCES IDENTITY				
Public name	Lithium	Lithium carbonate	Lithium hydroxide	Lithium chloride
EC number	231-102-5	209-062-5	215-183-4	231-212-3
CAS number	7439-93-2	554-13-2	1310-65-2	7447-41-8
Index number in Annex VI of the CLP Regulation	003-001-00-4	-	-	-
Molecular formula	Li	Li_2CO_3	LiOH	LiCl

[2] https://echa.europa.eu/documents/10162/0926d256-2ba7-3a02-3a54-91d7bfdbaf6f
[3] https://echa.europa.eu/documents/10162/e2a3c38e-85fe-505c-a325-293c70a74da5

Molecular weight		73.888 g/mol	23.947 g/mol (anhydrous) 41.962 g/mol (monohydrate)	42.394 g/mol

Type of substances: Mono-constituents

Structural formulas:



Lithium is the smallest metal atom in the periodic table. It is light, highly reactive, reacting with water, oxygen, carbon dioxide and nitrogen at room temperature. In contrast, the lithium salts covered by this RMOA are very stable. Lithium is a metal used more and more nowadays and the demand still grows. Lithium is never naturally found under its metallic form. It can be found in a solution, in fluids (in salar or brine, geothermal groundwater, etc.) or under a solid form in a mineral crystalline network.

1.3 Completed or ongoing process

Table 2: Completed or ongoing processes
Data consulted on October 2023:

EC/List number	Other REACH related work	RMOA	Evaluation			Authorisation		Restriction	CLH	Actions not under REACH/ CLP(*)
			CCH	TPE	SEV	Candidate List	Annex XIV	Annex XVII	Annex VI (CLP)	
231-102-5	ARN/ PACT	On going								
209-062-5	ARN/ PACT	On going							X	
215-183-4	ARN/PAC T	On going							X	
231-212-3	ARN/ PACT	On going							X	

2 Overview of the trans-sectorial regulations

2.1 Regulatory process under CLP regulation

Lithium has a harmonised classification as:

- Water-react 1 (H260);
- Skin Corr. 1B (H314).

Moreover, lithium is also self-classified as Acute Tox. 3 (H301), Eye Dam. 1 (H318), Flam. Sol. 1 (H228) and STOT SE 2 (H371).

Data consulted on October 2023:

EC/ List No	CAS No	Substance name	Harmonised classification	Classification in registrations	Classification in C&L notifications
231-102-5	7439-93-2	*Lithium*	Water-react. 1 (H260) Skin Corr. 1B (H314)		Acute Tox. 3 (H301) Eye Dam. 1 (H318) Flam. Sol. 1 (H228) STOT SE 2 (H371) Aquatic Chronic 4 (H413)
209-062-5	554-13-2	*Lithium carbonate*			Acute Tox. 4 (H302) Eye Irrit. 2 (H319) Skin Irrit. 2 (H315) Eye Dam. 1 (H318) STOT SE 3 (H335) Repr. 1 B (H360) STOT RE 1 (H372) Aquatic Chronic 3 (H412) Repr. 1 A (H360) STOT SE 2 (H371) STOT RE 1 (H372) Repr. 2 (H361) STOT RE 2 (H373) Acute Tox. 4 (H332)
231-212-3	7447-41-8	*Lithium chloride*	-		Acute Tox. 4 (H302) Acute Tox. 2 (H330) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Acute Tox. 4 (H312) Acute Tox. 4 (H332) STOT SE 3 (H335) Repr. 1A (H360) Aquatic Chronic 2 (H411) Lact. (H362) STOT RE 2 (H373) STOT SE 1 (H370) Aquatic Chronic 3 (H312) Carc. 1A (H350) Acute Tox. 3 (H301) Acute Tox. 3 (H311)
215-183-4	1310-65-2	*Lithium hydroxide*	-		Acute Tox. 4 (H302) Skin Corr. 1B (H314) Acute Tox. 3 (H301) Acute Tox. 3 (H311) Eye Dam. 1 (H318) Skin Corr. 1A (H314) Aquatic Chronic 3 (H412) Aquatic Chronic 2 (H411) Lact. (H 362)

A CLH dossier has been validated by RAC, in 2021, for the 3 lithium salts. The classification proposed is Repr. 1A (H360 FD) and Lact. (H362). On the 23rd August of 2023, the EU Commission requested ECHA to review new information in relation to the harmonised classification of the three salts. RAC confirmed by consensus its earlier opinion at the meeting from March 2024, recommending the above mentioned harmonised classifications[4]. They should now be accordingly implemented into an ATP. When it will be the case, the new classification will have numerous consequences:

- It will require company level risk management measures (RMM) for workers to be defined and implemented;

[4] Minutes of the 68th Meeting of the Committee for Risk Assessment (RAC-68) (https://echa.europa.eu/documents/10162/62900669/rac-68_final_minutes_en.pdf/09285010-2469-8644-0bd8-e812995bddf9?t=1711611039004)

- It would lead to add the corresponding substances to the generic restriction by means of restriction entry 30.

2.2 Worker protection

The principles of the European acquis on worker protection are set out in the overarching OSH "framework directive", which establishes duties on employers and workers to identify and manage workplace risks – including by prevention.

Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work ("CAD") and Directive 2022/431/EC on carcinogens, mutagens or reprotoxic substances at work ("CMRD") aim at protecting workers from chemical risks at the workplace. They are written in a "goal setting" approach with general principles (see next, on the employers' obligations). They set minimum requirements to protect and prevent workers from health and safety risks which might arise from exposure to chemicals (for Directive 98/24/EC - CAD) and to carcinogens, mutagens or reprotoxic substances specifically (for Directive 2022/431/EC - CMRD). They lay down limit values of exposure (namely Occupational Exposure Limits Values – OELs) and recommend the implementation of very similar risk management measures (RMMs) in order to control the risk in the workplace.

2.2.1 Chemical Agents Directive 98/24/EC (CAD)

CAD sets indicative or binding occupational exposure limit values (IOELs or BOELs) as well as biological limit values (BLs) at Community level (biological limit values are always binding contrary to OELs) that need to be translated at each national level. BOELs take into account socio-economic and technical feasibility factors as well as factors considered when establishing IOELs. For any chemical agent for which a BOEL or biological limit value is established at EU level, Member States must establish a corresponding national value, which may be more stringent, but cannot exceed the Community limit value.
For any chemical agent for which an indicative OEL value is established at Community level, Member States must establish a national OEL value (indicative or binding depending on the willingness of the Member State) taking into account the Community limit value.

The legal status of IOELs arises from the CAD and are implemented through the IOELs Directives.
Neither IOEL/BOEL nor BL is currently set for lithium and its salts.

Employers' obligations

Employers must determine whether any hazardous chemical agents are present at the workplace and assess any risk to the safety and health arising from their presence and all uses including those expected with higher exposure such as maintenance. On the basis of the general principles for prevention of risks associated with hazardous chemical agents, employers are required to ensure that the risk is eliminated or reduced to a minimum. Where the results of the risk assessment reveal a risk to the safety and health of workers, specific protection and prevention measures shall be applied. To this purpose, substitution shall by preference be undertaken. When substitution is not possible, further protection and prevention measures shall be applied, including in order of priority:

a. design of appropriate work processes and engineering controls and use of adequate equipment and materials, so as to avoid or minimise the release of hazardous chemical agents;

b. application of collective protective measures at the source of the risk, such as adequate ventilation and appropriate organizational measures;

c. where exposure cannot be avoided by other means, application of individual protective measures including personal protective equipment.

Such measures shall be accompanied by health surveillance if this is appropriate to the nature of the risk. When an indicative or binding OEL value established in the territory of a Member State has been exceeded, the employer shall immediately take remediation by implementing preventive and protective measures. Training of workers is also requested from employers.

2.2.2 Carcinogens, Mutagens or Reprotoxic substances Directive 2004/37/EC updated (CMRD)

CMRD sets BOELs for substances or mixtures that meet the criteria for classification as a Category 1A or 1B carcinogen, germ cell mutagen or reprotoxic substance set out in Annex I to the CLP Regulation. Unlike Directive 98/24/EC, OELs are always binding. For any carcinogen, mutagen or toxic for reproduction for which a BOEL is established at European level, Member States must establish a corresponding national BOEL that does not exceed the Community limit value but may be more stringent.

As a direct consequence of the future classification as Repro. 1A, lithium chloride, lithium hydroxide, lithium carbonate will be subject to control under the CMRD. To date, **no BOEL for these 3 salts and lithium has been adopted at EU level**.

BOELs are adjusted to the technical feasibility of European companies and Member States in order to ensure a harmonized implementation across Europe. Socio-economic aspects are taken into consideration whatever the status of the value is (especially for binding OEL). Setting an OEL in the existing legal framework is always a tripartite agreement with representatives of Employers, workers trade unions and Governments that allow its social acceptance and thus facilitates its implementation.

Employers' obligations

The Directive recommends substitution as a priority otherwise encourages to avoid exposure or to keep it as low as possible and below the binding limit that is set. Employers are required to "reduce the use of a carcinogen, mutagen or reprotoxic substance at the place of work, in particular by replacing it, in so far as is technically possible, by a substance, mixture or process which, under its conditions of use, is not dangerous or is less dangerous to workers' health and safety […]" (Article 4(1)). "Where the results of the assessment […] reveal a risk to workers' health and safety, workers' exposure must be prevented" (Article 5(1)). "Where it is not technically possible to replace the carcinogen, mutagen or reprotoxic substance by a substance, mixture or process, which, under its conditions of use, is not dangerous or is less dangerous to health or safety, the employer shall ensure that the carcinogen, mutagen or reprotoxic substance is, in so far as is technically possible, manufactured and used in a closed system" (Article 5(2)). "Where a closed system is not technically possible, the employer shall ensure that the level of exposure of workers to the carcinogen, mutagen or non-threshold reprotoxic substance is reduced to as low a level as is technically possible" (Article 5(3)).

2.2.3 OELs and lithium: state of the art

In Germany, a AGS value (as well as DFG value - MAK) for inorganic lithium compounds is set up at 0.2 mg/m^3 (inhalable fraction) for eight hours and same value for short-term exposures. The same values are set up in Switzerland.

2.2.4 Other legislations

In addition to the legislation on safety and health at work, risk at workplace arising from exposure to hazardous substances is also managed by the following Directives: ***Directive 89/656/EEC*** lays down minimum requirements for **personal protective equipment (PPE),** ***Directive 92/85/EC*** (**pregnant workers Directive),** ***Directive 94/33/EC*** (**young workers Directive**). They impose minimum standards for health and safety of workers and provide a framework of directions and safeguards to ensure that the occupational risk to health from hazardous substances is controlled. These Directives do not specifically address lithium, but cover it indirectly regarding its classification as a hazardous substance.

The future classification Repr. 1A of lithium salts will also directly influence worker's safety. Indeed, in France specifically, the recent decree n° 2024-307[5] defines modalities of the traceability of worker exposure to carcinogenic, mutagenic or reprotoxic chemical agents. The employers have to establish an updated list of workers likely to be exposed to carcinogenic, mutagenic or reprotoxic chemical agents. This list indicates, for each worker, the substances to which he is likely to be exposed as well as, when known, information on the nature, duration and degree of his exposure.

In addition to these legislative texts, ***Seveso III Directive 2012/18/EU*** related to the control of major-accident hazards involving dangerous substances (ex-Directive 96/82/EC) addresses risks for workers as well as risks for the general population located around sensitive industrial sites. The Seveso III Directive aims at controlling major-accident hazards involving dangerous substances and sets rules for the prevention of major accidents, which might result from certain industrial activities and the limitation of their consequences for human health and the environment.
It has to be taken into consideration that this directive, according to the article 2, shall not apply to, in particular:

- the exploitation, namely the exploration, extraction and processing, of minerals in mines and quarries, including by means of boreholes;
- waste land-fill sites, including underground waste storage.

2.3 EU sectorial regulations on substances and products (professionals and consumers)

When the classification as Repr. 1A will be implemented in the CLP regulation, these 3 salts will fall under the entry 30 of Reach Restrictions.

This entry 30 implies that substances which are classified as reproductive toxicant category 1A or 1B in Part 3 of Annex VI to Regulation (EC) No 1272/2008 and are listed in Appendix 5 or Appendix 6, respectively; are restricted.

[5] https://www.legifrance.gouv.fr/jorf/id/JORFTEXT000049366748 (consulted on 19/04/2024)

Indeed, the conditions of the restriction indicate that these substances shall not be placed on the market, or used, as substances, as constituents of other substances or in mixtures for supply to the general public when the individual concentration in the substance or mixture is equal to or greater than:

- either the relevant specific concentration limit specified in Part 3 of Annex VI to Regulation (EC) No 1272/2008, or,
- the relevant generic concentration limit specified in Part 3 of Annex I of Regulation (EC) No 1272/2008 (0.3%).

Regarding the 3 lithium salts and considering their future harmonised classification as Repr. 1A, if no specific concentration limit is specified, the relevant generic concentration will apply as in Part 3 of Annex I of the regulation No 1272/2008 meaning 0.3%. It implies finally that when the harmonised classification will be implemented in the CLP regulation, the access and the conditions of uses of these substances as constituent or in mixtures will be restricted for the general public.

In addition, there are numerous applications falling under specific sectorial regulations that will be displayed in Section 3 when describing these uses.

2.4 Regulations on waste

2.4.1 Waste framework Directive 2008/98/EC

This directive lays down measures to protect the environment and human health by preventing or reducing the adverse impacts on the generation and management of waste and by reducing overall impacts of resource use and improving the efficiency of such use.

According to article 7 of this directive, a Member State may consider waste as hazardous waste where, even though it does not appear as such on the list of waste, it displays one or more of the properties listed in Annex III. Annex III describes hazardous properties which render waste hazardous. The Member State shall notify the Commission of any such cases without delay. It shall record them in the report provided for in Article 37(1) and shall provide the Commission with all relevant information. In the light of notifications received, the list shall be reviewed in order to decide on its adaptation.

Thus, considering the current harmonised classification of lithium as Water-react 1 and Skin Corr. 1B, and the future harmonised classification as Repr. 1A for its three salts, waste containing lithium or its salts shall be classified accordingly provided that the conditions stated in Annex III are fulfilled (in terms of concentration limit for reprotoxicity and corrosion, according to a test method for flammability).

The Waste Framework Directive sets the basic concepts and definitions related to waste management, including definitions of waste, recycling and recovery.

The Waste Framework Directive lays down some basic waste management principles. It requires that waste be managed:

- without endangering human health and harming the environment;
- without risk for water, air, soil, plants or animals;
- without causing nuisance through noise or odours;
- without adversely affecting the countryside or places of special interest.

It explains when waste ceases to be waste and becomes a secondary raw material, and how to distinguish between waste and by-products. The Directive also introduces the "polluter pays principle" and the "extended producer responsibility".

2.4.2 RoHS Directive 2011/65/EU

The RoHS 2 Directive (Restriction of Hazardous Substances) is a European Union regulation aiming at restricting the use of certain hazardous substances in electrical and electronic equipment (EEE). It also aims at promoting the environmentally responsible recovery and disposal of waste EEE. Lithium and its salts are not covered by RoHS directive.

2.5 EU Regulation addressing emissions to environment

2.5.1 Industrial Emissions Directive 2010/75/EU (IED)

Covering some 52 000 large agro-industrial installations across the EU, the Industrial Emissions Directive is the main EU instrument regulating industrial pollutant emissions. This directive lays down rules on integrated prevention and control of pollution arising from industrial activities. It also lays down rules designed to prevent or, where this is not practicable, to reduce emissions into air, water and land and to prevent the generation of waste, in order to achieve a high level of protection of the environment taken as a whole.
This IED directive, aims at achieving a high level of environmental protection through the integrated prevention and control of pollution from a wide range of industrial and agricultural activities. It is the counterpart for chronic risks to Directive 2012/18/EU of July 4, 2012, known as the Seveso 3 Directive.

Its guiding principles are:

- the use of best available techniques (BAT) in the operation of the activities concerned. BAT must be the basis for setting emission limit values (ELVs) and other permit conditions;
- periodic review of permit conditions;
- restoration of the site to a condition at least equivalent to that described in a "baseline report" describing the state of the soil and groundwater prior to commissioning.

The activities covered by Chapter II of the IED are listed in Annex I.

The IED is currently discussed to be extended to large-scale battery production among which manufacture of lithium-ion batteries (with a production capacity >3.5 GWh or other than that corresponding exclusively to the assembly of cells into groups and modules, with a production capacity of 17,500 tonnes of battery cells (cathode, anode, electrolyte, separator and capsule)) and mining (extraction of industrial minerals and metals) (Annex I).

2.5.2 Water Framework Directive 2000/60/EC (WFD)

The Water Framework Directive 2000/60/EC is an EU directive which commits European Union member states to achieve good qualitative and quantitative status of all water bodies (including marine waters up to one nautical mile from shore) by 2015. It is a framework in the sense that it prescribes steps to reach the common goal rather than adopting the more traditional limit value approach. The Directive's aim for 'good status' for all water bodies will not be achieved, with 47% of EU water bodies covered by the Directive failing to achieve the aim.

Besides the set of Priority Substances laid down in Annex X of the WFD, which are regulated and to be monitored at EU level, the EU Member States (MS) need to identify pollutants of regional or local importance (in particular substances listed in Annex VIII) and provide environmental quality standards (EQS), monitoring schemes, and regulatory measures for them. This means that MS need to decide which are the candidate substances for further investigation and which are the substances then to be declared as River Basin-Specific Pollutants (RBSP). This requires assessments of impacts as well as prioritisation efforts and strategic screening for substances possibly causing concern. While this is a matter of discretion for each of the MS of concern, there is as yet no harmonization of the procedures involved. Therefore, JRC (European Commission, Joint Research Centre) and NORMAN (Network of Reference Laboratories for the Monitoring of Emerging Environmental Substances) organized a workshop in order to support MS. The objective of the workshop was to provide a common forum for MS and interested groups for presenting, discussing and streamlining approaches for a harmonised selection and monitoring of RBSP in the WFD context.

It should be noted that lithium was integrated within the annex 8b: List of river basin specific pollutants of the WFD reporting guidance 2022 (draft) published the 26th of October 2023 which indicates that this substance can be monitored to inform on the ecological state of certain river basins.

The integration of lithium and/or its salts in the priority list (appendix X) of this directive would allow to:

- introduce monitoring in waters;
- limit the contamination of environments by setting a limit value that takes into account the risks for the aquatic environment;
- protect water resources for the production of water intended for human consumption;
- provide means of action to limit emissions at the local level.

However, for this regulation to be applicable to all Member States, lithium and/or its salts would have to appear on the list of priority substances in the field of water (appendix X of the WFD), which is not the current case.

For a substance to be included in the list of priority substances of the WFD, it is necessary that:

- it is taken into account as a candidate substance in the prioritization carried out by the JRC. For this, sufficient data must be available, covering almost all Member States;
- it is prioritized;
- it is retained by the Member States and the Commission.

Since the entry into force of the directive, a watch list has been set up in France[6] and in Europe[7]: to date, lithium and/or its salts are not on any of these watch-lists. The substances appearing on this watch list must be selected from among those for which the available information indicates that they may present a significant risk, at European Union level, to or via the aquatic environment, but for which the monitoring is insufficient to reach a conclusion on the actual risk posed.

Finally, this directive would make it possible to establish an environmental limit value making it possible to regulate the discharges from the installations.

6 https://www.ineris.fr/sites/ineris.fr/files/contribution/Documents/Substances%20Pertinentes%20%C3%A0%20Surveiller%20%28SPAS%29%20v3.pdf

7 https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2020.257.01.0032.01.ENG&toc=OJ:L:2020:257:TOC

3 Information on tonnage and uses and their respective sectorial legislations

3.1 Overview of registration dossiers

Table 3: Overview of registrations (intermediate registrations versus article 10 full registration)

EC /List number	CAS Number	Substance name	REACH Annex	Article 10 Registrations (active)	Intermediate Registrations (active)	Not-updated NONS
231-102-5	7439-93-2	Lithium		1,000-10,000 t/y	x	-
209-062-5	554-13-2	Lithium carbonate		10,000-100,000 t/y	-	-
231-212-3	7447-41-8	Lithium chloride		1,000-10,000 t/y	-	-
215-183-4	1310-65-2	Lithium hydroxide		10,000-100,000 t/y	-	-

3.2 Tonnage and market of lithium

The mineralinfo website[8] (consulted in September 2022) indicates the main worldwide producers and the distribution of world lithium mining production in 2018 (Figure 1).

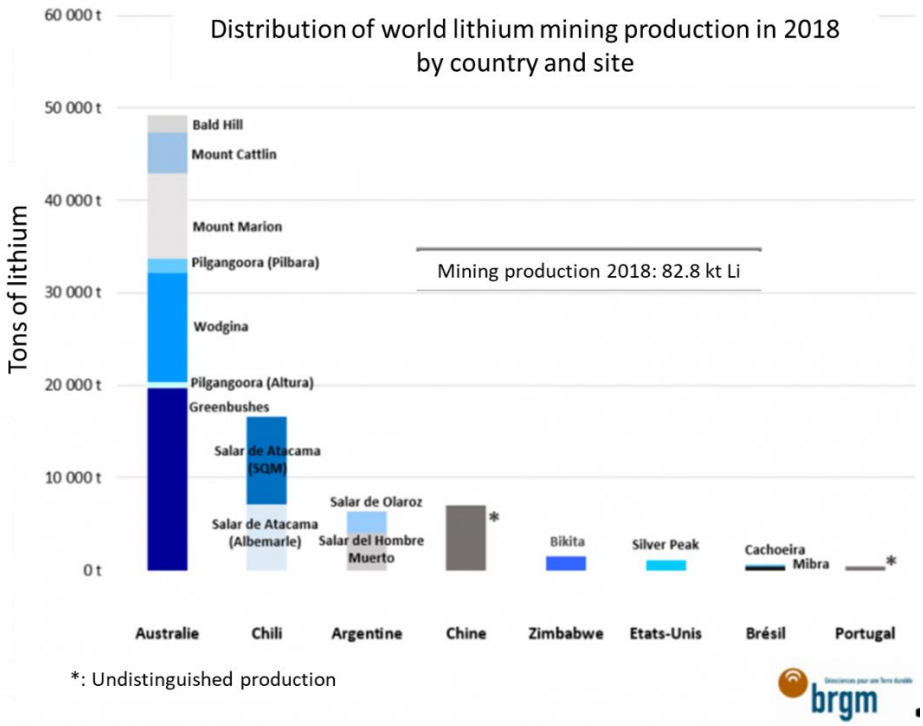


Figure 1: Distribution of world lithium mining production in 2018 (mineralinfo)

[8] https://www.mineralinfo.fr/fr/tag/lithium

In 2017, global lithium mining production was estimated between 41,000 and 44,000 t Li, 85% extracted from 3 countries: Chile, Argentina and Australia. By 2025, total lithium demand could triple (Albemarle Corporation, 2018 and Mineral info website) or quadruple (Roskill Information Services Ltd., 2016) compared to 2017, i.e. an average increase of 18%/year. In 2020, in Europe, one lithium mine is active, based in Portugal (Barroso) and exploited by Felmica. Around 348 tons of Li_2O/year were extracted, which represented 0.4% of the total amount of lithium extracted in the world in 2020 (82,500 kT) (Mines&Carrières, 2022).

The main driver of the growth prospects is the lithium consumption for rechargeable Lithium-Ion to develop electromobility and energy storage. This part of market continues to grow and it has a major impact on the global demand for lithium and therefore on prices. Total lithium consumption (expressed in lithium content) has doubled in 10 years, from 21,300 t Li in 2008 (Labbé and Daw, 2012) to an estimate around 43,000 t Li in 2017 (USGS, 2017). This increase was mainly linked to the battery sector, rising from a market share of 20% in 2008, i.e. 4,260 t Li, to nearly 40% in 2017, i.e. 16,400 t Li (BRGM, Infoterre). The share of the battery sector should be between 60% and 86 % of the total at this time, i.e. a tonnage greater than 100 kt Li, or more than 5-times the market overall in 2008 (BRGM, infoterre).

According to the ECHA website, the total tonnage for lithium carbonate and lithium hydroxide is between 10 000 and 100 000 tonnes; for lithium chloride it is between 1 000 and 10 000 tonnes.

3.3 Uses of lithium and their respective sectorial legislations

3.3.1 General information on uses as provided by different sources

Lithium can be processed to form a variety of chemicals, including lithium carbonate, lithium bromide, lithium chloride, butyl lithium and lithium hydroxide (ILIA website[9]).

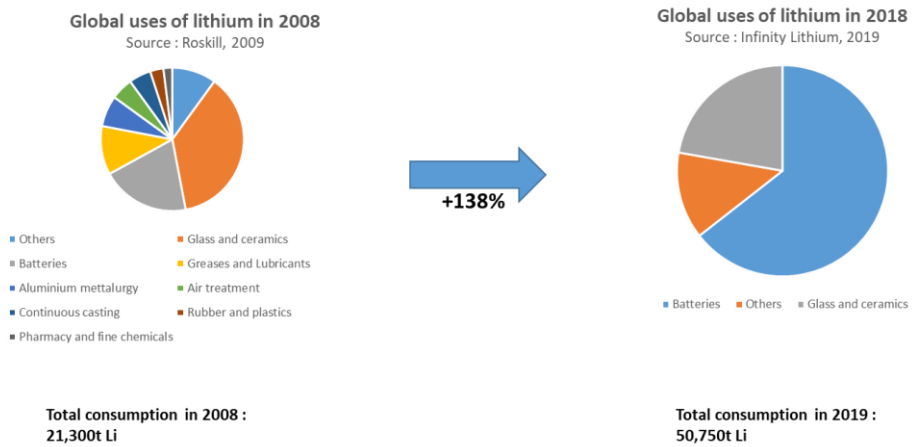


Figure 2: Worldwide uses of lithium between 2008 and 2018 (mineral info website)

Lithium has a wide range of uses and from 2015, it is dominated by the rechargeable battery sector (37%) since 2015 (see figure 2). Lithium is also widely

[9] https://lithium.org/lithium/ (consulted on 10/04/2023)

used in the field of glass and ceramics (30%) and to a lesser extent in the field of lubricating greases, metallurgy, polymers, air treatment, batteries and the medical field (BRGM 2018). Lithium can also be used as biocide enhancer for swimming pools, hot tubs and spas (US EPA 2008). According to Albemarle website[10], lithium carbonate is the building block for other lithium derivatives and is used in a variety of applications, such as frits for the ceramic and enamel industry, heat resistant glass, aluminium production, pharmaceuticals and batteries. Lithium hydroxide is mainly used as reagent in high-performance lithium greases, dyes and in lithium-ion batteries. Lithium chloride is used in fluxes, humidity control and zeolites; it is a raw material for the electrolysis of lithium metal.

ECHA website details the products in which lithium and the three salts are used according to the uses (consumer, professional or industrial uses). These products are described in Table 4.

Table 4: Consumer, professional and industrial uses of lithium[11], Li2CO3[12], LiOH[13] and LiCl[14]

	Consumer uses	Professional uses	Industrial uses
Lithium	No data indicating whether or in which chemical products the substance might be used.	Metals	Metals and pharmaceuticals Used for the manufacture of: chemicals, metals and machinery and vehicles
Li2CO3	Adhesives and sealants, fillers, putties, plasters, modelling clay, welding & soldering products, textile treatment products and dyes, adsorbents, pH regulators and water treatment products, pharmaceuticals and coating products	Coating products, adhesives and sealants, fillers, putties, plasters, modelling clay, textile treatment products and dyes, metals, welding & soldering products, pH regulators and water treatment products and paper chemicals and dyes	Adhesives and sealants, fillers, putties, plasters, modelling clay, coating products, non-metal-surface treatment products, metals, welding & soldering products, laboratory chemicals, paper chemicals and dyes and textile treatment products and dyes Used for the manufacture of: ineral products (e.g. plasters, cement), metals, textile, leather or fur, chemicals, fabricated metal products and electrical, electronic and optical equipment
LiOH	Adhesives and sealants, lubricants and greases, hydraulic fluids, metal working fluids, fillers, putties, plasters, modelling clay and biocides	Hydraulic fluids, lubricants and greases, metal working fluids, adhesives and sealants and fillers, putties, plasters, modelling clay	Lubricants and greases, metal working fluids, hydraulic fluids, inks and toners, polymers, adhesives and sealants and fillers, putties, plasters, modelling clay. Used for the manufacture of: chemicals, mineral products (e.g. plasters, cement), textile, leather or fur, electrical, electronic and optical equipment and machinery and vehicles.
LiCl	Coating products, metal	Laboratory chemicals, pH	Welding & soldering

[10] https://www.albemarle.com/offerings/lithium/products/lithium-salts (consulted on 10/04/2023)
[11] https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.028.274 (consulted on 10/04/2023)
[12] https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.008.239 (consulted on 10/04/2023)
[13] https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.013.804 (consulted on 10/04/2023)
[14] https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.028.375 (consulted on 10/04/2023)

	surface treatment products, non-metal-surface treatment products, adhesives and sealants, inks and toners, pH regulators and water treatment products, photo-chemicals, polishes and waxes and welding & soldering products	regulators and water treatment products, metal working fluids, pharmaceuticals, polymers, water treatment chemicals and welding & soldering products	products, laboratory chemicals, air care products, inks and toners, pH regulators and water treatment products, metal working fluids, pharmaceuticals, polymers and water treatment chemicals

The ECHA website also indicates that release of lithium into the environment can occur from industrial uses: in the production of articles, as an intermediate step in further manufacturing of another substance (use of intermediates) and of substances in closed systems with minimal release or from the manufacturing of the substance. Release to the environment of this substance is likely to occur from: indoor use, outdoor use resulting in inclusion into or onto a materials (e.g. binding agent in paints and coatings or adhesives), and outdoor use in closed systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oils and brake fluids).

Registered uses for lithium and the 3 salts

Table 5 : registered uses for lithium and the 3 salts

Chemical (EC number)				Product category
Lithium (231-102-5)	**Lithium carbonate (209-062-5)**	**Lithium hydroxide (215-183-4)**	**Lithium chloride (231-212-3)**	
	X	X	X	PC 20 : Products such as pH-regulators, flocculants, precipitants, neutralisation agents
		X	X	PC 37 : Water treatment chemicals
	X	X		PC 2 : Adsorbents
		X		PC 11 : Explosives
		X		PC 4 : anti-freeze and de-icing products
	X	X		PC 35 : Washing and cleaning products
		X		PC 8 : Biocidal products (disinfectants, pest control)
		X		PC 28 : Perfumes, fragrances
X	X	X	X	PC 29 : Pharmaceuticals
	X	X	X	PC 15 : Non metal surface treatment products
	X	X		PC 16 : Heat transfer fluids
	X	X	X	PC 1 : Adhesives, sealants
		X	X	PC 3 : Air care products
	X	X		PC 9b : Fillers, putties, platers modelling clay
	X	X	X	PC 9a : coatings and paints, thinners, paint removes
		X		PC 9c : Finger paint
	X		X	PC 26 : Paper and board treatment products
		X	X	PC 30 : Photo-chemicals
			X	PC 31 : Polishes and wax blends
	X	X		PC 34 : textiles dyes, and impregnating products
		X		PC 13 Fuels
	X	X	X	PC 14 : Metal surface treatment
		X	X	PC 32 : Polymer preparations and

				compounds
	X		X	PC 38 : Welding and soldering products, flux products
		X		PC 39 : Cosmetics, personal care products
X	X	X		PC 7 : Base metals and alloys
X	X	X	X	PC 21 : Laboratory chemicals
		X		PC 23 : Leather treatment products
		X		PC 24 : lubricants, greases, release products
		X	X	PC 25 : Metal working fluids
		X		PC 17 ; Hydraulic fluids
	X	X	X	PC 18 : Ink and toners
X	X	X		PC 19 : Intermediate
X	X			PC 42 : Electrolytes for batteries

3.3.2 Batteries

Batteries and accumulators, most of which are portable and used by households and professionals, contain certain substances that are hazardous to the environment and human health such as cadmium, mercury or nickel. These substances used in batteries, such as lithium, are obtained from rare resources that are not readily available in the European Union. They are also considered critical raw materials (CRM) by the Commission because they are crucial to Europe's economy.

Every year, over 1,500 million batteries and accumulators are placed on the French market, representing more than 270,000 tonnes.

Battery Li-ion:

Lithium has played a fundamental role in the development of high performance batteries called Lithium-ion (Li-ion). Li-ion batteries currently have the highest energy storage capacity per unit mass. This use now accounts for more than 50% of global lithium demand and is its main driver. Total lithium consumption for this use increased from a market share of 20% in 2008 (i.e. 4,260 t Li) to nearly 58% in 2018. According to forecasts, this share could rise to 85% in 2025 or even 2030. Estimates of the tonnages of lithium needed by this deadline vary according to the analyses, they will most likely exceed 150 kt Li in 2025, or even reach 300 kt Li in 2030[15] (MineralInfo website).

The first generations of batteries favoured cathode materials based on lithium cobaltate ($LiCoO_2$), which in 2007 represented 82% of Li-ion battery cathodes. The preferred lithium compound for the manufacture of these cathodes was lithium carbonate Li_2CO_3 of 99.5% purity. However, the evolution of cathode chemistry tends today to orient towards a growing demand for lithium hydroxide (LiOH) instead of Li_2CO_3[12]. The share of the battery sector is expected to be between 60% and 86% of the total around 2025. According to industrial data, lithium hydroxide is used at a concentration less than 10% (confidential source). Technology is progressing and if, to date, almost all of the electrodes of lithium-ion batteries are composed of nickel, manganese and cobalt, a new lithium-ion iron phosphate (LFP) technology is currently being developed.

[15] https://www.mineralinfo.fr/fr/ecomine/marche-du-lithium-2020-enjeux-paradoxes (consulted on 10/2023)

The two main lithium battery types are:

- primary (non-rechargeable): including coin or cylindrical batteries used in calculators and digital cameras. Lithium batteries have a higher energy density compared to alkaline batteries, as well as low weight and a long shelf and operating life. Lithium chloride and lithium metal seem to be used in non-rechargeable batteries according to industrial data (confidential source).
- Secondary (rechargeable): key current applications for lithium batteries are in e-mobility, powering cell phones, laptops, other hand-held electronic devices, power tools and large format batteries for electricity grid stabilisation. The advantages of the lithium secondary battery are its higher energy density and lighter weight compared to lead acid, nickel-cadmium and nickel-metal hydride batteries.

Battery LMP:

Lithium Metal Polymer Batteries are made of a lithium metallic anode. A LMP battery is composed of the successive stacking of four layers:

- a sheet of lithium;
- a polymer electrolyte based on POE (polyethylene oxide);
- a cathode;
- a current collector made of a metal strip.

To date, few industries use this technology and most of them are based in France. These batteries are mostly used in French electric buses.

According to Vandepaer, 2017, LMP is a promising technology which is advocated as more stable, safe and simple to manufacture than batteries with liquid electrolytes. It also indicated that while Li-ion batteries cause significantly more impacts than LMP units in terms of global warming and ozone depletion, LMP batteries are responsible for a bigger impact in terms of aquatic eutrophication originating from sulfidic tailings linked to mining activities. As the aim of this RMOA is not to compare the impact of different types of batteries on the environment, this statement has not been challenged.

Battery regulation: Battery Directive 2006/66/EC replaced by the regulation (EU) 2023/1542 from 2025

A specific collection and recycling system for batteries was set up in Europe in 1991, based on the principle of extended producer responsibility for managing the end-of-life of the batteries and accumulators they place on the market[16], whatever the types of batteries as defined in this branch:

- portable batteries defined as any accumulator or battery that can be handled by hand;
- automotive batteries, which are any accumulator or battery intended to power an automobile starting, lighting or ignition system;
- industrial ones (Anses 2019).

The directive 2006/66/EC on batteries and accumulators last amended in 2018 and repealed with effect from 2025 and replaced by regulation (EU) 2023/1542, was

[16] https://www.ecologie.gouv.fr/piles-et-accumulateurs

the main legal act regulating batteries at EU level until now. This Directive used to establish (Article 1):

"(1) rules regarding the placing on the market of batteries and accumulators and, in particular, a prohibition on the placing on the market of batteries and accumulators containing hazardous substances; and

(2) specific rules for the collection, treatment, recycling and disposal of waste batteries and accumulators to supplement relevant Community legislation on waste and to promote a high level of collection and recycling of waste batteries and accumulators.

It seeks to improve the environmental performance of batteries and accumulators and of the activities of all economic operators involved in the life cycle of batteries and accumulators, e.g. producers, distributors and end-users and, in particular, those operators directly involved in the treatment and recycling of waste batteries and accumulators."

The European Parliament and the Council adopted the new Batteries Regulation on July 12, 2023. The regulation (EU) 2023/1542 that entered into force on August 17, 2023 lays down the requirements on sustainability, safety, labelling, marking and information to allow the placing on the market or putting into service of batteries within the Union.

This regulation, partly replacing the battery directive 2006/66/EC and that must be applied from February 18, 2024, aims at ensuring recycling rates while minimising the environmental impact of this exponential growth in light of new socio-economic conditions, technological developments, markets, and battery usages.

This Regulation applies to all categories of batteries, namely portable batteries, starting, lighting and ignition batteries (SLI batteries), light means of transport batteries (LMT batteries), electric vehicle batteries and industrial batteries, regardless of their shape, volume, weight, design, material composition, chemistry, use or purpose. It also applies to batteries that are incorporated into or added to products or that are specifically designed to be incorporated into or added to products. This regulation does not apply to batteries incorporated into equipments intended to be sent into space, equipments connected to the protection of the essential security interests of Member States.

Annex I of this regulation lists the substances that shall not be contained (or with restrictions) in batteries. For now, the annex I only includes mercury, cadmium or lead. The Annex I could be amended by the EU Commission in case of an unacceptable risk to human or to the environment arises "*from the use of a substance in the manufacture of batteries or from the presence of a substance in the batteries when they are placed on the market, or arising during their subsequent life cycle stages, including during repurposing or the treatment of waste batteries" (Article 6(2)).*

"By the end of 2027, the EU Commission helped by ECHA will build a report on substances of concern, namely substances having an adverse effect on human health or the environment or hampering recycling for safe and high quality secondary raw materials, present in batteries or used in their manufacture. The Commission shall submit that report to the European Parliament and to the Council detailing its findings and shall consider the appropriate follow-up measures including the adoption of delegated acts" (Article 6(5)).

The substance(s) covered in this RMOA, which are reprotoxic category 1A and fulfil the ED criteria are reported to be used in batteries. The potential for release/exposure from the sealed batteries is considered to be low. Exposure and release from the production phase of the batteries at industrial sites and from the waste stage might require further investigation. It should take into account the exposures and releases occurring after accidents. The Regulation (EU) 2023/1542 will consider the need to further regulate this specific use of these substances.

3.3.3 Chemicals application

Lithium chemicals are also used in a variety of other applications.

3.3.3.1 Gas and Air treatment

Lithium may be used as an absorption medium for industrial refrigeration systems and for humidity control and drying systems[17].

Lithium hydroxide is used in air treatment to remove CO_2 from air closed systems such as submarines, mining rescue kids and space shuttles. Lithium hydroxide is used as an absorbant (Ahmadi et al. 2023).

Lithium hydroxide and chloride can also be used as an absorbent for absorption chiller systems used in laboratories, food processing, pharmaceuticals manufacturing etc…

No specific regulation has been found for this use.

3.3.3.2 Pharmaceuticals

Lithium carbonate is used as an active ingredient or as a raw material (intermediate/processing aid). Lithium hydroxide can be used as an active ingredient (confidential source). Lithium metal can be used as an intermediate use to manufacture fine chemicals as active pharmaceutical ingredients (ILIA-ANSES audition).

Lithium and its salts are used in the treatment for bipolar disorder as well as in other pharmaceutical products[18].

These uses do not fall under the REACh regulation but are regulated by the directive 2001/83/CE relating to medicinal products for human use.

3.3.3.3 Cosmetics

Lithium Magnesium Sodium Silicate is an ingredient used in cosmetics that comes from a combination of silicones, lithium, magnesium, and sodium. This ingredient is often found in makeup products such as foundations and eyeshadows, providing them with a silky and creamy texture. It acts as a dispersing agent, helping to blend different pigments and ingredients in a cosmetic product, resulting in smooth

[17] https://lithium.org/lithium/ (consulted on 10/2023)

[18] https://lithium.org/lithium/ (consulted on 10/2023)

application. Additionally, Lithium Magnesium Sodium Silicate is used to enhance the longevity of makeup products[19].

For makeup items like lip gloss or other products that provide shine, it serves as a base. It is also used in bath products, skincare, and makeup[20]. Lithium carbonate and hydroxide are used as pH regulators and curling or straightening agent[21].

It is to be noted that lithium hydroxide is on the list of substances which are restricted in cosmetic products (hair straighteners, pH adjuster for depilatories, other uses as pH adjuster (for rinse-off products only)) (Annex III of the Regulation (EC) No 1223/2009).

Regulation (EC) No 1223/2009 on cosmetic products is the main regulatory framework for finished cosmetic products when placed on the EU market. It strengthens the safety of cosmetic products and streamlines the framework for all operators in the sector. As indicated by recital 32, "*Given the hazardous properties of substances classified as carcinogenic, mutagenic or toxic for reproduction (CMR), category 1A, 1B and 2, pursuant to Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, their use in cosmetic products should be prohibited. However, as a hazardous property of a substance does not necessarily always entail a risk, there should be a possibility to allow the use of substances classified as CMR 2 substances where, in view of exposure and concentration, they have been found safe for use in cosmetic products by the SCCS and are regulated by the Commission in the Annexes to this Regulation.*"

The harmonised classification as reprotoxic cat. 1A will trigger regulatory action under the Cosmetic products regulation (EC) No 1223/2009 for uses as fragrance, since CMR cat. 1 are restricted by this regulation unless specifically derogated.

3.3.3.4 Lab chemicals

Lithium carbonate, hydroxide and chloride are also lab and processing chemicals. Indeed, it can be used in polymer, agrochemical and electronics. More specifically, it appears that lithium chloride can be used in processing chemical synthesis, pharmaceutical industry, gelatin industry, flavouring and fragrances, ironic liquids, polymers etc…

3.3.3.5 Construction materials

Lithium carbonate and hydroxide can be used in fast setting cement and mortars, e.g. self-leveling floor screeds (ILIA website[22], MELCC 2022)), adhesives for tiles. Lithium carbonate is used as an accelerator, to reduce construction time, and the maximum concentration in the end-product is less than 2% w/w.

The regulation (EU) No 305/2011 of 9 March 2011 lays down conditions for the placing or making available on the market of construction products. In the section 3 of the annex I, it is indicated that "*the construction works must be designed and*

[19] https://oma-and-me.com/blogs/principes-actifs/quest-ce-que-le-lithium-magnesium-sodium-silicate (consulted on 13/03/2024)
[20] https://www.lesielle.com/fr/lithium-magnesium-sodium-silicate-c-est-quoi-inci-cosmetique-929 (consulted on 13/03/2024)
[21] https://cosmeticobs.com/fr/articles/actus-59/classifications-clh-consultations-ciblees-sur-les-composes-de-lithium-et-le-methyl-methacrylate-7667 (consulted on 13/03/2024)
[22] https://lithium.org/lithium/ - accessed in December 2023

built in such a way that they will, throughout their life cycle, not be a threat to the hygiene or health and safety of workers, occupants or neighbours[…]:

(d) the release of dangerous substances into ground water, marine waters, surface waters or soil;
(e) the release of dangerous substances into drinking water or substances which have an otherwise negative impact on drinking water;
(f) faulty discharge of waste water, emission of flue gases or faulty disposal of solid or liquid waste;
(g) dampness in parts of the construction works or on surfaces within the construction works."

So according to the future harmonised classification of the 3 salts, it might have an impact on the use of these chemicals in construction materials.

3.3.3.6 Dye and pigments

Lithium carbonate and hydroxide can be used in additives to dyes or in fluxing agents to inorganic pigments. Lithium carbonate is used to increase brilliance.

CLH as a reprotoxic 1A will restrict the presence of the substances in clothing, other textiles, and footwear articles, by means of the restriction entry 72 of REACH Annex XVII (this would require addition of the relevant substances to Appendix 12 by the Commission through Article 68(2)).

3.3.3.7 Lubricant & grease

Lithium hydroxide can be used as an emulsifier used as an intermediate to manufacture lithium 12-hydroxystearate that is used as a thickener in the final grease formulation (ILIA website). Lithium greases represent 70% of greases produced worldwide and have unique properties with respect to water resistance and temperature range in which they can be used (MELCC 2022).

Lithium is used as a thickener in grease ensuring lubrication properties are maintained over a broad range of temperatures (ILIA website).

3.3.3.8 Chemical manufacturing

Lithium hydroxide can be used in chemical manufacturing as an intermediate or a processing aid. It can be used in: silicone manufacturing, gelatine manufacturing, wax additives, tanning additives, etc.

Lithium metal is used as raw material for chemical manufacturing.

3.3.3.9 Graphic art industry

Lithium chloride is used as a catalyst for toners, photographic developer solutions at a maximum concentration in the end use product of 2%.

3.3.3.10 Biocidal products

The harmonised classification as reprotoxic cat. 1A would render the substances unacceptable as co-formulants in biocidal products (but also Plant protection products) if present above the concentration limit leading to classification of the

mixture as CMR cat 1A according to the Biocidal product regulation (EU) 528/2012.

3.3.3.11 Fertiliser

Lithium fertilizers are used to "*meet the needs of crops in the trace element lithium. Lithium is a biologically important element in plant life and influences the content and heterogeneous composition of proteins and nucleic acids, enzymatic activity of enzymes associated with protein-nucleic acid metabolism*" "(The soviet school of farming, march, 2024)[23]. Nevertheless, at high concentrations, it inhibits plant growth (see Section 4.2.2).

Regulation (EU) 2019/1009, adopted by the European Parliament and the Council on June 5, 2019, lays down rules regarding the availability of EU fertilizing products in the market. This regulation does not mention Lithium.

3.3.3.12 Glass and ceramics

Based on ILIA's website, the addition of lithium increases the glass melt rate, lowers the viscosity and the melt temperature providing higher output, energy savings and moulding benefits. It also improves the glaze's colour, strength and lustre (ILIA, 2022, MELCC 2022). Similarly, it also lowers firing temperatures and thermal expansion and increases the strength of ceramic bodies. Lithium's extremely low co-efficient of thermal expansion makes these products resistant to thermal shock and imparts mechanical strength.

Based on the same source, lithium oxide can be used to create high-durability glass products where thermal shock resistance is important. Similarly, lithium carbonate is used as an additive in the manufacture of heat-shock-resistant cookware for freezer-to-oven use. It is an enhancer of mechanical and optical properties of the materials, strength, durability and brilliance of the glass. Very well-known are also flat-top cooking ranges with defined heating areas.

Lithium carbonate can also be used as an additive in optical glasses for telescopes.

3.3.4 Technical applications

Lithium is also used in a variety of metallurgical applications including steel or iron castings, welding, aluminium smelting (ILIA website).

Lithium metal is used in metal industry alloying element in manufacturing alloys together with other alloying elements.

3.3.5 Conclusion on uses

As explained in this section, Lithium and the 3 salts are used in a wide variety of sectors for professional, industrial or consumer uses.

Until 2020, uses except batteries were very diverse and represented the major part of the lithium uses. Glasses, ceramics were the most important uses (37%) followed by greases and lubricants (11%), air treatment (5%), the melting for steel production by continuous casting, pharmaceuticals (2%), aluminum metallurgy or

[23] https://universityagro.ru/en/agrochemistry/lithium-fertilizers/

other uses like cements, water treatment. Except for specialty uses, lithium degrees of purity were and remain relatively low meaning that impurities are tolerated (mineral info website). Since the last couple of years, the fastest growing and largest market for lithium globally is for use in batteries for the production of electric vehicles. Batteries Li-ion became the most important sector of use for lithium. It appears that the use of lithium in those batteries will continue to grow in the future.

3.4 Life cycle, recycling and waste treatment

According to the review from the Environmental Ministry of Quebec in 2022, pyrometallurgy is the traditional methodology used to recycle Li-ion batteries. The different parts of batteries are melt at high temperature to produce a thin powder containing cobalt, copper, nickel and iron. Other steps are necessary to extract these metals. Nevertheless Lithium is not part of this fraction and can't be valued through pyrometallurgy. Hydrometallurgy processes are necessary to recover lithium.

The first step is the deactivation of the batteries. This step being particularly technically delicate, pyrometallurgy is not the chosen route due to excessive energy release. Cryogenics is also not the preferred route due to excessive energy consumption, release of hydrogen and effluent management. The preferred route is rather grinding under a controlled atmosphere (nitrogen, CO_2 under vacuum). After grinding, there is sieving and separation by air flow to carry out an initial sorting of the materials. Eddy current separation is also used to separate non-ferrous metal waste from non-metallic waste. Finally, hydrometallurgy is used on black mass to obtain high purity salts. This phase includes a total leaching phase and separation of impurities (precipitation and solvent separation).

In France via the French Agency for Ecological Transition (ADEME), efforts are deployed to build a more sustainable economy by acting on the entire life cycle of products. For batteries and accumulators, a specific sector exist. For instance, indicators from 2022 produced from data from 2021 declared by eco-organisations and individual systems showed that for 304 635 tonnes of batteries and accumulators (36 244 portable batteries, 117 070 automotive batteries and 151 321 industrial batteries), 201 533 tonnes were collected (13 987 portable batteries, 172 775 automotive batteries and 14 771 industrial batteries) and 223 775 tonnes were recycled (71.96%) eliminated (28.03%) or energetically recovered (0.01%). Of the 223 775 tonnes processed, the majority corresponds to lead accumulators (205 372 t), 3261 t were lithium accumulators and 97 t were lithium batteries[24].

In Europe, via the new regulation (EU) 2023/1542 on batteries, efforts are also deployed to gradually introduce from 2024 onwards sustainability requirements on carbon footprint, recycled content and performance and durability. A more comprehensive regulatory framework on Extended Producer Responsibility will start applying by mid-2025, with higher collection targets being introduced over time. For portable batteries the targets will be 63% in 2027 and 73% in 2030, while for batteries from light means of transport, the target will be 51% in 2028 and 61% in 2031. All collected batteries have to be recycled and high levels of recovery have to be achieved, in particular of valuable materials such as copper, cobalt, lithium, nickel and lead[25].

[24] https://filieres-rep.ademe.fr/filieres-REP/filiere-PA/tableau-de-bord (consulted on 17/04/2024)
[25] https://ec.europa.eu/commission/presscorner/detail/en/ip_22_7588 (consulted on 17/04/2024)

4 Hazard information (including classification)

4.1 Human health hazard

In 2021, RAC adopted its opinion[26] on a dossier proposing a harmonised classification and labelling at EU level for 3 lithium salts: lithium carbonate, lithium hydroxide, lithium chloride. France, which was the MSCA in charge of this dossier proposed to classify these 3 salts as reprotoxic category 1A. RAC concluded its opinion by proposing the following hazard classes: Repr. 1A (H360FD); Lact. (H362).

This dossier is currently pending as, on request of the European Commission, ECHA requested the RAC to review an additional publication (Boyle et al. (2017)), which was provided by industry stakeholders at CARACAL and, if necessary, to amend its opinion in relation to the classification for reproductive toxicity of the three lithium salts. At a meeting in March 2024, RAC confirmed by consensus its earlier opinion, recommending the above mentioned harmonised classifications. They should now be accordingly implemented into an ATP.

In this section, a resume of the RAC opinion leading to the proposed harmonised classification is provided. For more details, please refer to the RAC opinion.

Moreover, this section contains a summary of data regarding the assessment of endocrine disruption properties of lithium performed after the CLH dossier and a summary of data regarding potential risks for human through environmental exposure. Both detailed analysis can be found in Annex 1 and 2, respectively.
They were not included in the CLH proposal as carried out later.

4.1.1 Toxicokinetics (absorption, distribution, metabolisation and elimination)

4.1.1.1 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 approximately 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.

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 disseminated website; 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).

[26] https://echa.europa.eu/documents/10162/e2a3c38e-85fe-505c-a325-293c70a74da5

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).

4.1.1.2 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 can substitute for sodium or potassium on several transport proteins that normally transport sodium or potassium, thus providing a pathway for lithium entry into cells (Timmer and Sands, 1999). 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. 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 (Lagerkvist and Lindell, 2002; Moore, 1995).

4.1.1.3 Metabolism

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

4.1.1.4 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 (Schou, 1958).

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-life 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).

4.1.2 Neural toxicity

Given the huge number of experimental studies addressing the neural effects of lithium at therapeutic doses, in particular during adulthood and puberty, the experts chose to focus the evaluation on the potential neurodevelopmental effects of this substance (see Annex 2, Section 13.1.2) in particular because this adverse effect could be a consequence of thyroid disturbance.

4.1.3 Genotoxicity

This section is based on the CLH report from June 2020 draft by France, leading to an opinion of the RAC in September 2021[27]. The section below is an extract of RAC opinion.

"*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 from in vitro acceptable tests 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."

4.1.4 Carcinogenicity

This section is based on the CLH report from June 2020 draft by France, leading to an opinion of the RAC in September 2021. The section below is an extract of RAC opinion.

[27] https://echa.europa.eu/fr/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e18270066e

"*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."

4.1.5 Toxicity to reproduction and development

This section is based on the CLH report from June 2020 draft by France, leading to an opinion of the RAC in September 2021[28]. As the toxicity to reproduction of lithium salts was agreed by the RAC, data were used in the framework of the endocrine disrupting potency assessment of lithium salts. Summaries presented below are the conclusions of the RAC opinion, completed by a focus on cardiodevelopmental toxicity

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, **Li^+ uses the sodium ion channels to reach target organs**.

In the RAC opinion (ECHA 2021), the evaluation of the reproductive toxicity is summarized as follow:

4.1.5.1 Toxicity on sexual function and fertility

Summary of the DS' proposal: "*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

[28] https://echa.europa.eu/fr/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e18270066e

*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.*"

Summary of RAC's opinion: "*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***."

4.1.5.2 Developmental toxicity

Summary of the DS's proposal: "*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."

Summary of RAC's opinion: "*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.*"

The study of Patorno et al. (2017), the key study for the classification of lithium salts, is a cohort study involving 1,325,563 pregnancies between 2000 and 2010, among which 663 women exposed to lithium during the first pregnancy trimester. 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.

4.1.5.3 Lactation

Summary of the DS's proposal: "*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*."

Summary of RAC's opinion: "*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***."

4.1.6 Endocrine disruption

Anses, through its expert committee, has investigated the endocrine disruption concern for human health for the three salts. The main conclusion of this assessment is the following:

''Human and experimental data unambiguously show that lithium has thyroid effects. Based on a weight of evidence analysis of the available data, Lithium alters thyroid hormone levels and leads to histopathological alterations of the thyroid. ***Therefore, Lithium fulfils the criteria of the WHO definition of an endocrine disruptor.***

These effects on thyroid are known to appear at therapeutic concentrations and few data exist on their occurrence at environmental concentrations. However, it is very likely that the environmental contamination by Li will increase due to a huge increase of lithium use, particularly with the production and recycling of batteries. Lithium dissemination has to be carefully scrutinized for proper management aiming at limiting the risks".

The whole assessment of the endocrine disruption effect on human health is available in Annex 1 and is also reported in Chevalier et al. 2024.

4.1.7 Additional human health hazard information at environmental doses

Considering the reprotoxic hazard and endocrine disrupting properties of lithium, France wanted to see if data is available regarding potential risks for human through environmental exposure. Thus, a literature search was conducted to identify epidemiological studies. The literature search methodology and all

identified adverse effects are detailed in Annex 2. The conclusions that could be drawn from this literature review are the following:

Overall, the available literature regarding human health effects from exposure to lithium at environmental doses is made of several studies on many outcomes but, except for those evaluating the protective effect of lithium on suicide prevention, there are not many studies for each given outcome.

Several studies are at high risk of bias because they do not have individual data (ecological study design) and/or measured lithium in tap water and thus missed exposure through other sources / lack internal doses.

Co-exposures were rarely taken into account while the few studies that have considered them reported changes in the associations after adjustment for other metals (Chai et al. 2022, Herlin et al. 2019).

The published literature might suggest a potential protective effect of lithium exposure from drinking water on the risk of suicide (suicide fatalities, suicide attempts at an emergency department, suicide rates); however, the designs of the studies and the observed publication bias preclude any causal assertion on the matter.

Regarding the thyroid, studies are cross-sectional and two have limited sample size. So despite the fact that they all suggested an **association with thyroid outcomes**, more studies with longitudinal design are needed to conclude.

Isolated studies of relatively good quality **indicated deleterious association between lithium exposure at environmental doses and the following health outcomes: autism spectrum disorders** (Liew et al. 2023)**, male and female reproduction** (Chai et al. 2022, Wu et al. 2021, Gonzalez-Martin et al. 2023), **growth and metabolism parameters** (Wang et al. 2020, Harari et al. 2015a; Harari et al. 2016), **and hypertension** (Zhong et al. 2021), in particular (but not limited) when exposure occurred during vulnerable windows of exposure such as the pregnancy. However, additional longitudinal studies, ideally with biospecimens to measure lithium exposure, are needed to confirm these results and approach a causal assertion.

4.1.8 Human health hazard linked to physico-chemical properties

4.1.8.1 Flammability

On ECHA disseminated website, it is indicated that the study on flammability regarding the solid is technically not feasible for lithium as the EU method A.10 for solids can only be applied to powdery, granular or pastelike substances. Lithium metal is stored in heavy oil in order to prevent it from reacting with air moisture and is also merchandised as such. Further, lithium is known to violently react with water. The reaction yields lithium hydroxide and flammable hydrogen gas. It is therefore labelled as flammable and classified in accordance with Regulation 1272/2008/EC as water react. 1 (category 1).

The study on flammability regarding the pyrophoric properties is technically not feasible for lithium as the EU method A.13 for solids can only be applied to powdery, granular or pastelike substances. Further, lithium does not meet the criteria for self-reactive substances and organic peroxides.

4.2 Environmental hazard

Considering the concerns for human health (reprotoxicity, endocrine disrupting properties of lithium) and the new projects on lithium mining appearing in Europe, France also wanted to see if data are available regarding potential risks for environmental species. Thus, three sources of data were screened:

1. ecotoxicological data from ECHA disseminated website and the CSR of the lead registrant of each studied substance (lithium, Li2CO3, LiCl and LiOH);
2. a recent report from the Ministry of the Environment from Quebec (MELCC 2022) assessing whether lithium is hazardous for terrestrial organisms (fauna and flora);
3. as this latter report only focused on the terrestrial compartment, a literature search was conducted up to 22 August, 2023 in order to identify data on other compartments, in particular the aquatic compartment.

Detailed information about these data and literature search methodology are provided in Annex 3. It is to be noted that ecotoxicity data of the three water-soluble[29] inorganic salts were combined to define the ecotoxicity of the metal ion, Li^+. Effects data (LC50, NOEC, etc.) were expressed as dissolved (bioavailable) metal ion concentration (mg Li/L)[30]. Besides, as the ecotoxicity of such substances is dependent on the physico-chemistry of the medium, the studies following OECD guidelines were carefully reviewed in terms of physical test conditions (pH, temperature, dissolved organic carbon, hardness…). The overall quality of each data is not discussed but limitations are pointed out.

It remains that the reliability and relevance of each study from the registration dossiers and literature will need to be further scrutinized to assess how the data may be considered in the purposes of classification and risk assessment, including PNEC derivation. In particular, the influence of the following parameters need to be assessed:

- pH effect: Some data from the registration dossiers showed variable pH. Some even exceeded the expected normal pH assay range recommended in OECD guidelines. Thus, it could be questionable if the observed toxicity in some studies is due to a pH increase, rather than the presence of lithium. Besides, studies from literature did not always provide pH information.

- Potential effect of the counterion: The potential effect of the counterion of the three lithium salts was not discussed, neither in the registration dossiers, nor in literature.

- Geochemical background: In studies investigating lithium toxicity to organisms directly collected in the environment, lithium concentration of the

[29] Water solubility of lithium salts (extracted from the CLH report (Anses 2020):
Li2CO3: 8.4 g/L (measured at 20°C)
LiCl: 569 g/L (measured at 20°C)
LiOH anhydrous: 71-125 g/L at 20°C
Li2SO4: 342 g/L (25°C) (from ECHA disseminated website)

[30] A molecular weight correction is applied to get the effects data (LC50, NOEC, etc.) as dissolved (bioavailable) metal ion concentration (mg Li/L).
However, it is to be noted that some publications were not enough precised on the test material (lithium or lithium salts). In such cases, effects data (LC50, NOEC, etc.) could not be expressed as dissolved (bioavailable) metal ion concentration (mg Li/L).

control conditions should systematically be provided as lithium naturally occurs in the environment and this results in background concentrations.

- Bioavailability and speciation: these concepts were not addressed in the set of available data. Yet, they are decisive to determine the degree to which metals are available and cause toxicity to aquatic, sediment-burying and terrestrial organisms.

The conclusions that could be drawn from the set of data review are described below.

4.2.1 Bioaccumulation potential

Some data of bioaccumulation for lithium were found in the report from the Ministry of Environment of Quebec (MELCC, 2022). However, it is to be noted that the assessment of bioaccumulation properties, and wider the assessement of PBT or vPvB criteria, is not applicable to inorganic substances according to ECHA guidance on PBT/vPvB assessment (ECHA 2023).

In the MELCC report (MELCC, 2022), it was reported that, although no information was identified through the literature review regarding the bioaccumulative potential of lithium in terrestrial invertebrates, amphibians, reptiles and birds, available studies showed that lithium may accumulate in various terrestrial organisms. It was reported that **accumulation is proportional to environmental levels and does not depend on the chemical form added**. In plants, it was reported that lithium is usually absorbed by roots, before being rapidly transported throughout the tissues. Lithium contents are generally higher in stems and leaves than in roots, while accumulations in grains and fruits are not significant. In the report, several data of accumulation were reported. For example, lithium concentrations in food range from 0.01 mg/kg in bananas to 55 mg/kg in oats. Plants native from arid basins in the western United States absorb much more lithium than plants native from humid areas and this can represent levels varying between 99.6 and 226.4 mg/kg in the boar thistle (*Cirsium arvense*) and the bulrush (*Holoschoenus vulgaris*). In mammals, following oral ingestion, lithium may be absorbed from the gastrointestinal tract and transported into cells by passive diffusion through sodium channels. Excretion of lithium mainly occurs by the kidneys, but a residual quantity is distributed in the organism, mainly in bones, endocrine glands (thyroid, pituitary and adrenal), brain and muscles. For example, 99.3 and 86.8 mg/kg lithium were measured in guinea pigs and cattle when 10.2 and 14.4 mg/kg were found in alpacas and llamas (MELCC 2022).

4.2.2 Conclusion on environmental hazard

Based on the registration dossiers and literature review, it is observed that lithium toxicity was studied in a large number of publications, and in a large number of different species, covering aquatic (fish, invertebrates, amphibians) and terrestrial species (plants, mammals, terrestrial invertebrates, birds). Data on micro-organisms were rarer. Various developmental stages were studied. It is also to be noted that acute or subacute exposure to lithium were more frequently tested than chronic exposure. A synthesis of available data and associated conclusions is provided below.

4.2.2.1 Acute toxicity

Based on the registration dossiers and their OECD guideline studies, data are available for all three trophic levels (fish, crustacean and algae). The lowest reliable

effect values calculated for lithium ion for the three trophic levels are: **96h-LC50 of 25.9 mg Li/L[31] for rainbow trout** based on mortality (Unnamed 1997), **48h-EC50 of 40.7 mg Li/L[32] for *Daphnia magna*** based on mobility and **72h-EC50 of 1.67 mg Li/L[33]** (**for *Raphidocelis subcapitata)*** based on growth rate.

Additional acute toxicity data were identified from the literature. Fish mortality data with 96h-LC50 values in the same order of magnitude than the registrants' data were found (Hamilton 1995). Lower effect values were observed for larval stages with a LC50 value of 1.24 mg/L Li for zebrafish larvae (Pruvot et al. 2012). Regarding aquatic invertebrates, 48h-EC50 values ranged between 2.3 and 41 mg/L Li for different crustacean species. Other invertebrates were studied (molluscs, echinoderms, annelids, amphoxius and cnidarians) but very few mortality data were available: LC50 of 2.5 mg/L Li for the ramshorn snail, *Marisa cornuarietis*, at the embryonic stage (Sawasdee et al. 2010), mortality at 0.55 g/L Li in sea urchin embryos (Ruocco et al. 2016), EC50 of 1.17 mg/L Li for *Tubifex tubifex* (Lenntech 2007, US EPA 2008, cited in Aral et al. 2008). Regarding algae, acute toxicity data from literature were much higher than the registrants' data.

Overall, all short-term toxicity values for the three trophic levels are > 1 mg Li/L (threshold for classification for short-term aquatic hazard according to the CLP regulation). The lowest effect value is the EC50 of 1.17 mg/L Li for *Tubifex tubifex* (Lenntech 2007, US EPA 2008, cited in Aral et al. 2008).

4.2.2.2 Long-term toxicity

The registration dossiers and their OECD guideline studies report data for the three trophic levels. For fish, the lowest NOEC calculated for lithium ion is **0.2 mg Li/L[34] for fathead minnow** based on growth (length) (Long et al. 1998). Regarding aquatic invertebrates, all data available in the registration dossiers show limitations. Thus, no long-term NOEC may be retained. Regarding algae, the lowest long-term NOEC is **0.2 mg Li/L[35] for *Raphidocelis subcapitata* based on growth rate**.

Data from literature allowed to fill the data gap for long-term toxicity on aquatic invertebrates. The lowest NOEC identified was **0.02 mg/L Li (measured concentration) for *Daphnia magna* based on living offspring** (Martins et al. 2022).

In amphibians, 2.5 mg/L lithium caused up-regulating effects on the thyroid gland of the premetamorphic American bullfrog which were the signs of an acceleration of the metamorphic process. No systemic toxicity was observed (Pinto-Vidal et al. 2021a).

It is to be noted that these values are lower than the lithium concentrations that may be found in Li-rich waters, for instance in rivers in the lithium-rich regions of northern Chile where the content in surface waters can reach 5.2 mg/L (no information is provided whether this high concentration has a natural origin or is due to anthropic activities). They are also lower than concentrations found in the

[31] Calculated from a geometric mean of LC50 (from measured concentrations) for LiCl
[32] Calculated from a geometric mean of EC50 (from measured concentrations) for LiCl
[33] Calculted from EC50 for LiOH (nominal concentration)
[34] Calculted from NOEC for LiCl (nominal concentration)
[35] Calculted from NOEC for LiOH (nominal concentration)

aquifer following a fire/explosion in Grand-Couronne in France that could reach 11.9 mg/L Li.

Overall, **the most sensitive species regarding long-term toxicity of lithium is *Daphnia magna*.**

4.2.2.3 Other endpoints unusually used for regulatory assessments

Besides the data above that are usually used in the frame of a regulatory assessment (classification, risk assessment), studies from literature investigated other multiple endpoints (developmental effects, haematological and biochemical effects, histopathological effects, oxidative damage, inflammatory response, etc.) and other developmental stages (embryo, larvae) that may not be used in a regulatory purpose.

Among the main effects caused by lithium, it can be highlighted that, following acute exposure in embryos or larvae, lithium commonly induces **developmental defects** in zebrafish, xenopus and sea urchins. Indeed, **lithium is a well-known teratogenic agent disrupting the development of the dorsal-ventral axis of organisms**, resulting in vegetalisation/dorsalisation. It is used as positive control in some studies at very high concentrations in order to observe this typical effect, for instance in zebrafish embryos at 2 g/L Li (Fairbairn et al. 2012). It is also used on purpose to investigate gene expression or signalling when development is perturbed by lithium, for instance in sea urchin embryos at 0.1 g/L Li (Kauffman et al. 2003) and in xenopus embryos at 0.7 g/L Li (Nagajski et al. 1989). Developmental abnormalities are also seen for example in starfish at 0.2 g/L Li (Kominami et al. 1984) and in ascidians at 0.14 g/L Li (Yoshida et al. 1998).

However, developmental toxicity is also observed at much lower concentrations: EC50 of 1.05 mg/L Li in zebrafish larvae based on pericardial edema and dorsal curvature, decrease in heart rate and locomotor response (Pruvot et al. 2012), malformations from 6.9 mg/L Li in sea urchin embryos based on affected arms, spicules and apex (Ruocco et al. 2016), developmental abnormalities (25%) at 3.3 mg/L Li in xenopus embryos based on microcephaly, microphthalmia, cyclopia, gut and tail malformation, bullae, decrease in length (Boga Pekmezekmek et al. 2020), NOEC of 4.3 mg/L Li in C. elegans based on reduction in larval growth (Inokuchi et al. 2015), developmental effects in hydras at 8 mg/L Li (no elongation of tentacles, no formation of hypostomes and body axis) (Johnson et al. 1982), slower growth in chicken embryos at 5 mg/L Li (Ikonomov et al. 2000, cited in the review from Kakhki and Ahmadi-Soleimani (2022)).

Lithium also causes biochemical, metabolic, histopathological and immune effects in various environmental species. For instance, lithium activated antioxidant and detoxification enzymes at 0.5 mg/L in mussels (Cunha et al. 2023) and caused histopathological effects of the digestive gland from 0.1 mg/L Li (Fraga et al. 2022).

Finally, lithium induces phytotoxicity in various plant species. At low concentration, it rather stimulates plant growth or has no effect, while at higher concentrations it inhibits plant growth. Nevertheless, these effects may vary among the species, with some that are more tolerant to lithium exposure than others (MELCC 2022). For example, germination of *A. pictum* was not affected by low litium concentration (0-100 mM) while it was gradually inhibited with increasing lithium exposure from 100 to 500 mM (Jiang et al. 2018b).

4.3 Conclusion on hazard information for human health and the environment

The Figure 3 below combines all relevant hazard information for human health and the environment.

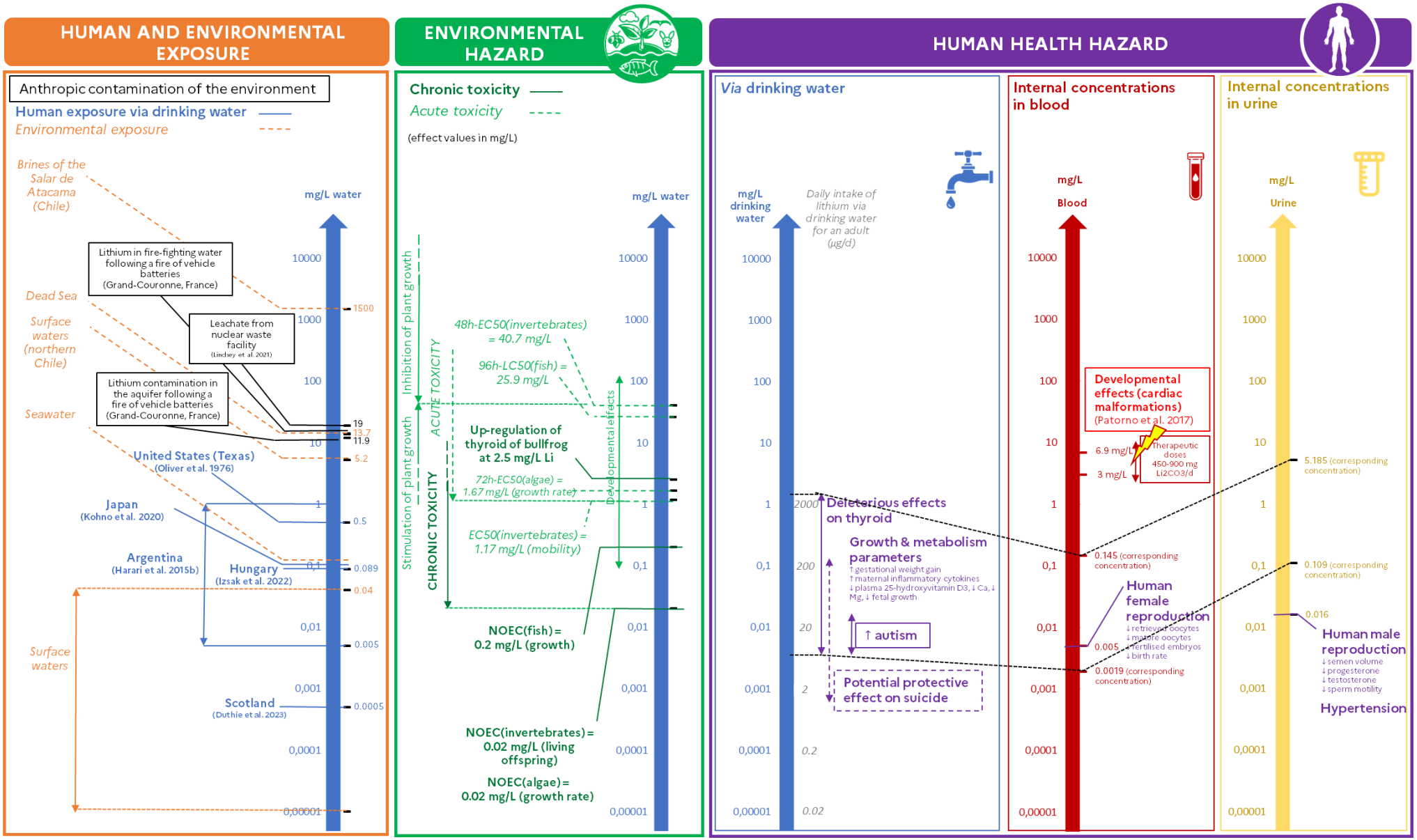
The range of therapeutic doses of lithium is 450-900 mg Li2CO3/d corresponding to serum concentrations between 0.5 and 1 mmol Li/L, i.e. between 3 and 6.9 mg Li/L. The study of Patorno et al. (2017) which is the key study for the classification of lithium salts showed that maternal use of lithium during the first trimester is associated with an increased risk of the cardiac malformation, particularly at high therapeutic doses, with a clear dose-response relationship.

Regarding environmental exposure via drinking water, isolated studies of relatively good quality indicated association between deleterious effects (autism spectrum disorders, male and female reproduction, growth and metabolism parameters, and hypertension) and lithium exposure from < 0.01 to > 1 mg Li/L drinking water. However, additional longitudinal studies with biospecimens to measure lithium exposure are needed to confirm these results.

Regarding environmental hazard, the main effects are indicated on the Figure 3, with chronic toxicity for the three trophic levels with effect concentrations < 1 mg/L. Acute toxicity (mortality) is observed at higher concentrations (> 1 mg/L) as well as developmental effects following acute exposure to lithium. Thus, lithium could fulfil the criteria for a harmonised classification for aquatic chronic toxicity.

Endocrine disrupting properties of lithium were evidenced in one good quality study showing up-regulation of thyroid in an amphibian species with no systemic toxicity at 2.5 mg Li/L which is considered as a relatively high concentration. Finally, lithium is also responsible for inhibiting plant growth at high concentrations while it stimulates it at lower concentrations.

Figure 3: Combined hazard characterization information for human health and the environment



4.4 Existing reference or guidance values

4.4.1 Human health

4.4.1.1 DNEL from the registration dossiers

The DNELs from the registration dossiers are reported in Table 6. In the CSR for Li, LiOH and LiCl, DNELs are based on the lower bound of the therapeutic serum lithium concentration range (point of departure) (1.2 mg Li/kg bw/d). According to the registrants, there is *"no evidence of concern with respect to repeated oral toxicity at medical doses",* and it is therefore considered as a NOAEL. However, this is questionable since adverse effects are observed in several organs and systems and are associated with the entire target therapeutic serum lithium concentration range (US EPA 2008). In particular, patients are monitored by their physicians, especially women during pregnancy. Thus, for deriving a p-RfD, US EPA considered the lower bound of the therapeutic serum lithium concentration range as a LOAEL (2.1 mg Li/kg bw/d) (see section 4.4.1.2). Thus, eMSCA recommends the registrants to revise the DNEL derivation for Li, LiOH and LiCl and therefore to update the associated risk assessments. In the CSR for Li2CO3 that was recently updated, the DNEL derivation appears to be more adequate. Nevertheless, it was not possible for the eMSCA to go further in the analysis and it would be necessary to confirm that the point of departure (PoD) was appropriate.

Table 6: DNEL for systemic effects from ECHA disseminated website

	Route	DNEL	PoD (NOAEL, NOAEC)	AF	Exposure	Comments from eMSCA[36]
Li (data are expressed in mg Li+/kg bw/d or mg Li+/m3/d)						
General population	Oral	1.2 mg/kg bw/d	1.2 mg/kg bw/d	1	Long-term	All PoDs were based on the lower bound of the therapeutic serum lithium concentration range (1.2 mg Li/kg bw/d). However, this is questionable since adverse effects are observed in several organs and systems and are associated with the entire target therapeutic serum lithium concentration range (US EPA 2008).
	Dermal	12 mg/kg bw/d	12 mg/kg bw/d			
	Inhalation	1.8 mg/m3/d	1.8 mg/m3			
Workers	Dermal	12 mg/kg bw/d	12 mg/kg bw/d			
	Inhalation	4.2 mg/m3/d	4.2 mg/m3			
General population and workers	All route	No hazard identified	-	-	Short-term	-
LiOH (data are expressed in mg LiOH/kg bw/d or mg LiOH/m3/d)						
General population	Oral	4.13 mg/kg bw/d	4.13 mg/kg bw/d	1	Long-term	For long-term DNELs, all PoDs were based on the lower bound of the therapeutic serum lithium concentration range (1.2 mg Li/kg bw/d). However, this is questionable since adverse effects are observed in several organs and systems and are associated with the entire target therapeutic serum lithium concentration range (US EPA 2008).
		12.4 mg/kg bw/d extrapolated from long-term DNEL	-	-	Short-term	
	Dermal	41.35 mg/kg bw/d	41.35 mg/kg bw/d	1	Long-term	
		50 mg/kg bw/d	LD0=2000 mg/kg bw/d	40	Short-term	
	Inhalation	6.21 mg/m3/d	6.21 mg/m3/d	1	Long-term	
		18.63 mg/m3/d extrapolated from long-term DNEL	-	-	Short-term	
Workers	Dermal	41.35 mg/kg bw/d	41.35 mg/kg bw/d	1	Long-term	
		100 mg/kg bw/d	LD0=2000 mg/kg bw/d	20	Short-term	
	Inhalation	10 mg/m3/d	14.47 mg/m3	1	Long-term	
		30 mg/m3/d extrapolated from long-term DNEL	-	-	Short-term	

[36] Proper DNELs derivation has not been carried out by eMSCA as the purpose of this document is not to demonstrate a risk and propose a restriction but rather to identify potential issues and discuss the possible risk management options.

LiCl (data are expressed in mg LiCl/kg bw/d or mg LiCl/m3/d)						
General population	Oral	0.38 mg/kg bw/d (a higher value is reported in the CSR)	15 mg/kg bw/d	40	Long-term	DNELs for LiCl reported on ECHA disseminated website are lower than the values reported in the CSR. For long-term DNELs reported in the CSR, all PoDs were based on the lower bound of the therapeutic serum lithium concentration range (1.2 mg Li/kg bw/d). However, this is questionable since adverse effects are observed in several organs and systems and are associated with the entire target therapeutic serum lithium concentration range (US EPA 2008).
		1.14 mg/kg bw/d extrapolated from long-term DNEL	-	-	Short-term	
	Dermal	3.75 mg/kg bw/d (a higher value is reported in the CSR)	150 mg/kg bw/d	40	Long-term	
		No hazard identified (a value is reported in the CSR)	-	-	Short-term	
	Inhalation	0.56 mg/m3/d (a higher value is reported in the CSR)	5.56 mg/m3	10	Long-term	
		No hazard identified (a value is reported in the CSR)	-	-	Short-term	
Workers	Dermal	9.9 mg/kg bw/d (a higher value is reported in the CSR)	238 mg/kg bw/d	24	Long-term	
		No hazard identified (a value is reported in the CSR)	-	-	Short-term	
	Inhalation	3.5 mg/m3/d (a higher value is reported in the CSR)	21 mg/m3	6	Long-term	
		No hazard identified (a value is reported in the CSR)	-	-	Short-term	
Li2CO3 (data are expressed in mg Li2CO3/kg bw/d or mg Li2CO3/m3/d)						
General population	Oral	6.43 mg/kg bw/d (a lower value is reported in the CSR)	6.43 mg/kg bw/d	1	Long-term	DNELs for Li2CO3 reported on ECHA disseminated website correspond to a former version of the CSR. They are higher than the values reported in the current version of the CSR. They were calculated from PoDs based on the lower bound of the therapeutic serum lithium concentration range (1.2 mg Li/kg bw/d). However, this is questionable since adverse effects are observed in several organs and systems and are associated with the entire target therapeutic serum lithium concentration range (US EPA 2008). The DNEL derivation in the updated version of the
		19.23 mg/kg bw/d extrapolated from long-term DNEL (a lower value is reported in the CSR)	-	-	Short term	
	Dermal	64.3 mg/kg bw/d (a lower value is reported in the CSR)	64.3 mg/kg bw/d	1	Long-term	
		50 mg/kg bw/d (a different conclusion is reported in the CSR)	LD0=2000 mg/kg bw/d	40	Short term	
	Inhalation	9.64 mg/m3/d (a lower value is reported in the CSR)	9.64 mg/m3	1	Long-term	

		28.92 mg/m3/d extrapolated from long-term DNEL (a different conclusion is reported in the CSR)	-	-	Short term	CSR appears to be more adequate. Nevertheless, it was not possible for the eMSCA to go further in the analysis and it would be necessary to confirm that the point of departure (PoD) was appropriate.
Workers	Dermal	64.3 mg/kg bw/d (a lower value is reported in the CSR)	6.43 mg/kg bw/d	1	Long-term	
		100 mg/kg bw/d (a different conclusion is reported in the CSR)	LD0=2000 mg/kg bw/d	20	Short term	
	Inhalation	10 mg/m3/d (a lower value is reported in the CSR)	22.5 mg/m3	1	Long-term	
		30 mg/m3 extrapolated from long-term DNEL (a different conclusion is reported in the CSR)	-	-	Short term	

4.4.1.2 Reference or guidance values from literature

Different reference or guidance values exist for lithium regarding oral exposure via water or the diet (Table 7).

Regarding oral exposure, the United States Environmental Protection Agency (U.S. EPA) has developed a provisional peer reviewed toxicity value (PPRTV) for lithium in relation to environmental exposures to lithium (U.S. EPA, 2008). It is also referred as provisional reference dose (p-RfD). Since a NOAEL could not be identified at the time of this assessment, U.S. EPA derived a PPRTV for chronic and subchronic exposure scenarios based on the lower bound of the therapeutic serum lithium concentrations in psychiatric patients (0.6 mM lithium/L or 4.2 mg lithium/L), which represents a LOAEL. Indeed, **the clinical literature and available animal data did not identify a NOAEL for adverse effects**. Considering whole-body clearance of 0.5 L/kg-bw/day and 100% oral bioavailability, U.S. EPA estimated that a daily lithium intake of approximately 2.1 mg lithium/ kg-bw/day would lead to a serum or plasma concentration of 0.6 mM lithium/L (i.e. 4.2 mg lithium/L serum or plasma) for a 70-kg individual. This blood level was considered as a PoD. Applying an uncertainty factor (UF) of 1,000 to this PoD gives a p-RfD of 0.002 mg/kg-bw/day or 2 µg/kg-bw/day for subchronic and chronic exposures.

This UF includes:

(1) use of a LOAEL rather than a NOAEL (factor of 10);

(2) inter-individual variability (factor of 10), based on pharmacokinetic and pharmacodynamics differences among people that might have pre-existing comorbidities; and

(3) database uncertainty (factor of 10) due to the absence of detailed information on effects of lithium on cardiovascular, neurological and endocrine systems and the lack of suitable subchronic or chronic exposure studies, well-controlled epidemiology studies, or multi-generation reproductive toxicity studies. US EPA considered a low-to-medium in this p-RfD.

Previously, it appears that US EPA had derived a higher tolerable daily intake of 20 µg/kg/d (Naeem et al. 2021).
Schrauzer (2002) proposed a provisional recommended dietary allowance (RDA) of 1.0 mg Li/d for a 70 kg adult, corresponding to 14.3 µg Li/kg/j. However, no detailed rational was provided to explain this proposal.

Anses in France and EFSA for Europe did not establish any dietary reference values for lithium.

Regarding lithium exposure via water consumption, different guidance values exist.

- The USGS proposed a non-regulatory Health-Based Screening Level of **10 µg/L** based upon the US EPA p-RfD of 0.002 mg/kg-day.

- A value of **60 µg/L**, based upon an assumption that the only source of lithium is from drinking water, was set (Lindsey et al. 2021).

- The US EPA has regulated lithium regional screening level of **40 µg/L** for residential tap waters.

- The Pennsylvania Department of Environmental Protection recently proposed a medium-specific concentration of **5 µg/L** for lithium (Bolan et al. 2021).

- The Eurasian Economic Union limit for lithium in drinking water is **30 µg/L**.

- Nevertheless, it is to be noted that the World Health Organization has not published limits on lithium in drinking water (Lindsey et al. 2021).

Finally, human biomonitoring reference values were derived by Ramoju et al. (2020) in order to interpret population-level biomonitoring data in health risk context. These values corresponded to whole blood biomonitoring equivalents (BEs) associated with the permissible daily exposure (PDE) of 560 µg/d from ICH adopted by Health Canada and the PPRTV of 2 µg/kg/d from US EPA. By using a simple kinetic relationship based on plasma clearance value (0.5 L/kg-bw/day) and the oral absorption fraction (100%), derived BE values in plasma and whole blood were respectively 4.2 µg Li/L plasma and 2.7 µg Li/L blood, based on the PPRTV, and 16 µg Li/L plasma and 10 µg Li/L blood, based on the PDE. The authors highlighted that these BE values should be considered as interim screening values that can be updated if there are changes in exposure guidance values for lithium by regulatory agencies. Moreover, these values do not represent diagnostic criteria and should not be used to assess the likelihood of an adverse health effect in an individual or within a population. **Human biomonitoring values for lithium in excess of the developed BEs may indicate exposures at or above the current exposure guidance value** underlying the BE derivation. However, in such cases, findings of lithium level above the BE should not be interpreted as indicative of risks of adverse lithium responses in individuals or of responses of individuals in tails of the distribution in population-monitoring studies.

Table 7: Existing reference or guidance values

Organism or country	Reference value						Reference
	Oral exposure		Water		Other		
	Type	Value	Type	Value	Type	Value	
Anses (France)		No recommended nutritional intake					Anses 2011
EFSA (Europe)		No dietary reference value					EFSA 2009
Europe				No potable water standards			Neves et al. 2020
USA				No regulatory threshold			Lindsey et al. 2021
USA			Drinking-water only threshold	60 µg/L (based on US EPA p-RfD of 2 µg/kg/d)			Lindsey et al. 2021
US EPA (USA)	TDI	20 µg/kg/d (1.40 mg/d for a 70 kg adult)					US EPA 2007, cited in Naeem et al. 2021
US EPA (USA)	PPRTV or p-RfD	2 µg/kg/d					US EPA 2008, cited in Ramoju et al. 2020
US EPA (USA)			Regional screening level for residential tap waters	40 µg/L			Bolan et al. 2021
USGS (USA)			HBSL (non-regulatory)	10 µg/L (based on US EPA p-RfD of 0.002 mg/kg/d)			Norman et al. 2018, cited in Lindsey et al. 2021
PADEPa (USA)			Medium-specific concentration for all grounwater within the Commonwealth	5 µg/L			Bolan et al. 2021
Australia			Agricultural irrigation water short and long-term trigger values[a]	2500 µg/L 75 µg/L for citrus crops			Australian and New Zealand Environment and Conservation Council 2000, cited in Neves et al. 2020
Russian Ministry of Health	RfD	20 µg/kg/d	Maximum permissible limit in drinking water[b]	30 µg/L			Sinitsyna et al. 2020

Eurasian Economic Union			Limit in drinking water	30 µg/L			Lindsey et al. 2021
-	Provisional RDA	14.3 µg/kg/d (1.0 mg/d for a 70 kg adult)					Schrauzer 2002
Iraq			Permissible limit in drinking water	5 µg/L			Jazza et al. 2022
WHO			No published limit on lithium in drinking water				Lindsey et al. 2021
EMA					PDE	560 µg/d[c]	EMA 2019
Health Canada					PDE	560 µg/d[c]	Health Canada 2016
USP					Permitted concentration	55 µg/g	USP 2017, Al-Thani et al. 2023

RfD: Reference Dose; p-RfD: Provisional Reference Dose; TDI: tolerable daily intake; RDA: recommended dietary allowance; PDE: permissible daily exposure; PPRTV: Provisional Peer-Reviewed sub-chronic and chronic Toxicity Value; HBSL: Health Based Screening Level

[a] The long-term trigger value (LTV) is the maximum concentration (mg/L) of contaminant in the irrigation water which can be tolerated assuming 100 years of irrigation. The short-term trigger value (STV) is the maximum concentration (mg/L) of contaminant in the irrigation water which can be tolerated for a shorter period of time (20 years). The LTV and STV values have been developed: (1) to minimise the build-up of contaminants in surface soils during the period of irrigation; and (2) to prevent the direct toxicity of contaminants in irrigation waters to standing crops (Australian and New Zealand Environment and Conservation Council 2000).

[b] SER 2.1.4.1074-01 (last amended on April 7, 2010). Drinking water. Hygienic requirements to quality of water taken from centralized drinking water supply systems. Quality control. Hygienic requirements to providing safety of hot water supply systems.

[c] International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) Guidance Document (specifically, ICH Q3D) derived permitted daily exposure (PDE) value for lithium (ICH, 2014). These PDEs are not typical exposure guidance values for environmental substances; rather, they represents the maximum permitted quantity of a material (as an impurity) permitted in drug products, as defined in ICH guidance document (ICH, 2014). Health Canada (2016) and EMA (2019) adopted ICH's PDE value of 560 µg/d for lithium.

4.4.1.3 Conclusions regarding the DNELs in comparison to other reference values

Among the existing reference and guidance values found in literature, there is no comparable value to the DNELs derived by the registrants for dermal and inhalation routes. For oral exposure, the DNELs proposed in the registration dossiers may be compared to the existing reference values found in literature (Table 8).

Table 8: DNELs from the registration dossiers and other existing reference values for long-term exposure by oral route for the general population

	Source	Reference values	PoD	AF	Exposure	Comments from eMSCA[37]
Li	Schrauzer 2002	Provisional RDA: 0.143 mg/kg bw/d			Long-term	No rational was provided to explain this proposal.
	US EPA 2008	p-RfD: 0.002 mg/kg bw/d	LOAEL: 2.1 mg/kg bw/d	1000	Long-term	eMSCA considers appropriate the PoD and AF used by US EPA. Hazard characterisation has not been performed in this dossier to discuss if this value is the most appropriate.
	US EPA 2007, cited in Naeem et al. 2021	TDI: 0.020 mg/kg bw/d			Long-term	eMSCA assumes that this value is no longer relevant and was replaced by the p-RfD of 2 µg/kg bw/d from US EPA in 2008. No rational for this former value of 20 µg/kg bw/d was found.
	Russian Ministry of Health	RfD: 0.020 mg/kg bw/d			Long-term	No rational was provided to explain this proposal.
	Registration dossier	DNEL: 1.2 mg/kg bw/d	NOAEL: 1.2 mg/kg bw/d	1	Long-term	PoDs were based on the lower bound of the therapeutic serum lithium concentration range (1.2 mg Li/kg bw/d). However, this is questionable since adverse effects are observed in several organs and systems and are associated with the entire target therapeutic serum lithium concentration range (US EPA 2008).
LiOH	Registration dossier	DNEL: 4.13 mg/kg bw/d	NOAEL: 4.13 mg/kg bw/d	1	Long-term	
LiCl	Registration dossier	DNEL: 7.32 mg/kg bw/d	NOAEL: 7.32 mg/kg bw/d	1	Long-term	
Li2CO3	Registration dossier	DNEL: 0.38 mg/kg bw/d	NOAEL: 15 mg/kg bw/d	40	Long-term	

4.4.2 Environment

4.4.2.1 PNEC from the registration dossiers

The PNECs derivation in the registration dossiers was examined by eMSCA. The registrants' values are reported in Table 9. Literature review on lithium effects on environmental species showed that **lower NOEC than the one considered for deriving some PNEC values exist** (0.02 mg/L Li (measured concentration) for *Daphnia magna* based on living offspring (Martins et al. 2022)). Other agencies have also proposed other values: **minimal and maximal provisional[38] $PNECs_{water}$ of 4.2 µg/L and 1650 µg/L respectively, have been reported by Ineris (Ineris 2020)**. No rational was provided for the derivation of these provisional PNECs for lithium in the report from Ineris. It is only indicated that these PNECs have uncertainties. Indeed, for metals, it is important to take into account

[37] Proper DNELs derivation has not been carried out by eMSCA as the purpose of this document is not to demonstrate a risk and propose a restriction but rather to identify potential issues and discuss the possible risk management options.

[38] Without a robust threshold value, Ineris uses provisional PNECs for the priorisation of substances carried out on a national scale for updating the lists of substances to be monitored in French surface waters.

the bioavailability and speciation of substances and the geochemical background. Nevertheless, it is noted that the order of magnitude between the provisional $PNECs_{water}$ of 4.2 µg/L from Ineris and the $PNEC_{freshwater}$ of 9 mg/L for Li2CO3 derived by the registrant is > 2000.

Thus, eMSCA recommends the registrants to revise the PNECs derivation, and therefore to update the associated risk assessments performed in the CSRs.

Table 9: PNEC from ECHA disseminated website

	PNEC	PoD	AF	Comments from eMSCA[39]
Li (data are expressed in mg Li+/L or mg Li+/kg sediment or soil)				
Freshwater	1.65 mg/L	NOEC: 10 mg/L for LiOH monohydrate (i.e. 1.65 mg Li/L) algue	1	Lower NOEC than the one considered for deriving the PNEC values exist in the literature (0.02 mg/L Li (measured concentration) for Daphnia magna based on living offspring (Martins et al. 2022)).
Marine water	0.165 mg/L	NOEC: 10 mg/L LiOH monohydrate (i.e. 1.65 mg Li/L)	10	
Intermittent releases to water	1.65 mg/L	-	-	
Sediments (freshwater)	44.2 mg/kg sediment dw (a lower value is reported in the CSR) (Equilibrium partitioning method)	-	-	
Sediments (marine water)	4.42 mg/kg sediment dw (a lower value is reported in the CSR) (Equilibrium partitioning method)	-	-	
Sewage treatment plant	22.95 mg/L	EC10: 79.2 mg/L for LiOH anhydrous (i.e. 22.95 mg Li/L)	1	-
Soil	6.29 mg/kg soil dw (a lower value is reported in the CSR) (Equilibrium partitioning method)	-	-	
Air	No hazard identified	-	-	
Secondary poisoning	No potential for bioaccumulation	-	-	
LiOH (data are expressed in mg LiOH/L or mg LiOH/kg sediment or soil)				
Freshwater	2.3 mg/L	NOEC: 2.3 mg/L for LiOH anhydrous	1	Lower NOEC than the one considered for deriving the PNEC values exist in the literature (0.02 mg/L Li (measured concentration) for Daphnia magna based on living offspring (Martins et al. 2022)).
Marine water	0.23 mg/L	NOEC: 2.3 mg/L for LiOH anhydrous	10	

[39] Proper DNELs derivation has not been carried out by eMSCA as the purpose of this document is not to demonstrate a risk and propose a restriction but rather to identify potential issues and discuss the possible risk management options.

Intermittent releases to water	3.44 mg/L (a lower value is reported in the CSR)	EC50(48h): 34.4 mg LiOH/L	10	
Sediments (freshwater)	153 mg/kg sediment dw (a lower value is reported in the CSR) (Equilibrium partitioning method)	-	-	
Sediments (marine water)	15.3 mg/kg sediment dw (a lower value is reported in the CSR) (Equilibrium partitioning method)	-	-	
Sewage treatment plant	79.2 mg/L	EC10: 79.2 mg/L for LiOH anhydrous	1	
Soil	28.22 mg/kg soil dw (a lower value is reported in the CSR) (Equilibrium partitioning method)	-	-	
Air	No hazard identified	-	-	
Secondary poisoning	No potential for bioaccumulation	-	-	
LiCl (data are expressed in mg LiCl/L or mg LiCl/kg sediment or soil)				
Freshwater	10.4 mg/L	NOEC: 1.7 mg Li/L (i.e. 10.4 mg LiCl/L)	1	Lower NOEC than the one considered for deriving the PNEC values exist in the literature (0.02 mg/L Li (measured concentration) for Daphnia magna based on living offspring (Martins et al. 2022)).
Marine water	1.04 mg/L	NOEC: 1.7 mg Li/L (i.e. 10.4 mg LiCl/L)	10	
Intermittent releases to water	10.4 mg/L	-	-	
Sediments (freshwater)	270 mg/kg sediment dw (a lower value is reported in the CSR) (Equilibrium partitioning method)	-	-	
Sediments (marine water)	27 mg/kg sediment dw (a lower value is reported in the CSR) (Equilibrium partitioning method)	-	-	
Sewage treatment plant	140.2 mg/L	EC10: 140.2 mg LiCl/L	1	
Soil	49.95 mg/kg soil dw (a lower value is reported in the CSR) (Equilibrium partitioning method)	-	-	
Air	No hazard identified	-	-	

Secondary poisoning	No potential for bioaccumulation	-	-	
Li2CO3 (data are expressed in mg Li2CO3/L or mg Li2CO3/kg sediment or soil)				
Freshwater	9 mg/L	NOEC: 1.7 mg Li/L (i.e. 9 mg Li2CO3/L)	1	Lower NOEC than the one considered for deriving the PNEC values exist in the literature (0.02 mg/L Li (measured concentration) for Daphnia magna based on living offspring (Martins et al. 2022)).
Marine water	0.9 mg/L	NOEC: 1.7 mg Li/L (i.e. 9 mg Li2CO3/L)	10	
Intermittent releases to water	0.3 mg/L	-	-	
Sediments (freshwater)	238.4 mg/kg sediment dw (Equilibrium partitioning method)	-	-	
Sediments (marine water)	23.84 mg/kg sediment dw (Equilibrium partitioning method)	-	-	
Sewage treatment plant	122.2 mg/L	EC10: 79.2 mg/L for LiOH anhydrous (i.e. 122.2 mg Li2CO3/L)	1	
Soil	44.11 mg/kg soil dw (Equilibrium partitioning method)	-	-	
Air	No hazard identified	-	-	
Secondary poisoning	No potential for bioaccumulation	-	-	

4.4.2.2 Reference or guidance values from literature

In the report from the Ministry of the Environment from Quebec, three types of toxicological reference values (TRV) corresponding to a certain acceptable level of adverse effects could be derived for plants and mammals (table 10) but it was not possible for microorganisms, terrestrial invertebrates, birds, amphibians and reptiles due to lacking information. The authors concluded that the derivation of TRV for these latter organisms would be necessary to perform risk assessment related to the presence of lithium in the environment for future mining projects.

Table 10: TRVs derived for terrestrial organisms exposed to Lithium

Terrestrial organisms	TRV 1*	TRV 2**	TVR 3***
Microorganisms	-	-	-
Plants	20.3 mg/kg	26.8 mg/kg	59.1 mg/kg
Terrestrial invertebrates		-	-
Birds		-	-
Mammals	-	15 mg/kg/d	28 g/kg/d

*TRV 1: TRV for sensitive environments corresponding to 10% of accepted adverse effects
**TRV 2: TRV for residential, recreational and institutional uses corresponding to 20% of accepted adverse effects
***TRV 3: TRV for commercial and industrial uses corresponding to 40% of accepted adverse effects

5 Exposure assessment

5.1 Human exposure

5.1.1 Exposure scenario for workers and consumers covered in the registration dossiers

eMSCA has reviewed the lead CSR for the 3 salts and for the lithium metal. While the CSR on lithium carbonate has been updated recently to take into consideration the batteries exposure scenarios, those of lithium chloride and lithium hydroxide have not since several years and do not take into consideration the evolution of the uses of lithium.

According to the registrants, all the exposure scenarios lead to RCRs < 1. Exposure concentrations for workers reported in the CSR of the registrant are mostly equal or higher than the median concentration reported with the occupational exposure levels as calculated by INRS (paragraph 5.1.2). Nevertheless, eMSCA cannot provide additional information as exposure concentrations are confidential data. Moreover, before going further in the assessment of exposure data, DNELs first need to be revised by the registrants.

5.1.2 Occupational exposure levels assessment tool from INRS

The web tool[40] provided by INRS for assessing occupational exposure levels reports 88 measurements for lithium from 1987 to 2022. The following statistics are provided:

- Regarding the industrial sector: 66% of the measurements have been performed in the manufacturing sector, 13% in "professional, scientific and technical activites", 9% in the construction sector, 8% in "wholesale and retail trade: repair of motor vehicles and motorcycles", 5% in mining and quarrying.
- Regarding the products associated with exposure: 40% of the measurements are related to welding fumes, 31% to dusts, 7% to surface treatment products, 5% to oils and greases, and 18% to unreported products.
- The 88 measurements include 64 personal measurements (5 short-term (]15,60[min) and 59 long-term ([60, 600] min) measurements) and 24 ambient measurements (long term - [60, 600] min). Only descriptive statistics for the long-term personal measurements are provided in Table 11 as the ambient measurements are not representative of occupational exposure.

Table 11: Descriptive statistics for personal measurements (long-term) of lithium from 1987 to 2022 (from INRS occupational exposure levels assessment tool)[41]

	Personal measurements [60, 600] min
Number of results	59
Number of results lower than the LQ	13 (22%)
Arithmetic mean	0.092 mg/m^3
Minimum	0.0005 mg/m^3
Median	0.014 mg/m^3
95th percentile	0.574 mg/m^3
Maximum	0.93 mg/m^3

5.1.3 Human exposure to lithium reported in literature

No specific literature search was performed in order to identify human exposure data. Data described below were extracted from publications identified via the literature search on lithium human health effects at environmental doses. It is to be noted that it is not exhaustive; data from epidemiological studies analysed in Section 4.1.7 was reported in priority. Additional exposure data from other publications were not described as long as it did not provide additional information than those already described. For transparency, these other publications were listed in Annex 2, Section 15.

[40] https://outil-expo-rch-rb.inrs.fr

[41] Descriptive statistics are not provided for the short-term personal measurements as the sample size is insufficient (n=5).

5.1.3.1 Presence of lithium in drinking water

- **Concentrations of lithium in drinking water**

The epidemiological studies identified from the literature search reported lithium concentrations in drinking water of various countries. **In Europe**, lithium concentrations ranged from **0.5** (lowest value measured in Scotland (Duthie et al. 2023)) **to 89.35 µg/L** (highest value measured in Hungary (Izsak et al. (2022)). **In the United States, lithium could be undetectable** (Oliver et al. 1976) or **reach 539 µg/L** (highest value measured in Texas (Fajardo et al. 2018)). In **Japan, concentrations were between 0** (Kugimiya et al. 2021) **and 130 µg/L** (highest value measured (Kohno et al. 2020)). Finally, in Argentina, lithium was observed between **5 and 1660 µg/**L (Harari et al. 2015b). All measured concentrations in drinking water are detailed in tables in Annex 2, Section 17.

Some publications reported lithium concentrations in bottled mineral waters. In the study from Seidel et al. (2019), lithium concentrations in analysed mineral and medicinal waters ranged from **0.6 to 865.1 µg/L (median: 31.2 µg/L)** in **Germany**. Neves et al. (2020) who quantified lithium concentration in **Portuguese** marketed bottled natural mineral water reported highly different lithium concentrations ranging from less than **1 to 2210 µg/**L. Two groups could be observed from the dataset: one group with low lithium concentrations (up to 11 µg Li/L) that represented 55.5% of the natural mineral water samples and a second group with lithium concentrations higher than 100 µg/L. Krachler et al. (2009) performed a quantitative evaluation of trace and ultra-trace metals in bottled waters from **28 countries**[42]. Lithium concentrations ranges from **0.057 to 5460 µg/L** (median: 4.80 µg/L). This wide range reflected the geology of the source region. Krachler et al. highlighted that **some bottled waters had lithium concentrations in the low mg/L range which is comparable to blood plasma levels of patients treated with Li-containing drugs against manic depression**.

- **Concentrations of lithium in groundwater used for drinking supply**

Maximum lithium concentrations in groundwater were **31 µg/L** (median 10.5 µg/L) and 97 µg/L, respectively in Denmark and Ireland where large-scale regional or national studies were performed. The U.S. Geological Survey (USGS) has reported lithium concentrations ranging from **1 to 650 µg/L in public-supply wells** (n=458 samples) as a part of the National Water-Quality Assessment (NAWQA) Project. Water from domestic supply wells sampled by the NAWQA Project (n=662 samples) showed lithium concentrations between <1 and 1200 µg/L (Lindsey et al. 2021).

The study from Lindsey et al. (2021) reported that lithium concentrations nationwide ranged from **<1 to 396 µg/L** (median of 8.1 µg/L) for public-supply wells and from **<1 to 1700 µg/L** (median of 6.9 µg/L) for domestic supply wells in the US. **The Health Based Screening Level (HBSL) of 10 µg/L and the drinking-water only threshold of 60 µg/L were respectively exceeded in 45 and 9% of the samples from public-supply wells and in 37 and 6% from domestic supply wells**. However, exceedances and median concentrations ranged broadly across geographic regions and principal aquifers. Concentrations were higher in arid regions than in humid regions and in older groundwater than in younger groundwater. This is consistent with a greater flux of freshwater through

[42] Australia, Belgium, Brazil, Canada, Czech Republic, Denmark, Dominican Republic, England, Finland, France, Germany, Hong Kong, Iceland, Israel, Italy, Japan, Kenya, Mexico, The Netherlands, Peru, Poland, Slovenia, Spain, Sweden, Switzerland, Trinidad, the U.S., and Yugoslavia.

aquifers in humid settings, leading to more extensive depletion of lithium from aquifer solids. Statistical and geochemical modeling approaches were used to identify potential causes of high lithium concentrations. **Multiple lines of evidence indicated that high lithium concentrations measured in the framework of this publication are most likely from natural sources and are derived from interaction of groundwater with aquifer materials and mixing with saline water sources**. Besides, proximity analysis and modeling strongly suggested that the highest concentrations were related to geothermal fluids. Local anthropogenic sources may be contributing to lithium concentrations in some groundwater samples, but there is no evidence for widespread effects. The authors indicate that lithium mines are not sufficiently widespread to influence the concentrations at the national scale. However, with the increase in the use of lithium for batteries and other purposes and the subsequent disposal of lithium in landfills, this study provides critical background on natural levels of lithium that can be used as a baseline to evaluate future changes that may be due to anthropogenic sources.

5.1.3.2 Presence of lithium in diet

Publications about lithium in diet were identified via the literature search. The review from Naeem et al. 2021 in particular reported a wide range of estimated **dietary intake of lithium by populations across different countries from 8.6 µg/d in Belgium to 1560 µg/d in China** (mean dietary intakes). According to WHO data, the **mean intake of lithium from typical diets in the USA has been estimated to be 60–70 µg/day (WHO 1996)**. Lithium intake through food depends on its concentration in food and varies due to variation in soil and drinking water lithium levels (Naeem et al. 2021). For instance, in the North of Chile where very high levels of lithium are found in surface waters, plant and animal tissues dedicated to diet also contain high lithium concentrations, thus reflecting those found in the surface waters in the nearby local water sources. Rough estimations of the lithium daily intake for the population in this region suggest that the daily consumption could reach 100 mg/d and then, may be within an order of magnitude of the dose for those undergoing maintenance treatment for bipolar disease (1-5 mg Li/kg/d) (Figueroa et al. 2012). To ensure some comparability between data, it was decided to focus on total diet studies (TDSs) that are conducted nationally and follow a standard method recommended by the World Health Organization (WHO). TDSs aim at monitoring exposure of the population to chemical substances present in food, including residues of plant protection products, environmental contaminants, newly-formed compounds, natural toxins, additives, substances migrating from food contact materials, trace elements or minerals. TDSs from France, Italy, UK and New Zealand were identified.

In France, a first TDS (TDS1) was conducted between 2000 and 2004 by the French National Institute for Agricultural Research (Inra) in collaboration with the French agency for food safety (Afssa). It provided an overview of exposure of the population, both adults and children, to inorganic and mineral contaminants, and to mycotoxins. In 2006, Anses performed a second TDS (TDS2) including 445 substances compared to 30 in TDS1.

In TDS1 (INRA 2004), it is reported that 679/998 (**68%) individual food composite samples display average lithium content > LOD of 3 µg/kg** of fresh weight. The main food groups containing lithium are shellfish (123 µg/kg) and drinking water (100 µg/L); other food groups contain less than 40 µg/kg (mean concentration). **The estimated average daily intake of the French population is 28.5 µg for adults aged 15 years or more and 14.5 µg for children aged 3–14.** The daily 97.5th percentile exposure is 144 µg for adults and 38 µg for

children. The intakes distribution of lithium for general population is provided in Table 12. Compared to existing French data, the results are on average higher by a factor of 3 (Noël et al. 2003b). The food groups including drinking waters and soups are the vectors contributing most (respectively 25–41% and 14–15%) to the exposure of the populations; other vectors contribute less than 10% of the total food exposure.

Table 12: Intakes distribution of lithium (µg/d) for general population – TDS1 (extracted from INRA 2004)

Nutritional reference value LTI	Toxicological reference value UL	Adults (≥15 years old, n=1474)					Children (3-14 years old, n=1018)				
		P2.5	P50	P97.5	%<LTI	%>UL	P2.5	P50	P97.5	%<LTI	%>UL
No recommendation		7.8	18	144	-	-	4.6	11	38	-	-

LTI: lowest threshold intake; UL: upper level

In TDS2 (Anses 2011), among the analysed samples, 8% display lithium content <LOD or LOQ (0.001 mg/kg). The highest average lithium levels are found in shellfish (0.07 mg/kg), dried vegetables (0.07 mg/kg), waters (0.07 mg/kg), coffee (0.04 mg/kg) and pasta (0.04 mg/kg). Other food groups contain less than 40 µg/kg (mean concentration). These average concentrations are around 3 times higher than the ones in TDS1, except for shellfish for which lithium level was lower in TDS2. **The estimated average daily intake of the French population is 48.2 µg for adults and 19.8 µg for children**. These average intakes are around 1.5 times higher than the ones in TDS1. Thus, exposure to lithium increased in TDS2 in comparision with TDS1. The intakes distribution of lithium for general population is provided in Table 13. In both adults and children, the vector that contributes the most to lithium exposure of the populations is waters (respectively 35 and 34%), followed by coffee (17%) and other hot drinks (14%) in adults.

As it is still unknown if lithium is nutritionaly required and thus, **as no recommended nutritional intake exists for lithium**, it is not possible to conclude on the coverage needs. Thus, additional data are required to define the nutritional needs in lithium. Nevertheless, one should note that based on lithium intake data in different countries, Schrauzer (2002) proposed a provisional recommended dietary allowance (RDA) of 1.0 mg Li/d for a 70 kg adult, corresponding to 14.3 µg Li/kg/j. However, no detailed rational justification was provided to explain this proposal.

Table 13: Intakes distribution of lithium (µg/d) for general population – TDS2 (extracted from Anses 2011)

No recommended nutritional intake, no safety limit	Adults				Children			
	P5	Mean	P95	%>TRV, SL, MOE, or %<ANN, RNI	P5	Mean	P95	%>TRV, SL, MOE, or %<ANN, RNI
	14.9	48.2	93.6	-	9	19.8	38.6	-

TRV: toxicological reference value; SL: safety limit; MOE: margin of exposure; ANN: average nutritional need; RNI: recommended nutritional intake

Following TDS2, Anses launched the infant TDS in 2010 in order to assess the dietary exposure of children under 3 years old. The overall lithium detection rate is 97% (excluding tap water). It is 100% in all matrices, except for sugars and derivatives and fruit baby jars (77%). The highest average lithium levels are found in pasta (45 µg/kg), vegetables (40 µg/kg), rice and durum or crushed wheat (36.5 µg/kg). **The estimated average daily intake of lithium (upper bound) is between 6.69 µg/d for infants from 1 to 4 months old and 8.72 µg/d for those from 13 to 36 months old.** The intakes distribution of lithium for infants

is provided in Table 14. For infants with the lowest intakes (<P10), the average lithium intake is between 2.66 and 4.26 µg/d. For infants with the highest intakes (>P90), the average Li intake is between 16.3 and 20.5 µg/d. In comparison to TDS2, the Li intake of infants under 3 years old is lower than the one for children between 3 and 6 years old. Up to 12 months, 1st and 2nd age preparations contribute the most to lithium intake, with 93% of intake in 1-4 month olds, 67% in 5-6 month olds, and 36% in 7-12 month olds. The water in these preparations contributes to the intake by 26 to 66%.

In terms of nutritional risks, the same conclusion was drawn than in TDS2. For infants under 3 years old, as neither nutritional reference nor upper safety limit exist for lithium, it is not possible to conclude on the coverage needs. It is not possible to conclude on the risk related to a potential excess or deficiency of lithium intake. Thus, additional data is necessary to define if lithium is nutritionaly required and then, to determine nutritional references and a safety limit.

Table 14: Intakes distribution of lithium (µg/d) for infants under 3 years old – infant TDS (extracted from Anses 2016)

Age class	Mean		P50		P10		P90	
	LB	UB	LB	UB	LB	UB	LB	UB
1-4 months	6.63	6.69	5.46	5.46	2.99	3.04	8.88	8.98
5-6 months	7.43	7.55	6.18	6.20	3.30	3.73	14.1	14.2
7-12 months	8.26	8.37	6.85	6.92	4.40	4.49	12.4	12.4
13-36 months	8.67	8.72	8.20	8.24	4.95	4.98	13.6	13.7

LB: lower bound; UB: upper bound

In Northern Italy, a TDS was performed in 2004 and lithium daily exposure of adult population (mean (range): 29.9 (8.2-42.4) µg/d) was in the range of the French TDS1 (Turconi et al. 2009).

In the 1994 UK TDS, the population dietary exposure estimate for lithium is 16 µg/d (it does not include the contribution from drinking water). The mean and upper range total adult exposure are 17 µg/d and 29 µg/d respectively (Ysart et al. 1999).

An additional study to the core programme of the 2016 New Zealand TDS reported overall intakes of lithium in the New Zealand diet in the range of those from the French TDS1 (New Zealand adults Upper Bound mean: 20–29 µg/day) (Pearson 2020). However, some food groups in New Zealand showed notable disparity with results from TDS2, for example, crustaceans and molluscs (NZ mean: 0.15 mg/kg, French TDS2 mean: 0.07 mg/kg) and water (NZ mean: 0.007 mg/kg, French TDS2 mean: 0.070 mg/kg). To characterise the hazard of dietary lithium, Pearson et al. selected the provisional subchronic and chronic reference dose (p-RfD) of 2 µg/kg/d from US EPA. All of the estimated New Zealand population intakes of Li fell below this health-based guidance value, with only the upper bound mean exposure for infants (1.59 µg/kg/d) exceeding 50% of this value. Thus, Pearson et al. concluded that dietary lithium is not a current food safety risk for the New Zealand population.

5.1.3.3 Biomonitoring data

Biomonitoring data from national surveys (ENNS and Esteban in France, HBM4EU in Europe, GeRES in Germany, NHANES in the US, ECMS in Canada) were screened to identify lithium impregnation in the general population.

Only the national cross-sectional study in the French general population, Esteban, reported data about lithium. This study estimated the impregnation of the general population between 6 to 74 years old to various environmental substances. The data collection phase of this study took place from April 2014 to March 2016. Results regarding the levels of impregnation of the French population (adults and children) by urinary metals including lithium were published in 2021 (Fillol et al. 2021). Lithium levels are presented in Tables 15-18. **In adults, the urinary median Li concentration is 28.92 µg/L** and the 95th percentile (upper limit of the 95% confidence interval of the P95) is 312.74 µg/L. **In children, the median is 28.69 µg/L** and the 95th percentile is 120.78 µg/L.

Table 15: Distribution of urinary Li concentrations (µg/L) of adults between 18 and 74 years old, Metropolitan France (2014-2016) (extracted from Fillol et al. 2021)

	n	GM	95%CI GM	P10	P25	P50	P75	P90	P95	95%CI P95
Total	2 419	33,13	[31,03 ; 35,38]	10,12	16,55	28,92	55,02	110,75	238,35	[201,97 ; 312,74]
Sex										
Men	1 060	34,97	[32,04 ; 38,16]	10,75	17,95	30,37	55,75	124,06	285,07	[202,28 ; 452,67]
Women	1 359	31,54	[28,83 ; 34,50]	9,49	15,57	27,90	54,02	103,52	212,89	[158,88 ; 271,26]
Age										
18-29	161	33,42	[27,97 ; 39,95]	10,93	17,59	29,16	55,28	95,38	203,24	[96,81 ; 503,71]
30-44	609	31,21	[27,65 ; 35,22]	9,39	16,33	28,70	49,76	100,68	207,39	[143,87 ; 306,89]
45-59	893	36,45	[32,68 ; 40,66]	10,42	17,53	31,66	63,95	131,28	273,65	[186,57 ; 422,18]
60-74	756	31,25	[27,82 ; 35,11]	9,72	14,96	26,43	49,50	122,70	278,29	[188,47 ; 440,36]

LOD = 0,03 µg. L^{-1} ; % > LOD = 100% ; LOQ = 0,05 µg. L^{-1} % > LOQ = 100%

Table 16: Distribution of urinary Li concentrations (µg/g creatinine) of adults between 18 and 74 years old, Metropolitan France (2014-2016) (extracted from Fillol et al. 2021)

	n	GM	95%CI GM	P10	P25	P50	P75	P90	P95	95%CI P95
Total	2 419	44,14	[41,31 ; 47,16]	13,12	20,50	36,12	72,79	185,49	366,30	[305,83 ; 466,13]
Sex										
Men	1 060	38,37	[35,27 ; 41,74]	12,54	18,68	31,67	57,37	165,36	331,29	[236,10 ; 507,19]
Women	1 359	50,21	[45,68 ; 55,20]	14,24	23,49	40,09	84,87	209,56	386,83	[324,82 ; 542,33]
Age										
18-29	161	32,56	[27,26 ; 38,87]	12,06	15,31	27,41	51,05	121,06	232,64	[131,44 ; 378,47]
30-44	609	35,43	[31,94 ; 39,31]	11,44	17,87	29,14	61,08	130,51	247,16	[163,16 ; 313,49]
45-59	893	51,64	[46,09 ; 57,87]	14,68	23,71	41,43	83,74	243,19	499,29	[343,32 ; 730,11]
60-74	756	57,89	[51,01 ; 65,70]	17,94	29,17	44,32	94,10	241,64	458,36	[322,42 ; 655,87]

Table 17: Distribution of urinary Li concentrations (µg/L) of children between 6 and 17 years old, Metropolitan France (2014-2016) (extracted from Fillol et al. 2021)

	n	MG	IC 95% MG	P10	P25	P50	P75	P90	P95	IC 95% P95
Total	1 052	31,33	[29,06 ; 33,77]	13,36	19,40	28,69	46,61	78,81	107,62	[93,00 ; 120,78]
Âge (ans)										
6-10	477	39,35	[35,67 ; 43,41]	18,29	24,48	35,25	55,17	89,72	127,83	[96,02 ; 214,77]
11-14	389	29,33	[26,19 ; 32,83]	12,29	18,31	27,02	45,55	75,46	96,75	[78,36 ; 117,62]
15-17	186	22,73	[20,11 ; 25,69]	10,39	13,92	20,34	31,81	45,96	85,50	[47,81 ; 149,54]
Sexe										
Garçon	535	32,38	[29,12 ; 36,01]	12,92	19,48	29,78	48,24	82,77	118,31	[88,99 ; 150,05]
Fille	517	30,32	[27,77 ; 33,10]	13,65	19,25	27,77	45,30	73,23	99,40	[82,40 ; 111,52]

LOD = 0,03 µg. L⁻¹ ; %>LOD = 100% ; LOQ = 0,05 µg. L⁻¹ ; %>LOQ = 99,4 %

Table 18: Distribution of urinary Li concentrations (µg/g creatinine) of children between 6 and 17 years old, Metropolitan France (2014-2016) (extracted from Fillol et al. 2021)

	n	MG	IC 95% MG	P10	P25	P50	P75	P90	P95	IC 95% P95
Total	1 052	31,85	[29,78 ; 34,07]	13,46	20,07	30,31	47,17	75,21	93,49	[84,77 ; 115,06]
Âge (ans)										
6-10	477	31,26	[28,22 ; 34,63]	14,16	19,15	28,88	45,79	75,50	103,13	[83,36 ; 188,37]
11-14	389	32,92	[29,78 ; 36,40]	13,51	20,70	31,48	50,59	77,85	90,75	[81,61 ; 109,15]
15-17	186	31,35	[27,52 ; 35,72]	11,33	20,84	31,97	44,70	65,10	106,28	[72,50 ; 204,09]
Sexe										
Garçon	535	33,40	[30,22 ; 36,92]	13,60	19,81	30,64	53,72	80,67	108,58	[86,30 ; 189,97]
Fille	517	30,40	[28,01 ; 32,99]	13,29	20,23	30,13	41,92	65,67	86,79	[74,65 ; 95,60]

Other publications identified via the literature search provided Li biomonitoring data in different biospecimens (urine, blood, hair, breast milk) in various countries. Data are described in Annex 2, Section 17.

Four studies reported urinary Li concentrations that could be compared to Esteban's results. **While Heitland et al. (2021) showed Li levels in a German population that were quite similar to Esteban's results, Figueroa et al. 2014 (Chile) and Igra et al. (2016) (Argentina) reported much higher Li levels**. In Brasil, Australia and Canada (Barbosa et al. 2023, Callan et al. 2013, Ratelle et al. 2020), urinary Li levels were lower than Esteban's results (Table 19).

Regarding biomonitoring data in blood, P95 Li concentrations mainly ranged between 1 and ~3 µg/L (Callan et al. 2013 (Australia), Cesbron et al. 2013 (France), Heitland et al. 2021 (Germany)). Much higher values were especially observed in Chile (range: 1050-1410 µg/L in maternal venous blood, Figueroa et al. 2014) (Table 20).

Li concentrations in hair were mainly between 0.001 and 1 µg/g. Biomonitoring data in Chile standed out with a mean concentration of 5.3 µg/g (Figueroa et al. 2014) (Table 21).

Few biomonitoring data were collected from breast milk, with very high concentrations in Chilian women (Figueroa et al. 2014), placenta and umbilical cord blood (Tables 22, 23, 24).

High levels of lithium found in biospecimens of the population from Chile reflect the exceedingly high lithium levels found in water and food (Figueroa et al. 2014).

Finally, the epidemiological studies described in Annex 2, Section 17 also reported biomonitoring data in the same order of magnitude as the above data according to each country.

Table 19: Concentrations of Li in urine from several publications

Country	Region	Study type, population		µg/g creatinine					µg/L					Reference
			Number of participants /samples (n)	Mean	Median	P95	P95 (95% CI)	Range	Mean	Median	P95	P95 (95% CI)	Range	
Brasil		Longitudinal study of Brazilian Adult Health (ELSA-Brasil)	996	-	6.4	18.8	20.9	0.02-146	-	-	-	-	-	Barbosa et al. 2023
Germany		Adult	102	35	-	80	-	-	27.6	-	103	-	2-416	Heitland et al. 2021
Chile	Valle Camarones	Adult	67 14	-	-	-	-	-	2822[a] 5754[b]	-	-	-	-	Figueroa et al. 2014
	Valle Lluta	Children	44 35	-	-	-	-	-	703[a] 1713[b]	-	-	-	-	
	Valle Azapa	Children	8 37	-	-	-	-	-	140[a] 198[b]	-	-	-	-	
Argentina	Puna	Adult (women)	198							340-4550				Concha et al. 2010
Argentina		Mother-child cohort - 3rd trimester	152							1465	3732			Igra et al. 2016
Canada			198				48 (43-50)					51 (41–66)		Ratelle et al. 2020
Australia		Australian Maternal Exposure to Toxic Substances (AMETS) study	157 (µg/g creatinine) 173 (µg/L)	11.58	9.19	29.2	-	2.95-43.5	8.85	7.34	22.6	-	1.04-36.6	Callan et al. 2013

[a] lowest average concentration reported; [b] highest average concentration reported

Table 20: Concentrations of Li in blood (whole blood, plasma, serum) (µg/L) from several publications

Country	Region	Study type, population	Number of participants /samples (n)	Biospecimen	Mean	Median	P95	P95 (95% CI)	Range	Reference
Australia		Australian Maternal Exposure to Toxic Substances (AMETS) study	172	Whole blood	0.77	0.63	2.00	-	<0.08-3.39	Callan et al. 2013
France		Adult	106	Whole blood	na	<1.2	<1.4	-	-	Cesbron et al. 2013
France		Adult	106	Plasma	na	0.64	1.88	-	-	Cesbron et al. 2013
France		Children (under 5 years to < 18 years)	99	Whole blood		<1.0	3.1			Goullé et al. 2015
				Plasma		<1.0	3.4			
Chile	Valle Camarones	Adult	125	Serum	-	-	-	-	9-175[a]	Figueroa et al. 2014
	Arica	Adult	22	Maternal venous blood	-	-	-	-	1050-1410	
Argentina		Mother-child cohort - 3rd trimester	152	Whole blood		26	63			Igra et al. 2016
Germany		Adult	102	Whole blood	0.93	-	1.4	-	0.41-9.9	Heitland et al. 2021
			102	Serum	1.3	-	3.2	-	0.3-16.9	
			102	Erythrocytes	0.46	-	1.1	-	<0.3-4	
Kazakhstan	Akzhar	Adult	26	Whole blood		3.413[b]				Semenova et al. 2019
Canada			276	Whole blood				5.9 (4.1–8.4)		Ratelle et al. 2020
Italy		Adult enroled as part of the FAST study "Effects of Lifestyle Changes on Semen Quality in Healthy Young Men Living in Highly Polluted Areas"	317	Serum	-	12.5	-	-	0.7-371	Nunzio et al. 2022
-		Adult		Serum					0.2-0.8*	WHO 1996, cited in Nunzio et al. 2022
-		Adult		Whole blood					0.4-1*	WHO 1996, cited in Nunzio et al. 2022
Italy		Adult		Serum					0.36-2.20*	ISS 10/22 2005, cited in Nunzio et al. 2022

[a] no range was described in the publication but it was extracted the lowest and highest individual concentration; [b] highest median reported in the publication; * Reference concentrations in adult human clinical specimens (pooled data from several publications)

Table 21: Concentrations of Li in hair (µg/g) from several publications

Country	Region	Study type, population	Number of participants /samples (n)	Mean	Median	Range	Reference
Tatarstan		Children	-	0.04		0.018-0.076	Sitdikov et al. 2011
Chile	Valle Camarones	Adult	22	5.3		ND-9.21	Figueroa et al. 2014
USA	New York	Adult	206			0.009-0.228	Schrauzer et al. 2002, cited in Figueroa et al. 2014
-		Adult				0.010-0.1*	WHO 1996, cited in Nunzio et al. 2022
Ethiopia		Children	81	0.32	0.2	0.02-1.6	Astolfi et al. 2020
China		Adult – female	30		0.051	0.019-0.16	Pan et al. 2015
		Adult – male	27		0.041	0020-0.25	Pan et al. 2015
Italy		Adolescents (from 11 to 14 years old)	943		0.02		Tamburo et al. 2016
Poland		Students (aged 19-25)	95	0.123	0.104	0.037-0.374	Szynkowska et al. 2015
Poland		Polish students	117	0.108	0.075	0-0.320	Chojnacka et al. 2010

* Reference concentrations in adult human clinical specimens (pooled data from several publications)

Table 22: Concentrations of Li in breast milk (µg/L) from several publications

Country	Region	Study type, population	Number of participants /samples (n)	Mean	Median	Range	Reference
Chile	Arica	Adult	20			2498-4379	Figueroa et al. 2014
Jordan			76	3.31+/-1.60	3.15	nd-11.3	Tahboub et al. 2021

Table 23: Concentrations of Li in placenta (µg/g) from several publications

Country	Study type, population	Number of participants /samples (n)	Mean	Range	Reference
Spain	Adult – women	62	0.47	0.03-2.34	Cerrillos et al. 2019

Table 24: Concentrations of Li in umbilical cord blood (µg/g) from several publications

Country	Region	Study type, population	Number of participants /samples (n)	Mean	Reference
USA	Kentucky, Ohio and West Virginia	Adult - women	rural: 79 urban: 93	rural: 0.49 urban: 0.57	Cottrell et al. 2018

5.1.4 Conclusion regarding human exposure

The general population may be exposed to lithium from diverse sources (drinking water, food). It is observed that concentrations in drinking water are geographically variable. This is due to the natural occurrence of lithium which is geographically variable. The contribution of anthropic activities in exposure concentrations is also possible. There is an urgent need to determine to what extent these activities, especially with the increase in the use of lithium, contribute to human exposure.

Occupational exposure to lithium also occurs. Nevertheless, very few data are available. There is a need to gather such data.

5.2 Environmental exposure

5.2.1 Environmental exposure data from literature

No specific literature search was performed in order to identify environmental exposure data. Data described below were extracted from publications identified via the literature search on the potential effects of lithium on human health at environmental doses and on environmental species. It is to be noted that it is not exhaustive; data from reviews (MELCC 2022, Bolan et al. 2021, Adeel et al. 2023) were reported in priority. Additional environmental exposure data from other publications were not described as long as it did not provide additional information than those already described. For transparency, these other publications were listed in Annex 2, Section 15.

In the following sections, the sources of the presence of lithium in the environment are described. It originates from both geogenic and anthropogenic sources.

5.2.1.1 Geogenic origin of lithium

Lithium is the 30th most abundant element in the upper continental crust, similar to the abundance of lead and copper (Lindsey et al. 2021). Lithium is naturally found in aquatic and terrestrial environments, in more than 150 minerals, in clays, brines, geothermal waters and in seawater. Lithium concentrations may vary from 5 mg/kg in limestone rocks to 65 mg/kg in igneous rocks. Concentrations measured in soils near deposits may reach up to 1600 mg/kg in South America and up to 40 000 mg/kg in Australia (MELCC 2022). **Natural weathering processes in geological deposits, hot springs arising from geothermal activities, and volcanic eruptions are the major geogenic sources releasing Li into the environment, and play an important role in balancing the global Li cycle** (Bolan et al. 2021). Weathering of rocks also leads to a major release of Li, which upon chemical processes dissolves in rivers and by physical transport processes eventually resides in sediments of rivers and oceans (Adeel et al. 2023). Lithium also occurs naturally in freshwater, groundwater, oceans, soil, and the atmosphere (Bolan et al. 2021, see below for further details).

Lithium is considered as a highly mobile element in the environment. The review from Naeem et al. (2021) in particular reported Kd values from 0.13 to 3.0. Its mobility/availability in soils mainly depends on its physico-chemical properties and the prevailing conditions in the soil (pH, soil composition). Once solubilized, the Li+ ions may be washed out of the ground by the rains and reach groundwaters and the aquatic environment where lithium will then be found in ionic form. The most common compounds found are lithium chloride (LiCl) and lithium sulphate

(Li2SO4); they are the most soluble[43] and they dissociate in an aqueous medium (MELCC 2022).

Lithium concentrations are generally higher in seawater (about 170 µg/L) than in freshwater (between 0.01 and 40 µg/L). These concentrations are generally higher in regions where lithium-rich brines and minerals are found**. The lithium content varies from 13.7 mg/L in the Dead Sea to 1500 mg/L in the brines of the Salar de Atacama, in Chile.** In rivers in the lithium-rich regions of northern Chile, the content in surface waters can reach 5.2 mg/L.

Lithium concentrations in groundwater can reach 500 µg/L, while in saline groundwater, which is common in arid and semi-arid regions, lithium concentrations vary from 10 to 16000 µg/L and can reach 100 mg/L in Salar de Atacama in Chile (MELCC 2022).

Adeel et al. (2023) provided a global geographical distribution of Li in various water bodies including surface and groundwaters (Figure 4). It appears that lithium concentrations in these water bodies in Europe are < 0.06 µg/L.

Few data is available regarding Li concentrations in sediments. The average concentrations measured are 17 mg/kg in sandstones, 46 mg/kg in shales and 26 mg/kg in limestones. A high concentration of 1500 mg/kg was reported in Nevada (MELCC 2022).

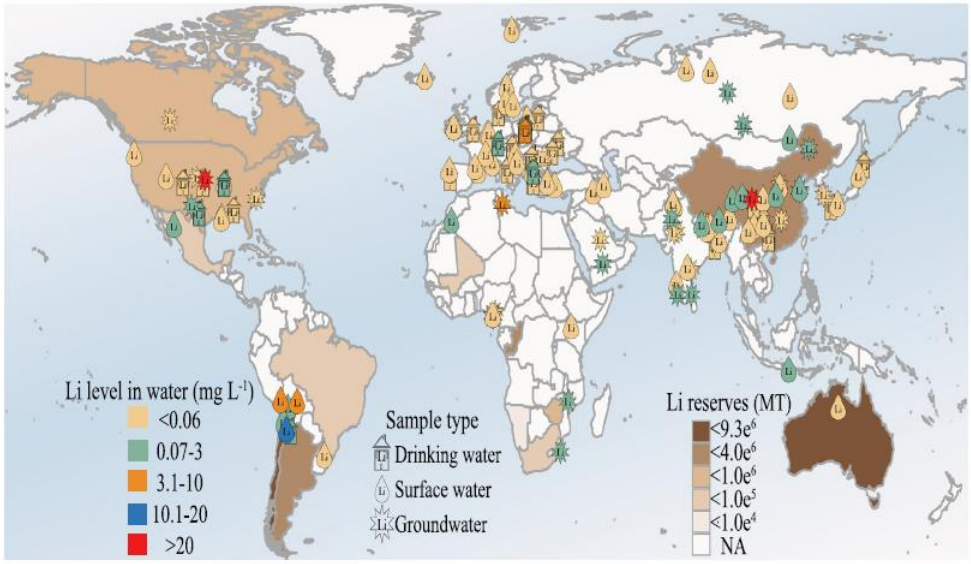


Figure 4: Global geographical distribution of Li in various water bodies* (extracted from Adeel et al. 2023)
***Data sets were collected for 17 countries from published reports. The categorization (<0.06 – >20 mg/L) used is based of the recommendation by the United States Geological Survey (USGS). Three types of datasets (Li level in water (<0.06, 0.07-3, 3.1-10, 10.1-20, and >20), sample type (drinking water, surface water, and groundwater), and Li reserves in the world) are presented. Surface water includes lakes, rivers, ponds, and glaciers. Different colors inside the icons highlight the different Li levels in**

[43] Water solubility of lithium salts (extracted from the CLH report (Anses 2020):
Li2CO3: 8.4 g/L (measured at 20°C)
LiCl: 569 g/L (measured at 20°C)
LiOH anhydrous: 71-125 g/L at 20°C
Li2SO4: 342 g/L (25°C) (from ECHA disseminated website)

water, various icons show the sample type, and color inside the map denotes the Li reserves in the world.

5.2.1.2 Anthropogenic sources of lithium

Environmental Li contamination also originates from anthropogenic sources. For example, improper disposal of Li-based products, e.g. in landfills, may lead to the leaching of the chemical content into soil and contaminate surface and groundwaters. **The concentration measured in leachate from a waste disposal site was reported at 330 µg/L** (Lindsey et al. 2021). Due to medicinal use of lithium and excretion from users into wastewater, septic systems and sewage treatment plants have been noted as potential sources of contamination (Lindsey et al. 2021). Besides, in the study from Furlong et al. 2017 aiming at describing the presence, persistence and concentrations of contaminants of emerging concern in source and treated drinking waters of the US, **lithium was the most frequently quantified pharmaceuticals (maximum concentration: 46 µg/L) and the results suggested minimal or no removal of this substance during water treatment**, consistent with lithium's being characterized as a conservative inorganic tracer. However, Furlong et al. indicated that it could not be determined if the percentage of lithium was attributable to pharmaceutical-derived wastewater or petrogenic contributions to source-water samples. Chemical manufacturing facilities, spills from manufacturing and recycling facilities, and industrial effluents may also be a source of contamination. **Leachate from a nuclear waste facility was 19 mg/L** (Lindsey et al. 2021). The lubricating greases used in vehicles manufactured from LiOH·H2O can release lithium into the environment through surface-water runoff from roads. Agricultural and soil amendments are another potential sources of lithium contamination, because biosolids (sludge) from wastewater treatment facilities are used as soil amendments. Smelting and mining of ores are also a major source of Li contamination during the exploitation and processing of ore deposits, via the disposal of tailings and discharge of tailing effluents containing lithium, contaminating air, water and soil. Moreover, tailing water is repeatedly used without any additional treatment intensifying the dissolved Li content in water systems. Due to the presence of ores from actively mined areas in Northern Chile, lithium contents in surface-water sources of the region were remarkably high (Bolan et al. 2021).

In addition, accidental releases occur (see paragraph 6.3 for further details) and can lead to release of lithium in fire-fighting water (14 mg/L), in groundwaters with high spatial variability and in air.

Due to anthropogenic sources and the increasing demand for Li, in particular for portable energy storage devices, several reviews are in accordance with the fact that the release of lithium to the environment is expected to increase significantly in the coming decades (Chow et al. 2022, Bolan et al. 2021, Adeel et al. 2023). Nevertheless, eMSCA notes that exposure data due to anthropogenic sources remain limited.

5.2.2 Norman EMPODAT database

Norman EMPODAT is a database of geo-referenced monitoring and bio-monitoring data in Europe on emerging substances in the following matrices: water, sediments, biota, SPM[44], soil, sewage sludge and air. This database cannot be used as

[44] SPM: suspended particulate matter

representative of contamination areas associated to industrial activities, but rather as an initial overview of the state of the various environmental media. Moreover, the variability of the detection and quantification limits restrains the data exploitation.

Data about lithium was only available for the surface water compartment in France from 2016 to 2020 (Table 25)[45]. The range of the quantification limit is [0.1; 10] µg/L.

Table 25: Results for surface water compartment (Norman)

Descriptive data analysis for surface water (Total number of analysis: 48541)	
Surface water (µg/L) 2016 – 2020	**Total individual values > LOQ: 41683 (85.87% of total analysis)**
90th Percentile	9.40
Mean	5.74
Median	3.00
Maximum	$2.88.10^{3}$

5.2.3 Naïades database

Naïades is a database collecting French data only. It is an observatory on the quality of river and water bodies. 69719 samples are implemented in the database for lithium between 1978 and 2023, including 60133 values above the LOQ[46]. Samples (> LOQ) were analysed by 24 different laboratories, in different compartments but mainly in surface water, in 16 different French regions[47] and between 2016 and 2022 (Figure 5).

[45] https://www.norman-network.com/nds/empodat/chemicalSearch.php (accessed on 18/10/2023)

[46] https://naiades.eaufrance.fr/ (accessed on 04/07/2023)

[47] Auvergne-Rhône-Alpes, Bourgogne-France-Comté, Bretagne, Centre-Val de Loire, Corse, Grand Est, Guadeloupe, Guyane, Hauts-de-France, Ile-de-France, La Réunion, Martinique, Normandie, Nouvelle-Aquitaine, Occitanie, Provence-Alpes-Côte d'Azur

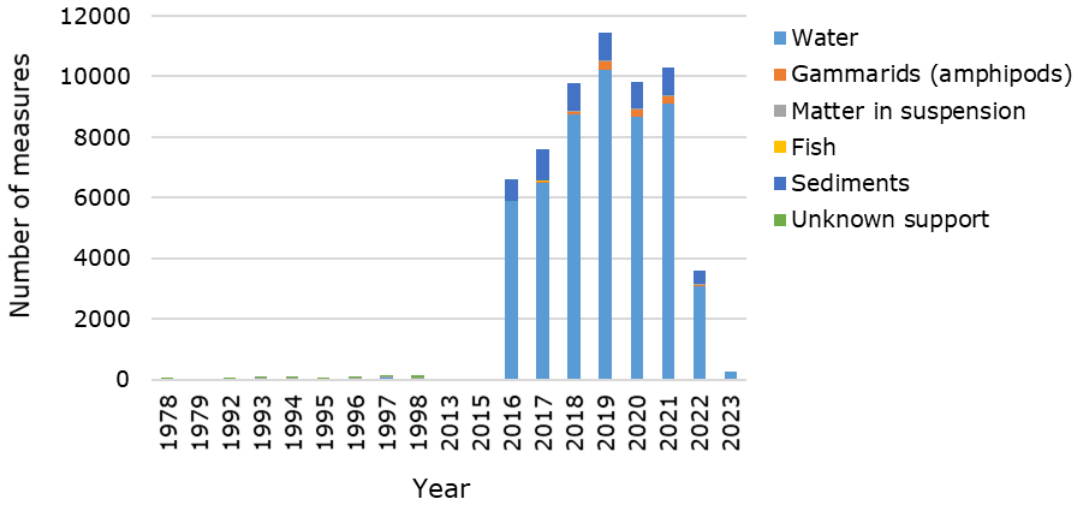


Figure 5: Data (> LOQ) distribution per compartment and year (Naïades)

Table 26 and Figure 6 are presenting data (> LOQ) in water compartment according to each studied region. Most of the concentrations measured in this compartment range between 0 and 30 µg/L. Maximum concentrations exceeding 1 mg/L are observed in Normandie and Grand Est, those exceeding 0.1 mg/L are observed in Ile-de-France, Bourgogne, Nouvelle-Aquitaine, Occitanie and Auvergne.

In sediments, Li concentrations range between 10 and 150 mg/kg (data not shown).

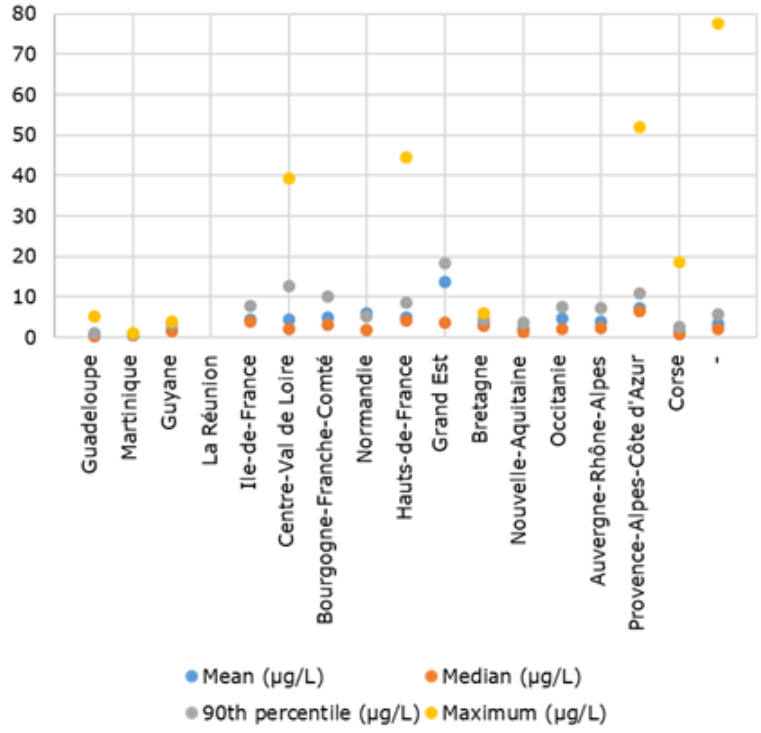


Figure 6: Data distribution in water compartment according to each studied region*
*Ordinate scale is reduced to better distinguish data distribution.

Table 26: Data (> LOQ) in water compartment according to each studied region (Naïades)

	Guadeloupe	Martinique	Guyane	La Réunion	Île-de-France	Centre-Val de Loire	Bourgogne-Franche-Comté	Normandie	Hauts-de-France	Grand Est	Bretagne	Nouvelle-Aquitaine	Occitanie	Auvergne-Rhône-Alpes	Provence-Alpes-Côte d'Azur	Corse	Unknown region
Year of sampling	2018-2021	2017-2020	2017-2019	-	1978-2023	2016-2022	2016-2023	1992-2023	2016-2022	2016-2023	2016-2020	2016-2021	2016-2023	2016-2023	2018-2023	2018-2023	2016-2023
Total number of analysis	397	39	39	-	4528	929	4257	5813	4580	24060	22	4723	6899	2557	1276	225	1322
Total individual values > LOQ	334	26	7	-	4362	897	3357	5001	4402	21832	22	4047	3465	2290	1265	150	1263
Mean (µg/L)	0.63	0.49	1.77	-	4.55	4.47	4.92	5.97	4.94	13.76	3.30	2.17	4.61	3.84	7.35	1.69	3.35
Median (µg/L)	0.4	0.5	1.6	-	3.8	2.22	3.13	1.8	4.28	3.57	2.95	1.4	2.17	2.4	6.5	0.8	2.01
90th percentile (µg/L)	1.2	0.75	3.36	-	7.81	12.78	10.04	5.2	8.6	18.5	4.49	3.7	7.506	7.3	10.9	2.57	5.8
Maximum (µg/L)	5.3	1.1	3.9	-	158	39.4	112	1350	44.5	3080	5.9	229	200	321	51.9	18.6	77.7

For the period 2016-2018, the frequency of quantification of lithium in water and sediment in Metropolitan France was respectively ~90% and 100%. In overseas departments and regions of France for the same period, the frequency of quantification was ~65% in water and ~92% in sediments (Ineris 2020). Est of France contains the biggest mean value of Li concentrations.

5.2.4 ADES database

Over 2014-2017, data from the ADES groundwater quality data indicate concentrations ranging from 0.1 to 762 (Li) µg/L, with an average value of 5.4 µg(Li)/L[48].

5.2.5 Conclusion regarding environmental exposure

Lithium has geogenic sources, thus it is naturally found in the environment in freshwater, groundwater, oceans, soil, and the atmosphere at variable concentrations. The presence of lithium in the environment is also due to anthropogenic sources but the extent of the effect of human activities contributing to current and future increasing environmental lithium contamination is unknown.

6 Risk characterisation

6.1 Risk characterisation in the registration dossiers

No analysis of the risk characterisation provided in the CSR for human health and the environment was performed by eMSCA. However, some critics were formulated regarding the DNEL and PNEC derivation in Section 4.4 and should conduct the registrants to revise these reference values. Then, the associated risk assessments should be adapted.

6.2 Human health risk assessments reported in literature

More than 50 risk assessment studies have been identified through the literature search that was conducted up to 28 June, 2023 to identify potential human health effects from exposure to lithium at environmental doses (search methodology detailed in Annex 2, Section 15).

More than half of these studies (n=33) were excluded because 1) they did actually not assess the health risks regarding Li as no toxicological reference value was provided for this metal, or 2) calculation errors or imprecisions/inconsistency in the results were observed.

The remaining studies (n=25) were described in Annex 4. They correspond to human health risk assessments regarding exposure to different trace elements including lithium, either *via* oral ingestion or dermal absorption of water (drinking water, surface water, rainwater from rain-fed cisterns, groundwater) from various sources (river, lake, well, tap water) (Table 61), or in the diet (Table 62), in various countries. **Six studies identified risks related to lithium exposure via drinking water** (HQ > 1) (Lu et al. 2022; Sadeghi et al. 2021; Chen et al. 2020; Al-Khatib et al. 2019; Elumalai et al. 2017; Vetrimurugan et al. 2017) **while five other studies did not identify a risk**. Regarding Li exposure through the diet, only 1 of 13 studies identified a health risk to the population (Otachi et al. 2015).

[48] https://www.seine-maritime.gouv.fr/contenu/telechargement/57649/405239/file/20230704%20rapport%20cellule%20post%20accident%20HIGHWAY.pdf

It was not possible to compare this set of studies as they did not use the same parameters for calculating the risks, and in particular not the same toxicological reference values. For example, some studies used the last updated p-RfD of 2 µg/kg/d from US EPA, while other used a former value of 20 µg/kg/d.

One other remark is that the potential origin (natural/geogenic or anthropogenic) of Li presence in drinking water and diet was not always indicated.

None of the studies evaluated the risks linked to the different uses of lithium salts (in metals, coating, glass, ceramics…) neither for workers and consumers, nor for the environment.

6.3 Risks arising from the physico-chemical properties of lithium and battery use

Based on the hazards described in 4.2, lithium uses leads to various risks. In particular, throughout its life cycle, from manufacturing, transportation, storage, handling to recycling, a battery exposes the user to different risks. While some risks are always present because they are inherent to the battery (electrical risks or risks associated with handling heavy electrical vehicle batteries), other risks are linked to the battery components (chemical risks, fire/explosion) and only occur in case of dysfunctions. The causes of these dysfunctions may be of internal origin (e.g. manufacturing defects) or due to a use that does not comply with the defined use by the manufacturer (e.g. shock or overload) (INRS 2021). **Lithium batteries are responsible for a 25% increase in fires in sorting centers** (US and UK data)[49]. In January 2023 in France (Grand-Couronne), a fire broke out in a warehouse storing automobile parts, including several thousand vehicle batteries and lithium battery cells located in the commune of Grand-Couronne in France[50]. In February 2024 in Aveyron, another warehouse of an industrial site authorised to recycle and store batteries was confronted to a fire destructing 900 t of Li batteries[51].

Main occupational risks are summarised in Table 27.

Table 27: Main occupational risks[20]

Risks and/or health consequences	Sources
Electrical risk • Electrification, even electrocution (internal burns, muscle contractions, heart rhythm disturbances, death...) • Flash arc (external burns, splashing of molten metal, eye and ear damage...)	• Direct or indirect contact with battery terminals or connectors • Short-circuit • Arcing during battery disconnection operations
Chemical risk • Presence of hazardous electrolyte and electrodes containing toxic metal oxides (consequences depending on composition: skin burns, adverse effects	• Battery thermal runaway due to malfunction: electrolyte leaks and releases of hazardous compounds • Unsuitable charger or misuse • Release of toxic dust during battery

49 https://www.inrs.fr/metiers/energie/utilisation-batteries-lithium/utilisation-batteries-lithium-risques.html (accessed on 26/02/2024)

50 https://www.seine-maritime.gouv.fr/contenu/telechargement/57649/405239/file/20230704%20rapport%20cellule%20post%20accident%20HIGHWAY.pdf

51 https://www.lemonde.fr/societe/article/2024/02/18/en-aveyron-un-incendie-dans-un-entrepot-de-stockage-detruit-900-tonnes-de-batteries-au-lithium_6217180_3224.html (accessed on 26/02/2024)

on some organs in case of ingestion, inhalation or skin contact, serious eye damage, skin allergy, etc.). • Discharge or off-gassing of hazardous compounds in case of misuse (exposure through inhalation and skin contact)	recycling processes
Fire/explosion • Formation of explosive atmospheres • Creation of an ignition source, outbreak of fire (heating, flames, short-circuit, etc.) • Projection of ignited material (electrodes, electrolyte)	• Thermal runaway of the battery following a malfunction: gas release • Unsuitable charger • Use of combustible or flammable electrolytes and electrodes (including lithium and its alloys)

Due to these risks, Li batteries require both specific storage conditions and labeling imposed by Dangerous Goods Regulations[52]. If this regulatory labeling cannot be affixed directly to the item, bulk sales should not be possible.

Besides, it is to be highlighted that this **fire/explosion risk may lead to environmental risks**. For instance, following the accident in Grand-Couronne, measures were taken, in particular management of fire-fighting water and waste, waste disposal and environmental measurements**. Lithium was detected in fire-fighting water with a very high concentration > 14000 µg/L**. Initial analysis of the treatment of rainwater collected on site showed difficulties in treating lithium. Measured lithium concentrations in groundwaters showed a high degree of spatial variability and a contamination of the aquifer: 22.6 µg/L; 13.1 µg/L; 11900 µg/L; 129 µg/l; 2,460 µg/L. Measurements in air showed an increase in lithium levels one week after the fire in comparison with weekly measurements performed before the accident.

6.4 6.4 Risks arising from the extraction of lithium contained in geothermal fluids

eMSCA's report does not aim at assessing the risks. This has been covered in a recent report from Ineris (Ineris 2024). Besides the unspecific risks that also concern other activities such as the exploitation of hydrocarbons or underground storage (loss of confinement, accidental releases or ignition of combustibles), Ineris reported potential risks linked to the extraction process of lithium:

- the risk of induced seismic tremors, loss of confinement, bursting related to any process under pressure;
- the risk of burn for the workers due to the temperature of the brine;
- the risk of toxicity due to the exposure to LiCl or due to the exsolution of dissolved gas (CO2, H2S);
- the risk of fire in case of electrochemical separations;
- the risk of environmental pollution in the event of release into the air, soil or water.

[52] European agreement concerning the International Carriage of Dangerous Goods by Road

6.5 Environmental risk assessments

Grey literature on overall environmental risks related to the impact of lithium mining (deterioration, disturbance, destruction of species habitats linked to the development of infrastructure…) was identified but was not considered in this report as it does not refer to toxicological effects.

A comparison of the ecotoxicological values observed for aquatic species in available studies with environmental exposure data shows that lithium environmental levels may exceed hazard levels. As a result, risks may exist for aquatic species. This is confirmed by the report from Ineris (Ineris 2020) indicating that, for water compartment in Metropolitan France, lithium is considered as a very critical substance with concentrations exceeding the minimal provisional PNEC (4.2 µg/L) in 50% of the stations by 5.7 fold.

7 Uncertainties

First of all, other salts of lithium than LiCl, LiOH and Li2CO3 exist and were not considered in the report. Nevertheless, it is to be noted that human toxicity seems to be triggered by Li ion and not the counterion. Depending on the bioavailability, some of the effects observed might apply to other salts.

Regarding the environment, uncertainties remain how the counterion is involved in the observed toxicities (cf. section 4.2).

7.1 Hazard information

7.1.1 Human health hazard at environmental doses

As shown in section 4.1.7, the literature review regarding human health hazard at environmental doses provided some epidemiological studies showing an association between Li exposure and thyroid outcomes, autism spectrum disorders, male and female reproduction, growth and metabolism parameters, and hypertension. However, they were isolated studies. Animal data confirm the observed effects on thyroid and reproduction. Nevertheless, for the other effects identified, no animal data exist, and in particular at low lithium doses.More studies are needed to confirm these results.

Although the literature search strategy aimed at identifying any kinds of human health hazard at environmental doses, it is not excluded that lithium causes other specific effects than the ones that were identified.

7.1.2 Environmental hazard

As shown in section 4.2, concentrations in the order of ≥ 1 mg/L (representative of specific environmental situations or resulting from anthropic activities) were more frequently tested than lower concentrations (µg/L). Very few studies investigated the ED effects of Li on environmental species and the one available were carried out at high doses (2.5 mg/L Li).

7.1.3 Exposure assessment and risk characterisation

As described in section 5.2.1, while it is acknowledged that mining of ores results in Li contamination of the surrounding environment, this contamination appears not to be sufficiently quantified. Only one study determining lithium concentrations in man-made waters of mining facilities of Li deposits in Russia was identified (Abramova et al. 2022). Abnormally high Li concentrations were noted in this study, with maximum values of 3877 µg/L in the neutral quarry waters of the Zavitinskoye and 3740 and 869 µg/L in the acidic sub-basement waters of the Orlovskoye and Etykinskoye deposits, respectively.

No study investigating occupational exposure to lithium during mining was identified from the literature review. To overcome this lack of information, it was envisaged to compare environmental concentrations around Li deposits, that could approximate occupational exposure due to mining activities, with therapeutic doses to anticipate certain adverse effects. However, adequate information to apply this methodology were missing.

Regarding other anthropic activities, few data were available. Moore et al. 1995 reported that **occupational exposures take place mainly through the inhalation of airbone lithium compounds and the ingestion of inhaled particles that are transported from the airways and swallowed**. The authors cited a 1981 NIOSH study of occupational lithium exposure reporting that workers in a **lithium processing plant experienced estimated daily doses of 0.00016 to 0.82 mg Li/kg/d**, with a geometric mean dose of 0.025 mg Li/kg/d. eMSCA highlights that this latter value is only 2 order of magnitude of the higher bound of the therapeutic lithium doses range for the treatment of bipolar disorder (1.2-2.4 mg Li/kg/d).

Regarding risk characterisation, the registrants did not identify risks for workers, consumers and the environment, but the DNEL and PNEC upon which the risk assessments were based were questionable (sections 4.4.1.1, 4.4.2.1).

8 Recommendations

- **Need for revising CSRs**

The battery use has only been included in the Li_2CO_3 CSR, but not in the LiOH and LiCl CSR. Accordingly, an update version of the LiCl and LiOH CSR must be integrated on IUCLID in order to fulfil the article 22 of REACH that stipulates that *"a registrant shall be responsible on his own initiative for updating his registration without undue delay with relevant new information".*

No risk assessment specifically addressing the risk regarding the reprotoxic properties of lithium for workers and general population induced by the extraction of lithium contained in geothermal fluids, has been identified. The same observation applies to risk assessment regarding environmental hazards. These should be included in the CSR under REACH regulation.

For human health hazard assessment, considering the remarks regarding the DNELs derived by the registrants (section 4.4.1.1), the eMSCA recommends an update of the values for Li, LiOH and LiCl, and therefore an update of the associated risk assessment.

For environmental hazard assessment, the CSRs for lithium and the three salts should take into account all available ecotoxicological data from the literature. Thus, considering the remarks regarding the PNECs derived by the registrants (section 4.4.2.1), the eMSCA recommends an update of the values for Li and the three salts, and therefore an update of the associated risk assessment.

- **Need for monitoring data**

Measurements and monitoring data in water, soil and air are needed in order to monitor lithium contamination (Bhattacharyy et al. 2021, Bolan et al. 2021, Chow et al. 2022, Adeel et al. 2023). In particular, monitoring programs are required to evaluate the temporal and spatial trends of lithium in different ecosystems, in particular the natural and agricultural resources near major cities. The city runoffs and municipal wastewater facilities could have significant levels of lithium in the near future, and it is important to establish the baseline level to understand the trends and loading of lithium in the environment Chow et al. (2022).

The implantation of mining projects or battery manufacturing plants shoud be used as opportunities to gather data on how these activities impact the exposure of their environment to this alkali metal and its salts.

- **Need for establishing toxicological reference values**

Several publications highlighted the need for the derivation of toxicological reference values by health and environmental agencies regarding diet and drinking water in order to identity safe limits (Naeem et al. 2021, Adeel et al. 2023). In France in particular, a toxicological reference value related to the inhalation route will be derived.

- **Need for improving wastewater treatment plants for efficient Li elimination**

There is a need for improving wastewater treatment plants for efficient lithium elimination (Barbosa et al. 2023a).

- **Need for implementing water retention areas around battery storage areas**

Due to the increase in fire/explosion risk related to lithium batteries and the potential consequences in terms of environmental contamination, there is an urgent need for implementing water retention areas around battery storage areas.

9 Justification for the (no) need for regulatory risk management action at EU level

Considering:

- human health hazard assessment reported and/or performed in this report showing deleterious effects of lithium at therapeutic doses but also through environmental exposure at low doses (e.g. < 1 mg/L Li in drinking water) on different outcomes (autism spectrum disorders, male and female reproduction, growth and metabolism parameters and hypertension), although there is a need for confirming these results;

- environmental hazard assessment showing quite consistently adverse effects on diverse environmental species;

- exposure data showing that environmental risks cannot be excluded;

a need for further regulatory action is identified. Several RMOs have been assessed by eMSCA in order to cover all the concerns raised above.

9.1 Harmonised classification of substances under CLP regulation (EC) No 1272/2008

France MSCA had fulfilled a CLH dossier for 3 lithium salts: lithium carbonate, lithium chloride and lithium hydroxide and proposed a classification of Reprotoxic 1A (H360FD) which was approved by the RAC in 2021 with an added hazard class of Lact (H362), and confirmed recently in March 2024.
France MSCA also assessed, in the frame of this RMOA, the endocrine disruption potential of the 3 salts and concluded that lithium fulfils the criteria of the WHO definition of an endocrine disruptor based on its effects on thyroid. Thus, lithium is deemed to fulfil the CLP criteria for endocrine disruption. Classification under CLP can therefore be considered.

Based on the future classification Repr. 1A, many downstream regulations will be impacted and it should lead to major market modifications.

Considering the ecotoxicological effects observed on various environmental species with chronic effect values < 1 mg/L, lithium could fulfil the criteria for a harmonised classification for aquatic chronic toxicity. As a result, registrants should update their registration dossier.

9.2 Restriction

For a restriction proposal, an "unacceptable" risk has to be demonstrated. No risk assessment was performed by eMSCA in this report but a comparison of exposure data and hazard levels showed that exposures may exceed hazard levels for environmental species. The major use as componant of batteries will be dealt within the appropriate regulatory framework. For the others, the possibility to restrict them should be appreciated depending on various parameters among which:

- their contribution to the overall leachage to the environment and exposure of workers but also;

- their essentiality[53]. It should be phased out of consumer products and minimised and substituted as far as possible in all uses.

Nevertheless, the first recommendation from eMSCA is the update of DNEL and PNEC in CSRs to provide demonstration that risks are adequately controlled for all registered uses of lithium and its salts.

To strengthen the implementation of RMM according to the S-T-O-P[54] principle and protect workers, setting of OELs appears to be an appropriate way of regulation.

[53] Guiding criteria and principles for the essential use concept in EU legislation dealing with chemicals, C(2024) 1995 final

[54] Substitution – Technical protective measures – Organisational protective measures – Personal protective measures

Risks linked to the physico-chemical, toxicological and ecotoxicological properties of Lithium when used in batteries should be taken into account in the dedicated regulation 2023/1542 (see below).

9.3 SVHC identification /Authorization

As the classification Repr. 1A is not yet implemented in the CLP Annex VI, lithium salts are not yet eligible for SVHC identification. As soon as the classification will be included into an ATP, this will lead to employees obligations to consider and favour substitution. Depending on the outcomes of the efforts made by the users, SVHC identification could be envisaged. Inclusion in annex XIV would force applicants to submit authorisation's requests, depending on the status of lithium salts during their processes: this does not apply to applications where lithium and its salts are used as intermediates. This possibility could be explored further taking into account the diversity of uses.

9.4 Occupational Exposure Limit

Due to the future classification Repr. 1A, the setting of BOEL for lithium would be necessary to improve worker's safety. This shoud be used as opportunity to gather occupational exposure data that are currently lacking.

9.5 Emission limit for lithium in water

- **Drinking water directive (EU 2020/2184):**

In relation to its capacity to contaminate water resources, lithium might be addressed within the revised EU Drinking Water Directive (EU 2020/2184). This regulation allows to better know, control and limit the human exposure to lithium through drinking water.

However to be of regulatory relevance, substance specific regulations need to be implemented on a national level in all Member States.

- **Water Framework Directive (2000/60/EC):**

Considering the potential risks for the aquatic environment linked to lithium, lithium could be considered in the Water Framework Directive (WFD) for community action in the field of water policy (2000/60/EC) and/or its daughter directives. The integration of lithium in this directive would allow to:

- introduce monitoring of lithium in waters,
- limit the contamination of environments by setting a limit value that takes into account the risks for the aquatic environment,
- protect water resources for the production of water intended for human consumption,
- provide means of action to limit emissions at the local level.

However, for this regulation to be applicable to all Member States, lithium would have to be on the list of priority substances in the field of water (appendix X of the WFD).

For a substance to be included in the list of priority substances of the WFD, it is necessary that:
- it is taken into account as a candidate substance in the prioritization carried out by the JRC. For this, sufficient data must be available, covering almost all Member States;
- it is prioritized;
- it is retained by the Member States and the Commission.

Since the implementation of this directive, a vigilance list ("watch list") has been introduced, both in France and in Europe.

9.6 Battery regulation

As explained in section 3.3.2, the new regulation 2023/1542/EC concerning batteries and waste batteries was put into place in 2023. Besides, an ECHA report is expected in 2026 to help the EU Commission to build a report on substances of concern, namely substances having an adverse effect on human health or the environment or hampering recycling for safe and high quality secondary raw materials, present in batteries or used in their manufacture.

Lithium is for now, not included in the Annex I of this regulation that restrict the presence of some chemicals in batteries. This Annex could be amended by the EU Commission in case an unacceptable risk to human or the environment should arise from the use of a substance in the manufacture of batteries or from the presence of a substance in the batteries when they are placed on the market, or should be arising during their subsequent life cycle.

Therefore, France MSCA recommends the risk assessments (to human and/or the environment) due within relevant regulatory frameworks to take into account the new hazard characteristics established in the frame of this RMOA report, in order to achieve robust and clear results to be considered accordingly.

10 Conclusions and proposed actions

To be developed at a later stage (after public consultation).

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Annex 1: Assessment of the endocrine disruption concern for human health

The endpoints for lithium exposure investigated by ANSES included neurodevelopment and reproductive, renal and thyroid functions.

This work has been performed by the expert group on Endocrine disruptor (GT-PE) at Anses from 2019 to 2022 and is on its way to be published in Environment International.

12 Literature search

Literature search was conducted in the PubMed and Scopus databases, and divided by endpoint of interest.

Thyroid effects (Search date: up to August 2021 for human data. Up to September 2021 for *in vitro* data)

- Human data

A first bibliographic search was performed in 2019.

Titles, key words and abstracts were screened with the following key words:

- Scopus

((TITLE (lithium) OR TITLE (lithium AND carbonate)) AND TITLE-ABS-KEY (thyro*)) AND (LIMIT-TO (SUBJAREA , "MEDI") OR LIMIT-TO (SUBJAREA , "BIOC") OR LIMIT-TO (SUBJAREA , "NEUR") OR LIMIT-TO (SUBJAREA , "PHAR") OR LIMIT-TO (SUBJAREA , "PSYC") OR LIMIT-TO (SUBJAREA , "AGRI") OR LIMIT-TO (SUBJAREA , "IMMU") OR LIMIT-TO (SUBJAREA , "CHEM") OR LIMIT-TO (SUBJAREA , "HEAL"))

A second bibliographic search was performed later with more specific key words:

- Scopus

TITLE-ABS-KEY (lithium AND (thyroid OR thyroxine OR t4 OR triiodothyronine OR t3 OR "Thyroid-stimulating hormone" OR thyrotropin OR tsh OR hypothyroxinemia OR "thyroid hormones") AND ("clinical trial" OR "Randomized Controlled Trial")) AND (LIMIT-TO (DOCTYPE , "ar"))

- Pubmed

((lithium AND (Thyroid OR thyroxine OR T4 OR triiodothyronine OR t3 OR "Thyroid-stimulating hormone" OR thyrotropin OR TSH OR hypothyroxinemia OR "thyroid hormones")) – filters: RCT + clinical studies
((lithium AND (Thyroid OR thyroxine OR T4 OR triiodothyronine OR t3 OR "Thyroid-stimulating hormone" OR thyrotropin OR TSH OR hypothyroxinemia OR "thyroid hormones")) – filter: Meta-analysis

- *In vivo* data

Titles, key words and abstracts were screened with the following key words:

- Pubmed

((lithium AND (Thyroid OR thyroxine OR T4 OR triiodothyronine OR t3 OR "Thyroid-stimulating hormone" OR thyrotropin OR TSH OR hypothyroxinemia OR "thyroid hormones")) AND (animal[Filter])) NOT (review[Publication Type])

- Scopus

TITLE-ABS-KEY (lithium AND (thyroid OR thyroxine OR t4 OR triiodothyronine OR t3 OR "Thyroid-stimulating hormone" OR thyrotropin OR tsh OR hypothyroxinemia OR "thyroid hormones") AND (animal* OR rodent* OR rats OR mice)) AND (LIMIT-TO (DOCTYPE , "ar"))

After this search, additional studies have been identified confirming the findings displayed here: El-Mahalawayet al. (2019), Maiti et al. (2010), Jaffer et al. (1993) and Mohammed et al. (2020).

- *In vitro* data

Titles, key words and abstracts were screened with the following key words:

  - Pubmed

((lithium) AND (Thyroid OR thyroxine OR T4 OR triiodothyronine OR t3 OR "Thyroid-stimulating hormone" OR thyrotropin OR TSH OR hypothyroxinemia OR "thyroid hormones")) AND (vitro[Title/Abstract] OR cell*[Title/Abstract] OR "ex vivo"[Title/Abstract] OR culture[Title/Abstract])

  - Scopus

TITLE-ABS-KEY (lithium AND (thyroid OR thyroxine OR t4 OR triiodothyronine OR t3 OR "Thyroid-stimulating hormone" OR thyrotropin OR tsh OR hypothyroxinemia OR "thyroid hormones") AND ((vitro) OR (cell*) OR ("ex vivo") OR (culture)) AND (LIMIT-TO (DOCTYPE , "ar"))

<u>Neurodevelopmental effects (Search date: Up to November 2021)</u>

Titles, key words and abstracts were screened with the following key words:

  - Pubmed

Lithium and nervous system and (development OR prenatal OR postnatal OR infant OR child)

The table below summarised the inclusion/exclusion criteria used for human and animal studies.

Table 28: Literature search inclusion and exclusion criteria

Publication type	IN	Primary research studies
	OUT	Secondary studies (e.g. reviews, editorials, conference)
Language	IN	English
	OUT	Other languages
Study design	IN	Human experimental volunteer studies, Cohort studies, Cross-sectional studies, Case-control studies Experimental animal studies (For the nervous system, we focused on experimental studies addressing developmental exposure to lithium) *In vitro* studies
	OUT	Case studies *in silico* studies Studies without control group
Population	IN	Adult male and female volunteers, men and women, under lithium treatment All mammalian animals Euthyroid subjects
	OUT	Previously reported thyroid dysfunction
Exposure	IN	Mono exposure
	OUT	Mixtures, Co-exposure Doses/concentrations > 12 mEq/kg/d
Outcome	IN	Human/animals health endpoints
	OUT	Protective/positive effects Li used for aversive test Not addressing specifically adverse effects

13 Human Health hazard assessment

13.1 Subchronic and chronic toxicity linked to potential ED adverse effect

13.1.1 Thyroid effect

- **Human information**

Numerous studies investigated the effect of lithium on thyroid function. Most of them were performed on patients under lithium treatment, i.e. with an exposure of participants corresponding to therapeutic dosages (mainly between 600 and 1200 mg/d).

Publications of interest were described in Table 29. Among these publications, seven studies fulfilled the research criteria (see above Table 28) and were used as key studies.

Table 29: Summary of human data

References	Study design (population (age), number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustement factors	Results	Study quality, risk of biais
Key studies (fulfilling inclusion criteria)						
Broberg K. (2011)	Cross sectional environmental study N = 202 women in four Andean villages in northern Argentina No control group (Exposure biomarker in conitnuous: can be considered as having a control group (compare the most and the less exposed participants)), no inclusion/exclusion criteria for study participation other than sex.	Lithium exposure assessed based on concentrations in spot urine	Thyroid dysfunction: samples Plasma fT4 and TSH	Selected elements (arsenic, boron, cesium, iodine), age, BMI and parity	Urine li concentration inversely associated with fT4 before and after adjustment [adjusted β for a 1,000-µg/L increase in urinary li concentration = 0.17; 95% (CI), −0.32 to −0.015; p = 0.032] and positively associated with TSH (adjusted β = 0.089; 95% CI, 0.024 to 0.15; p = 0.007).	3 of 202 women reported ongoing use of medication: 1 being treated for gastritis and 2 for high blood pressure.
Ozpoyraz N. (2002)	Cohort study N = 95. 49 in lithium group, Exclusion criteria: thyroid dysfunction before lithium treatment, use of a mood stabilizer other than li, treatment with thyroid hormone, antithyroid drugs, or glucocorticoids; current or prior substance or alcohol abuse or dependence and systemic or neurologic disorders. 46 in control group. Age- and sex matched patients in control group had newly diagnosed somatoform disorders, had not received any psychotropic drugs prior to	Mean serum lithium level on entry: 0.69 ± 0.13 mEq/L, with an average dose of 1016 ± 147 mg/d (range, 600–1200 mg/d) for the entire group.	Serum concentrations of fT3, fT4, and TSH used to classify patients as having clinical or subclinical hypothyroidism. The normal laboratory ranges were as follows: tT3, 85–185 ng/dL; fT3, 2.3–4.2 ng/dL; tT4, 4.5–12.5 µg/dL; fT4, 0.89–1.8 ng/dL; and TSH, 0.47–5.01 µIU/mL.	No relationship apparent between thyroid function and sex, concomitant psychotropic drug use, duration of li treatment, or family history of thyroid disorder. Li-treated smokers had a significantly higher fT3 level than their nonsmoking counterparts (3.0 ± 0.4 vs 2.5 ± 0.4 ng/dL, P<.0001). Patients older than 50 years of age had significantly lower	Among li-treated patients, 12% had clinical hypothyroidism and 2% subclinical hypothyroidism. No case of clinical or subclinical hypothyroidism in control group. Compared with control group values, fT3 and tT4 levels significantly decreased and TSH levels significantly increased in li group. Despite these differences, all thyroid values were within normal ranges for both groups	Relative small sample size No comparison with prevalence of hypothyroidism in global population High level of Li_2CO_3

References	Study design (population (age), number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustement factors	Results	Study quality, risk of biais
	study entry, and had no history of thyroid disorders or thyroid treatment.			fT4 levels compared with patients younger than 50		
Lombardi G. (1993)	Longitudinal study N = 12 (8 females, 4 males) followed for 12 months. Control group: 6 euthymic bipolar patients without li administration Normal thyroid function and absence of thyroid antibodies before beginning the therapy. No other medication interfering with the HPT axis function and/or with the hormone assays, administered during the li therapy in the patients.	900 mg li/d resulting in li serum levels of 0.6-0.9 mEq/l	Plasma T4, T3, fT4 and fT3, basal TSH or ΔTSH (TSH response to TRH, calculated by the net increase (maximum values reached minus basal values)). During the week preceding the start of the therapy, plasma T4, T3, fT4, fT3, TSH and Tg levels determined in all the patients and the TRH test performed. Thyroid follow-up repeated under lithium treatment on the 10th day and after the 1st, 3rd, 6th, 9th, and 12th month of therapy.	No adjustment factors	Average plasma T4, T3, fT4 and fT3 values remained within the normal range, substantially unchanged with respect to pretreatment values. Variable percent of patients showed higher basal TSH (8-41%) or ΔTSH (25-75%) than the upper limit of the normal range. In particular, mean TSH level increases significantly 10d after the beginning of the therapy ($p<0.05$) and remained significantly higher than pretreatment values throughout li treatment	Small sample size
Perrild H. (1984)	Longitudinal study n= 16 (8 men, 8 women) exposed for 4 weeks. All euthyroid with no history of thyroid disease. No other medication Study participants are their own control	300 mg of Li_2CO_3given twice a day Median serum lithium levels on day 14: 0.28 10^{-6} mEq/l (0.28 nmol/l (0.07-0.37 nmol/l)) in males and 0.29 10^{-6} mEq/l (0.29 nmol/l (0.07 — 0.47 nmol/l)) in females, (unchanged on day 28).	Hypothyroidism: serum levels of thyroid hormones (TSH, fT4 and fT3) determined before, after 2 and 4 weeks of treatment and 1 month after termination of lithium intake	No adjustment factors	Compared to values before treatment, significantly, TSH levels rise, and T4 and fT4 decrease in both male and female. The T3 and fT3 levels decrease only in male.	Small sample size. Not real control group Intra and inter individual TRH test variability

References	Study design (population (age), number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustement factors	Results	Study quality, risk of biais
Lambert C.G. (2016)	Retrospective cohort study N = 24574 Li group (monotherapy): 3629 Control groups: 8 other monotherapies Criteria for inclusion: one year of no prior hypothyroid diagnosis nor bipolar disorder (BD) drug treatment	Nine monotherapeutic exposures considered: lithium carbonate, aripiprazole, carbamazepine, lamotrigine, olanzapine, oxcarbazepine, quetiapine, risperidone, and valproate. They required that drug exposures had no gaps of 30 days or longer to increase the likelihood that prescriptions were not only filled, but also continuously taken by the patients	Hypothyroidism was captured as the observation of any subcategory of hypothyroidism or prescription of thyroid medication	Following covariates examined for their effect on the CIF: Treatment, Age at treatment, Sex, Patient visit days in the year preceding monotherapy, Whether thyroid testing was performed in the 14d preceding monotherapy, Nonparametric thyroid testing rank during Monotherapy, Pretreatment prescriptions and comorbidities	Modest (statistically significant) effect of li on hypothyroidism in BD compared to alternate therapies. Estimates on the four-year cumulative hypothyroidism incidence for li ranged from 1.06-fold higher than quetiapine up to 1.39-fold higher than oxcarbazepine	Retrospective study Control groups: patients under various treatments (always monotherapies)
Johnston AM & Eagles JM. (1999)	Retrospective study N = 718 patients on li treatment. No control group. Comparison with local population. 18 patients excluded because introduction of thyroxine treatment pre-date Li 5 other excluded due to missing information on temporality between Li and thyroxine treatment	The mean serum li level for the whole sample in 1995 was 0.64 mEq/L (s.d. = 0.22), with 73% having levels between 0.4 and 0.8, 18% levels above 0.8, and 9% below 0.4 mEq/L.	Hypothyroidism: TSH above 3.3 mU/l, Total T4 below 70 mmol/l and doctor's judgement that thyroxine treatment necessary	No adjustment factors	72 patients (60 women, 12 men) developed clinical hypothyroidism. Prevalence: 10.4% (women 14% vs. men 4.5%). Overall prevalence in the area: 3.2% in women, 0.66% in men	Potential co-treatment not precised No control group but authors compared results with prevalence in local population
Kusalic M. & Engelsmann F. (1999)	Prospective cohort study n = 101 patients attending the Affective Disorders	Li therapy for 1 to 32 years.	Hypothyroidism: TSH value of above 4	Independent	Hypothyroidism developed in 40 patients under li (40%) (Among	Potential co-treatment not precised/evaluated.

References	Study design (population (age), number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustement factors	Results	Study quality, risk of biais
	Clinic at St. Mary's Hospital, Montreal, Que., given a diagnosis of bipolar disorder (28 men, 73 women) in li group, receiving maintenance li therapy for 1 to 32 years. 8 patients who were hypothyroid at baseline excluded. 82 in control group (no psychiatric or endocrinological diagnoses) matched for age and sex (30 men, 52 women)	Serum lithium levels analyzed at 3-month intervals and maintained at 0.75 to 0.85 mEql/L	mU/L as the criterion for grade II hypothyroidism	variables entered into the regression equation: sex, current age, age at the beginning of li therapy, duration of li therapy, elevated calcium level and family history of thyroid illness.	which 9 of 26 women from the older patient group and 15 of 47 women from the younger patient group). Number not provided for control group. Women in control group had significantly lower TSH values compared with women in li-treated group (p = 0.003). Men in control group had significantly lower TSH values compared with men in li-treated group (p = 0.0001).	Prevalence in control group not given by authors. The repeated multivariate analyses did not identify significant predictors of the elevated TSH level.
Weight of evidence studies						
Studies with no control group						
Bocchetta A. (2007)	Prospective cohort study (15 years) N = 150 (45 subjects completed 15-yr follow-up) No control group	Lithium serum levels within the desired limits of 0.5-1.0 mEq/l.	Hypothyroidism: cases requiring hormone replacement with levothyroxine	No adjustment factors	Annual rates of newly developed hypothyroidism: 1.5%. Non-significant increased risk in women (2.1% annual rate, 0.3% in men; RR: 6.2; 95% CI: 0.8-47.0).	No control group. Medication other than lithium prescribed according to the patient's mental state. Previous thyroid dysfunction not precised
Caykoylu A. (2002)	Cross-sectional study N = 42 patients No control group Any patient who had had thyroid dysfunction before lithium therapy excluded Any patient who had taken psychotropic agents, in addition to lithium, for	Lithium maintenance therapy ranged from 4 to 156 months, and the lithium dosage was between 600 mg/d and 1200 mg/d.	Based on serum concentrations of T4, T3 and TSH, patients classified as having subclinical hypothyroidism, subclinical hyperthyroidism, hypothyroidism,	No adjustment factors	3 patients (all males), i.e. 7.1%, have subclinical hypothyroidism, 3 (all females) subclinical hyperthyroidism and 1 hyperthyroidism No case of clinical hypothyroidism	Cross sectional study. No control group. Small sample size. Only one measurement

References	Study design (population (age), number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustement factors	Results	Study quality, risk of biais
	more than 1 week excluded. Other exclusion criteria: history of receipt of thyroid hormone, anti-thyroid drugs or glucocorticoid therapy		hyperthyroidism or euthyroid			
Studies with other limitations						
Shine B. (2015)	Retrospective cohort study n = 4678 patients and 689 228 controls Inclusion criteria: all patients in the laboratory information system aged at least 18 years and with at least 2 measurements of serum creatinine, thyrotropin, calcium, glycated haemoglobin (HbA1c), or lithium taken between Oct 1, 1985, and March 31, 2014. Control group: participants having no laboratory lithium measurement	Main exposure: lithium therapy, defined as more than 2 detectable serum lithium measurements	Hypothyroidism: thyrotropin activity >5.5 mU/L hyperthyroidism: thyrotropin activity <0.2 mU/L	Adjustment for age (cut-off 60 years), sex, and evidence of diabetes	After adjustment, presence of lithium significantly associated with hypothyroidism (HR = 2.31 (2.05–2.60); p<0.0001)	Primary psychiatric diagnosis, polypharmacy, medical comorbidities of patients unknown
Ozerdem A. (2014)	Retrospective, cross-sectional, naturalistic study n = 3,204 patients. Patients with BD (n = 469), unipolar depression (n = 615), other psychiatric diagnoses (n = 999), patients from endocrinology clinics (n = 645), and from dermatology clinics (n = 476)	Lithium-treated patients (n = 240). Serum lithium levels available for 203 patient. Mean serum lithium level: 0.71 ± 0.24 mEq/L. Authors divided the group into those with values above and below the 0.8 mEq/L cut-off value.	2 different normal ranges for TSH: a high normal range (0.4–5.0 μIU/mL) and a low normal range (0.3–3.0 μIU/mL)	No adjustment factors	Patients with BD had significantly higher TSH levels than the others (3.14 μIU/L vs 2.56 in endocrinology and 1.94 in dermatology; p <0.022). Patients with BD had higher TSH levels than patients with other psychiatric diseases. Among bipolar patients, 430 records available to	Potential previous thyroid dysfunction not precised. 93 patients (21.6%) on monotherapy, and 240 (55.8%) of the 430 patients taking lithium either alone (n = 42, 9.0%) or in combination with other psychotropic medications.

References	Study design (population (age), number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustement factors	Results	Study quality, risk of biais
					assess the possible impact of the treatment used (240 patients taking li treatment in mono- or multi-therapy). No correlation between li levels and TSH levels. Nevertheless, a significantly lower proportion of li-treated patients (n = 188, 78.3%) had serum TSH levels in the 0.4–5.0 µIU/mL normal range compared to non-li users (n = 168, 88.4%) (χ^2 = 8.228; df = 2; p = 0.016). 150 (62.5%) li-treated patients, as opposed to 140 (73.7%) non li-treated patients, had TSH values within the 0.3–3.0 µIU/mL normal range. Difference also significant (χ^2 = 6.046; df = 2; p = 0.049)	

Broberg et al. (2011) evaluated the potential effects of chronic exposure to lithium through drinking water and other environmental sources on the concentrations of free T4 and TSH in plasma via urinary lithium levels. Urine lithium concentration was inversely associated with free T4 before and after adjustment (on elements elevated in water of the same region, age, parity, and BMI) and positively associated with TSH.

Ozpoyraz et al. (2002) reported in 49 lithium-treated patients (salt not precised) a prevalence of 12% of clinical hypothyroidism and 2% of subclinical hypothyroidism, while there was no case of clinical or subclinical hypothyroidism in control group (46 patients).

Lombardi et al. (1993), in a longitudinal study, assessed the thyroid function during lithium (carbonate) therapy over a period of at least 12 months comparing basal and TRH-stimulated TSH in euthymic bipolar patients (12 [8 females, 4 males] in the treated group and 6 in the control group). During lithium therapy, plasma T4, T3, fT4 and fT3 remained within the normal range, substantially unchanged with respect to the pretreatment values. Anti-Tg and microsomal (anti-TPO) antibodies remained undetectable throughout the study. Ten patients out of the 12 had higher basal TSH levels at least in one of the check-ups performed during lithium therapy. Mean TSH level increased significantly 10 days after the beginning of the therapy ($p<0.05$) and remained significantly higher than the pretreatment values throughout lithium treatment. Similarly, a transitory TRH-stimulated TSH increase was found in 11 patients during lithium therapy.

Perrild et al. (1984) have followed a small number of young healthy subjects (n = 16; 8 men, 8 women) who received $Li_2CO_3$for 4 weeks at the dose of 300 mg twice a day, leading to a low blood concentration of Li around 0.28 10^{-6} mEq/L. The TSH levels rose, and T4 and fT4 decreased in both men and women. The T3 and fT3 levels decreased only in men.

Lambert et al. (2016) in a retrospective cohort study considered only adult patients with at least two diagnosis of bipolar illness in their medical history and at least one thyroid hormone evaluation. Among the 24 574 patients (65.3% of women, average age 39.5 years), 3629 were treated with Li_2CO_3, while other patients were treated by aripirazole, carbamazepine, lamotrigine, olanzapine, oxcarbazepine, quetiapine, risperidone or sodium valproate. During a mean follow-up of 269 days (1-3255), clinical hypothyroidism occurred in 7.5% of the sample (n=1850), but in 10.7% of patients treated with Li_2CO_3. Estimates on the four-year cumulative hypothyroidism incidence for lithium ranged from 1.06-fold higher than quetiapine up to 1.39-fold higher than oxcarbazepine.

Johnston and Eagles (1999) reported a prevalence of clinical hypothyroidism of 10.4% (72 patients) especially among women (14% vs 4.5%) in a retrospective study of 718 patients under lithium therapy (salt not precised). Compared to the overall prevalence in the area (number of patients receiving repeat prescription of thyroxine), which was 3.2% in women and 0.66% in men, the differences were significant in both sexes for all age groups (Women 20-39 years: RR = 11.3 [CI 5.6-22.7]; 49-59 years: RR = 5.65 [CI 3.7-8.7]; 60-79 years: RR = 1.98 [CI 1.24-3.13]; Men 40-59 years: RR = 9.5 [CI 4.38-20.7]; 60-79 years: RR = 4.25 [CI 1.69-10.6]). The calculated annual incidence of hypothyroidism in this study was 2.17% and 0.68% among women and men under lithium therapy respectively.

Kusalic et al. (1999) reported, in a cohort of 101 patients under lithium (salt not precised), 40 cases of hypothyroidism (39.6%), diagnosed on TSH level. Prevalence of hypothyroidism was not given by authors for the control group (82 patients without psychiatric or endocrinological diseases).

Limitations can be noted in some of these studies:

- Small sample size (Perrild et al., 1984; Lombardi et al., 1993) ;
- No "real" control group:
    - comparison of prevalence with local population (Johnston & Eagles, 1999)
    - comparison of prevalence with various other monotherapies (Lambert et al., 2016)
    - prevalence in control group not given by Kusalic et al. (1999);
    - pre-post study design (Perrild et al., 1984)
- Definition of hypothyroidism:
    - based only on TSH levels (Kusalic et al., 1999)
    - based on TSH and T3 and T4 levels (Johnston & Eagles, 1999; Ozpoyraz et al., 2002) compared to threshold values
    - based on comparison of the hormones levels to the levels before treatment (Perrild et al., 1984).

However, despite these differences and limitations, all these studies consistently showed that lithium therapy or lithium environmental exposure induced thyroid disturbance, and particularly a hypothyroid effect.

Other studies, not specifically meeting the research criteria, can nevertheless be used as supportive studies in a weight of evidence (WOE) approach, as they present interesting features as e.g.: a follow up of several years of subjects exposed to lithium, the assessment of the effect of bipolar disorder itself on thyroid dysfunction, or a robustness in the protocol and the selection criteria of patients.

Two of them had no control group:

Bocchetta et al. (2007) followed during up to 15 years patients under lithium treatment (n =150) who had undergone a cross-sectional evaluation of their thyroid function in 1989 when they were at different stage of lithium treatment. Compared to the beginning of the study, fourteen additional patients (13 women, 1 man) were prescribed levothyroxine replacement treatment during follow-up. The annual incidence rate of hypothyroidism was 1.5%. There was a non-significant increased risk in women (2.1% annual rate compared to 0.3% in men; relative risk: 6.2; 95% confidence interval: 0.8-47.0).

Çayköylü et al. (2002) reported, in a cross sectional study on 42 patients under lithium therapy (salt not precised), 3 cases (all males) of subclinical hypothyroidism (7.1%), no case of clinical hypothyroidism, 3 cases (all females) of subclinical hyperthyroidism (7.1%) and 1 clinical hyperthyroidism (2.4%). In addition, increased conversion of T4 to T3 was detected in 20 subjects.

Two other studies lack some information on previous thyroid function before lithium therapy:

Shine et al. (2015) investigated the prevalence of hypothyroidism in a retrospective cohort study including 4678 patients under lithium treatment (doses and formulations not available) and 689 228 controls (all other patients of the same sex and age group considered as not taking lithium therapy if they had no detectable lithium measurement or never tested for lithium concentrations). After adjustment for age, sex, and evidence of diabetes, presence of lithium was significantly associated with hypothyroidism (HR = 2.31 (2.05–2.60); $p<0.0001$), but not with development of hyperthyroidism (1.21 (0.96–1.55); $p=0.1010$).

Ozerderm et al. (2014) assessed the prevalence of thyroid abnormalities in a population of patients (either inpatient or outpatient) in psychiatry compared to a population in either endocrinology (considered to have a high prevalence of thyroid disease) or dermatology (considered to have a low prevalence of thyroid disease). They included a total of 3204 adult patients (2083 in psychiatry, 645 in endocrinology and 476 in dermatology). Patients with bipolar disorder had significantly higher TSH levels than the others (3.14 mIU/L vs 2.56 in endocrinology and 1.94 in dermatology; $p < 0.022$), even than patients with other psychiatric diseases. There was no correlation between lithium levels and TSH levels. Nevertheless, there were significantly fewer patients in the TSH ranges considered as normal among lithium users as among non-users.

In conclusion, these supportive studies confirm the observations reported with key studies, i.e. that lithium induced thyroid disorders, and more specifically hypothyroidism.

- **Experimental data**

The search of articles was performed using the keywords and exclusion and inclusion criteria presented in Table 28. This allowed a first identification of 371 experimental studies based on title and abstract. A first screening allowed to remove duplicates and articles that did not address specifically adverse effects of exposure to lithium. Then, based on the full text articles, 27 publications were analysed in details (Table 30).

Table 30: Summary of in vivo animal experimental data

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
Key studies (fulfilling inclusion criteria)							
Männistö et al., 1971	In vivo Non GL Single dose	Males Sprague-Dawley (SD) adult rats 5/group: 6 groups - Euthyroid ± Li (ET) - Hypothyroid (Carbimazole inhibition TPO) ± Li (HypoT) - Hyperthyroid T4 ± Li (HyperT)	LiCl: 4 mg/d/rat 0.5 mEq/kg/d Drinking water 2 weeks	Statistical model? Comparison + Li vs - Li	Organ weights: thyroid, anterior pituitary, adrenal	NS	Minor limitation: Uncertainty on statistical analysis Overall good
					Protein Bound Iodine (PBI) - cholesterol	NS	
					Plasma LI	0.53 ± 0.02 mEq/L	
					Radioactive iodine uptake: Whole neck (% of Li-euthyroid animals)	↘ over 72h post 131I	
					whole thyroid lobe at 4h post 131I	↘	
					/mg of tissue) at 4h post 131I	NS (huge variability)	
					Iodine Thyroid hormone incorporation		
					Inorganic iodine	↗	
					In MIT et DIT	NS	
					In T4 + T3	↘	
Kumar et al., 2019	In vivo Non GL Multiple doses	Female wistar rats 110-120 g age (~7 weeks of age) N=7/group	Li_2CO_3 0.27, 0.54, 0.81 mEq/kg/d Oral 9 days iodine uptake performed after 1 week exposure	One way ANOVA + post hoc	Radioiodine uptake Whole body or thyroid	↗at 4 and 24h at 3 doses (not dose dependent)	Good quality
					Thyroid iodine half life	↗ from 0.54 higher effect with higher dose	
					T3 T4 TSH	↘T3 T4 and ↗ TSH from 0.54.	

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
Petrov et al., 1985	In vivo non GL Dose–response (3 doses)	Male albino Rat (220g) N=11/groups: 2 subgroups in each dose group: Basal and TSH stimulated	LiCl 0.5-1-2 mEq/kg/d Oral 6 weeks	The only reported comparison is each group vs negative control Method? No pharmacodynamic analysis for iodine half-life determination	Basal and TSH-induced TSH-T3-T4-Calcitonin-Parathormone-Calcemia Iodine uptake Thyroid iodine half-life Thyroid histology (not quantitative)	**Basal** ↘ T3 and T4 at the highest dose only.↗ TSH, calcitonin, and Ca all doses. ↘ parathormone 2 last doses. ↘ **TSH-induced T3 and T4 secretion** NS ↗ Histological characteristic of thyroid hyperactivity at low dose Hypoactivity at higher dose	Some limitations Uncertainty on data analysis
Berens et al., 1970	Non GL	In vivo Male SD (about 100 g at start, ~ 6 weeks) Intact and hypophysectomized + TSH 2 diets: Low iodine (LID) Normal iodine (NID) Li exposure: acute or chronic N = 5-12 Only reported results: NID animals	Acute IP LiCl - 4 meq/kg ip 4h before sampling - 2 meq/kg 16 and 4h before sampling Chronic oral 3 meq/kg? (food at 30 meq/kg) Li_2CO_3 - NID 15 and 53 d	Method not specified	Serum LI Relative Thyroid weight Thyroid/serum Iodine ratio TSH Radioiodine uptake at 9, 21 and 36h post ^{131}I Organically bound iodine Iodine release rate from the thyroid Thyroid iodine contents Histology	Acute ip 2.19 (1 injection) 3.59 (2 doses) chronic oral 0.41 meq/L ↗ only after 25d exposure NS at 15 or 53 days NS NS at 25 and 101 days No effect: no impact on iodine organification ↘with 25 and 101 d exposure (↗ half-life) ↗ total and tissue concentration at 101 but not 53 days NS	Limitation: no description of statistical analysis Multiplicity of conditions for radioiodine uptake measurements

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
					Effects of Acute administration Thyroid/serum iodine ratio in intact animals Thyroid/serum iodine ratio in hypophysectomized + constant TSH Thyroid uptake single 4 mEq dose 12h before ^{131}I measure at 8h after ^{131}I Thyroid uptake 2 mEq at 9 and 0h before ^{131}I measure at 11h after 131I	Single dose 4h: no effect two doses at 4 and 16h ↘ Idem at 16h => effect no dependent upon TSH plasma concentration ↘ ↘	
Toplan et al., 2013	Non GL Two doses	Male wistar rats 160-200 g adults (n=8/group)	Li_2CO_3 0, 3, 6 mEq/kg bw Drinking water 30 days	Non parametric Mann-Whitney U test	Plasma LI T3 T4 TSH Oxidative stress systemic biomarkers	0.3 and 0.8 mEq/L ↘ high dose ↗ high dose ↗	OK
Frankenfeld et al., 2002	Non GL Single dose	Male Isogenic Dutch-Miranda rats 3 months old N = 3/group or > 5? Not clear	LiCl 1.25 mEq/kg bw Drinking water 8 weeks	TSH: logarithmic transformation Method: Analysis of variance randomised block experimental design + post hoc test on multiple comparisons	Weekly serum T4, T3 and -TSH End of the experiment serum TSH Pituitary TSH content Thyroid DOI activity (T3 formation) Pituitary deiodinases activity	No time-dependent effect of LI NS ↗ but NS ↗ NS on either DOI1 or DOI2.	Limitations: number of animals involved unclear Huge interindividual variability for DOI activities
Bolaris et al., 1995	Non GL Single dose	Males and females Wistar rats 3 to 6 months old N = 2 sex/group (total brain)	LiCl 5 mEq/kg bw Single administration 1h exposure	Student T test (males et females pooled) Pharmacodynamic analysis.	T4 concentrations **Single 1h exposure:** ^{-125}I-T3 binding on nuclear binding in vivo (4h post ^{125}I) liver and brain	NS ↗	

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
		N=10 to 18 for 1 and 7 days exposure	Single administration 1 d repeated 7 d		^{125}I-T3 cytosolic binding in vivo (4h liver brain) **1 and 7d exposure:** Cerebral nuclear binding	↗ ↗	
Caberlotto et al. 2013	LiCl effect on glycogen synthase kinase 3 (GSK3) in central nervous system.	Adult SD rats. n=7-8/group	IP injection 200 mg/kg (4.6 mEq/kg). Exposure 1.5 hours.	Data log transformed before analysis when necessary. ANOVA test followed by Dunnett's tests as post hoc test.	TSH measurement	No effect on TSH level. Effect on central nervous system.	Limitation: short treatment (1.5 hours)
Eravci et al. 2000	LiCl acute treatment with IP injection and subchronic treatment with food.	Adult male euthyroid SD rats. N= 6 rats/condition.	Li acute 1: 1 IP injection 7.5 mEq/kg yielded highly toxic serum concentration of Li (>2 mEq) and measure at 12h and 24h. Li acute 2: 1 IP injection 3 mEq/kg yielded 0.44 mEq serum concentration and measure at 12h. Li subchronic: One group with 0.15% Li diet (0.7 mEq in serum) and a second group with 0.3% Li	Mann-Whitney U test. Authors highlight that some significant results may reflect statistical artefacts	TH measurement (T3 and T4 in serum and tissues), serum TSH concentration and deiodinase activity (5'D-I, 5'D-II and 5D-III)	Li acute 1: ↗ of 5'D-II activity in the frontal cortex at 12h and 24h post injection (no effect in hypothalamus and pituitary). No effect on 5D-III activity. 25% ↘ of T4 in the cortical area at 12h and 24h (T3 ↘ with a lesser extent). Drastic ↘ of T4 and T3 in serum. No effect on TSH. Li acute 2: ↗ of 5'D-II activity in cortex, amygdala, hypothalamus and cerebellum. T4 ↘ in the 4 tissues. T3 ↗ except in hypothalamus. No effect on 5D-III activity. Li subchronic: in cortex, 0.15% Li ↘ and	OK

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
			diet (1.3 mEq in serum) for 2 weeks (14 d). Controls received saline injection.			0.3% Li ↗ 5'D-II activity, 5D-III activity ↘ with both concentrations, 0.15% Li ↗ and 0.3% Li ↘ T4, T3 not affected. Tissue specific effect of Li on deiodinase activity, tissue TH concentrations and serum TH levels.	
Morley et al., 1981	Effect of Li_2CO_3 supplemented diet on TRH in rats	Male SD rats (n = 33)	Serum Li 0.4 to 0.6 mEq/L. Chronic treatment 2 weeks.	Student's t test	Measurement of TRH, TSH, somatostatin, T3, T4, FT3 and FT4.	↗ hypothalamic TRH and ↘ T4 and FT4. No effect on T3 and FT3. Li_2CO_3 ↗ hypothalamic TRH.	OK
Pinto Vidal et al., 2021	LiCl effect on American bullfrog metamorphosis	The American bullfrog (Lithobates catesbeianus) tadpoles at the Gosner's stage 25 were collected in a frog farm	6d after the tadpoles arrived at the laboratory the exposure lasted for 21d (AMA like exposure) to 2.5 mg/L (0.06 mEq)	For the assessment of mortality: log-rank Test. For data concerning the external development (TWW, HLL, and SVL) authors used two-way ANOVA, considering the time (7 or 21 d) vs treatment (exposed or non-exposed) as variables. Authors tested for normality using the Kolmogorov Smirnov test and	Survival; Total Wet weight (TWW) ; Snout-Vent Length (SVL) ; normalized Hind Limb Length (HLL); Thyroid histology measurements	LiCl treatment induced mortality of 2/20 animals at day 19 and 20. TWW, SVL and HLL showed no significance. Histology: The total gland area in relation to the CT showed a 26% for Li treatment. The total follicular area of the gland after 21 d of exposure was 0.034 ± 0.015 mm2 for the CT, while the LI group (0.0153 ± 0.0150 mm2) presented a ↘ of 55% ($P < 0.05$) in relation to the CT. Number of follicles for the CT group: 19.41 ± 9.41 follicles; LI (10.13	Study well documented, low doses and similar to the OECD validated AMA (TG 231)

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
				homoscedasticity by the Brown-Forsythe test. The Bonferroni post hoc test. Fro thyroid histology Kruskal-Wallis test followed by Dunn's test used as a post hoc test		± 9.50 follicles) showed a 48% ↘ ($P < 0.05$)	
Law et al., 2017	In situ cellular level Raman spectroscopy of the thyroid. Mice exposed to Li_2CO_3 via drinking water for 2 weeks	10 C57 strain male mice (6 – 8 weeks old, 25 – 30 g)	Mice on average consumed 100 mL of water during the 2 weeks experiment. Each of the lithium treated subjects consumed on average 11.1 mg of Li_2CO_3 before they were sacrificed.	Statistical analysis of the spectra performed across the 2 subject groups (controls and li treated) using the two-tailed distribution and unpaired testing.	Thyroid dissected and snap frozen. Before performing Raman spectroscopy, thyroid epithelial cells and smooth muscle removed and the remaining tissue flattened into a thin layer tissue. Then Raman spectrometry was performed	The Raman spectra of the thyroid contained 7 main peaks at 563, 1087, 1265, 1301, 1440, 1656 and 1746 cm-1. The 3 significantly different peaks at 1440, 1656 and 1746 cm-1 related to significant components of tyrosine. Therefore, Raman spectroscopy data indicates ↗ tyrosine in the follicular lumen following li treatment → li induces loss of iodination of tyrosine residues in the thyroid gland. Li effect on NIS or TPO is suggested by the authors	Good quality
Singh et al., 2015	histomorphological changes in thyroid gland after administration of Li_2CO_3.	30 Adult Wistar rats (150-250 g)	40 mg/kg bw/d (1.08 mEq/kg bw/d) Li_2CO_3 by ip injection for 30 d.	One way ANOVA	Routine histological studies (follicular size)	Size of follicles ↗ and showed an abundance of colloid. Statistically significant ↘ in epithelial cell size.	Good quality low concentration

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
Pradhan et al., 2012	Role of li on circadian rhythms of other hormones and glucose profiles in light–dark (12L:12D), constant light (12L:12L) and constant dark (12D:12D) regimens in rats	Adult male albino rats (100 days old)	Controls were maintained. LiCl IP 2 mEq/kg bw/d 15 hours after the last injection, animals sacrificed.	two-way analysis of variance followed by post hoc t test	Effect of lithium on circadian rhythms of TSH, T3, T4, corticosterone, norepinephrine, epinephrine, insulin and blood glucose levels in L–D cycle	Li treatment significantly ↘ thyroid and serum T3 and T4 levels but ↗ pituitary and serum TSH profiles at all the 4 time points (06.00 h, 12.00 h, 18.00 h and 24.00 h) of a 24h period, and significantly sustained circadian rhythms without any change in their pattern in L–D cycle. Li treatment showed 2 peaks (06.00 and 18.00 h) of TSH both in the pituitary gland and blood serum	Good quality low concentration
Li et al., 2017	Non GL Single dose	Female SD rat 110-140 g n=10/group CT LI Also Zn and Zn+LI groups not considered	Li_2CO_3 3 mEq/kg bw Diet 1-2 and 4 months	One way anova post hoc comparison	Iodine uptake Iodine thyroid half-life Thyroid Na/K ATPase activity Oxidative stress thyroid biomarkers and lipid peroxidation Serum TT4 Serum TT3	↘ 2h post ^{131}I for 2 and 4 mths, ↘ 24h uptake at 1-2-4 mths ↗ all conditions ↗ after 4 months ↗ after 2 and/or 4 months ↘ after 2 and 4 mths ↗ at 1 mths ↘ at 2 and 4 mths	
Ahmed et al., 2021	Li_2CO_3 breast feeding model in rat	26 pregnant female SD rats. Pups: Equal number male and female. There was a total of 26×12=312 subjects in this study with n=18	Human therapeutic levels. Mother treated with 27.2 mEq/12h/50Kg resulting in	ANOVA and Post-hoc testing with Tukey's test.	Thyroid function: laser analytical spectroscopy, blood and immunohistochemical tests.	Treatments lead to gain weight, higher TSH level and reduced blood T4, elevated blood urea nitrogen (Slight effect on kidney), inhibition of iodine uptake,	Temporary mild change in kidney function

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
		(n=7 control, n=7 Li, and n=4 Li+iodine) dams rearing P18 pups, n=6 (n=3 control and n=3 Li) rearing P25 pups, and n=2 (n=1 control and n=1 Li) rearing P60 pups.	plasma Li level arround 0.5 mEq (= lower end of the therapeutic window for human). Solution fed by gavage through the nursing period P4 to P21. Pups around 0.075 mEq. Controls received gavage with water only.			inhibition of iodination of tyrosine, thyroglobuline cleavage and TH production. Reversion by idodine supplementation.	
Weight of evidence studies							
Hullin et al., 1970	Non GL Multiple dose and duration	Male wistar (150-260 g) N = 6 to 15 depends on the evaluated parameter	Li Lactate 1-3 mEq/kg bw: Acute single IP Acute 5-8 days IP Chronic in drinking water: ~1 mEq/kg bw/d	Rank Sum test (non parametric test)	**Acute single experiment (1.5-5 mEq/kg):** -Urinary ^{125}I excretion -Blood ^{125}I concentration -Thyroid ^{125}I uptake **Acute multiple injections (5-8d 1-3.5 mEq/kg):** -Thyroid ^{125}I uptake **Oral Chronic exposure 10-25 weeks** ^{125}I serum ^{125} I thyroid uptake	 ↘ ↗ ↗ ↗ ↘ ↘	Low level of reporting/ animal numbers, doses and duration of exposure Serious renal toxicity from 5 mEq/kg bw/d
Glumova et al., 1979	Non GL 2 doses	In vivo Albinos adult rats	LiCl	Not specified	Accumulation of iodine in the thyroid	↘ compared to control for the highest dose	Low level of reporting:

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
		Males and females N= 8-10	0, 0,5 or 1 mEq/kg bw 3 weeks		gland (time course of radioactivity of the thyroid post ^{131}I over 72h) Plasma PBI at 72h post ^{131}I Serum TT3 – Serum TT4-FT4 Thyroid glucose-6-dehydrogenase activity Thyroid histology Blood LI concentration	only (similar disappearance rate but lower level in early time point) ↘ in highest dose => ↘ iodine incorporation no effect for lowest NS for any doses ↘ ↗ follicle size. Flat epithelium enlarged colloid – degenerative lesions From 0,05 to 0,65 mEq/l	considers NS effect as established modification Units of serum hormone and histologic parameter unclear
Downie et al., 1977	Non GL Single dose	Japanese quail (3 to 6 months old)	LiCl Male: 8.9 mEq/kg Female: 6,9 mEq/kg Once im	Not specified	Intrathyroidal ^{125}I incorporation at 1-6 and 8h after ^{125}I administration Plasma iodine levels	Females: ↘ in for all 3 periods Males: ↘ at 1 h and ↗ at 18 h ↗ in both sexes (at 18 h females- 6 and 18 h for males)	Low level of reporting no p value
Ljunggren et al., 1971	LiCl IP injection effect on T4 and thyroglobuline synthesis in rats	White male Wistar rats. n between 7 and 14.	IP injection 0.5 to 1.5 mL 0.3N LiCl (in therapeutic range) 3 times/d, 15 d. Dose of Li adjusted with regard to the concentration of Li in the serum (1.09 mEq/L).	Not available	Blood: Li, PBI, T4. Thyroid: histology, T4 and its precursors, thyroglobulin and its precursors.	Li treatment leads in the serum to ↘ of PBI and T4 (no effect on T3 levels). Slightly ↘ iodine uptake in the thyroid. No effect on biosynthesis of T4 or thyroglobulin. ↘ of tyrosine iodination. Histology normal. Short Li treatment ↘ TH content in serum.	Limitation on statistical analysis

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
Lazarus and Muston, 1978	LiCl treatment for 0, 2, 4 and 8 weeks. Measure iodine concentration in salivary gland where the iodine uptake process is thought to be identical to thyroid gland.	Male TO strain mice aged 16 weeks. At least n= 6/ conditions.	Acute: IP injection 0.1 mEq and stopped after 2 hours. Serum Li level reaches therapeutic range in man. Chronic: 12 to 28 mEq in drinking water leading to different groups with serum Li concentration 0.1-0.5 mEq 0.6-1 mEq, 1.1-2 mEq and > 2 mEq. Li treatment for 0, 2, 4 and 8 weeks.	Student's t-test	Content of $^{125}I^-$ in salivary gland	No effect on salivary gland weights. High Li level animals lose weight and died prematurely. Acute: Li injection leads to ↘ of iodine concentration in submandibular gland. Chronic: Li treatments lead to ↗ of iodine concentration in submandibular gland at 2 and 4 weeks. High concentration leads to ↘ of iodine in salivary gland at 8 weeks. Li treatments affect iodine uptake by salivary glands.	Limitation on statistical analysis
Bagchi et al. 1982	Chronic treatment of rats fed with an iodine deficient Li supplemented diet	Four week old female SD rats. N = 7-10.	Duration 16 weeks with low iodine Remington diet + Li_2CO_3 1.1 g/kg (serum Li concentration 0.52 mEp/L)	Student's unpaired t-test	Hypothalamic and pituitary function. Serum hormones measurements (T3, T4, TSH). Pituitary and thyroid weighed. TSH content of the pituitary determined	No effect on bw. Thyroid enlarged in both group but less in Li treated group. Pituitary weight ↘ in Li treated group. T3 and T4 below normal range for both group and not different between the 2 groups. Li treatment ↘ TSH response to iodine deficiency. No effect on TSH content in pituitary	Limitation on statistical analysis

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
						and TSH release (stimulation by TRH).	
Aydin et al., 1996	Effects of Li_2CO_3 treatment in drinking water on the morphology and the function of thyroid gland.	Male and female New Zealand albino rabbits n=10/group.	2mg/kg iodine. 0.544 mEq/kg/d Li_2CO_3 in drinking water for 45 days.	Student's t-test	Endpoints at 0, 7, 15, 30, 45 and 60 d. Serum antithyroid antibodies. T3, T4 and TSH concentration. Thyroid histology.	T3 and T4 concentration lower at 45 and 60 d. Antithyroid antibodies negative in all groups. Thyroid weight ↗. Thyroid has macrofollicular colloidal goitre, hyperplastic microfollicular goitre and follicular degeneration and fibrosis.	Limitation on statistical analysis
Takasugi et al., 1989	Effects of Li_2CO_3 treatment in drinking water on iodine uptake by thyroid gland.	ddY Mice n= 5 or 6/group	Li_2CO_3 0.01% (2.72 mEq/L) or 0.1% (27.2 mEq/L) in drinking water for 4 weeks. Treatment with 0.01% leads to 8 nEq/thyroid. Test co-treatment with PTU (0.5 mg/ml) or T4 (0.5 µg/d) in drinking water.	Student's t-test	Iodine concentration in thyroid. T3 and T4 concentration in serum.	↘ of bw with the highest Li concentration (toxic effects and refusal of drinking the solution with treatment). With the lowest Li concentration: ↘ of iodine uptake, no effect on serum T4, ↘ of serum T3.	Limitation on statistical analysis

Reference	Method/ guideline/deviation	Type of study, animals (n)	Dose level/ Exposure duration	Statistical analysis	Endpoints	Results	Study quality (Klimisch, other)
Singh et al., 1994	Biokinetics of 131-I in Rat Thyroid Following Lead and Lithium Supplementation	Female Porton rats 130-150 g	Li_2CO_3 administrated to rats in the powdered pelleted diet containing 1.1 g li/kg. Duration of exposure: 1, 2, 3, 4 mo	No mention of statistical assay	I-131 uptake / radioactivity in the neck area measured at intervals 2h 24h	after li treatment, overall significant ↘ in 2 and 24 h thyroidal 131-I uptake in the 4 treatment periods, and both 2 and 24 h uptake showed the maximum ↘ when li treatment given for 2 mo.	Limitation on statistical analysis
Baumgartner et al., 1994	Effects of lithium on thyroid hormone metabolism in rat frontal cortex	Adult SD rats (250g)	Li given via diet (3% LI in pellets) for 14 days Rats euthanatized at 3 time points (4am, 1pm, 8 pm)	Ad hoc statistics	5' DII deiodinase activity 5 DIII deiodinase activity	Rat cortex inhibition of D3 at all time points and increase of D2 activity at 8pm only	Good quality but no measurements of Li
Burrow et al., 1971	Patients and animal studies	SD female rats (250g) placed in a low iodine diet	SD rats received IP injections of 0.75 mmole of LiCl or NaCl in divided doses over varying time periods, terminated by the injection of 5 µCi of ^{131}-I	Statistics but not explained	Iodine discharge, T3, T4, TSH levels in blood after lithium treatment	Li concentration in pooled rat thyroid glands: 10.1 mEq/kg compared to a mean serum level of 1.9 ±0.6 mEq/L.	

Although the corpus of available publications included very old studies some of which not fulfilling quality requirements of actual standards for scientific publications, it is remarkable that the effect of Lithium on the thyroid function are quite consistent. Over the sixteen *in vivo* studies finally retained, all showed quite consistently an effect of Li (whether chloride, carbonate, hydroxide or lactate) on different markers of the thyroid function (see table 31).

Table 31: summary of the different thyroid-related effects examined in the different *in vivo* studies and the frequency and sense of results for each endpoint.

Endpoint	Total number of studies evaluating the endpoint	Number of studies showing a decrease with Li treatment	Number of studies showing an increase with Li treatment	Number of studies showing equivocal or no modification
« thyroid Iodine uptake »*	6	3	1	2
Iodine half life	3		3	
serum protein-bound iodine	1			1
Tyrosine iodination	2	1		1
Thyroid Histology*	2	1	1	1
T4 (tissular)	1	1		
T3 (tissular)	1		1	
T4 (circulating)	6	4		2
T3 (circulating)*	5	4	1	1
TRH	1		1	
TSH	4			
TSH feedback				
Deiodinase activity	1		1 In thyroid	1 In pituitary
T3 binding to Thyroid hormone receptor	1		1 brain and liver	

* Li effects are time-dependent and/or dose dependent in the same study

The nature of the effect varies between studies **but none of these studies concluded on an absence of effect on one or another thyroid parameters**. The effects on thyroid markers were expressed for a dose range of 0.06 to 8.9 mEq/kg bw/day via oral route and/or 0.4 to 1 mEq/l li serum concentrations when given. The most frequently described alteration *in vivo* is a modification of iodine uptake. In some instances, this was associated with decreased thyroid hormone (TH) and/or increased TSH systemic concentration (Mannisto et al., 1971; Kumar et al., 2019; Berens et al., 1970; Pradhan et al., 2012). An increase of thyroid iodine half-life was also consistently described. One study showed that the effect of Li on iodine uptake could be at least in part independent of feedback regulation of TSH secretion (study in hypophysectomized TSH-treated rats, Berens et al., 1970). These effects are regularly associated with modifications of the histopathological structure of the gland, when investigated. Particularly, increased thyroid weight, goitre, decrease in the number of follicles, increase in the size of follicles but decrease of the epithelial cells size, degenerative lesions, fibrosis were observed (Petrov et al., 1985; Pinto Vidal et al., 2021; Singh et al., 2015; Glumova et al., 1979; Aydin et al., 1996).

Interestingly, the **effect of Li on iodine uptake seems to be, at least in part, dependent upon the duration of exposure**. Early effects are rather characterized by an increased iodine uptake while in the longer term a decreased iodine uptake was most frequently reported (Table 8). This latter observation could be viewed as a compensatory mechanism aiming at protecting the thyroid gland from tissue iodine excess triggered by an initial increase in iodine uptake.
A relatively recent article studied, in mouse exposed for 2 weeks, the effect of lithium on iodine intake and TH synthesis using Raman spectrometry (Law et al. 2017). This innovative technique allowed authors to conclude that lithium induces a loss of iodination of tyrosine residues in the thyroid gland. This suggests that Li might have an effect on NIS and/or TPO activity. An effect on NIS is consistent with data showing a decrease of iodine capture.

Livingstone et al. (2006), in their review, propose a figure summarising the actions of lithium on thyroid cells (figure 7).

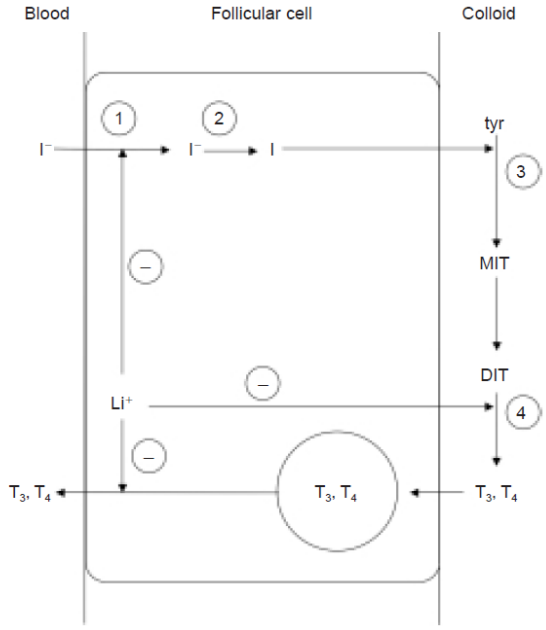


Figure 7: Mechanism of action of lithium on thyroid (Livingstone et al., 2006)

Li is frequently used as an inhibitor of protein G-associated signaling pathways PI3, AMPc. Consistently, several *in vitro* and *ex vivo* data showed that Li can inhibit TSH signaling implicating these pathways. This suggests that the inhibitory effect of Li on iodine uptake might proceed at least in part from a direct action of Li on follicular cell responsiveness to TSH thus leading to a decreased NIS expression. In some studies, Li increased iodine serum concentrations. The mechanisms underlying such effect and whether this systemic iodine excess contributes to early increase of iodine incorporation and/or increased intrathyroidal iodine content remain unclear. One study suggests that nephrotoxicity can occur with Li (Ahmed et al., 2021). This could in turn results in decreased renal clearance of iodine. This, however, was observed for doses regimen at 5 mEq/kg/d or more. Here, in Ahmed et al. manuscript (2021), Li concentration in rat pups was 0.075 mEq and Li treatment has only mild and temporary effects on kidney function (slight increase of blood urea). This work has the advantage of showing the consequences on newborns of the effect of a treatment of the mother through breast feeding. Compare to control animals, treatment induces higher TSH level, as well as inhibition of iodine uptake, of tyrosine iodination and thyroglobuline cleavage leading to the decrease of TH production and T4 serum concentration.

Interestingly, Li "antithyroid" effect was confirmed in a study from Pinto Vidal et al. (2021) on a modified Amphibian metamorphosis assay (TG 231) using American bullfrog. A dose of 0.06 mEq was used for 21 days. The total follicular number and area of the gland after 21 days presented a reduction of 55% ($P < 0.05$) in Li treated animals compared to the controls.
Li showed a tissue specific effect on deiodinase activity (Eravci et al., 2000) in thyroid-responsive tissues in rodents. Li acute treatment following IP injection at non-toxic dose (0.44 mEq) increased 5'D-II activity in the cortex, amygdala, hypothalamus and cerebellum, but did not affect 5'D-II activity in the pituitary. T4 levels were decreased in the four tissues showing increased 5'D-II activity, while T3 levels increased except in the hypothalamus. Variation of 5D-III activity was never measured with acute treatment. When sub-chronic treatments were performed, low dose led to reduced 5'D-II activity and increased level of T4 while higher dose increased 5'D-II activity and decreased level of T4. T3 and TSH were not affected. The absence of Li effect on TSH was also confirmed by Caberlotto et al. (2013). In contrast, Li treatment increased hypothalamic TRH associated with decreased T4 and fT4 serum levels and without any effect on T3 and fT3 serum levels (Morley et al., 1981).
Limitations can be noted in some of these studies:

- Lack of statistical analysis (Ljunggren et al., 1971)
- Inappropriate statistical analysis (Lazarus and Muston, 1978 ; Bagchi et al., 1982 ; Aydin et al., 1996 ; Takasugi et al., 1989)

However, despite these limitations, all these studies consistently showed that lithium exposure induced thyroid disturbance.

Unfortunately, most of the analysed studies on the thyroid effects were conducted in adult animals and none examined the effect on vulnerable developmental stages, such as gestation or critical periods such as foetal or postnatal development, except the recent study from Ahmed et al. (2021) (with an exposure via lactation).

Li exerts proapoptotic and antimitogenic effects on tumoral thyroid cells at very high concentration (>10mM) not relevant for the sake of the present exercise. *In vitro* and *ex vivo*, Li is quite consistently associated with a proliferative effect on thyroid cells as well as to an inhibition of TSH-mediated activation of signaling pathways and functional endpoints.

13.1.2 Focus on the neurodevelopmental toxicity

Given the key role of thyroid hormones in neurodevelopmental processes, we focused at studies of the neural effects of developmental exposure to lithium in humans and animals. In the framework of this dossier, a new bibliographic search focusing specifically on neurodevelopmental effects of lithium was performed.

The search of articles was performed using the keywords and exclusion and inclusion criteria presented in Table 28. This allowed a first identification of 9 experimental studies on the basis of title and abstract (Abu-Taweel et al., 2012; Dixit et al., 1988; Li et al., 2021; Messiha et al., 1988; Mohammed et al., 2020; Rabin et al., 2000; Rastogi and Singhal, 1977; Wajda et al., 1983; Youngs et al., 2006) plus an additional article gathered using keywords on the thyroid system (Mohammed et al., 2021). A detailed analysis of these 10 articles led to the inclusion of 5 articles (Abu-Taweel et al., 2012; Mohammed et al., 2020, 2021; Rastogi and Singhal, 1977; Youngs et al., 2006) addressing neurodevelopmental effects of exposure to lithium. Appart from the publication of Abu-Taweel (2012), none of the other publications was presented in the CLH report. They are developed here because this is the adverse effect of interest within the evaluation of the endocrine properties of Li. These selected studies are described in Table 32 below.

Table 32: Summary of *in vivo* animal experimental data on neurodevelopmental toxicity

Reference	Method/ guideline/deviation	Type of study, animals (n), sexe	Dose/period/duration	Endpoints /age of analyses	Statistical analysis	Results	Study quality (Klimisch, other)
Abu-Taweel, 2012.		Swiss-Webster mice 7 analyzed litters per treatment group: 21 pups analyzed (3 from each litter) per treatment group.	Drinking water: **15 and 30 mg/kg bw of lithium chloride = 0.35 and 0.7 mEq/kg**. Dams exposed from GD1 to PND15.	Offspring analyzed from PND1 to PND22. Body weight, aye opening and hair appearance. Behavioral tests: Righting reflex, cliff avoidance, rotating reflex, 21 pups belonging to seven litters from each treatment category were analyzed. Locomotor activity: assessed on PND22 males (10 per treatment group). Acetylcholinesterase (AChE) activity on PND22 males.	Described in the "Methods" section	**Body weight analyzed every 2-days**: reduced in the treated groups, with a more important effect in the group exposed to 30 mg/kg. **Eye opening and hair appearance**: Significantly delayed in the two treated groups. **Sensory motor reflexes**: Li had an inhibitory effect on all tested tested reflexes. **Locomotor activity of weaned males**: reduced rearing, locomotion duration and increased immobility in an open-field test for the two Li doses. **AChe activity in the brain of weaned males:** significant inhibition in a dose-dependent manner.	

Reference	Method/ guideline/deviation	Type of study, animals (n), sexe	Dose/period/duration	Endpoints /age of analyses	Statistical analysis	Results	Study quality (Klimisch, other)
Rastogi and Singhal, 1977		Sprague-Dawley rats No sex distinction between analyzed offspring 8 animals per group for spontaneous activity	Control group: Veh for 30 days T3-group : T3 sc (10 µg/100 g) from PND1-PND31 T3-LI-group: sc T3 (10 µg/100 g) from PND1-PND31 plus ip Li carbonate (60 mg/kg = 1.6 mq/kg) from PND25-PND31. Li-group: Veh from PND1-PND31 plus ip Li carbonate (60 mg/kg = 1.6 mq/kg) from PND25-PND31.	PND31: Brains collected for analyses 24h after the last injection of T3 and/or Li. Norepinephrine and dopamine levels measured. Activities of MAO, TH and COMT were also evaluated.		Hyperthyroid rats: Increased locomotor activity (X2) Increased DA levels in the HT (+50%), pons medulla (+45%), midbrain (+35%), striatum (+36%) and cerebral cortex (+23%). Unchanged NE levels. Hyperthyroid rats + Li: Reduced sponatneous activity to control levels Reduced DA levels in all these regions (to control levels) at the exception of the pons medulla. Li restored the activity of catechol-0- methyl transferase to normal limits. In control rats, lithium reduced DA levels in the striatum but had no effects on the other regions. Increased NE levels in the HT, midbrain, striatum and cerebral cortex.	

Reference	Method/ guideline/deviation	Type of study, animals (n), sexe	Dose/period/duration	Endpoints /age of analyses	Statistical analysis	Results	Study quality (Klimisch, other)
Mohammed et al., 2020.		Wistar rats: 36 dams mated with 18 males. Analyzed offspring: 6 per treatment group. No sex distinction between analyzed offspring	Pregnant dams orally administered **50 mg LiCl/kg bw** (1 ml LiCl/100 g rat) by gavage = **1.18 mEq/kg**. Exposure from GD1 to LD28.	Analyses at LD14, LD21 and LD28. Serum FT3, FT4 and TSH measured in dams and offspring. Serum cytokines (GH, TGF-b, TNF-a, INFg, IL1-b, leptin, resistin and adiponectin measured in dams and offspring. Analyses of monoamine (5-HT, NE and DA) levels in the cerebrum of offspring (Spectrofluorometry)	Described in the "Methods" section	**Body weight:** Reduced body weight in both Li-treated dams (-21%, -5%, -18%) and offspring (-25%, -30%, -37%) at LD14, LD21 and LD29, respectively. **Thyroid markers:** hypothyroid state in dams and neonates at LD14, 21 and 28 (reduced levels of FT3 and FT4 and increased levels of TSH). Growth and cytokine markers at PND14, 21 and 28: Altered levels of serum levels of GH (-55 to -72%), TNF-α and TGF-β (+210% and +280% at PND28), IL-1β (elevation at PND21), INF-γ (+120% and +263% at PND21/28), leptin (increased at PND21/28), adiponectin and resistin. **Brain monoamine levels in offspring**: LD14: Increased 5-HT (+13%), reduced NE and DA levels (-60% and -43%, respectively). LD21: Increased NE (+65%), reduced 5-HT and DA levels (-34% and -54%, respectively). LD28: Increased NE (+36%), reduced 5-HT and DA levels (-40% and -23%, respectively). **General observations**: Abortion in one case at GD17, neonatal mortality observed in 3 cases at birth.	

Reference	Method/ guideline/deviation	Type of study, animals (n), sexe	Dose/period/duration	Endpoints /age of analyses	Statistical analysis	Results	Study quality (Klimisch, other)
Mohammed et al., **2021**.		Wistar rats: 36 dams mated with 18 males. Number of analyzed offspring not mentioned in this study, but same protocol as in the former author's study (2020). No sex distinction between analyzed offspring	Pregnant dams orally administered **50 mg LiCl/kg b.wt.** (1 ml LiCl/100 g rat) by gavage = **1.18 mEq/kg**. Exposure from GD1 to LD28.	Analyses at LD14, LD21 and LD28. Thyroid histopathology. Gene expression of deiodinases (DII and III). Brain histology: eosin/heamatoxyllin staining. GSH, t-SH, CAT and SOD assays. Oxidative stress markers: H2O2, MDA, NO assays.	Described in the "Methods" section	**Cerebral cortex histology**: nuclear pyknosis, degeneration, gliosis, vacuolization at all tested ages. **Thyroid histopathology**: follicular dilatation and degeneration, hyperplasia, lumen obliteration and colloid vacuolation in the maternal and neonatal thyroid gland at LD14, LD21, LD28. Gene expression of desiodinases (DII and III): increased mRNA levels at PND 21 for DII and 14 for DIII. Cerebral prooxidant/antioxidant levels: Reduced levels of GSH (-29 to -52%), total thiol content (-33 to -36% at PND21 and PND28), catalase activity (-26% to -68% at all stages), and SOD activity (increased at all stages). Significant increase in lipid peroxidation marker (MDA), H2O2 (+55% at PND14, +130% at PND21, +552% at PND28), MDA (+40.03%, +21.57% and +36.54%, at PNDs 14, 21 and 28), NO increased at all stages (+59.53% at PND 21).	

Reference	Method/ guideline/deviation	Type of study, animals (n), sexe	Dose/period/duration	Endpoints /age of analyses	Statistical analysis	Results	Study quality (Klimisch, other)
Youngs et al., **2006**		Sprague Dawley rats, males n = 10 to 54 males per treatment group	Males fed with 0.15% Li_2CO_3chow (**40 mg/kg rat/d = 1.08 mEq Li/kg/d**): equivalent to therapeutic dose in children and adolescents: 0.4 to 1.2 mEq/L) or control chow (Co). Exposure from PND20 to PND41 (3 weeks exposure). Because of polydypsia/polyurea induced by Li diet, cages were equiped with bottles of 450 mM NaCl solution.	Li/Co group: analyses at PND36 while still on Li or control diet. Prior-Li/Co [2w] group: analyses 2 weeks after Li or Co treatment arrest. Prior-Li/Co [6w]): analyses 6 weeks after Li or Co treatment arrest.	Appropriate statistical analyses shown in the text and figure legends	**Li levels**: After 1 week on Li diet, Li serum levels at 1.2 mEq/L and decrease to 0.4 mEq/L at the end of 3-week exposure comparable to what is described in adolescents. Body weight gain: 50% lower weight gain for adult rats on Li chow, in a control experiment, compared to their matched controls (control rats, from 250 to 369 g; Li chow rats, from 250 to 312 g over 3 weeks; n 8 per group). Normal weight gain for adolescent Li rats on Li chow and after exposure arrest. **Increased innate anxiety but not learned fear:** *Anxiety-related behavior:* Li-treated rats spent less time in the interior zone (open-field) for all three groups. Also less time spent in the open arms (elevetaed plus maze) for the prior-Li[6w], a tendancy for the prior-Li[2w]. No potential effect on locomotor activity in the open-field, but no assessment of locomotor activity in a dedicated paradigm was performed. *Conditioned fear response*: normal response assessed in the fear-potentiated startle paradigm for the Li and prior-Li[2w] groups. *Spatial memory in the Morris water maze*: No difference between Li and prior-Li[2w] rats and their respective control groups in learning and remembering the location of the	

Reference	Method/ guideline/de viation	Type of study, animals (n), sexe	Dose/period/durati on	Endpoints /age of analyses	Statistical analysis	Results	Study quality (Klimisch, other)
						platform, nor in relearning a new position of the platform. **Gene expression microarray analysis in the amygdala in on-Li and prior-Li[2w] rats (Affymetrix arrays):** Li affected the expression of gene transcripts of the synapse and the cytoskeleton (synaptic adjustements).	

In the study of Abu-Taweel (2012), Swiss-Webster dams were exposed from GD1 to PND15 to LiCl in drinking water at 0.35 and 0.7 mEq/Kg of lithium. The analyses of their offspring from PND1 to PND22 showed reduced body weight with a more important effect at the higher dose. At the behavioral level, the two doses of lithium treatment reduced sensorimotor reflexes while assessed by righting reflex, cliff avoidance and rotating reflex tests. Eye opening and hair appearance were also delayed. This delay together with reduced body weight suggest that Li treatment possibly induced growth retardation. At PND22, locomotor activity assessed in the open-field test was reduced while immobility was increased.
Rastogi and Singhal (1977) investigated the effects of ip injection of Li_2CO_3 at 1.6 mEq/kg of lithium to Sprague Dawley rats from PND25 to PND31. These animals were previously administered T3 subcutaneously (10 μg/100 g) from PND1 to PND31 to induce hyperthyroidism. Interestingly, while T3 treatment increased locomotor activity, cotreatment with Li_2CO_3 during the last 6 days of T3 administration reduced it to control levels (Table 32).
These two studies indicate that lithium is able to reduce activity in both basal and stimulated (T3-treated) conditions. The reduced activity observed in rodents reminds of the floppy infant syndrome, which has been described in babies born of mothers treated during pregnancy with lithium (Kieviet et al., 2013). This syndrome consists of symptoms including hypotonia, hypothermia, respiratory depression, cyanosis, arrhythmias, decrease sucking reflex neonatal thyroid toxicity, nephrogenic diabetes insipidus, cardiovascular and renal dysfunctions. It was reported that the incidence of the floppy infant syndrome is low and the risk of neonatal complications increased at lithium doses higher than 0.64 mEq/l, but some studies show that symptoms can also occur at low maternal lithium levels (Kieviet et al., 2013).
In the literature search described above, no experimental studies using lithium at low environmental doses were found. A non-exhaustive search was thus conducted to see whether this aspect was analysed in zebrafish, one of the most studied aquatic models for environment. Nery et al. (2014) reported that exposure of zebrafish embryos to LiCl reduced locomotion at 500 and 5000 μM in a dose dependent manner at 10 day post fertilization (dpf). Accordingly, a decreased activity and velocity were also described in 6 dpf larvae, which were exposed at 2-dpf for 24 h to 150 μM LiCl (Pruvot et al., 2012).

The study of Youngs et al. (2006) evaluated the behavioral effects of oral exposure of Sprague Dawley rats to Li_2CO_3 at 1.08 mEq/Kg from PND20 to PND41 on anxiety state level using the open-field and elevated plus maze tests (Table 32). The conditioned fear response and spatial memory were also analysed. Reduced exploration of the anxiogenic areas behavior was reported in the open-field and elevated plus maze test for Li-treated rats 2 or 6 weeks after the exposure arrest. No effects were observed on conditioned fear response or spatial memory. Thus, lithium treatment selectively induced long-term effects on basal anxiety-related behaviour. These changes were associated with modifications in the expression of gene transcripts of the synapses and cytoskeleton in the amygdala, a key brain area underlying the modulation of the anxiety state level (Ressler, 2010).

Therefore, the experimental rodent studies together with the data presented on zebrafish suggest that developmental exposure to lithium may reduce activity, and that this effect may occur at low dose levels. Peripubertal exposure to lithium seems also to increase the anxiety-state level in rodents.

13.2 Analysis of the mode of action and the biological plausibility of a link between the adverse effects and endocrine activity

A/Human adverse effect data and/or Experimental animal (In vivo study (level 4 and 5))

- Human information

An effect of lithium on thyroid function is consistently described in human. Lithium being used as a treatment for BP for many years, numerous data (among which 11 considered of interest in the framework of this dossier), all coming from patients on therapy are available, except one study with an environmental exposure (Broberg et al., 2011). Protocols, investigated parameters and criteria vary substantially between studies. However, despite these differences and limitations noted for some studies, a thyroid disturbance, and particularly hypothyroidism, induced by lithium is consistently reported.

- Non-human information

Numerous studies investigating thyroid effects in experimental animals are available. Observations are very consistent with those in human. Although the corpus of available publications included very old studies, some of which not fulfilling quality requirements of actual standards for scientific publications, it is remarkable that results on the effect of lithium on the thyroid function are consistent. Indeed, all the studies selected based on quality criteria (defined in section 12) showed effects of lithium salts (whether chloride, carbonate, hydroxide or lactate) on different markers of the thyroid function:

- T4 circulating levels decrease in the majority of the studies;
- TSH circulating levels are also often significantly increased;
- When investigated, an impact on the deiodinase activities, which seems to be tissue-specific;
- An increase in thyroid size/weight and/or modification of the thyroid histological structure in several studies

Other effects of interest were observed in experimental animals and epidemiological studies and could potentially be linked to thyroid disruption.
First, effects were reported on neurodevelopment. Indeed, behavioral alterations, in particular reduced activity, were described in rodents after developmental exposure to lithium. Two developmental studies on zebrafish showing reduced locomotor activity in a dose dependent manner are also available. Therefore, altogether these studies suggest that developmental exposure to lithium reduces activity. However, these effects have been observed at high doses. Whether this effect may occur at low dose level of exposure still needs to be investigated.
The summary of product characteristics of lithium based medications reports reversible neonatal disorders, including neurological effects. Consequently, an appropriate monitoring of the newborns is recommended.
Effects on cardiovascular function, and specifically on cardiodevelopment were also reported. Particularly, a recent and robust cohort study highlighted a dose response relationship between a lithium exposure during the first trimester of pregnancy and cardiac malformation in newborns. Moreover, as for the neurodevelopmental effects, the summary of product characteristics of lithium-based medications reports cardiovascular adverse effects among which T-wave flattening (or possibly inversion), ventricular ectopics, congestive myopathy, bradycardia, ECG changes and conduction disturbances e.g. sinus node dysfunction.

B/ Endocrine mode of action of Li underlying thyroid disruption

The most frequently described alteration *in vivo* is a modification of iodine uptake. In some instances, this was associated to decreased thyroid hormone (TH) and/or increased TSH systemic concentration (Mannisto et al., 1971; Kumar et al., 2019; Berens et al., 1970; Pradhan et al., 2012). An increase of thyroid iodine half-life was also consistently described. One study showed that the effect of Li on iodine uptake could be at least in part independent of feedback regulation of TSH (study in hypophysectomized TSH-treated rats, Berens et al., 1970).
Interestingly, this effect of Li on iodine uptake seems to be dependent upon the duration of exposure. Early effects are rather characterized by an increased iodine uptake while in the longer term a decreased iodine uptake was most frequently reported. This latter observation could be viewed as a compensatory mechanism aiming at protecting the thyroid gland from tissue iodine excess triggered by an initial increase in iodine uptake.
A relatively recent publication studied the effect of lithium on iodine intake and TH synthesis in mouse exposed for 2 weeks using Raman spectrometry (Law et al. 2017). This innovative technique allowed showing that Lithium induces a loss of iodination of tyrosine residues in the thyroid gland. This suggests that Li could have an effect on NIS and/or TPO activity. An effect on NIS is consistent with data showing a decrease of iodine capture.

Lithium is a well-known inhibitor of protein G-associated signaling pathways typical of trans-membrane receptor such as TSH receptor among many other ones. The cAMP second messenger pathway in particular is an important target of Li. Not only, Li has been shown to decrease **TSH-induced** cAMP production in different thyroid *in vitro* and *ex vivo* models but it also seems to act downstream to cAMP in these models. **This MoA of Li is important as adenylate cyclase pathway is particularly well represented in thyroid cells and tissue.** In some instances, but not in all studies, **Li has been shown to be specifically associated with decrease response to TSH. Decrease of iodine uptake is quite consistently described.** In some studies, Li increased iodine serum concentrations. The mechanisms underlying such effect and whether this systemic iodine excess contributes to early increase of iodine incorporation and/or increased intrathyroidal iodine content remain unclear. The inhibitory effect of Li on iodine uptake might proceed at least in part from a direct action of Li on follicular cell responsiveness to TSH that might result in a decrease of NIS expression
In conclusion, the effect of Li on *in vitro* and *ex vivo* thyroid systems appears to proceed at least in part through a non-specific MIE, i.e. inhibition of protein G/adenylate cyclase/PK signalling pathways, which are common to several signal transduction pathways involving GPCRs and to several organs/cell types. However, data on these systems clearly show that this MIE can be specifically involved in lowering TSH-mediated events and as a consequence a tissue specificity exist. Some of these events, iodine uptake for example, have functional significance in terms of thyroid hormone production. This is consistent with the fact that most studies monitoring T4 and/or T3 systemic concentrations reported a decreased in both or either of these hormones. Modification of functional state of the thyroid gland are also illustrated by histopathological modifications of the thyroid structure. To conclude, Li might activate different MIEs leading to thyroid disruption.

B/ Mode(s) of action underlying neurodevelopmental effects

The behavioral alterations described in rodents following developmental exposure to lithium were associated with changes in several neurotransmitter pathways. Reduced acetylcholine esterase activity was measured in brain homogenates of

PND male mice treated with lithium (Abu-Taweel et al. 2012). In the study of Rastogi and Singhal (1977), the increased locomotor activity induced by T3 treatment in rat pups was associated with higher dopamine levels in several brain areas, and cotreatment with Li2CO3 during the last 6 days of postnatal T3 administration reduced to control levels dopamine in rats (Table 32). Lithium treatment reduced dopamine levels in the striatum of postnatal euthyroid control rats (Rastogi and Singhal, 1977). Whether lithium exerts its effects on locomotor activity and brain catecholamines directly or by targeting the thyroid system is not clear.

Levels of monoamines (serotonin, norepinephrine, dopamine) were also measured in the cerebrum of Wistar rats orally exposed to LiCl at 1.18 mEq/kg from GD1 to LD28 (Mohammed et al. 2020). In agreement with the study of Rastogi and Singhal (1977), altered monoamine levels were observed at all studied postnatal stages together with reduced dopamine. It was shown in a further study by the same authors that under similar experimental conditions, lithium treatment altered cerebral cortex histology and ratio of prooxidant/anti-oxidant balance (Mohammed et al., 2021).

Lithium has broad effects including inhibition of excitatory neurotransmitters (dopamine, glutamate) and stimulation of inhibitory ones (GABA). One of the key mechanisms thought to underly the therapeutic effects in bipolar disorders is related to the inhibition of dopamine neurotransmission (Malhi et al., 2013). Therefore, the reduction of dopamine levels at both basal and T3-induced conditions (Mohammed et al, 2020; Rastogi and Singhal, 1977) in particular in the striatum, one of the key brain areas modulating locomotor activity, is of interest since it could explain at least in part the observed reduced activity induced by lithium treatment.

Given the thyroid effects reported above for lithium and the crucial role of thyroid hormones in neurodevelopmental processes, it is possible that the two mode of action may be linked. However, there is no clear evidence for such a link as mentioned above.

Furthermore, it is important to note that in two studies (Abu Taweel., 2012; Mohammed et al. 2020) over the three reporting body weight of offspring (Table 32), reduced body weight was observed. This suggest that growth retardation issues may possibly also participate to the observed effects induced by lithium. It remains to be established whether the effects of lithium on locomotor activity and the underlying brain catecholaminergic pathways are direct (i.e. by acting on neural cells) or indirect (via thyroid hormone system disruption).

B/ Mode(s) of action underlying developmental cardiotoxic effects

Thyroid hormones modulate every component of the cardiovascular system necessary for normal cardiovascular development and function (Chattergoon et al., 2005).

Thyroid hormones are critical regulators of cardiac growth and development, both in foetal life and postnatally. They activate the transcription of genes involved in cardiac contractility and contribute to the regulation of hemodynamic changes associated with the rapid increase in body size. Thyroid hormones indeed stimulate a burst of cardiomyocyte (CM) proliferation in the murine heart in preadolescence; a response required to meet the massive increase in circulatory demand predicated by an almost quadrupling of body weight during a period of about 21 days from birth to adolescence. Given the postnatal cardiomyocyte mitogenic potential of T3, its ability to enhance cardiac function by promoting cardiomyocyte proliferation warrants further consideration. During postnatal cardiac development, TH mediates direct transcriptional activation of genes involved in cardiac contractility, regulates hemodynamic changes such as stroke volume, blood volume, heart rate and blood pressure that are associated with the rapid increase in body size during this period,

and it initiates a brief burst of subendocardial CM proliferation in preadolescence that involves activation of the IGF-1/IGF-1R/Akt pathway.
The effects of T3 on the heart are due to the transcriptional regulation of a number of contractile and calcium handling genes (Maillet et al., 2013). These effects have been most widely studied with respect to their role in postnatal heart development, and under the premise that CMs exit the cell cycle and become terminally differentiated soon after birth. It has been evidenced that the proliferative competence of CMs may be retained until well after the neonatal period, allowing murine CMs to undergo a proliferative burst during preadolescence in response to a T3 surge (Naqvi et al., 2014); that T3 can induce DNA synthesis in terminally differentiated adult CMs (Ledda-Columbano et al., 2006), and that remodeling post-myocardial infarction in mice is associated with local hypothyroidism of spared myocardium due to re-expression of D3, a thyroid-inactivating enzyme normally expressed only in the foetus (Janssen et al., 2013).

Thyroid hormones supplementation is critical for cardiomyocytes maturation from pluripotent stem cells in vitro. (Tampakakis et al., 2021; Ahmed et al., 2020; Huang et al., 2019; Yang et al., 2014; Parikh et al., 2017).
Klein et al. described that the relationship of thyroid hormonal abnormalities and cardiovascular disease goes well beyond the risk of atherosclerosis in association with hypothyroidism. Indeed, the two organ systems are intimately linked by their embryological anlage, and the ubiquitous effects of thyroid hormones on the major components of the entire circulatory system: the heart, the blood vessels and the blood as defined by the flow law. As previously described (Fazio et al., 2004), thyroid hormones cause a myriad of hemodynamic effects and all can be related directly or indirectly to the flow law. Thyroid function influences every structure of the heart and its specialized conducting system. Moreover, thyroid hormones, in addition to their direct effects on cardiovascular function also have indirect effects mediated through the autonomic nervous system, the renin-angiotensin-aldosterone system (RAAS), vascular compliance, vasoreactivity and renal function.

13.2.1 Conclusion of the biological plausibility between the adverse effect and the endocrine mode of action for human health

It is well established, based on case-control study where exposed groups were compared to control groups or in which symptoms were reversed when exposure to lithium was stopped, that exposure to lithium affects the regulation of the thyroid function leading to clinical hypothyroidism in humans.

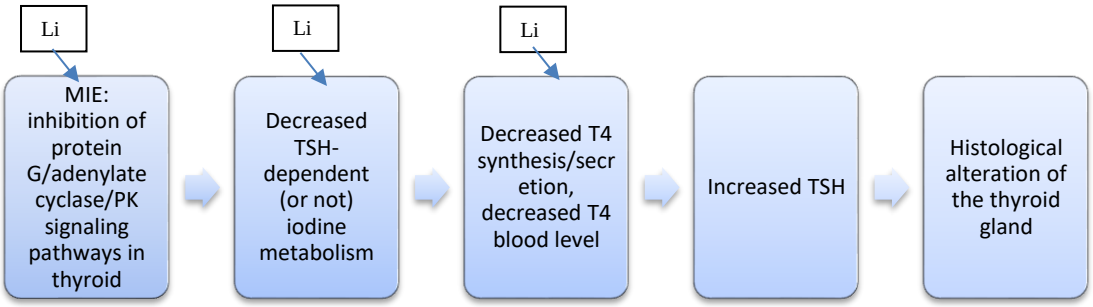
Findings are consistent in numerous experimental studies, with either acute or chronic exposures, using mainly oral route. These findings, in particular histopathological changes in the thyroid and decrease circulating TH concentrations are considered as adverse effects in themselves, in line with ECHA/EFSA recommendations (2018) on the interpretation of experimental data. Therefore, these findings are consistent to fulfil the WHO definition for EDCs.

Altogether, the effects observed in humans and in experimental animals are fully consistent with a MIE involving inhibition of protein G/adenylate cyclase/PK signaling pathways, leading to a decrease of iodine uptake as an endocrine mode of action (MoA). A causal effect relationship between such a thyroid MoA and the most convincing adverse effect noticed in *in vivo* studies, i.e. alteration of the thyroid gland anatomy and histological structure, is beyond doubt.

Regarding the neurodevelopmental effects described, that might be an additional adverse effect emphasising the ELoC of Li, uncertainty remains on its thyroid origin and the specificity of this MoA as discussed in section 13.1.2. Indeed, there is evidence for a reduction of dopamine levels in both basal and T3-induced conditions in particular in the striatum, one of the key brain areas modulating locomotor activity. This could explain at least in part the observed reduced activity induced by lithium treatment.

It is well established that neurodevelopmental effects could result from a thyroid dysfunction. Unfortunately, regarding experimental studies investigating thyroid effects of lithium, all studies but one (Ahmed et al., 2021) were conducted in adult animal and none examined the effect on vulnerable developmental stages, such as gestation or critical periods such as foetal or postnatal development. Although thyroid hormones are known to regulate dopamine levels, there is currently not enough evidence to dissociate between a potential endocrine mode of action targeting the thyroid and a non-endocrine MoA involving a direct effect on neurotransmitters in the observed neurobehavioral outcomes. Nevertheless, there is a high level of evidence for a link between thyroid disruption and neurodevelopmental alterations and existing AOP linking alteration of iodine trafficking through modulation NIS and/or TPO activities (AOP 134 and 42 respectively) and neurodevelopmental outcomes. Therefore, a thyroid-mediated neurodevelopmental adverse effect of Li cannot be ruled out even if it cannot currently be discriminated from a direct neurotoxic effect of lithium.

Finally, regarding the human relevance of plausible endocrine MoA and the biological link with the effect, it is stated in the ECHA/EFSA guidance (2018) that humans and rodents have to be considered equally sensitive to thyroid-disruption.



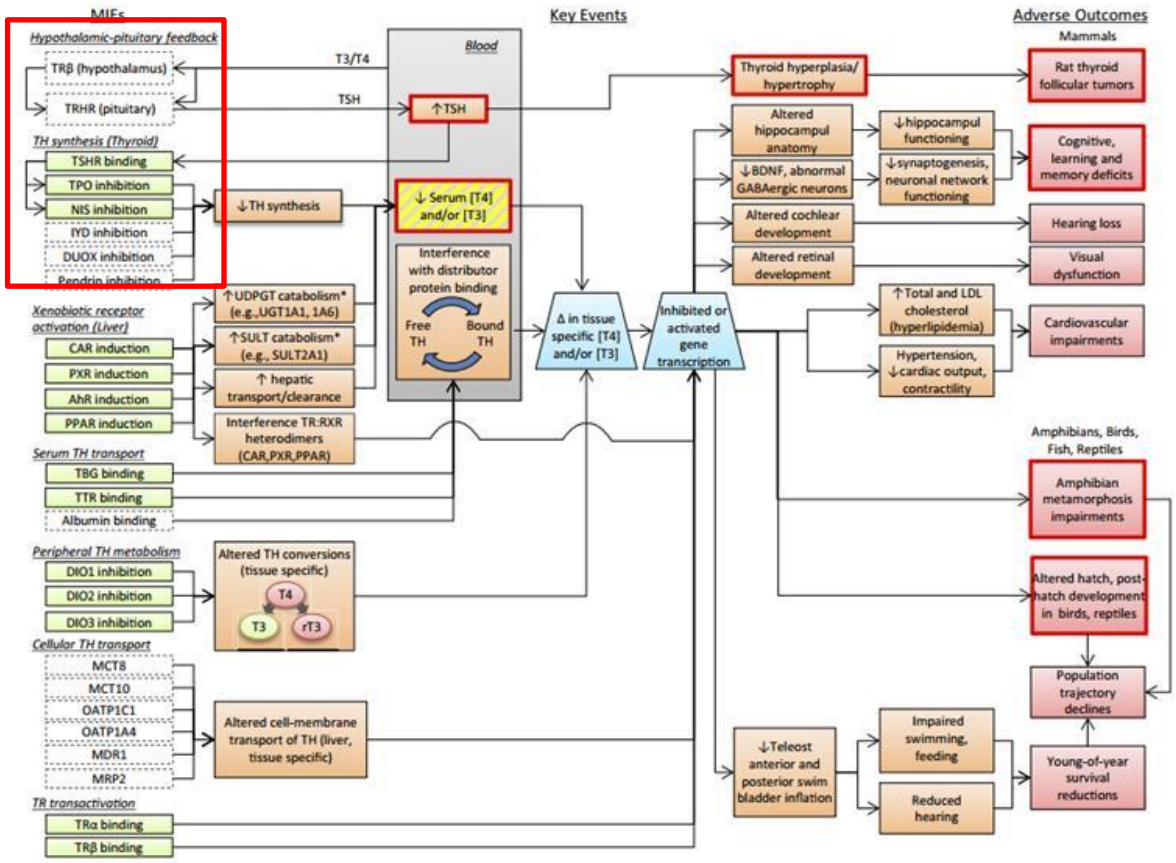


Figure 2. Adverse outcome pathway (AOP) network for chemically induced thyroid activity showing the integration of multiple individual AOPs under development and proposed. Biological linkages described may be informed by *in vitro*, *in vivo*, or computational data and may be causal, inferential, or putative, depending on the strength of the evidence. Boxes with thick, red borders represent *in vivo* end points that are targeted by U.S. EPA and OECD test guidelines. In the left-hand column, MIE boxes with solid borders (shaded green) represent current MIEs with *in vitro* high-throughput screening (HTS) assays that have demonstrated reliability and are available for use in thyroid activity screens, whereas those with dashed borders represent putative MIEs in the thyroid axis cur-

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Annex 2: Human health hazard information at environmental doses

15 Search methodology for the literature search

15.1 First literature search

Objective: identify potential human health effects from exposure to lithium at environmental doses
Bibliographic databases searched for relevant studies: PudMed and Scopus
Searches were run on 28 June 2023.
Search equations were as followed:

PubMed:

((lithium[Title/Abstract] OR "lithium carbonate"[Title/Abstract] OR "lithium chloride"[Title/Abstract] OR "lithium hydroxide"[Title/Abstract]) AND (human[Title/Abstract] OR population[Title/Abstract] OR women[Title/Abstract] OR men[Title/Abstract] OR pregnancy[Title/Abstract] OR child[Title/Abstract] OR infant[Title/Abstract] OR adolescent[Title/Abstract])) AND ("environmental exposure"[Title/Abstract] OR "drinking water"[Title/Abstract] OR "tap water"[Title/Abstract] OR "potable water"[Title/Abstract] OR "bottled water"[Title/Abstract] OR "water pollution"[Title/Abstract] OR food[Title/Abstract] OR diet[Title/Abstract] OR "water pollutant"[Title/Abstract] OR "environmental pollution"[Title/Abstract] OR "environmental pollutant"[Title/Abstract] OR "environmental contamination"[Title/Abstract] OR "environmental contaminant"[Title/Abstract])	257 results

Scopus:

TITLE-ABS-KEY(lithium OR "lithium carbonate" OR "lithium chloride" OR "lithium hydroxide") AND TITLE-ABS-KEY(human OR population OR women OR men OR pregnancy OR child OR infant OR adolescent) AND TITLE-ABS-KEY("environmental exposure" OR "drinking water" OR "tap water" OR "potable water" OR "bottled water" OR "water pollution" OR food OR diet OR "water pollutant" OR "environmental pollution" OR "environmental pollutant" OR "environmental contamination" OR "environmental contaminant")	3167 results

3424 scientific papers were retrieved and downloaded in the electronic reference management software Endnote and reference duplicates were removed (256). Afterwards, the remaining references (3168) were imported into the web-tool Rayyan to organise the review. These references were screened on title and abstract considering the following inclusion/exclusion criteria (Table 33).

Table 33: Literature search inclusion and exclusion criteria

Publication type	IN	Primary research studies, reviews
	OUT	Secondary studies (e.g. editorials, conference, commentary)
Language	IN	English, French
	OUT	Other languages
Study design	IN	Human experimental volunteer studies, cohort studies, cross-sectional studies, case-control studies Human health risk assessment
	OUT	Experimental animal studies In vitro studies in silico studies
Population	IN	Humans
	OUT	Humans under lithium treatment All mammalian animals

Exposure	IN	Mono exposure
	OUT	Mixtures, co-exposure, wrong drug
Outcome	In	All health endpoints (including protective/positive effects, studies not addressing specifically adverse effects)

The exposure data from the following publications were not detailed in this report as they do not provide additional interesting information than those already described:

- Presence in water

Abramova, V. A. 2022. "Rare Alkali Metals in the Waters of Lithium-containing Deposits in Eastern Transbaikalia." IOP Conference Series: Earth and Environmental Science.

Ahmed, G., M. A. Miah, H. M. Anawar, D. A. Chowdhury, and J. U. Ahmad. 2012. "Influence of multi-industrial activities on trace metal contamination: An approach towards surface water body in the vicinity of Dhaka Export Processing Zone (DEPZ)." *Environmental Monitoring and Assessment* 184 (7): 4181-4190. https://doi.org/10.1007/s10661-011-2254-9.

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Al-Thani, G., A. E. Ibrahim, M. Alomairi, B. I. Salman, M. M. Hegazy, A. Al-Harrasi, and S. El Deeb. 2023. "Toxic Elemental Impurities in Herbal Weight Loss Supplements; A Study Using ICP-OES Microwave-Assisted Digestion." *Toxics* 11 (3). https://doi.org/10.3390/toxics11030272.

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Baloch, S., T. G. Kazi, H. I. Afridi, J. A. Baig, F. N. Talpur, and M. B. Arain. 2017. "Correlation of lithium levels between drinking water obtained from different sources and scalp hair samples of adult male subjects." *Environmental Geochemistry and Health* 39 (5): 1191-1199. https://doi.org/10.1007/s10653-016-9886-1.

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15.2 Second literature search

Objective: identify additional literature focusing on adverse effects identified in good quality studies via the first literature search (autism, male reproduction and cardiovascular effects)
Bibliographic databases searched for relevant studies: PudMed and Scopus
Search equations were as followed:

	Lithium and autism	
Pubmed	(lithium[Title/Abstract] OR "lithium carbonate"[Title/Abstract]) AND (autism[Title/Abstract] OR "autistic disorder"[Title/Abstract])	92 results
Scopus	TITLE-ABS-KEY (lithium OR "lithium carbonate") AND TITLE-ABS-KEY (autism OR "autistic disorder")	706 results
	Lithium and male reproduction	
Pubmed	(lithium[Title/Abstract]) AND (reproducti*[Title/Abstract] OR "testosterone"[Title/Abstract] OR "fertility"[Title/Abstract] OR "infertility"[Title/Abstract] OR "sperm"[Title/Abstract]) Restriction: Human	137 results
Scopus	TITLE-ABS-KEY (lithium) AND TITLE-ABS-KEY (reproducti* OR testosterone OR fertility OR infertility OR sperm) Restriction: Human, female, male, humans, adult, pregnancy, adolescent	1067 results
	Lithium and cardiovascular effects	
Pubmed	(lithium[Title/Abstract]) AND ("blood pressure"[Title/Abstract] OR "hypertension"[Title/Abstract] OR "cardio*"[Title/Abstract] OR "cardia*"[Title/Abstract]) AND ("environmental exposure"[Title/Abstract] OR "drinking water"[Title/Abstract] OR "tap water"[Title/Abstract] OR "potable water"[Title/Abstract] OR "bottled water"[Title/Abstract] OR "water pollution"[Title/Abstract] OR food[Title/Abstract] OR diet[Title/Abstract] OR "water pollutant"[Title/Abstract] OR "environmental pollution"[Title/Abstract] OR "environmental pollutant"[Title/Abstract] OR "environmental contamination"[Title/Abstract] OR "environmental contaminant"[Title/Abstract])	104 results
Scopus	TITLE-ABS-KEY(lithium) AND TITLE-ABS-KEY("blood pressure" OR hypertension OR cardio* OR cardia*) AND TITLE-ABS-KEY("environmental exposure" OR "drinking water" OR "tap water" OR "potable water" OR "bottled water" OR "water pollution" OR food OR diet OR "water pollutant" OR "environmental pollution" OR "environmental pollutant" OR "environmental contamination" OR "environmental contaminant")	651 results

Regarding literature search on lithium and autism, 798 scientific papers were retrieved and downloaded in the electronic reference management software Endnote and reference duplicates were removed (80). Afterwards, the remaining references were screened on title and abstract considering the following inclusion/exclusion criteria (Table 34).

Regarding literature search on lithium and male reproduction or cardiovascular effects, 1204 and 755 scientific papers were respectively retrieved and downloaded in the electronic reference management software Endnote and reference duplicates were removed (resp. 104 and 80). Afterwards, the remaining references were imported into the web-tool Rayyan to organise the review. These references were screened on title and abstract considering the same inclusion/exclusion criteria (Table 34).

Table 34: Literature search inclusion and exclusion criteria

Publication type	IN	Primary research studies, reviews
	OUT	Secondary studies (e.g. editorials, conference, commentary)

Language	IN	English, French
	OUT	Other languages
Study design	IN	Human experimental volunteer studies, cohort studies, cross-sectional studies, case-control studies
	OUT	Experimental animal studies In vitro studies in silico studies
Population	IN	Humans
	OUT	Humans under lithium treatment All mammalian animals
Exposure	IN	Mono exposure
	OUT	Mixtures, co-exposure, wrong drug
Outcome	In	Autism / male reproduction / cardiovascular effects

16 Human health hazard information at environmental doses

16.1 Mental health in adults

16.1.1 Suicide

Two meta-analysis investigated the relationship between lithium content in public drinking water and suicide mortality in the general population (Barjasteh-Askari et al. 2020; Memon et al. 2020). A third meta-analysis studied the association between lithium in drinking water and neuropsychiatric outcomes including suicide and psychiatric hospital admissions (Eyre-Watt et al. 2021). These 3 meta-analyses included each more than 13 studies from Japan, Europe and America and reported lithium concentrations in drinking water ranging from "0" (that is not clearly identified as no exposure in the publications) to 123 µg/L. They all reported a possible benefit of a higher lithium concentration in drinking water on suicide incidence rate but with a low level of certainty. On the one hand, while Barjasteh-Askari et al. (2020) suggested a statistically significant protective effect of lithium on suicide in men but not in women, Memon et al. (2020) suggested the contrary (statistically significant protective effect in women but not in men). On the other hand, both Barjasteh-Askari et al. (2020) and Eyre-Watt et al. (2021) reported that the overall inverse association between lithium exposure and suicide rates was subject to a publication bias, and that the association was not significant any more after correcting for the publication bias. It is to be noticed that Memon et al. (2020) indicated potential conflicts of interest and Eyre et al. (2021) included clinical studies with potential conflicts of interest. Ten other studies, not included in the meta-analyses, were identified.

Two (overlapping) Japanese case-control studies (Kurosawa et al. 2018; Kanehisa et al. 2017) assessed the correlation between serum lithium concentrations and suicide attempts among patients presenting to emergency departments. Their quality seems to be 'fair' according to the Newcastle-Ottawa Scale (NOS) but the sample size is limited (<200 patients in total) and there is a risk of selection bias. They reported a lower serum lithium concentration in patients with suicide attempts (n=33) compared to no suicide attempts (n=147) among patients presenting to emergency. Patients diagnosed as 'self-harm' rather than 'suicide attempts' (n=18) did not seem to differ from the controls. These studies support the relationship between the "internal dose of in vivo exposure to lithium and the risk of suicide in a coherent manner (the lower the lithium, the higher the risk of suicide) but their designs limit the conclusions that can be drawn from such studies.

Eight cross-sectionnal studies (Kugimiya et al. 2021; Terao et al. 2009; Kozaka et al. 2020; Izsak et al. 2022; Helbich et al. 2012; Liaugaudaite et al. 2022; Lopez Steinmetz et al. 2021) and one transversal study (Ando et al. 2022) were identified but are not reported here because of their low sample size and high risk of bias (the exposure was mostly assessed by sampling posterior to the period of the suicide rates measure, the risk of false positive results was mostly not controlled, potential confusion factors were more or less adjusted for). For complementary information, the reader can look at the cross-sectional study of Kozaka et al. which reported a "meta-regression like" graph summarizing several cross-sectional studies. However, it should be noted that this approach is not protected against the publication bias highlighted previously.

Conclusion: The published literature might suggest a potential protective effect of lithium exposure from drinking water on the risk of suicide (suicide fatalities, suicide attempts at an emergency department, suicide rates); however, the designs of the studies and the observed publication bias preclude any causal assertion on the matter.

16.1.2 Mental health in adults other than suicide

Various outcomes were studied including depression, hyperthymic temperament, dementia and Alzheimer.

Several references (5) were excluded, due to 1) their low sample size (N = 34, (Shiotsuki et al. 2008), N = 20 cases and 5 control (Velthorst et al. 2017)), 2) inadequate designs (comparison of mental score before and after a stay in a ressort (Shiotsuki et al. 2008), ecological studies (Fajardo et al. 2018; Muronaga et al. 2022)), 3) minimal adjustment for confounders (Duthie et al. 2023).

One study reported a positive **beneficial association between lithium levels in drinking water and hyperthymic temperament scores** (Matsuzaki et al. 2017) in a cohort of 609 residents in Japan.

Another study among 364 randomly selected adults from Washington county did not report associations between lithium measured in tap water collected at home and mental scores obtained from questionnaires evaluating depression, unhappiness, dysfunctioning and aggression, except when they restricted their population to housewives and retired women (with the hypothesis that they were more likely to spend time at home, N = 131). In this subsample, **the proportion of women with high depression score increased with increased lithium concentrations** in drinking water (Oliver et al. 1976).

Conclusion: The epidemiological studies with satisfying design evaluating the effect of environmental concentrations of lithium in drinking water on adult mental health other than suicide were scarce. Results of the two studies mentioned above are not comparable since they evaluated two different aspects of mental health (hyperthimic temperament (Matsuzaki et al. 2017) versus depression (Oliver 1976). Taking together, this prevented us to draw conclusion regarding the potential effect of exposure to environmental concentrations of lithium and impairment of mental health in adults.

16.2 Mental health in young individual

16.2.1 Autism Spectrum disorders (ASD)

Studying environmental risk factors for ASD is challenging because of 1) the prevalence of these disorders (around 1%) requiring large sample size, 2) the onset in early life requesting data on exposure prospectively collected during fetal life and infancy, which are not always available. In addition, for compounds with multiple sources and routes of exposure, relying on biomarkers of exposure assessed in biosamples collected during sensitive windows is usually recommended but most of the time not the way exposure was assessed in the available studies.

Several studies looking at the associations between lithium and ASD were identified. With one exception (Liew et al. 2023) considered as key study, most studies were cross-sectional case-controls studies and assessed lithium concentrations in biospecimens collected after diagnosis. Only few of these studies adjusted for confunders and / or matched cases and controls on sex and gender.

Key study

One case control study conducted in Denmark included 8842 ASD identified from nationwide registries and 43864 controls. Lithium exposure during pregnancy through drinking water was estimated based on residential addresses of the participants and kriging interpolation from 151 waterworks measurements of lithium across all regions in Denmark (Liew et al. 2023). This study reported an increased ASD risk with increased lithium exposure (OR[95%CI]: 1.23 [1.17-1.29] for an interquartile range (IQR) increase in lithium concentrations in drinking water). Neither interaction with child sex, nor with birth year was observed. Strengths included the large sample size, the reliance on ASD diagnosis, and the consideration of relocation during pregnancy. As discussed by the authors and commentaries (Barbanti Zancheta 2023, Strawbridge 2023, Bellinger 2023), several limitations should be acknowledged including 1) no estimation of internal doses, 2) lack of data regarding exposure through other sources (e.g. diet), 3) residual confounding cannot be ruled out, 4) exposure assessed based on the home address.

While this study suggests a deleterious association between lithium exposure and ASD, replication of this finding is required to be able to draw strong conclusions.

Supportive studies

A few cross-sectional studies with smaller sample size assessed lithium in serum or hair of ASD and control children.

Two studies reported difference (either decrease (Wu et al. 2022) or increase (De Palma et al. 2012)) in lithium concentration between ASD and controls, that did not remain significant after adjustment for covariates.

Four studies matched cases and controls on age and / or sex and thus did not adjust their statistical analysis for these two factors nor as for any other covariates. One reported decreased serum lithium among ASD cases (Zhang et al. 2022) while the three others conducted by the same first author did not show any difference between ASD and controls (Skalny et al. 2017a, 2017b, 2020).

Excluded studies

A few cross-sectional studies relied on < 30 ASD cases and/or did not account for confunders or did not match controls and cases and thus were excluded.

Conclusion: One study with a large sample size and some limitations, suggested an increased ASD risk with increased lithium exposure through drinking water during pregnancy (Liew et al. 2023). Supportive studies did not allow to confirm such effects (Wu et al. 2022, De Palma et al. 2012). However, these studies were of moderate quality. Thus, further large studies with longitudinal design are needed to confirm the deleterious effect of lithium on ASD suggested by Liew et al. (2023).

16.2.2 Attention-Deficit/Hyperactivity Disorder (ADHD)

Thygesen et al. (2021) investigated the associations between lithium exposure (through drinking water, estimated based on residential addresses at the 5th birthday of the children) and ADHD diagnosis (identified from a registry) among 284,309 Danish children, including 9,500 ADHD cases. After accounting for potential confounding factors, such as region, the authors reported **protective associations between lithium concentrations in drinking water and ADHD**, only in the model including all the covariates and the region. The effect of lithium varied by regions. After stratification, a protective association with lithium was observed in two regions, a deleterious association in one region, and no significant effect in the remaining two regions.

Conclusion: Two studies with similar designs reported either a deleterious association with ASD or a protective one with ADHD. Due to the limited number of studies retrieved in this literature search, additional data is needed.

16.3 Thyroid

In a cross-sectional study conducted in China (He et al. 2022), 585 cases of thyroid cancer were compared to 585 controls, matched on sex and gender. The authors reported lower lithium urinary concentrations (standardized for creatinine) in thyroid cancer patients than in controls.

In another cross-sectional study involving 171 pregnant women from the Argentinean Andes (Harari et al. 2015b), the authors repeatedly collected blood samples during pregnancy (a total of 255 measurements was performed, including 27 in first, 82 in second, and 146 in third trimester) and reported that increased blood lithium concentration was associated with **elevated levels of TSH** (thyroid-stimulating hormone) and **decreased levels of T3** (triiodothyronine).

Broberg et al. (2011) conducted a cross-sectional study involving 202 women from the Argentinean Andes and reported that **urine lithium concentration was inversely associated with free T4 (thyroxine) before and after adjustment** (on elements elevated in water of the same region, age, parity, and body mass index (BMI)) and **positively associated with TSH**.

Conclusion: Studies about thyroid are cross-sectional and two have limited sample size. So despite the fact that they all suggested an **association with thyroid outcomes**, more studies with longitudinal design are needed to conclude.

16.4 Growth and metabolism

Three studies of good quality were related to effects of lithium exposure on growth and metabolism. All of them relied on prospective mother-child cohorts with collection of repeated biological samples (blood, urine or both) across the pregnancy to assess exposure to lithium by biomarkers (Wang et al. 2020; Harari et al. 2015a; Harari et al. 2016).

The study from Wang et al. (2020) performed among 234 pregnant women from the Shanghai Maternal-Child Pairs cohort reported statistically significant associations between higher blood level of lithium and **higher gestational weight gain** of the mothers, both cross-sectionally (i.e., by trimester) and over the course of pregnancy. In addition, statistically significant associations between lithium and **maternal inflammatory cytokines** during the 3rd trimester were also reported: higher blood level of lithium was associated with higher levels of TNFa and IL-6, and with lower levels of GDF-15 (Wang et al. 2020).

The other two publications studied a population-based mother-child cohort in the Argentinean Andes which included around 200 pregnant women (Harari et al. 2015a; Harari et al. 2016). Harari et al. (2016) evaluated the associations between blood lithium levels and calcium homeostasis during pregnancy and reported that higher blood lithium level was associated with **lower plasma 25-hydroxyvitamin D3 level**, and **lower calcium and magnesium levels** in urine. Harari et al. (2015a) evaluated the associations between maternal exposure to lithium and fetal growth parameters. They reported associations between higher maternal blood lithium level and a **decrease in all fetal growth parameters**, statistically significant for femur length (β[95%CI] = -0.23 cm [-0.43; -0.042]) and size at birth (β[95%CI] = -0.53 cm [-1.0; -0.052]).

Two other studies were not considered in this report due to methodological limitations (Wang et al. 2019, Enderle et al. 2020). Wang et al. (2019) evaluated the link between exposure to lithium (indirect assessment; living in lithium contaminated area or not) and the urine metabolome in children (n=214) and in smoker and non-smoker elderly (n=352). The authors reported a number of associations with various metabolomic markers but did not adjust for any potential confounder (Wang et al. 2019). Enderle et al. (2020), in a cross-sectional study that included 928 adults, explored the potential predictors of plasma lithium levels. In univariate analyses, the authors reported that higher plasma lithium levels were associated with lower BMI, glomerular filtration rate, and low-density lipoproteins, and with higher prevalence of diabetes, HbA1c levels, and higher high-density lipoproteins (Enderle et al. 2020). However, among these markers, only the glomerular filtration rate was identified as one the main predictors of lithium level in the multivariate analysis.

Conclusion: Isolated studies of relatively good quality suggested **deleterious effects of lithium on growth and metabolism parameters,** in particular (but not limited) when exposure occurred during vulnerable windows of exposure such as the pregnancy. However, additional longitudinal studies with biospecimen to measure lithium exposure are needed to confirm these results.

16.5 Male reproductive health

One study performed in 666 young male students, with data and biological sample collection both at baseline and 2-year later, reported that higher urine level of lithium was associated with **lower semen volume, lower progesterone, and lower testosterone**. Only the association observed for testosterone (Q4 vs Q1: -6.45% [-10.49; -2.23], p=0,003) persisted after considering potential co-exposure to other metals (Chai et al. 2022). No association has been observed with other sperm quality parameters nor with other sex hormones.

Another study performed in 622 sperm donors evaluated the associations between daily concentrations of PM2.5 and its constituents (including lithium) and sperm quality (Wu et al. 2021). After controlling for a number of potential confounders, the authors reported that higher concentration of lithium during 0–90 days before the semen collection date was associated with a **decrease in sperm motility** (but

not in sperm concentration, nor in sperm count) at some specific percentiles (60th and 70th) of the distribution.

A third study, of lower quality due to its small sample size and no adjustment for potential confounders, was also identified. Using a cross-sectional study design performed among men consulting for infertility, Karabulut et al. (2022) compared the levels of lithium measured in seminal plasma in a group of men with normal sperm parameters (n=60) and a group of men with abnormal sperm parameter (n=53) according to WHO criteria. The authors observed **no difference in lithium between the two groups** (mean lithium in the normal group = 32.89 ng/mL, mean lithium in the abnormal group = 48.16 ng/mL, p-value=0.28). However, it is questioned if the test used to compare the lithium levels between the two groups is adequate in view of the lithium distribution. This cannot be sorted out without access to individual data.

Finally, a fourth study (Skalnaya et al. 2015) was not considered due to incomplete reporting of the results (all tables are missing in the article).

Conclusion: The few epidemiological studies available are coherent in demonstrating that exposure to environmental doses of lithium can be related to impairment of male fertility.

16.6 Female reproductive health

One study performed in 60 women attending an in vitro fertilization (IVF) treatment measured lithium (together with other essential trace elements) in urine, follicular fluid and plasma and evaluated their association with ovarian response and reproductive outcomes (Gonzalez-Martin et al. 2023). After adjusting for potential confounders, the authors reported that higher lithium level in follicular fluid was associated with lower number of retrieved oocytes (mean difference (95% CI) between p20 vs. p80 = 0.82 [0.68, 0.98]; p trend = 0.03), lower proportion of mature oocytes (0.80 [0.67, 0.95], p trend = 0.015), and lower proportion of fertilized embryos (0.78 [0.65, 0.94], p trend = 0.011). In addition, higher lithium level in urine was associated with lower birth rate (OR [95% CI]: 0.33 [0.11, 0.89]; *p* value = 0.036).

Three other studies of lower quality due to no adjustment for any confounders or low sample size (n≤30) were not considered in this report (Skalny et al. 2018, Sun et al. 2019, Syrkasheva et al. 2021).

Conclusion: The isolated study of relatively good quality (but with a small sample size) suggested **deleterious effects of lithium exposure on IVF outcomes**. However, additional longitudinal studies not only limited to sub- or infertile women are needed to confirm the potential impact of lithium exposure on female reproduction.

16.7 Cardiovascular effects

One longitudinal study performed in 1300 adults enrolled in the Wanjiang cohort (Zhong et al. 2021) evaluated the association between urine lithium level (among other metals) and the risk of hypertension. An **increased risk of hypertension was suggested** with lithium exposure when treated in quartiles: Q3 vs Q1, OR [95%CI] = 1.45 [1.01; 2.06]. However, this association was not tested in continuous and the authors focused more their analyses on other metals.

Five additional studies of lower quality due to no or minimal adjustment for confounders were also identified.

In a cross-sectional study that included 928 adults, Enderle et al. (2020) explored the potential predictors of plasma lithium levels. They identified five predictors of lithium levels including **diastolic blood pressure that was inversely associated with lithium**: plasma lithium concentration decreased by -0.0016 µg/L per 1-unit increment in diastolic blood pressure.

Two case-control studies reported higher blood level of lithium in cases with cardiovascular diseases than in controls (Skalny et al. 2017, Ilyas et al. 2017). In a small case-control study (n=21 cases and n=21 controls), Skalny et al. (2017) reported a **significantly higher lithium levels in serum of men with ischemic stroke** (median = 2.1 µg/L) than in controls (median = 1.7 µg/L). While cases and controls were matched for age- and body mass index at enrolment, no other potential confounders were considered in this study. Similarly, in another small case-control study (n=70 cases with valvular heart disease and n=66 controls), Ilyas et al. (2017) reported **significantly higher blood lithium levels in cases** (0.325 µg/g wet weight) than in controls (0.281 µg/g wet weight), but did not control for potential confounders.

Two ecological studies with no individual data, reported a **lower cardiovascular mortality in association with lithium exposure**: Voors et al. (1970) observed this association between the level of lithium measured in water supply and arteriosclerotic heart death at the city level (n=99) and Dawson et al. (1978) between lithium measured in tap water and in urine of volunteers and three types of cardiovascular diseases mortality rates at the county level (n=24).

Conclusion: Isolated studies of relatively good quality suggested **deleterious effects of lithium on hypertension**. However, additional longitudinal studies with biospecimen to measure lithium exposure and on other cardiovascular indicators are needed to confirm these results.

16.8 Aging

One study performed in 169 pregnant women measured lithium and telomere length, both in maternal blood, cord blood, and placenta tissue (Herlin et al. 2019). No association was observed between lithium level and telomere length, when lithium was studied alone. However, after adjusting for other metals (boron, arsenic and antimony), lithium was associated with higher telomere length in maternal blood (results are not reported for cord blood nor placenta).

16.9 Other studies

Other studies explored the potential effect of lithium exposure on various other outcomes: pediatric morbidity (Karakis et al. 2021), juvenile idiopathic arthritis (Kindgren et al. 2019), all-cause mortality (Fajardo et al. 2018b), homicide and crime (Kohno et al. 2020; Giotakos et al. 2015). However, these studies have strong methodological limitations (small sample size, limitations in statistical analysis, ecological design, no adjustment for potential confounders) that limits the interpretation of the findings and are therefore not detailed here (see Section 17).

17 Detailed information on human health hazard at environmental doses

17.1 First literature search

Table 35: Summary of epidemiologic studies investigating suicide and homicides

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Meta-analyses with limitations							
Eyre-Watt et al. (2021)	Meta-analysis 27 articles: 20 longitudinal studies, 3 case–control studies, 4 cross-sectional studies Sample size from 43 to 17.2 million 11 studies from Europe, 9 from USA, 7 from Japan	Indirect (drinking water) Time of expo : NA The mean lithium concentration ranged from 0.48 to 27.4 µg/L	**Neuropsychiatric outcomes** (including suicide)	Has been changed between the registered protocol and the final publication without explanation Meta-analysis of correlation	Studies commonly adjusted for age, gender and population size, although these were inconsistently applied and varied widely. Less common confounders included population density, urbanicity, annual mean temperature and availability of mental health services.	Higher lithium concentrations were associated with **reduced suicide rates (r [95%CI]: −0.191 [−0.287; −0.090], p<0.001) but not significant after taking into account the publication bias** (Egger's test; t value=2.90, p=0.013); adjusted values (r [95%CI]: −0.080 [−0.188; 0.029])	High risk of bias of included studies Publication bias at the meta level Overall quality: low The meta-analysis includes individual studies with potential conflict of interest (Ando et al. 2017: an author received payments from pharmaceutical industries (consulting, grants, honoraria); Kessing et al. 2017: an author has been a consultant for

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
							pharmaceutical industries)
Barjasteh-Askari et al. (2020)	Meta-analysis Populations from geographical regions for which the relationship between lithium in drinking water and suicide has been investigated for a period of time 14 studies used in the meta-analysis with a total sample size of 939 regions in 13 ecologic studies and 3740,113 people in one cohort study	Indirect (drinking water) Time of expo: NA Li concentration in drinking water: from 0 to 123 (µg/L)	**Suicide**: annual mean suicide incidence	Crude relationship between the lithium content of drinking water and suicide incidence in the general population Subgroups analysis women/men (10 studies)	Gender	Relationship between the lithium concentration in drinking water and **reduced suicide mortality** (**OR [95%CI]: 0.42 [0.27; 0.67]; p-value <0.01**) **but not significant if taking into account the publication bias** observed (Egger's test p value <0.05; Trim & Fill corrected OR [95%CI]:0.62 [0.38; 1.01]) OR [95%CI]: 0.54 [0.35; 0.84]; p-value <0.01 for men, OR [95%CI]: 0.70 [0.48; 1.01]; p-value=0.057 for women	High risk of bias of included studies Publication bias at the meta level Overall quality: low
Memon et al. (2020)	Meta-analysis 15 ecological studies fulfilled the selection criteria and were included in the synthesis, 4 studies were conducted in Japan, 4 in Austria, 3 in USA, and 1 each in England, Greece, Italy and Lithuania Study populations ranged from 1 109 261 to 22 097 948	Indirect (drinking water) Time of expo : NA Mean lithium levels in the drinking-water samples ranged from 3.8 µg/L to 46.3 µg/L	**Suicide**: suicide mortality rate per 100 000 per year	Where both adjusted and unadjusted regression coefficients were presented, the unadjusted regression coefficient was used in preference.	Male and female, high vs low suicide rate country, high vs low lithium concentration country	**Protective association** between lithium levels/concentration in publicly available drinking water and total suicide rates (**pooled β [95%CI]: −0.27 [−0.47; −0.08]; p=0.006**, I^2=83.3%) Male: (pooled β [95%CI]: −0.26 [−0.56; 0.03]; p=0.08, I^2=91.9%) Female: (pooled β [95%CI]: −0.13 [−0.24; −0.02]; p=0.03, I^2=28.5%)	High risk of bias of included studies Publication bias at the meta level Overall quality: low Potential conflict of interest: some authors received payments from pharmaceutical industries (for lectures, advisory board membership, speaking at events)

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Key studies (fulfilling inclusion criteria)							
Ando et al. (2022)	Case-control study Japan, Tokyo 12 suicides (44% of female) and 16 non-suicides (56% of female) examined or dissected at the Tokyo Medical Examiner's Office from March 2018 to June 2021 Mean age: 40.4 years (SD 17.6) (suicides); 61.4 years (SD 14.0) (non-suicides).	Aqueous humor lithium concentration measured twice 16h apart Mean aqueous humor lithium concentration: 0.50 µg/L (suicides); 0.92 µg/L (non-suicides)	**Suicides vs non-suicides**	t test, ANCOVA and model including age and sex as confounder	Age, sex	t-test: the **mean concentration in the aqueous humor was significantly lower in suicides** (0.50 µg/L (variance s^2=0.04)) than in non-suicides (0.92 µg/L (s^2=0.07)). ANCOVA : **significant relationship between suicide and lithium concentration in the aqueous humor**, even after controlling for age and sex (F(1, 24)=8.57, P=0.007). Partial correlation between suicide and lithium concentration in the aqueous humor was calculated as 0.51 (95% CI=[0.17, 0.74]. Random and slope model showed the significant effect of suicide on aqueous humor lithium concentration (b=−0.344, SE=0.089, 95% CI=[−0.528, −0.161], t(26)=−3.855, P=0.001). When age and sex were included as confounders, only the random intercept model was identified and it showed the significant effects of suicide (b=−0.261, SE=0.102, 95% CI=[−0.471 to −0.051], t(24)=−2.568, P=0.017) as well as postmortem interval (b=0.0025, SE=0.0012, 95%CI=[0.00002 to 0.00489], t(70)=2.011, P=0.048) on aqueous humor lithium concentration.	NOS evaluation: fair Risk of selection bias

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Kurosawa et al. (2018)	Case-control study Japan Between Apr. 1, 2013 and Sept. 30, 2017, 5030 consecutive patients were ascertained at a university emergency department. Among them, 1997 patients suffering from intoxication or injury were included because intoxication and injury may be at least partially derived from suicide attempt and deliberate self-harm. 197 of the 1997 patients gave informed consent with the measurement of serum lithium levels and plasma EPA, DHA and arachidonic acid levels. Among the 197 patients, 51 suffered from deliberate self-harm (18) or suicide attempts (33) and 146 were control patients. 123 males and 74 females Mean age: 52.2 years	Direct routine blood sampling was performed at the initial visit. After individual patients recovered, written informed consent was taken and, if they agreed, a remnant of the blood was used to measure plasma EPA[55], DHA[56], arachidonic acid and serum lithium levels. Alternatively and thereafter, serum lithium levels were measured by two of the authors who were blind to the data of the patients using mass spectrometry, where the minimum amount of lithium which can be measured was 0.15 µg/L. The distribution of lithium levels was considerably skewed. Mean serum lithium level: 5.9 µg/L (SD=4.0, range=0.65–23.0)	**Suicide attempt and deliberate self-harm**	A] ANOVA Log-transformed lithium levels: Suicide attempts / self-harm / Control B] Multivariate logistic regression analyses of the suicide attempts group and deliberate self-harm group with control group as a reference	B] adjusted for: Gender, age, EPA, DHA, arachidonic acid	A] Log-transformed lithium levels (mean (SD, 95%CI)): - **Suicide attempts (n=33): 0.56 (0.32, 0.45–0.67)** - Self-harm (n=18): 0.75 (0.24, 0.63–0.87) - Control (n=146): 0.70 (0.28, 0.65–0.75) p=0.024 B] Odds ratio (95% CI) for Log-transformed lithium levels and p value: - **Suicide attempts vs control: 0.156 (0.038–0.644), p=0.010** - Self-harm vs control: 3.094 (0.380–25.187), p=0.291	Good quality but risk of selection bias NOS evaluation: fair Potential conflict of interest: some authors received payments from pharmaceutical industries (lecture fees)

[55] Eicosapentaenoic acid
[56] Docosahexaenoic acid

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Kanehisa et al. (2017)	Case-control study Japan 4414 consecutive patients at a university emergency department between April 1, 2013, and December 31, 2016 Among them, 1725 patients suffering from intoxication or injury were screened. 1355 of the 1725 patients were eligible. 1118 of the 1355 patients did not give consent. => 237 participating patients Among them, 38 patients were excluded due to the lack of measurement of serum lithium levels. Finally, N=199 patients analysed: - 31 patients with suicide attempts - 21 patients with self-harm - 147 control patients	Serum lithium levels At the university emergency department, routine blood sampling was performed at the first visit. A remnant of the blood was used to measure serum lithium levels. Measurements were done by a third party using mass spectrometry (minimal measurable amount: 1 µg/L (if under: =1). The distribution of lithium levels was considerably skewed.	**Suicide attempts** The suicide attempt group (n=31) and self-harm group (n=21) were re-categorized into an injury group (n=22) and an intoxication group (n=30). All patients of the control group were categorized into the injury group (n=147)	Multivariate logistic regression, age, gender, and log-transformed lithium levels A] "suicide attempts" group, "self-harm" group, and the "control" group B] "injured patients", "intoxicated", control C] five groups: self-harm with intoxication, self-harm with injury, suicide attempts with intoxication, suicide attempts with injury, and control D] sub-groups analysis of A] restricted to patients with depression or bipolar disorder	Adjustment for age and gender	Compared to 147 control, adjusted for age and gender: A] 1) **lower lithium for suicide attempts** (n=31 ; p=0.032, OR [95%CI]: 0.23 [0.06; 0.88] 2) no significant difference for self harm (n=21) B] 1) **lower lithium for "injured patients"** (n=22, p= 0.026, OR [95%CI]: 0.17 [0.04–0.81] 2) no significant difference for "intoxicated" (n=30) C] 1) **significant difference between the five groups** (F=3.37, p=0.011) 2) **"suicide attempts with injury" with significantly lower lithium levels than the other** (four p < 0.01) D] 1) **"suicide attempt" with significantly lower lithium than the "self-harm"** (p=0.004) **and control group** (p=0.001) 2) no significant between "self-harm" and control	Good quality but risk of selection bias NOS evaluation: fair Potential conflict of interest: an author served as consultant to a pharmaceutical industry and received payments from several pharmaceutical industries (lecture fees and grants)
Studies with limitations							
Izsak et al. (2022)	Cross sectional study Hungary	Public drinking water supply systems using their own water source (n=1325) were	**Suicide cases** (2005–2015)	District-level data Gender- and age-	Income, alcohol consumption and religiosity (confounding variables	**Total population: suicide rates decreased as lithium level increased in 3 models/3, in a significant manner in 2**	High risk of bias

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
	Hungarian data at the level of districts (n=197) 25 571 completed suicide cases were documented among those above 15 years of age in Hungary (male=19 595; female=5 976).	sampled between January 2016 and July 2018 (resampled between April 2018 and August 2020). Areas were selected based on the lithium concentrations in drinking water: in one group, the concentrations were typically low (n=43), while in the other group they were typically high (n=41) Lithium levels aggregated to the level of districts ranged from 0.71 to 89.35 µg/L (mean=11.2; SD=11.9).		standardised (with the indirect method using 5-year age-groups) mortality ratios for suicide (sSMR) in the total population	previously reported to affect suicide risk)	**models/3**. Male population: suicide rates decreased as lithium level increased in 3 models/3, in a significant manner in 2 models/3. Female population: suicide rates decreased as lithium level increased in 3 models/3, but not in a significant manner in 3 models/3.	
Liaugaudaite et al. (2022)	Cross sectional study Lithuania 54 municipalities	Samples from the main public drinking water systems (June-July 2017) Median lithium concentration was calculated (7.0 µg/L). Municipalities with lithium concentration in drinking water greater than the median were assigned to the high lithium exposure group (n=26), the remaining	**Suicide rate** data were collected from the Health Information center of the Institute of Hygiene. Suicide data comprised all registered suicide events across all age groups and gender within the 5-year period (from January 2012 to December 2016). Characteristics per 100,000 Suicide	Multiple linear regression analysis with the suicide SMR (total, men, and women) as the dependent variable was conducted adjusting for potential confounding factors of suicide risk.	Municipalities' sociodemographic characteristics such as local population size 2012–2016, unemployment rate, visits to psychiatrist, divorce rate, women/men proportion, affective disorders, mental and behavioral disorders (MBD), MBD due to use of alcohol,	**Suicide SMR was inversely associated with lithium level in drinking water in municipalities with high lithium levels.** Multiple regression analysis of lithium predicting suicide SMRs: - in the low lithium exposure group (n=27): β=0.141, p=0.482 (crude model), no data reported for adjusted model - in the high lithium exposure group (n=26): Total: β=-0.462, p=0.017 (crude model), β=-0.363, p=0.044	High risk of bias

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
		municipalities were assigned to the low lithium exposure group (n=27). Mean lithium concentration in 53 drinking water samples was 11.5 (SD 9.9) µg/L ranging from 1.0 to 39.0 µg/L, median – 7.0 (IQR 3.5–20) µg/L.	SMRs total Overall 42.54±11.43		schizophrenia, nervous system diseases, attempted suicide, antidepressant use	(adjusted model) Women: β=-0.343, p=0.087 (crude model), β=-0.455, p=0.015 (adjusted model) Men: β=-0.452, p=0.020 (crude model), β=-0.465, p=0.009 (adjusted model)	
Kugimiya et al. (2021)	Ecological in its design Japan 808 cities and wards (785 cities of 46 prefectures and 23 wards of Tokyo)	From 2010 to 2015, collection of 988 tap water samples (usually from the main rail station or municipal office) of each city and ward Categorisation of lithium levels into four groups (that is, 0 to less than 10 µg/L, 10 to less than 20 µg/L, 20 to less than 30 µg/L, 30 µg/L or more) Mean lithium level in drinking water: 2.39 µg/L (SD=4.0; range, 0-43.0)	**Suicide standardized mortality ratios** (SMRs) (number of observed deaths in an individual city and ward population divided by the number of expected deaths, compared with the gender- and age-matched general population) from 2010 to 2016, calculated for each individual city and ward for each year and averaged over the 7 years. Collected from the Ministry of Health, Labor, and Welfare and from the Statistics Bureau, Ministry of Internal Affairs and	Multiple regression analyses adjusted for the size of each population	Model 1: adjusted for proportions of elderly people, proportions of one-person households, proportions of people in primary industry employment, overall unemployment rates, annual marriage rates, annual mean temperatures, and annual total sunshine hours. Model 2: further adjusted for the mean proportion of residents who continued to live in the same city Tentative model:	Adjusted models showed **significant inverse associations of lithium levels with total and male SMRs, but not with female SMRs.** Limited to the 47 cities that had a prefectural office and provided data on the consumption of bottled water, the inverse association between lithium levels and total suicide SMRs was not significant. 30 µg/L or more was associated with lower suicide SMRs less than 100.	High risk of bias The authors proposed an arbitrary cut off but did not seems to have assess it or search for cut off value using specific statistical methods. Conflict of interest: not indicated but an author received payments from pharmaceutical industries (cf. Kurosawa et al. 2018, Kanehisa et al. 2017)

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
			Communications of Japan.		bottled instead of tap water as further adjustment factor		
Lopez Steinmetz et al. (2021)	Cross sectional study Argentinean Andes: Cochinoca, Susques (within the plateau region), and Tumbaya (east of the plateau, in the Quebrada de Humahuaca Valley) The plateau and the Quebrada de Humahuaca Valley are low-income rural areas.	Water samples were collected during the dry season (August 2015) due to two main reasons: i) the lack of rainfalls results on low flows and the relative increase of Li values in water, which are then detectable during chemical analysis; and ii) during the rainy season the flow of the rivers increases, making some roads inaccessible to vehicles. Seven samples were collected in Cochinoca, 8 in Susques, and 3 in Tumbaya. Li grade ranges were 1.01 to 2.98 mg/L in Cochinoca, 0.14 to 2.43 mg/L in Susques, and 0.05 to 0.08 mg/L in Tumbaya. The altitude of sampling sites was comprised between 2192 m asl and 4200 m asl	**Suicide mortality rates** considered available official data on suicide deaths and population. It was considered all the recorded cases of deaths by suicide in Cochinoca, Susques, and Tumbaya, in all age groups and in both sexes, for the period 2003–2013	Multiple linear regressions		**Lithium but not altitude was positively correlated with suicide mortality** when analysing bivariate correlations (Li: rho=0.76, p-value < 0.001). However, when generalised linear models were calculated, a significant interaction effect was found between lithium and altitude (p-value < 0.001). No significant differences were found between the Li concentrations in treated and no treated waters (K-W=3.33, df=1, p-value=0.07), nor between the Li concentrations by altitude of the sampled sites (K-W=12.45, df=7, p-value=0.09).	High risk of bias

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Kohno et al. (2020)	Cross sectional study Japan: 274 municipalities of Kyushu Island	Between 2010 and 2013, 434 drinking water samples were taken in the 274 municipalities (mainly from the main railway station or the municipal offices). Resampling was performed in the same places 1 year later and showed very little fluctuation. Mean level: 4.2 µg/L (s.d.=9.3; range 0–130)	**Criminal offences** (homicide, rape, robbery, arson, violence, fraud and gambling but excluded negligence resulting in traffic accidents). Crime rates: number of recognised criminal offences divided by the total populations per municipality in 2009	A] crude model B] adjusted model weighted least squares regression analysis adjusted for the size of each population	B] adjusted for the proportion of 1-person households, overall unemployment rate and the proportion of elderly people	A] No association between lithium levels in drinking water and crime rates in the crude model. B] **Lithium levels were significantly and inversely associated with crime rates after adjusting for the proportion of elderly people, the proportion of 1-person households and the overall unemployment rate.**	High risk of bias Conflict of interest: none declared but an author received payments from pharmaceutical industries (cf. Kurosawa et al. 2018, Kanehisa et al. 2017)
Kozaka et al. (2020)	Cross sectional study Japan: 26 municipalities of Miyazaki Prefecture	Tap water samples from water treatment plants located in Miyazaki Prefecture's 26 municipalities (n=78) Mean lithium level in the tap water: 2.8 µg/L (SD, 3.1; median, 1.7 µg/L; range, 0.2– 12.3)	**Standardized mortality ratio (SMR) for suicide** in each municipality and the average suicide SMRs over 5 years (2009–2013) Obtained from the Ministry of Health, Labor and Welfare	Weighted least-squares regression analysis, adjusted for the size of each municipality's population crude model + adjusted model	Proportion of elderly people, proportion of one-person households, annual marriage rate, annual mean income, unemployment rate, the density of medical doctors per 100,000 people, annual total rainfall, proportion of people with a college education or higher	**No association between lithium levels in tap water and suicide mortality rates** was found in Miyazaki Prefecture.	High risk of bias The study might lack power for lithium range. Conflict of interest: none declared but an author received payments from pharmaceutical industries (cf. Kurosawa et al. 2018, Kanehisa et al. 2017)
Giotakos et al. (2015)	Cross sectional study Greece: 34 prefectures	149 samples of drinking water collected from 34 out of the 51 prefectures of Greece in 2012	Data for **homicides** taken from the National Statistic Service of Greece for the period 2007–2011	The index of homicides was the mean of homicides per prefecture.	No confounders taken into account	**Decreased risk of homicide with increased lithium levels** suggested in 2 out 4 analyses.	High risk of bias

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
		Average lithium level: 11.10 µg/l (SD=21.16)		Four regressions (linear, exponential, inverse, linear weighted for prefecture population) were performed between lithium levels and mean number of homicides			
Helbich et al. (2012)	Cross sectional study Austria: 99 districts	6,460 water samples collected from drinking water supplies from 99 Austrian districts (from 1 to 312 samples, mean: 65.3 samples per district) Lowest measurable threshold lithium level: 0.0033 mg/L Mean lithium level: 0.0113 mg/L (SD ±0.027)	**Suicide rates** adjusted as standardized mortality ratios Outcome measurement: 2005–2009	Lithium levels were averaged per district. Step-by-step approach: 1) exploratory spatial data analysis, 2) global non-spatial regression model, 3) global spatial regression model, 4) local spatial regression model	Proportion of Roman Catholics, population density, per capita income, density of psychiatrists, general practitioners and psychotherapists, unemployment rates	**Inverse association between lithium levels in drinking water and suicide mortality** (global spatial regression model)	High risk of bias
Terao et al. (2009)	Japan 18 municipalities of Oita prefecture, an average (economically, culturally, and politically) prefecture in Japan	Lithium levels in drinking water (tap water) Range: from 0.7 to 59 µg/L	**Standardised mortality ratio (SMR) of suicide rate** in Oita for 2002–2006	Weighted least squares regression analysis adjusted for the size of each population		SMRs of suicide rate across the 18 municipalities were **significantly and negatively associated with lithium levels in drinking water** (β=-0.65, $p < 0.004$)	High risk of bias

Table 36: Summary of epidemiologic studies investigating mental health effects in adults

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Key studies (fulfilling inclusion criteria)							
Matsuzaki et al. (2017)	Cohort Japan N = 609 residents (270 males and 339 females) in Sapporo, Obihiro, Takaoka, Koshigaya, and Oita cities Mean age: 33.4 years +/- 9.1	Lithium in tap water samples (chiefly from the main rail station, the city office, or water purification plant) - correlation coefficient between the lithium levels and those re-measured after 1 year in the same places was 0.998 Mean (SD) lithium levels (µg/L): Sapporo: 20.0 +/- 17.3 Obihiro: 0.1 +/- 0.1 Koshigaya: 2.6 Takaoka: 1.5 Oita: 43.0 +/- 5.0	**Temperament:** by Temperament Evaluation of Memphis, Pisa, Paris and San Diego-auto questionnaire (TEMPS-A) - 110 items	Adjusted linear regression	Selected using stepwise regression: depressive temperament, cyclothymic temperament, irritable temperament, anxious temperament, age, latitude	A **significantly positive association of lithium levels with hyperthymic temperament scores**	Measurement error: Li only measured in tap water (no individual level) Conflict of interest: not indicated but an author received payments from pharmaceutical industries (cf. Kurosawa et al. 2018, Kanehisa et al. 2017)
Oliver et al. (1976)	Cohort Washington county, USA N = 384 individuals (164 males and 220 females) Random selection of households, random individual adults from each household	Li in drinking water samples collected at home (from every rural or small town resident, and one of every three town residents) Li concentration in drinking water: not	**Depression** assessed by questionnaires: CES[57] depression, Lubin depression adjective check list, unhappiness score		Age, sex, education, income, marital status, occupational status, life events, church attendance	Main population: slight tendency for the proportion of persons with high CES-depression scores to increase with increasing lithium (but not significantly). In analysis restricted to 131 housewives and retired women (likely to spend much of their time at home): the association becomes significant. No association with other scores.	Comments from the authors: relatively low variability in Li levels - may limit power to detect associations. Questionnaires (not a validated diagnosis of depression)

[57] Center for Epidemiologic Studies

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
		detectable to 32 ppb 38% of samples < LOD; 10% > 10 ppb					
Studies with limitations							
Duthie et al. (2023)	Cohort (Scottish Mental Survey 1932 (SMS1932)) Scotland Including almost all people born in 1921 and at school in Scotland in June 1932 (N = 87,498 schoolchildren). Data available for 37,597 (43.6%) (18,325 women and 19,272 men) including 3605 with record of having developed dementia	Li in drinking water: 285 sampling sites across Scotland dating from 2014 obtained from the sole public water provider (Scottish Water) Mean lithium level at all sampling sites: 1.45 µg/L (SD 1.83, range 0.5-18.2)	**Dementia:** dementia cases (all cause dementia) were identified by linkage to electronic medical records: from the general or psychiatric hospital or death certificates. + 32 individuals identified from primary care records	Geocoding: Residential location identified from the record which first mentioned dementia. For those without dementia, residential location at the first hospital admission after the age of 60 years. For those not admitted to hospital, location of death. Cox model	Age in years was the timescale and all effect estimates were additionally adjusted for age-11 IQ at age-11 Stratified for sex	Lithium levels were **positively associated with the risk of dementia in women** (highest in second quartile, HR 1.17, 95%CI 1.04–1.32), but there was no relationship in men.	Only one time point for exposure (2014) while medical record stopped in 2012. Comments from the authors: our study was unable to take into account change of residential location. Only one adjustment factor (IQ at 11)
Muronaga et al. (2022)	Ecological in its design 808 Japanese cities: 785 cities of 46 prefectures and 23 wards of Tokyo ≥65 year old population Prevalence data	From 2010 to 2015, collection of 988 tap water samples (usually from the main rail station or municipal office) of each city and ward	**Prevalence of AD** during 5 years from 2010 to 2014 from the national database of Ministry of Health, Labor, and Welfare of Japan.	To calculate the prevalence of AD: number of AD patients estimated by the anonymous claims registered as G30 in the two major medical insurances for	Proportions of one-person households as a family factor, proportions of people in primary industry employment as a job factor, annual total sunshine hours as a	The adjusted model showed a **significant inverse association of lithium levels with female, but not with male, or total prevalence of AD**.	Measure of exposure concomitant with prevalence data Conflict of interest: none declared but an author received payments from pharmaceutical industries (cf.

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
		Mean lithium level in drinking water: 2.39 µg/L (SD 4.0)		≥65 year old from 2010 to 2014 divided by the number of population ≥65 years old in the individual 808 cities and wards from 2010 to 2014. Multiple regression analyses adjusted for the size of each population	meteorological factor, and total number of beds in psychiatric hospitals as a medical factor.		Kurosawa et al. 2018, Kanehisa et al. 2017)
Fajardo et al. (2018)	Ecological in its design Texas	6,180 water samples from public wells since 2007 obtained and averaged for 234 of 254 Texas counties. Mean Li concentration (95%CI): 0.056 (0.048, 0.064) mg/L	**Changes in Alzheimer's disease (AD) mortality:** To calculate the change in AD mortality across the Texas counties, the age-adjusted AD mortality rates was obtained (per 100,000) for each county between 2000–2006 and between 2009–2015 from the Center for Disease Control Wonder's Compressed Mortality Database using the code 'G30'.	Correlation + Partial correlation to adjust for confounders	Percent of population represented by females, Hispanics, and African Americans within each county from 2011–2015, percentage of adults having some post-secondary education, air pollution (PM), percent of population living in rural areas, prevalence of adult (>20 years) obesity and diabetes, estimates on physical inactivity	**Protective effect:** Texas counties below the median level of lithium concentration (0.04 mg/L) have greater increases in AD mortality over time. Statistical significance was lost after controlling for physical inactivity, type 2 diabetes, and type 2 diabetes and obesity combined.	Not considering moving / death due to AD may not be the best indicators of prevalence

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
			The change in AD mortality was then calculated by subtracting the rate obtained between 2000–2006 from those obtained between 2009–2015.				
Velthorst et al. (2017)	20 patients with a psychotic disorder (18 males and 2 females) (mean age = 24.35, SD = 3.78) and 5 controls (whose siblings had schizophrenia) (1 male and 4 females) (mean age = 28, SD = 8.40)	Lithium in teeths (reflect pre and post natal exposure)	**Psychotic symptom severity** in patients diagnosed with schizophrenia	Distributed lag model (DLM) adjusted for sex and age to study the associations between Li and Positive and Negative Symptom Scale (PANSS) scores that evaluates symptoms severity	sex and age	Patients had higher lithium levels than controls specifically during −12 to 4 weeks before birth and the first month after birth	Low sample size / only 20 cases and 5 non affected patients
Shiotsuki et al. (2008)	43 subjects (26 females and 17 males) (mean age: 65.7+/-10.8 years) visiting cold-spring resorts. They stay at an inn near the spring for several days. They wake in the very early morning (around 2-3 AM) and continuously drink from the cold spring until 6-7 AM. Japan	One cold spring with 6.1 mg/L Li, the other with 15.7 mg/L Li Blood sampling of Li: increase from 0.026±0.032 to 0.073±0.049 mEq/L ($p<0.0001$)	**Mental effects** (the State-Trait Anxiety Inventory (STAI) and Profiles of Mood States Test (POMS))	Comparison of Li levels in blood, STAI and POMS scores before and after drinking the water rich in Li	None	After drinking, serum Li increased and ratings of POMS significantly improved.	Low quality study: The change in POMS scores observed cannot really be related to an increase in serum Li since many things were affected during the stay at the resort -> results not considered

Table 37: Summary of epidemiologic studies investigating mental health effects in young individual

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Key studies (fulfilling inclusion criteria)							
Liew et al. (2023)	Nationwide case-control study Denmark N = 8842 children diagnosed with autism spectrum disorders (ASD) born from 2000 through 2013 and 43 864 control participants matched by birth year and sex from the Danish Medical Birth Registry	Geocoded maternal residential addresses during pregnancy were linked to lithium level (range, 0.6 to 30.7 µg/L) in drinking water estimated using kriging interpolation based on 151 waterworks measurements of lithium across all regions in Denmark.	**ASD diagnoses** extracted from the Danish National Patient Register and the Danish Psychiatric Central Register	Analysis in the whole population + stratified for child sex	Maternal age, maternal smoking during pregnancy, location of residence (large cities/provincial cities and rural towns/communities), neighbourhood socioeconomic status measures, including the community employment status and a housing index, ambient air pollution levels for NO_2 and PM2.5 during pregnancy.	Increased maternal lithium exposure associated with **increased risk of ASD** (**OR[95%CI]: 1.23 [1.17-1.29]** for an IQR increase in geocoded maternal exposure to natural source of lithium in drinking water). No significant interaction with child sex or birth years ($p > 0.1$). Association were slightly stronger for mothers and children living in urban areas than for those residing in provincial towns/rural area.	Strength: large nationwide population, ASD diagnoses from registry, account for moving during pregnancy. Weaknesses: unmeasured confounders (e.g. other elements of water such as iodine), measurement error (e.g. missing information on water consumption), did not account for childhood exposure, exposure at home and not at work.
Thygesen et al. (2021)	Cohort Denmark N = 284,309 individuals born between January 1, 1994 and December 31, 2007, living at a	Level of exposure of the geogenic trace elements in the water supply area at the person's residential address at the 5th birthday as a proxy	**ADHD diagnosis**: from the Danish Psychiatric Central Research Register and from the Danish National Patient Register from which only	Survival analyses, using Poisson regression to estimate incidence rate ratios (IRRs) Trace elements	Age, sex, calendar year, parental socio-economic status, neighbourhood level socio-economic status and parental psychiatric illness + region	In model 3 (additionally adjusted for region) -> **protective effect of Li (IRR[58][95%CI]: 0.90 [0.85; 0.97])**. No association in the model not adjusted for region.	Results differed if it is adjusted or not for region (+ effect varied by region) Strength: large nationwide

[58] Incidence rate ratio

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
	residential address in an area covered by a drinking water sampling campaigns, followed for incidence of Attention-Deficit/ Hyperactivity Disorder (ADHD) from the age of five until the end of study, December 31, 2016. 9500 ADHD cases	of the intake of the specific elements from drinking water during the first five years of life. Li (range): 0.61-30.69 µg/L	contacts to departments of psychiatry, pediatrics, and neurology were included.	concentrations were modeled as categorical quartiles and as continuous trend variables with the lowest quartile as the reference category.		After stratification by region, protective effect seen in 2 region (IRR[95%CI]: 0.81 [0.72; 0.91] (Central Denmark); 0.73 [0.63; 0.86] (South Denmark)) while deleterious effect reported in another one.	population, ADHD diagnosis from registry, account for region. Weaknesses: exposure in drinking water (geolocalisation) at home but not at daycare, missing of other sources of exposure
Studies with limitations							
Fiore et al. (2020)	Cross-sectional study Province of Catania (Sicily, South Italy) N = 48 subjects with ASD (70.8% male), aged from 2 to 17 years old No control	Measurement of metals including Li in hair Median (IQR): 0.025 (0.025-0.025) µg/L	**Severity of autism:** Calibrated Severity Score (CSS) from 4 to 10 was computed to provide a continuous measure of overall ASD symptom severity. Developmental quotient (DQ) and/or Intellectual quotient (IQ)	Spearman Correlation	No adjustment	**No correlation between lithium in hair and ASD**	Poor quality: low sample size, no control, no adjustment
Qureshi et al. (2020)	Cross-sectional study USA 28 mothers who had young children with ASD and 29 mothers who had young typically developing (TD) children -> inclusion in the study of 21 children with ASD and 26 TD Child ages: 2-5 years old	Measurement of 18 essential (including lithium) and 20 toxic elements in urine (mean) Children with ASD: $1.08.10^{-1}$ µg/mL TD children: $1.33.10^{-1}$ µg/mL	**ASD diagnosis** verified by the Autism Diagnostic Interview-Revised (ADI-R)	Mann-Whitney or t-test (with false discovery rate (FDR) correction)	None with the exception of the other element assessed in hair for classification	**No difference in Li urinary concentration between ASD child group and TD child group**	Poor quality: low sample size, no adjustment

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
		Mothers with children with ASD: $6.23.10^{-2}$ µg/mL Mothers with TD children: $6.97.10^{-2}$ µg/mL					

Table 38: Summary of epidemiologic studies investigating effects on thyroid

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Key studies (fulfilling inclusion criteria)							
He et al. (2022)	Case-control study China N = 585 newly diagnosed thyroid cancer patients and 585 healthy controls	14 urinary trace elements, including lithium, adjusted for creatinine (IQR, µg/g creatinine) Case: 0.80-11.48 Control: 3.62-15.87	**Case of thyroid cancer + thyroid hormones** (fT3[59], fT4[60], TSH[61]) measured in fasting blood samples	Logistic regression with Li coded in quartile and BKMR[62]	Model 2: age, gender, body mass index, household annual income, physical activity, smoking status, passive smoking, sleeping quality, history exposure of X-ray, family history of thyroid diseases, family history of malignant tumor. Model 3: additionally adjusted for FT3, FT4, TSH, and lnI (continuous variables)	**Increased lithium urinary concentration was associated with a lower risk of thyroid cancer**	Weakness: Cross sectional study

[59] Free triiodothyronine
[60] Free thyroxine
[61] Thyroid-stimulating hormone
[62] Bayesian kernel machine regression

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Harari et al. (2015)	Cross-sectional study (Li and thyroid hormones measured at the same time) Argentinean Andes N = 171 pregnant women (exclusion of women who tested positive for anti-TPO antibodies)	Li in blood at different time points. Between 1 to 3 measurements per women (n=27 in first, n=82 in second, and n=146 in third trimester; in total 255 measurements) The lithium concentrations in blood [median 25 µg/L (0.0036 mmol/L); range 1.9–145 µg/L (0.000027– 0.021 mmol/L) correlated significantly with those in urine and drinking water (rs = 0.84, p < 0.001, and rs = 0.40, p < 0.001, resp.). Lithium concentrations in drinking water: 530-830 µg/L (San Antonio de los Cobres) 5.0-324 µg/L (surrounding villages) 958-1660 µg/L (Aguas Blancas)	**Thyroid function:** TSH, free/total thyroxine (fT4/T4), free/total triiodothyronine (fT3/T3), thyroglobulin, and transthyretin in serum, sampled at the same time than Li concentration (non-fasting blood was not possible)	Quantile regression	Gestational age, parity, height, urinary iodine, serum selenium, urinary arsenic, and urinary cesium	**Positive association with TSH, especially in the lower tail of TSH distribution** Also, the **blood lithium concentrations were associated with a decrease in transthyretin, particularly in the highest percentiles** **Decrease in Total T3** (not dependant of the percentile)	Longitudinal design but cross sectional analysis, low sample size (N = 171 with a total of 255 measurements)
Broberg et al. (2011)	Cross sectional environmental study 4 Andean villages in northern Argentina	Lithium exposure assessed based on concentrations in spot urine	**Thyroid dysfunction:** Plasma fT4 and TSH	Multivariate models	Selected elements (arsenic, boron, cesium, iodine), age, body mass index and parity	**Urine Li concentration inversely associated with fT4 before and after adjustment** [adjusted β for a 1,000-µg/L increase in urinary Li	3 of 202 women reported ongoing use of medication: 1

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
	N = 202 women No control group (exposure biomarker in continuous: can be considered as having a control group (compare the most and the less exposed participants)), no inclusion/exclusion criteria for study participation other than sex.	Median urinary lithium concentration (P5-P95): 3910 µg/L (270–10400 µg/L)				concentration = **0.17; 95% (CI), −0.32 to −0.015; p=0.032]** and **positively associated with TSH [adjusted β = 0.089; 95% CI, 0.024 to 0.15; p=0.007]**.	being treated for gastritis and 2 for high blood pressure.

Table 39: Summary of epidemiologic studies investigating other endpoints (related to growth, metabolism, male reproductive health, cardiovascular effects and aging)

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Key studies (fulfilling inclusion criteria)							
Chai et al. (2022)	Cohort of young male students (Male Reproductive Health in Chongqing College students) Mean age: 21 years old China N = 666 2-years follow-up	Urine biomarkers of 18 metals collected at baseline and 1-2 years later. Li was detected in 99.0% of the urine samples. Median (Q1, Q3) = 16.5 µg/L (7.76, 30.47) at baseline and 16.69 µg/L (9.6, 27.28) 2 years later.	- **Semen quality** at baseline and 1-2 years later: semen volume, sperm concentration, sperm total number, progressive motility, morphology. - **Sex hormones** at baseline and 1-2 years later: serum estradiol, LH, FSH, testosterone,	Mixed model Covariates were considered mainly based on prior knowledge and published literature.	Urinary creatinine, abstinence periods, age, body mass index, status of tobacco smoking, alcohol consuming, tea drinking	- Higher Li was associated with **lower semen volume** (p=0.007) and **lower progesterone** in single-exposure models but not after controlling for other metals (Q4 vs Q1: **β [95% CI] = -1.1% [-4.09; 1.99%] for semen volume**, not reported of progesterone). - Higher Li was associated with **lower testosterone** (Q4 vs Q1: **β [95% CI] = -6.45% [-10.49; -2.23%], p=0.003**) and this association persists after controlling for other metals.	NOS evaluation: good quality (longitudinal design, multi-pollutants, sample size) No information about urine sampling (first morning void?). Regression coefficient not provided (forest plot only).

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
			prolactin, progesterone				
Zhong et al. (2021)	Wanjiang cohort of adults Area on the Yangtze River, China N = 1303 Follow-up 4-5 years later	Biomarkers: 22 metals in urine. Standardized on creatinine. Not clear if metals were assessed in urines collected at baseline, at the time of follow-up, or both. Median (IQR), Li = 13.55 µg/g creatinine (8.08; 20.84)	**Hypertension** defined by: (1) SBP[63] ≥ 140 mmHg and/or DBP[64] ≥ 90 mmHg or (2) diagnosed with hypertension by physicians or (3) currently using antihypertensive medications	Logistic regression with exposure in quartiles. Single-metal models and multi-metals models. Correction for multiple testing. Confounders identified by a directed acyclic graph. Spline to explore non-linearity. Interaction with covariates or between metals tested. BKMR[65]	Age (continuous), sex (male or female), smoking (yes or no), drinking (yes or no), body mass index (underweight, normal weight, overweight, obesity) and BP[66] at baseline (continuous)	Crude comparison showed **higher Li in hypertensive group (median (IQR) = 14.46 (9.31-21.69) µg/g creatinine** vs. 13.08 (7.59-20.37) µg/g creatinine, p=0.03). Adjusted logistic regression with Li in quartiles **OR[67] [95%CI]:** **- Q2 vs Q1: 1.28 [0.90; 1.84]** **- Q3 vs Q1: 1.45 [1.01; 2.06]** **- Q4 vs Q1: 1.35 [0.94; 1.93]** p-trend=0.08 and p-FDR=0.66.	NOS evaluation: good quality but advanced statistical analysis performed only for 4 metals (that passed the p-value correction) so all details not available for Li, e.g., testing Li in continuous +/- splines + interaction.
Wang et al. (2020)	Shanghai Maternal-Child Pairs cohort Shanghai, China N = 234 Repeated measures across the pregnancy	Biomarkers: 27 metals measured in whole blood collected during the 1st, 2nd, and 3rd trimesters of pregnancy. Median (25-75th) Li = 1.5 µg/L (1.0; 2.0)	**Gestational weight gain (GWG)**: weight and height self-reported at baseline and measured by trained professional during pregnancy. Cytokines: TNF-α,	Linear mixed-effects models with a random intercept to estimate associations between each ln-transformed metal concentrations and GWG or GWG rate over	Maternal age, gestational age, pre-pregnancy body mass index, energy intake in pregnancy, education, household income, physical activity, passive smoking, parity,	**Li associated with GWG (β [95% CI]: 0.10 [0.005; 0.195])**, especially during the 3rd trimester (**β [95% CI]: 0.366 [0.065; 0.666]**). Li in 3rd trimester **positively associated with TNFα** (**β [95% CI]: 0.606 [0.193; 1.018]**, FDR-ajusted p[68]=0,008) and **IL-6 (β [95% CI]: 0.222 [0.087; 0.357]**, FDR-ajusted p=0,004),	NOS evaluation: good quality (good design, repeated measurements during pregnancy, biomarkers, dose-response relation, good statistical analysis)

[63] Systolic blood pressure
[64] Diastolic blood pressure
[65] Bayesian kernel machine regression
[66] Blood pressure
[67] Odds ratios

[68] False discovery rate

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
			IL-6, IL-1β, IL-10, GDF-15 in serum	the course of pregnancy. Correction for multiple testing. Linear regression for cross-sectional associations. Confounders selected based on the univariate analysis and previous literature	gestational anemia, gestational diabetes mellitus, and infant gender	but **negatively with GDF-15 (β [95% CI]: -1.294 [-2.493; -0.096]**, FDR-ajusted p=0,045)	Relatively small sample size. For cytokines, not sure if measured during 3rd trimester only and if the associations reported are with Li during 3rd trimester or overall during pregnancy.
Herlin et al. (2019)	Mother-child cohort Maternal age: 12-41 years old Andean part of the Salta province, northern Argentina N = 169	Biomarkers: maternal and cord whole blood + placenta tissue Median (range) of Li in: - maternal whole blood = 24 µg/L (1.9–145) - placenta tissue = 38 µg/kg (5.2–143) - cord blood = 48 µg/L (9.5–156)	**Telomer length** (TL) in maternal blood leukocytes (late pregnancy), cord blood leukocytes, and placental tissue	Linear regression, p-value correction, muti-pollutant, sex-interaction	Maternal age, education (years at school), and pre-pregnancy body mass index + gestational week at birth for TL in cord blood and placenta + birth weight for TL in cord blood	**No association between Li and TL** (maternal blood, cord blood and placenta) in single-exposure models (e.g., for TL in maternal blood: **β [95%CI]: 0.030 [-0.016; 0.076], p=0.195**). **Higher TL reported with higher Li in maternal blood after considering co-exposure to boron, arsenic and antimony** (**β [95%CI]: 0.107 [0.039; 0.176], p=0.002**). No sex-interaction	NOS evaluation: fair to good quality study. Few confounding factors considered.
Harari et al. (2016)	Mother-child cohort Maternal age: 13-41 years old San Antonio de los Cobres and surrounding nine small villages in the Andean part of the Salta province, northern Argentina N = 178	Blood and urine biomarkers measured once or repeatedly during pregnancy. + levels in drinking water The highest water lithium concentration was observed in the largest village San Antonio de los Cobres (mean 718	Total and albumin-adjusted serum calcium concentrations, during 1st, 2nd and 3rd trimester	Mixed model with random intercept to study calcium trajectory through the pregnancy + linear regression to study 3rd trimester calcium level. + Quantile regression to	Adjusted for gestational age and season of sampling (model 1) + maternal age and urinary arsenic metabolites (model 2) + serum boron (model 3).	Higher exposure to lithium is associated with **lower 25(OH)-Vitamin D3** (**-6.1 nmol/L [-9.5; -2.6] for a 25 µg/L increment in blood lithium, p=0.001**), **lower urine calcium, and lower urine magnesium**. Adjustment for urine albumin attenuated the association between Li and Vitamin D3 but it is still significant.	NOS evaluation: good quality (longitudinal study using biomarkers of exposure during pregnancy, good statistical analysis). Small sample size.

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
		µg/L, range 528–837 µg/L), as compared with 5.0–324 µg/L in the other 9 villages. Median blood lithium concentration: 25 µg/L (range 1.9–145 µg/L) Median urine lithium concentration: 1491 µg/L (range 105–4598 µg/L)		explore the strength of the effect at different level of calcium. + Logistic regression when outcome is dichotomized. Models were adjusted for covariates known to affect the calcium homeostasis or that influenced the estimates more than 10%.	Maternal education level, maternal height, parity, parental monthly income, coca chewing, hemoglobin concentrations, lean body mass, cesium exposure and infections during pregnancy as well as ethnicity did not influence the associations between blood lithium and the outcomes.	Quantile regression shows stronger effect at higher level of Vitamin D3.	
Harari et al. (2015)	Mother-child cohort Maternal age: 13-41 years old Andean part of the Salta province, northern Argentina N = 136	Blood and urine biomarkers measured once or repeatedly during pregnancy. Biomarkers associated cross-sectionally with fetal growth and averaged for birth weight. + levels in drinking water Lithium concentrations in drinking water: 6.5-958 µg/L Median blood lithium concentration: 25 µg/L (range 1.9–145 µg/L)	**Fetal growth** (ultrasound during 2nd and/or 3rd trimester) + birth weight, length, head circumference	Ultrasound: linear mixed models with random slope and intercept. Birth weight, length: linear regression. Exposure both in tertiles and continuous. Counfounders: a priori selection of known risk factors for low fetal or birth size and on results from exploratory stepwise regression	Adjusted for gestational age (+square), parity, family monthly income, maternal height (cm), fetal sex, maternal urinary arsenic (continuous), blood cesium (continuous) and serum boron (< or ≥80 µg/L).	- Fetal growth: no significant association with mixed model. Cross-sectionally, both **blood and urine lithium concentrations seem inversely associated with all fetal measurements**. The strongest association was observed between urine Li level and **lower femur length** during the 2nd trimester (3rd vs 1st tertile: **-0.23 cm [-0.43; -0.042], p=0.018**) - Birth length: Every 25 µg/L increase in blood lithium concentration was associated with a **decrease in birth length: -0.53 cm [-1.0; -0.052]**. Similarly, newborns in the highest tertile of lithium exposure (mean blood lithium 47 µg/L) were 0.81 cm shorter compared with those in the lowest tertile (<18 µg/L).	NOS evaluation: good quality (longitudinal study using biomarkers of exposure during pregnancy, good statistical analysis). Small sample size.

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
		Median urine lithium concentration: 1491 µg/L (range 105–4598 µg/L)					
Studies with limitations							
Wang et al. (2019)	N = 566 participants (352 elderly and 214 children) divided into two groups: "exposed" vs "controls" based on residential region (living closed to a coking plant or in an unpolluted area 70km away) "Exposed" group: - 142 elderly non-smokers (52 men, 90 women) (average age: 61.9 ± 7.6, 79) - 79 elderly smokers (male) (average age: 59.1 ± 7.0) - 148 children (84 boys, 64 girls) (average age: 7.0 ± 1.4) "Control" group: - 96 elderly non-smokers (28 men, 68 women) (average age: 58.8 ± 8.6) - 35 elderly smokers (male) (average age: 59.7 ± 6.9) - 66 children (average age: 7.2 ± 1.4) South of Shanxi province, China	Biomarkers: 17 metals in urine. Standardized on creatinine	**Metabolomic data**	Logistic regression and linear regression analyses No comparison of exposed vs control group, no control for any difference between groups, no justification for stratification by age and smoking status...)	None	Li associated with: - in children: higher nonenedioylcarnitine level (β [95%CI]: 0.51 [0.13; 0.89], p=0.009) - in elderly non-smokers: higher nonanoylcarnitine (0.42 [0.16; 0.68], p=0.001), hydroxydodecenoylcarnitine (0.37 [0.07; 0.67], p=0.015) and dodecanedioylcarnitine (0.57 [0.29; 0.85], p<0.001) - in elderly smokers: higher pyroglutamic acid (0.61 [0.08; 1.14], p=0.028), nonanoylcarnitine (0.50 [0.14; 0.86], p=0.007), octenedioylcarnitine (0.59 [0.16; 1.01], p=0.008), azelaic acid (0.53 [0.08; 0.99], p=0.024) and hydroxytetradecanedioic acid (0.89 [0.08; 1.70], p=0.04)	NOS evaluation: poor quality; no control for any varying factor between the groups
Voors et al. (1970)	Ecological study USA N = 99 cities	Li in water supply	**Arterosclerosic mortality**	Partial correlation between Li and mortality, controlling for water hardness. Stratification by sex and ethnicity	Water hardness	Correlations range from -0.426 (for white males) to -0.215 (for non-white females) and are all significant	Poor quality: ecological study, no control for individual data
Skalny et al. (2018)	N = 208 pregnant women: 50 women with IVF pregnancy and	Biomarker of 25 metals and essential	**Toxic levels in hair of women**	Mann-Whitney U test + linear	No detail about confounders	Crude comparison showed higher hair Li levels in the IVF group	NOS evaluation: poor quality

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
	158 control cases with spontaneous pregnancy Siberian Federal District of the Russian Federation	elements: maternal hair collected during the 3rd trimester of pregnancy and child hair collected at the age of 9 months. Li in controls: median=0.008 ug/g (0.004–0.013) Li in cases: median=0.011 ug/g (0.006–0.020)	**with IVF pregnancy and its association with their children's hair element levels**	regression but no detail exposure distribution…	No data on correlation between metals.	compared to spontaneous pregnancy group. Crude correlation between Li and duration of infertility (rho=0.20, p=0.03)	Selection bias: only pregnant women + exclusion of the most exposed women (exclusion criteria=(i) the presence of metal implants (including dental amalgam fillings), (ii) occupational exposure to heavy metals, (iii) habitation near heavy metal emission sources (heavy industry); (iv) smoking (both former and present); (v) using infant formulas instead of breastfeeding
Kindgren et al. (2019)	Nested case-control study from a mother-child cohort Sweden N = 17,055: - n = 42 cases of Juvenile idiopathic arthritis (JIA-group) (including n = 11 children positive for antinuclear antibodies (ANA-group)) - n = 40 controls (randomly selected, age- and sex-paired)	Biomarkers: 10 metals in cord blood. + fish consumption Median (range) of Li (ug/L): Controls=1.00 (1.00–1.22) JIA=1.00 (1.00–39.40) ANA=2.08 (1.00–39.40)	**Juvenile idiopathic arthritis** (+/- Antinuclear antibodies): medical record linkage + confirmation by paediatric rheumatologists in local hospitals	Logistic regression to study the association between fish consumption and JIA Mann-Whitney/Student to study the metals	Heredity for rheumatism (JIA or rheumatoid arthritis in first- and second-degree relatives), parity, mode of delivery, preterm birth, duration of (exclusive and total)	Higher cord blood Li levels in ANA-group than in controls Higher cord blood Li levels in JIA-group than in controls (higher mean but not higher median), no detail about the test/the distribution…)	NOS evaluation: good quality Good study design, data collection, etc. but poor statistical analysis.

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
					breastfeeding, introduction of formula, parental age, education, smoking habits, and whether or not the parents were born outside Sweden		
Karakis et al. 2021	Birth cohort Study sample n = 111 (initial cohort: n = 1823)	Biomarkers: urinary concentrations of 25 metals at delivery. Geometric mean Li = 9.64 ppb (p40 = 8.15 and p60 = 11.2)	Medical records: **preterm delivery and small-for-gestational age (SGA), malformations, asthma and cardiovascular morbidity, behavioral and developmental disorders, obesity, and malignancies**	Poisson regression, exposure categorized into quintiles but considered as a continuous variable. Relative Risk reported without confidence interval (but reporting standard deviation)	Maternal age, parity, newborn gender, and preterm birth	**Prenatal exposure to Li associated with higher risk of behavioral and developmental disorders**: - preterm delivery=0.92 [-0.18; 2.02] - asthma=1.01 [-0.66; 2.68] - cardiovascular=1.09 [-0.23; 2.41] - behavioral or developmental=1.82 [1.8; 1.84] - obesity=0.85 [-0.07; 1.77] - malformations=1.00 [-0.96; 2.96]	NOS evaluation: poor quality Small sample size, rare and heterogeneous outcomes, limitations in statistical analysis
Fajardo et al. (2018)	Ecological study Texas counties N = 234 counties (n = 135 with data on suicide)	Indirect: water samples from public wells Mean Li levels in the Texas counties: 0.003-0.539 mg/L	Age-adjusted **all-cause mortality** and years of potential life lost	Correlations + partial correlation controlling for suicide mortality and socioeconomic status	Adjustment for suicide mortality and socioeconomic status: median household income, % of unemployment, and % of adults with college education	Negative correlation between Li and all-cause mortality (rho=-0.26) and lower number of years of life lost (rho=-0.17). No longer significant for all-cause mortality after adjusting for suicide mortality (but limited to a sub-population with available data)	Poor quality: ecological design, limitations in statistical analysis

17.2 Second literature search

Table 40: Summary of epidemiologic studies from the second literature search investigating autism spectrum disorder

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Supportive studies (fulfilling inclusion criteria) of the key study from Liew et al. (2023) (table 37)							
Wu et al. (2022)	Cross-sectional case-control study China Children between 2 and 8 years old Cases: 92 children, comprising 78 boys and 14 girls, were enrolled with ASD from autism rehabilitation institutions in Beijing Controls: 103 typically developing individuals (92 boys and 11 girls, aged 2–8 years) from kindergartens in Beijing.	Serum concentrations of 9 chemical elements (lithium, calcium, iodine, iron, magnesium, potassium, selenium, strontium and zinc -> cross sectional (exposure measured after diagnosis)	**ASD diagnosis:** Children who scored 30 or more points on the Childhood Autism Rating Scale (CARS)	Logistic regression model The participants were divided into low-, medium-, and high-level groups based on the tertiles of the element concentrations in the control group.	Yes (some): Adjusted for: age (continuous), sex, family history of dementia, low intelligence, or any other known mental problems, and history of intestinal disorders caused by antibiotic use.	Univariate analysis (Mann-Withney test): Li concentrations were lower in cases compared to controls / Lower Li associated with increased severity score among cases. Multivariate analysis: no significant association with Li while some associations reported for other elements (zinc, potassium, strontium, selenium)	Strength: Li measured in serum Weaknesses: cross-sectional study
De Palma et al. (2012)	Cross-sectional case-control study Verona, Italy Cases: 44 children with diagnosis of autism (37 males), mean aged 9.00 ± 4.05 years Controls: 61 healthy children (25 males), mean aged 8.4 ± 3.1 years (age-balanced controls (but not sex))	Concentrations of metallic elements including lithium in hair	**ASD diagnosis:** children consecutively attending the Centre for Diagnosis, Research and Therapy of Autism in Verona, Italy. Diagnosis by a skilled physician specialized in child neurology and psychiatry, following the DSM IV diagnostic criteria	Adjusted analyses for age, gender	None	Unadjusted comparisons showed higher concentrations of molybdenum, lithium and selenium in autistic children. No associations after adjustement for age and gender.	Limited number of confounders considered (gender and sex), sex not balanced between ASD cases and controls, cross-sectional study

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Zhang et al. (2022)	Age- and gender-matched cross-sectional case-control study China Cases: 30 children diagnosed with ASD Controls: 30 typically developing children Recruitment from the Maternal and Child Health Hospital of Baoan in Shenzhen, China Age: 2 to 6 years	23 trace elements including lithium in fasting blood	**ASD diagnosis**	Shapiro–Wilk test or Wilocoxon-Mann-Whitney test (if non normal distribution). No adjustment	None, but cases and controls were matched on sex and gender	Decreased Li in serum in cases (median = 19.31 µg/L) compared to controls (median = 28.18 µg/L), p-value < 0.0001	Limited number of cases and controls (N = 30 in each), unadjusted comparaison but age and sex were similar in both groups Cross-sectional study
Skalny et al. (2017)	Age-matched cross-sectional case-control study Moscow, Russia Boys only, matched on age: 33 with communication disorders (CD) / 33 with ASD / 33 neurotypical boys Age: 3-8 years Exclusion criteria: In order to prevent the influence of side factors on hair trace element content, the following exclusion criteria were used: the presence of neuropsychiatric disorders (except CD and ASD for the respective groups) including abnormal eating behaviors (ICD-10: F50), vegetarianism, endocrine disorders, metallic implants (including dental fillings), acute traumas and	Hair levels of trace elements including Li	**ASD diagnosis** verified in the scientific Center for Mental Health, Russian Academy of Medical Sciences (Moscow, Russia)	Mann-Whitney U test to compare median	None	No difference in Li concentration between cases and controls	Statistical analyses not adjusted, cases and controls matched on age, only boys included, cross-sectional study

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
	inflammatory diseases, and the use of mineral supplements.						
Skalny et al. (2017)	Sex and age-matched cross-sectional case-control study Moscow, Russia 74 ASD children and 74 sex and age-matched controls divided into two age groups (2–4 and 5–9 years, number of cases > 30 in both group) Mean age (ASD and controls resp.): 3.20 ± 0.87, 3.17 ± 0.92 years (first age group); 6.85 ± 1.90, 6.85 ± 1.81 years (second age group) Same exclusion criteria as Skalny et al. (2017)	Hair content of trace elements including lithium	**ASD diagnosis:** ASD children diagnosed in Scientific Center for Mental Health, Russian Academy of Medical Sciences (Moscow, Russia).	Mann-Whitney U test to compare median	None	No difference in Li concentration between cases and controls in the whole cohort, nor as in the 2 age groups	Statistical analyses were not adjusted Cross-sectional study
Skalny et al. (2020)	Cross-sectional case-control study Boys only, 53 ASD (5.18 ± 1.00 years old) and 52 controls (5.13 ± 1.05 years old), height and weight matched Exclusion criteria: presence of other neuropsychiatric disorders; endocrine disorders; metallic implants (including dental amalgam fillings); using trace element-enriched shampoos and hair care products; habitation near sources of industrial metal exposure; exposure to passive smoking; acute infectious, surgical and traumatic diseases.	Li in hair collected while children were already diagnosed	The **diagnosis of ASD** (ICD-10: F84.0) was extracted from the clinical record of the outpatient department. ASD was also diagnosed using ICD-10 criteria (impaired development before the age of 3 years; impairment in social interaction; abnormalities in communication; restricted, repetitive, and stereotyped behavior and	Mann-Whitney U test	None	No significant group difference in age, height, or weight values between the groups were observed. No difference in Li concentration between groups (no adjustment for confounders).	Statistical analyses were not adjusted Only boys included Cross-sectional study

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
			interests; being not attributable to other pervasive developmental disorders). In addition, ASD was also verified by Childhood Autism Rating Scale (CARS). Only newly diagnosed children with ASD before specific treatment were involved in the present study.				
Studies with limitations not included in the analysis (low sample size (< 30 cases), no adjustment for confounders)							
Adams et al. (2006)	Cross-sectional case-control study Arizona, USA Children with autism spectrum disorders (n=51) (7.1 ± 3.0 years old), a subset of their mothers (n=29), neurotypical children (n=40) (7.5 ± 3.0 years old), and a subset of their mothers (n=25). Control recruitment: Parents of the participants with ASD asked friends and neighbors to act as controls for the study.	Toxic metals and essential mineral (including Li) in hair	**ASD diagnosis** by a psychiatrist or developmental pediatrician of Autism Spectrum Disorder (ASD), including autism, PDD/NOS, and Asperger's syndrome.	Unmatched t-test	None, cases and control were not matched either	Average Li lower among cases of ASD; difference only significant when restricted to children aged 3-6 years (23 ASD and 26 controls)	There was an attempt to match the ages and genders as closely as possible -> but it was just an attempt, it was not successfull
Wegmann et al. (2023)	Cross-sectional case-control study Sweden	Measurement of metal levels (Al, Cd, Hg, Li, Pb and Zn) in the cord blood of newborns and in the serum of	**ASD** cases identified from The Swedish National Patient Register (ICD codes)	Bayesian multivariate log-normal model	None	Larger standard deviations of Al and Li in the ASD group	Number of cases < 30

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
	For cord blood exposure: 20 ASD cases and 40 controls For the serum at 5-year-old: 11 ASD and 24 controls. Samples from a biobank related to All Babies in Southeast Sweden (ABIS) registry	the same children at 5 years of age					
Wecker et al. (1985)	Case-control study 12 ASD (boys) and 22 controls New Orleans area Only males	Li in hair	ASD cases: The experimental groups were comprised of children enrolled at the Louisiana State University Medical Center Therapeutic Nursery School. Controls: The control group consisted of children from throughout the metropolitan New Orleans area.		None	The concentration of lithium was significantly greater in samples from autistic children as compared to the normals (p=0.042)	Number of cases < 30
Domingues et al. (2016)	Case-control study Italy Cases: 29 ASD children (26 males and 3 females) (mean age: 7.3 years) Controls: 36 (26 males and 10 females) (mean age: 8.4 years) Recruitment by the Laboratorio NovEra srl, Civitanova Marche, Italy.	Concentrations of 41 elements including Li in hair	Clinical information was obtained from medical records supplied by parents. All subjects had been evaluated for DSM-IV-TR classification and assignment of ADOS parameters.	t-test or Mann-Whitney test	None	No difference in Li concentration between cases and controls	Number of cases < 30

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Zhang et al. (2021)	Meta-analysis studies included in the meta-analysis: Al-Farsi et al. 2013 (27 children with ASD), Wecker et al. 1985 (N=12 ASD)					In autistic children, the overall levels of barium (Ba), mercury (Hg), lithium (Li), and lead (Pb) were higher. The levels of Hg, Li, Pb and selenium (Se) in the hair of autistic children were higher than those of healthy children.	The mata-analysis only included 5 studies, some of them did not pass the inclusion criteria, e.g. number of cases < 30.

Table 41: Summary of epidemiologic studies from the second literature search investigating male reproduction

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Key studies (fulfilling inclusion criteria)							
Wu et al. (2021)	Human sperm bank China, 2019 n=622 men (n=2314 semen samples) Ages: adults	Li in air: Daily concentrations of PM2.5 and its constituents (including Li) were obtained from three monitoring stations. The median concentration of Li during 0–90 days before the semen collection date, based on the nearest monitoring station was calculated to assess individual exposure level. Median level: 0.44 ng/m3 +/- 0.19	**Semen quality**: semen volume, sperm count, concentration, and motility.	Quantile regression to estimate exposure-response associations over the entire distribution of each semen quality parameter from the 10th through 90th percentiles, with a 10% increment: model 1 not adjusting for PM2.5 in order to estimate the constituent-specific effect,	adjusted for age, BMI, ethnic, education, marital status, childbearing history, career, percent of body fat, smoking status, drinking status, abstinence period, ambient temperature and season	No associations between Li and sperm concentration and sperm count. Li associated with a decrease in sperm motility (total and progressive motility) at some specific percentiles of the distribution (60th and 70th percentiles) in model 2	Good quality study but limited to Li exposure through air pollution.

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
				and model 2 adjusting for PM2.5 to estimate the constituent-specific effect holding other constituents constant			
Studies with limitations							
Karabulut et al (2022)	Cross-sectionnal study Turkey, 2022 n=113 men consulting IVF center Ages: 18-52 years old	Biomarkers: 24 metals in seminal plasma Li levels in seminal plasma: mean +/- sd = 40.15 ng/mL +/- 70.16; median = 6.91 ng/mL	Participants divided into 2 groups according to **semen quality** (volume, concentration, motility): group 1 (n=60) with normal semen parameters, and group 2 (n=53) with at least one abdnormal semen parameter according to the WHO criteria	Univariate analysis comparing the levels of the 24 metals in the two groups.	None	No difference in mean seminal Li levels between the two group (p-value = 0.28): mean Li in group 1 = 32.89 ng/ml ± 62.85 and mean Li in group 2 = 48.16 ± 80.26.	NOS evaluation: Low quality due to no adjustment for any confounders. Mean and median Li are different suggesting a non normal distribution but no information was reported about the test used to compare the exposure levels between the two groups.

Table 42: Summary of epidemiologic studies from the second literature search investigating cardiovascular health

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Studies with limitations							
Enderle et al. (2020)	Cross-sectional study Northern Germany, 2010-2012 n=928 Ages: 18-80 years old	Biomarkers: Li plasma levels Blood samples collected at the time of the visit Median (Q1-Q3) Li levels = 0.96 µg/L (0.70, 1.37)	From clinical examination: - **anthropometric measurements**: BMI, waist-to-hip ratio - **blood pressure**: systolic and diastolic, hypertension - **type 2 diabetes**: HbA1c, plasma glucose - **estimate glomerular filtration rate** (eGFR) - other biological markers: high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, C-reactive protein (CRP), Glutamate-Oxalacetate-Transaminase (GOT), Gamma-Glutamyl-Transferase (GGT) and Glutamate-Pyruvate-Transaminase (GPT)	Mostly univariate analyses with Li treated in tertiles (T1: <0.78 µg/L; T2: 0.79 to 1.21 µg/L; T3: >=1.22 µg/L) Multivariate analysis: Least Angle Regression (LAR) algorithm to identify the best predictors of Li levels. Splines to evaluate the dose-response between the predictors selected by LAR and Li levels	The following variables were considered as potential predictors of Li in the LAR model: Age, sex, waist circumference regressed on BMI, systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, triglycerides, HbA1c, eGFR, CRP, GOT, GGT, GPT, physical activity, education, smoking status and daily alcohol intake	Univariate analyses by tertile of Li with p<0.05: - Body mass index (kg/m^2): T1=28.2 ± 5.4, T2=26.9 ± 4.4, T3=27.3 ± 4.8 (p-value=0.004) - prevalent hypertension: T1=64%, T2=58%, T3=67% (p-value=0.04) - HbA1c (%): T1=5.65 ± 0.60, T2=5.73 ± 0.54, T3=5.82 ± 0.67 (p-value=0.002) - Prevalent diabetes: T1=6%, T2=7%, T3=12% (p-value=0.01) - HDL (mg/dL): T1=62.7 ± 16.8, T2=66.6 ± 18.6, T3=65.1 ± 18.6 (p-value=0.03) - LDL (mg/dL): T1=134.8 ± 33.2, T2=131.4 ± 31.7, T3=127.9 ± 35.1 (p-value=0.04) - eGFR (mL/min/1.73 m^2): T1=89.0 ± 14.3, T2=84.1 ± 14.1, T3=80.2 ± 16.4 (p-value <0.0001) - Creatinine (mg/dL): T1=0.87 ± 0.17, T2=0.88 ± 0.16, T3=0.91 ± 0.21 (p-value=0.02) Multivariate analysis: 5 predictors were selected including age, smoking status, alcool consumption, eGFR and diastolic blood pressure. Both eGFR and diastolic blood pressure were associated with lower Li levels: decreased plasma lithium concentration (µg/L) by -0.0069 and by -0.0016 per 1-unit increment in eGFR and diastolic blood pressure, respectively.	NOS evaluation: poor quality due to cross-sectional design. Study aiming at exploring the predictors of Li plasma levels. Potential confounders are not well controlled since considered as potential predictors.

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Skalny et al. (2017)	Case-control study Russia n=42 men (21 acute ischemic stroke patients and 21 controls) Age: 50 to 67 years old	Biomarkers: 15 trace elements, including Li, measured in blood serum (median Li = 0.0021 µg/mL in cases and 0.0017 µg/mL in controls). Effect biomarkers: thyroid, steroid, gonadotropin hormones, and stroke risk and brain damage markers Blood collection perfomed 1 day after diagnosis for cases; no information about timing of blood collection for controls.	**Ischemic stroke** diagnosed by medical doctor (neurologists). No information about the source population to select the controls. A number of exclusion criteria: endocrine pathology or hormonal therapy; neurodegenerative diseases; positive smoking status; metal implants; occupational exposure in heavy industry in the past; excessive alcohol consumption; acute infectious and traumatic diseases; vegetarian diet; administration of mineral supplements.	Univariate analyses: (1) comparison of effect biomarkers between cases and controls, (2) comparison of trace element levels between cases and controls, (3) correlation between trace elements and effect biomarkers significant in (1). Multivariate analysis: (4) multivariate linear regressions for effect biomarkers significant in (1), including all trace elements as independent variables (but with no other adjustment).	None but cases and controls were matched for age and BMI at enrolment. Multivariate regressions adjusted for co-exposure to other trace elements.	(1) Univariate analyses showed significantly lower TSH levels and higher total and free T3, anti-TPO-Ab, prolactin, and cortisol levels in cases than in controls. (2) Univariate analysis showed **higher Li levels in cases (0.0021 µg/mL [0.0018–0.0032]) than in controls (0.0017 µg/mL [0.0013–0.0022]), p-value=0.046.** (3) Crude correlations between Li and effect biomarkers that differed between cases and controls showed positive and significant correlations between Li levels and TSH (rho=0.40), free T3 (rho=0.70), and Anti-TPO-Ab (rho=0.36) but no correlation with total T3, prolactin, cortisol, and brain damage and stroke risk biomarkers. (4) the multivairate regressions confirm the associations between **higher Li levels and higher TSH (beta=0.63, p-value=0.024) and higher free T3 (beta=0.58, p-value=0.041)**	NOS evaluation: fair quality with limitations due to the small sample size (n=21 cases and n=21 controls) and no adjustment for potential confounders (although cases and controls were comparable in terms of age and BMI, and a number of exclusion criteria may prevent the risk of confounding).
Ilyas et al. (2017)	Case-control study Pakistan n=70 cases with valvular heart disease and n=66 controls Ages: 16-76 years old	Biomarkers: 14 trace elements including Li measured in blood Median Li: 0.325 µg/g wet weight in cases vs 0.281 µg/g	**Valvular heart disease** diagnosed by a medical doctor. No information about the source population to	Univariate analysis to compare Li levels in cases and controls. Some additional plots to describe Li levels in cases	None. More vegetarians and less smokers in cases than in controls	Significantly higher Li levels in cases (0.325 µg/g wet weight) than in controls (0.281 µg/g wet weight). Also observed by age groups.	NOS evaluation: poor quality due to no adjustment for confounders and small sample size.

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
		wet weight in controls	select the controls.	and controls, stratified by other characteristics (gender, urban vs rural habitat, diet, smoking habits, and occupation). + PCA and clustering to identify metal exposure profiles?			
Dawson et al. (1978)	Ecological study USA, 1968 (24 Texas communities) Age: 5-97 years old	7 metals including Li measured both in drinking tap water and urine samples of volonteers from each community.	**Mortality rate** over 45 years old: Central nervous system (CNS), arteriosclerotic and degenerative diseases of the heart (ADH), hypertension with heart disease (HH), other diseases of the heart (OD), hypertension (H), and general arteriosclerosis (GA)	Product-moment correlation	None	Positive correlation between Li measured in urine and in drinking tap water (rho=0.41, p-value=0.02). Negative correlation between Li in drinking tap water and ADH (-0.42, p<0.01), HH (-0.47, p<0.001), and H (-0.44, p<0.01) mortality. Negative correlation between Li in urine and HH (-0.38, p<0.01), and H (-0.37, p<0.01) mortality. Positive correlation between Na/Li ratio in drinking water and HH (0.42, p<0.01), and H (0.50, p<0.001) mortality. Positive correlation between Na/Li ratio in urine and H (0.37, p<0.02) mortality.	NOS evaluation: poor quality study due to its ecological study design that didn't allowed to control for individual characteristics.

Table 43: Summary of epidemiologic studies from the second literature search investigating female reproduction

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
Key studies (fulfilling inclusion criteria)							
Gonzalez-Martin et al. (2023)	Cohort USA, 2018-2019 n=60 women with IVF treatment Mean age = 33 years old	Biomarkers: 8 trace elements, including Li, measured in urine, follicular fluid, and blood plasma collected on the day of the oocyte retrieval. Geometric mean Li in urines: 23.3 ng/mL Geometric mean Li in follicular fluid: 1.34 ng/mL Geometric mean Li in plasma: 5.91 ng/mL	**Ovarian response and reproductive outcomes**: anti-Müllerian hormone (AMH), trigger day Estradiol, number of retrieved oocytes, relative proportion of mature oocytes, fertilized embryos, blastocysts and euploid embryos + implantation, clinical pregnancy, live birth and reproductive goal	Logistic regression accounting for potential confounders	Adjusted for age, BMI, race/ethnicity, and smoking status	Negative associations between Li in follicular fluid and number of oocytes retrieved (Mean differences (95% CI) between p20 vs. p80 = 0.82 (0.68, 0.98); p trend = 0.03), relative proportion of matured oocytes (0.80 (0.67, 0.95), p trend = 0.015), and fertilized embryos (0.78 (0.65, 0.94), p trend = 0.011). Li in urine associated with lower probability of a live birth (OR 0.33; 95% CI: 0.11, 0.89; p value = 0.036)	NOS evaluation: good quality study but with small sample size and lack of details about the statistical analysis.
Studies with limitations							
Syrkasheva et al. (2021)	Cross-sectional study Russia, 2017-2018 n=30 women who applied for assisted reproductive technologies (ART) infertility treatment Age: 18-39 years old	Biomarkers: 31 chemicals measured in blood, including Li	**Hormonal parameters**: anti-Müllerian hormone (AMH) and free thyroxine T4 blood levels	Crude correlation	None	Negative correlation between Li and AMH (rho=-0.37, p-value=0.046)	Low quality due to small sample size and no adjustment for potential confounders
Sun et al. (2019)	Case-control study China, 2017 n=114 controls, n=145 with fallopian tube obstruction, and n=89 with polycyclic ovary	Biomarkers: 13 trace elements measured in follicular fluid collected on the day of the oocyte retrieval.	**Reproductive outcomes**: Number of retrieved oocytes and high quality embryos rate	Univariate comparison of Li levels in the 3 groups of women. Multivariate	None	No difference in Li measured in follicular fluid of controls (Li = 2.78 ug/L ± 0.67), women with PCOS (Li = 2.75 ± 0.52 µg/L), and women with fallopian tube obstruction (Li=2.24 ± 0.56 µg/L)	Poor quality study reporting results for copper only (crude mean comparison for Li with no

References	Study design (population (age), country, number of subjects)	Exposure assessment (direct/indirect), time of exposure, median exposure level	Health outcome (self-reported, diagnosis by medical doctor, biomarkers)	Statistical analysis and adjustment factors	Confounders	Results	Study quality, risk of biais
	syndrome (PCOS)	Mean Li in follicular fluid of controls: 2.78 ug/L		analysis performed for copper only.			control for any confounders)

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Annex 3: Environmental hazard

19 Search methodology for the literature search

Bibliographic databases searched for relevant studies: PudMed and Scopus
Searches were run on 22 August 2023.
Search equations were as followed:

PubMed:

((lithium[Title/Abstract] OR "lithium carbonate"[Title/Abstract] OR "lithium chloride"[Title/Abstract] OR "lithium hydroxide"[Title/Abstract]) AND (ecotox*[Title/Abstract] OR toxic*[Title/Abstract] OR hazard[Title/Abstract] OR mortality[Title/Abstract] OR fate[Title/Abstract] OR bioaccumulation[Title/Abstract] OR biodegradation[Title/Abstract] OR persistance[Title/Abstract])) AND (animal[Title/Abstract] OR fauna[Title/Abstract] OR mammal*[Title/Abstract] OR wildlife[Title/Abstract] OR vertebrat*[Title/Abstract] OR aquatic[Title/Abstract] OR marine[Title/Abstract] OR freshwater[Title/Abstract] OR fish[Title/Abstract] OR fishes[Title/Abstract] OR amphibia*[Title/Abstract] OR mollusc[Title/Abstract] OR coral[Title/Abstract] OR zebrafish[Title/Abstract] OR "danio rerio"[Title/Abstract] OR "fathead minnow"[Title/Abstract] OR "rainbow trout"[Title/Abstract] OR medaka[Title/Abstract] OR mussel[Title/Abstract] OR carp[Title/Abstract] OR frog[Title/Abstract] OR "sea urchin"[Title/Abstract] OR snail[Title/Abstract] OR "terrestrial organisms"[Title/Abstract] OR bird[Title/Abstract] OR duck[Title/Abstract] OR quail[Title/Abstract] OR insect[Title/Abstract] OR chiron[Title/Abstract] OR gastropod[Title/Abstract] OR arthropod[Title/Abstract] OR reptile[Title/Abstract] OR worm[Title/Abstract] OR nematode[Title/Abstract] OR eisenia[Title/Abstract] OR hare[Title/Abstract] OR bee[Title/Abstract] OR flora[Title/Abstract] OR algae[Title/Abstract] OR alga[Title/Abstract] OR seaweed[Title/Abstract] OR invertebrat*[Title/Abstract] OR crustacea[Title/Abstract] OR crustacean[Title/Abstract] OR daphni*[Title/Abstract] OR macroorganism[Title/Abstract] OR microorganism[Title/Abstract] OR larva[Title/Abstract] OR larval[Title/Abstract] OR embryo[Title/Abstract] OR sediment[Title/Abstract] OR soil[Title/Abstract] OR stickleback[Title/Abstract] OR "sheephead minnow"[Title/Abstract] OR xenopus[Title/Abstract] OR mallard[Title/Abstract] OR bobwhite[Title/Abstract] OR anas*[Title/Abstract] OR colinus*[Title/Abstract] OR api*[Title/Abstract] OR bumble*[Title/Abstract] OR typhlodromus[Title/Abstract] OR aphidius[Title/Abstract] OR collembol*[Title/Abstract] OR folsomia[Title/Abstract] OR springtail[Title/Abstract] OR mite*[Title/Abstract] OR hypoaspis[Title/Abstract])	280 results

Scopus:

TITLE-ABS-KEY(lithium OR "lithium carbonate" OR "lithium chloride" OR "lithium hydroxide") AND TITLE-ABS-KEY(ecotox* OR toxic* OR hazard OR mortality OR fate OR bioaccumulation OR biodegradation OR persistance) AND TITLE-ABS-KEY(animal OR fauna OR mammal* OR wildlife OR vertebrat* OR aquatic OR marine OR freshwater OR fish OR fishes OR amphibia* OR mollusc OR coral OR zebrafish OR "danio rerio" OR "fathead minnow" OR "rainbow trout" OR medaka OR mussel OR carp OR frog OR "sea urchin" OR snail OR "terrestrial organisms" OR bird OR duck OR quail OR insect OR chiron OR gastropod OR arthropod OR reptile OR worm OR nematode OR eisenia OR hare OR bee OR flora OR algae OR alga OR seaweed OR invertebrat* OR crustacea OR crustacean OR daphni* OR macroorganism OR microorganism OR larva OR larval OR embryo OR sediment OR soil OR stickleback OR "sheephead minnow" OR xenopus OR mallard OR bobwhite OR anas* OR colinus* OR api* OR bumble* OR typhlodromus OR aphidius OR collembol* OR folsomia OR springtail OR mite* OR hypoaspis)	2689 results

2969 scientific papers were retrieved and downloaded in the electronic reference management software Endnote and reference duplicates were removed (273). Afterwards, the remaining references (2696) were imported into the web-tool Rayyan to organise the review. These references were screened on title and abstract considering the following inclusion/exclusion criteria (Table 44).

Table 44: Literature search inclusion and exclusion criteria

Publication type	IN	Primary research studies, reviews
	OUT	Secondary studies (e.g. editorials, conference, commentary)
Language	IN	English, French
	OUT	Other languages
Study design	IN	Toxicological studies on environmental species
	OUT	Toxicological studies on human Toxicological studies on rat/mice In vitro studies Non toxicological studies Wrong drug Studies about trace elements concentrations in species, soil, water

Grey literature (e.g., reports by national agencies) were also included in the literature search.

20 Environmental hazard

20.1 Aquatic compartment (including sediment)

20.1.1 Fish

20.1.1.1 Short-term toxicity to fish

Data from the registration dossiers

Three studies following the OECD TG 203 are reported in the registration dossier (Unnamed 1996, Unnamed 1997, Unnamed 2010). They respectively tested the acute toxicity of Li_2CO_3, LiCl and $LiOH*H_2O$ on two fish species under static exposure conditions. They respectively reported 96h-LC_{50} values of 5.69, 25.9 mg Li/L (measured concentrations) for rainbow trout (*Oncorhynchus mykiss*) (Unnamed 1996 and Unnamed 1997 resp.) and 18.0 mg Li/L (nominal concentration) for zebrafish (*Danio rerio*) (Unnamed 2010). The registrants judged the three tests to be Klimisch reliability 1. However, the studies from Unnamed 1996 and Unnamed 2010 showed methodological limitations that may affect the results of the tests (unstable pH and exceeding expected normal pH assay range comprised between 6.0-8.5 as recommended in the OECD TG 203, no information on water hardness). The difference in 96h-LC_{50} values observed for rainbow trout could be explained by an effect of the pH due to the different lithium salts tested in both studies. In the study from 1997, LiCl was tested and the pH of the solution was relatively stable whereas in the study from 1996 (Unnamed 1996), Li2CO3 was tested and pH increased with increasing concentrations of the test substance. This pH increase may be due to the dissociation of Li2CO3 into Li+ and CO_3^{2-}, this latter hydrolising to yield HCO3- and OH- leading to an increase in pH. Thus, it is questionable if the highest toxicity observed in the study from 1996 could be due to this increase in pH, rather than the presence of lithium.

Overall, based on the data available in the registration dossier, only one study with no methodological limitations may be retained regarding acute toxicity of lithium to fish with a 96h-LC50 of 25.9 mg Li/L for rainbow trout.

Data from literature search

Literature search provided few short-term data (i.e. mortality data) on fish that may be used in a regulatory hazard assessment and further risk assessment. Indeed, according to ECHA guidance R7b, "*acute toxicity related to waterborne exposure is generally expressed in terms of a concentration which is lethal to 50% of the test organisms*". Other publications were identified but they investigated endpoints (fish developmental effects, circadian rhythm, visual function, gene expression, locomotion, haematological and biochemical effects, oxidative damage and inflammatory response) or developmental stages (embryo, larvae) that may not be used for assessing acute toxicity to fish in the frame of a regulatory assessment. Thus, they are described separately.

- **Relevant publications in a regulatory purpose**

The studies are presented first and compared one to another later in the paragraph.

Kszos et al. 2003 evaluated the toxicity of lithium to *Pimephales promelas* (fathead minnow), *Ceriodaphnia dubia* and a freshwater snail (*Elimia clavaeformis*). Results regarding *Ceriodaphnia dubia* and *Elimia clavaeformis* are described in the section 19.1.2 dedicated to aquatic invertebrates. *Pimephales promelas* larvae were exposed to LiCl for 7 days in dilute mineral water (DMW) (0.32 (measured), 0.93 (measured), 1.87 (measured), 3 (nominal), 4 (nominal) mg Li/L) or East Fork Poplar Creek water (EFPC) (0.36 (measured), 0.98 (measured), 1.90 (measured), 3.0 (measured), 4 (nominal) mg/L). The pH was assessed daily for the control water only; detailed values were not reported. The results showed that lithium in DMW was 5 to 10 times more toxic than in EFPC water (no EC50 reported in the publication). For lithium in DMW, the concentrations inhibiting 25% or 50% of the fish growth were 0.38 mg Li/L and 0.57 mg Li/L respectively. For lithium in EFPC water, these concentrations were 1.99 and 2.47 mg Li/L respectively. The authors hypothesized that this difference of toxicity between DMW and EFPC water is due to their content of Na, 2.8 and 17.4 mg/L respectively, as they showed that lithium was better tolerated when more Na was present. eMSCA agrees that lithium toxicity in the environment appears to depend on the presence of other salts.

Kszos et al. 2003 also published a review providing a case-example evaluating the toxicity of Li-contaminated groundwater on fathead minnow and *Ceriodaphnia dubia*. The results regarding the latter species are described in the section 19.1.2 dedicated to aquatic invertebrates. Regarding fathead minnow, a short-term toxicity test was conducted with larvae (≤48h old at initiation) using a method very similar to "EPA method 1000.0, Fathead minnow, *P. promelas*, larval survival and growth test" (EPA 1994). The larvae were exposed to various concentrations of groundwater treatment facility (GWTF) effluent (control, 3, 6, 12, 25, 50 and 100%) that was non-treated or resin-treated to remove lithium. The 25% concentration of the non-treated GWTF effluent contained 3.1 mg/L Li; the 100% concentration of the treated GWTF effluent contained 1.62 mg/L Li. The control water had an average pH of 7.9. It was observed that the removal of lithium from the effluent clearly reduced the effluent's toxicity. Although minnow survival in the controls associated with the treated effluent (62.5%) was less than acceptable per EPA methods (EPA, 1994), the differences in survival and growth in the treated versus non-treated effluent clearly show the benefits of removing lithium.The NOECs for fathead minnows in the non-treated and treated effluent were 6% and 12%, respectively (no corresponding lithium concentration was reported). The review also reported the toxicological values from other studies but their experimental conditions were not provided and should be examined to assess their reliability: LOEC (10d, juvenile survival, rainbow trout): 0.6 mg/L Li; LC50 (96h,

fathead minnow, embryo-larval): 42 mg/L Li; LC50 (96h, *Morone saxalitis* (1.8 g)): >105.0 mg/L Li; embryo formation (*Fundulus heteroclitus*): 1.7 mg/L Li.

Hamilton (1995) investigated the acute toxicity of boron, lithium (as lithium chloride), selenate, selenite, uranium, vanadium and zinc to early stages of development (swimup fry, juveniles and larger juveniles) of Colorado squawfish (*Ptychocheilus lucius*), razorback sucker (*Xyrauchen texanus*), and bonytail (*Gila elegans*) in a reconstituted water quality simulating the middle Green River of Utah. Dissolved oxygen and pH were measured at the beginning and end of the tests in the control, low, medium and high treatments with live fish present. Dissolved oxygen concentrations were maintained at or greater than 40% saturation in most tests. The pH of the test solutions ranged from 7.0 to 8.5 at 96h of exposure. For Colorado squawfish, the LC50(96h) values were 17, 28 and 41 mg/L Li, respectively for each stage of development (swimup fry, juveniles and larger juveniles). For razorback sucker, corresponding LC50(96h) values were 25, 53 and 186 mg/L Li. For bonytail, corresponding values were 22, 62 and 65 mg/L Li. Thus, the LC50(96h) geometric mean calculated based on these individual LC50(96h) values was 42 mg/L Li. For the 3 species studies, the swimup life stage was more sensitive to lithium than the 2 older life stages. Additionnally, the values of LC50 at this stage are rather similar when razorback sucker larger juveniles appear much less sensitive than the two other species at the same stage.

The review from Aral et al. (2008) reported the results from Lenntech 2007 and US EPA 2008 indicating a LC50 of 9.2-62 mg/L LiCl (1.5-10 mg/L Li) for the white cloud mountain minnow (*Tanichthys albonubes*) (no information provided on developmental stage) following 48h exposure. It is to be noted that the study was funded by Talison Minerals, which is the world's largest lithium mineral producer in Australia. Moreover, these data were reported with no detail on the experimental conditions of the tests that are required to assess the reliability.

ThangaMalathi et al. 2020 investigated the acute toxicity of lithium and its toxicological effects on survival, physiological, haematological and biochemical parameters of the widely consumed spotted sneak head *Channa punctatus* and Nile Tilapia *Oreochromis niloticus*. The fishes were exposed for 96h to the following LiCl concentrations: 0, 80 mg/L (the study reports the following information: Li as 0.29 mg/L and 0.22 mg/L without explaining why there are 2 values), 100 mg/L (Li as 0.32 mg/L and 0.42 mg/L), 120 mg/L (Li as 0.53 mg/L and 0.44 mg/L) and 150 mg/L (Li as 0.61 mg/L and 0.13 mg/L). The pH during experiments was 7.4. Mortality ranged from 10% to 95% and dose-dependently increased with additional signs of overtoxicity.

- **Other publications that may not be used in a regulatory purpose**

Fairbairn et al. 2012 studied the morphological abnormalities that resulted in zebrafish embryos when axis determination was disrupted by environmental contaminants (polycyclic aromatic hydrocarbons and dibutyl phthalate). In the study, LiCl was used as a positive control because it is a well-known disruptor of axis determination. Embryos exposed to 300 mM LiCl (corresponding to high dose of 12.7 g/L LiCl or 2.08 g/L Li) for 10 min from 2.5 hour post fertilisation (hpf) showed morphological abnormalities at 12.5 hpf including incomplete epiboly and mild and severe hyper convergence-extension. 90% of LiCl exposed embryos exhibited phenotypes indicative of hyper-dorsal development. At 36 hpf, the embryos showed twisted and expanded posterior region situated above the plane of the yolk. LiCl disrupts development of the dorsal-ventral axis in zebrafish embryos via perturbation of the *Wnt/beta-catenin* signaling pathway. LiCl inhibits GSK-3 preventing beta-catenin degradation throughout the embryo and resulting in increased, ectopic, nuclear accumulation of beta-catenin.

Selderslaghs et al. 2009 developed a screening assay to identify teratogenic and embryotoxic chemicals using the zebrafish embryo. Known positive, including LiCl, and negative developmental toxicants were used to assess the assay. The embryos were exposed to 5.76 x 10^{-2}, 0.23, 0.92, 3.69, 14.7, 58.9 and 235.9 mM LiCl (corresponding to concentrations between 0.40 mg/L Li and 1.64 g/L Li (very high concentration)) under static exposure conditions from 2 hpf to 144 hpf. The pH was checked for all solutions and adjusted to 6.8-8 when necessary and oxygen levels of the solutions were always higher than 80%. Control embryos showed a survival rate of 90% or higher and normal development up to 144 hpf. At 24 and 48 hpf, mortality is observed at the highest concentration tested, while no teratogenic effects were noted. Kinks in the chorda were occasionally observed but this malformation was not observed in a systematic manner at different concentrations. Lithium chloride exerted its major teratogenic potential at later time points. At 72 hpf, a slight retardation in development was observed, manifested as a delay in hatching after exposure to 58.9 mM lithium chloride (i.e. 409 mg/L Li). At 144 hpf skeletal deformities and reduced swimming behavior were most prominent. At 144 hpf, the LC50 value was 53.43 mM LiCl (i.e. 371 mg/L Li) and the EC50 for teratogenic effects was 10.71 mM (i.e. 74.3 mg/L Li). Thus, the teratogenic index was 4.99.

Selderslaghs et al. 2012 established a standard operating protocol in which the embryotoxic and teratogenic potential of 6 compounds, including LiCl, was assessed. The objective was to consolidate the conclusion of the former work (Selderslaghs et al. 2009). The experimental conditions were the same as in the study from Selderslaghs et al. 2009. Zebrafish embryos were exposed to 5.76x10^{-5} – 0.24 mM LiCl (corresponding to 0.3x10^{-3} - 1.6 mg/L Li). The LC50 value at 144 hpf was 0.05 mM (0.35 mg/L Li) and the EC50 value for developmental effects was 3.96x10^{-3} mM (0.027 mg/L Li) leading to a teratogenic index of 13.40. It is to be noted that these toxicological values were extremely different from the previous study from Selderslaghs et al. (2009) with LC50 and EC50 values more than 1000 times lower whereas the experimental procedures were similar. One could assume a unit error in the study from 2009. Malformations observed in LiCl-exposed zebrafish were in agreement with defects observed in animals in the former work (delayed hatching, skeletal deformities, side-wise position, lack of swimming, kinks in the chorda) (Selderslaghs et al. 2009).

Conde-Vancells et al. 2018 performed several assays using mouse embryonic stem cells and zebrafish embryos to evaluate the potential developmental toxicity of industrial and pharmaceutical chemicals. Eight known mammalian teratogens including LiCl and 3 non-teratogenic compounds were tested. Zebrafish embryos were exposed to 10 nM, 100 nM, 1 µM, 10 µM, 100 µM, 1 mM LiCl (i.e. from 6.9x10^{-5} to 6.9 mg/L Li) from 3 hpf to 72 hpf. At 72 hpf, no mortality was observed. At 100 µM, 8.8% of embryos showed axial curvature (statistically significant) but no more effect was seen at 1 mM. The authors indicated that the result for LiCl was a false negative.

Pruvot et al. 2012 assessed various biological effects of psychotropic drugs, including LiCl, on zebrafish larvae. The larvae were exposed to 0, 50, 100, 150 and 200 µM LiCl (i.e. from 0.35 to 1.4 mg/L Li) from 48 hpf for 24h. The LC50 value for LiCl was 208 µM (1.4 mg/L Li) at 72 hpf. The surviving larvae were further exposed to LiCl in fresh medium for 72h (until 144 hpf). At 144 hpf, the LC50 value was 179 µM (1.24 mg/L Li). If the exposure was started from 4 hpf until 24 or 48 hpf, LiCl exerted only minimal effects at the highest tested concentration (higher than 20 mM). Thus, early developmental stages were less sensitive to LiCl than at 3dpf. Regarding developmental defects, LiCl exposure induced pericardial edema and dorsal curvature, with an EC50 value of 232 µM (1.6 mg/L Li) and 151 µM (1.05 mg/L Li), respectively for the exposure from 2 to 3 dpf and from 2 to 6 dpf. Thus,

the teratogenic index was 0.89 and 1.19, respectively for both exposure periods. Regarding the heart rate analysis, at 3 dpf, LiCl caused a dose-dependent decrease in heart rate from 50 µM LiCl. Regarding the effects of LiCl on zebrafish larvae locomotion, at 6 dpf, LiCl exposure at 150 µM led to a significant decrease of activity (18%) and velocity (0.4mm/s) when compared to untreated larvae.

Baro-Camarasa *et al.* (2023) determined the concentrations of 13 trace elements including lithium in the muscle and liver of a pregnant female Pacific sharpnose shark (*Rhizoprionodon longurio*) fished near a copper mine of Santa Rosalia and its embryos. Higher lithium concentrations in embryo tissues than the pregnant female tissues were found, which indicate an easy maternal transfer of this trace element.

Summary regarding short-term toxicity to fish

Both data from the registration dossiers and literature (only useful data for a regulatory purpose) are synthetised in Table 45 below.

Few mortality data are available. From the registration dossiers, the lowest **acute fish 96h-LC50 was 25.9 mg Li/L** for rainbow trout (juvenile) (Unnamed 1997). Lower LC50 are available from the registration dossiers, but were not used because it could not be excluded that the observed toxicity was due to an increase in pH. Few studies from literature also reported mortality data, with 96h-LC50 values in the same order of magnitude than the registrants' data (Hamilton 1995). Lower mortality data were identified (LOEC(10d, juvenile survival, rainbow trout) of 0.6 mg/L) (review from Kszos et al. 2003) but experimental conditions were not provided preventing from assessing the reliability. Besides, mortality was observed in zebrafish larvae with a LC50 value of 179 µM LiCl for an exposure from 48 to 144hpf, i.e. 1.24 mg/L Li (Pruvot et al. 2012). **Overall, the reliability and relevance of these studies from the registration dossiers and literature will need to be further scrutinized to assess if the data may be considered in the purposes of classification and risk assessment**.

Besides, although the following endpoints may not be used to assess the acute toxicity in a regulatory purpose, **developmental defects were reported at embryonic or larval stages of fish following acute exposure to lithium**. Indeed, lithium is a well-known teratogenic agent disrupting the development of the dorsal-ventral axis of fish. It is used as positive control in some studies at very high concentrations (2 g/L Li, Fairbairn et al. 2012) in order to observe this typical effect. But developmental defects, such as pericardial edema and dorsal curvature, decrease in heart rate and locomotor response, could be observed at lower concentrations (EC50 of 151 µM LiCl, i.e. 1.05 mg/L Li in zebrafish larvae for an exposure from 2 to 6 dpf.

Table 45: Short-term effects on fish

Substance, exposure conditions	Species	Developmental stages[69]	Test duration	Value	Calculated value for Li ion	Endpoint	Effect parameter	pH of the test solutions	Reference	Guideline
Li2CO3	*Oncorhynchus mykiss*	Juvenile	96h	30.3 mg/L (measured conc.)	5.69 mg/L	LC50	Mortality	Initiation: 8.7-10.4 Remainder of the test: 6.7-9.8	Unnamed, 1996	OECD TG 203
LiOH*H2O	*Danio rerio*	Juvenile	96h	109 mg/L (nominal conc.)	18.0 mg/L	LC50	Mortality	Initiation: 10.7	Unnamed, 2010	OECD TG 203
LiCl	*Oncorhynchus mykiss*	Juvenile	96h	158 mg/L (measured conc.)	25.9 mg/L	LC50	Mortality	Initiation: 7.5-8.7 Remainder of the test: 7.0-7.5	Unnamed, 1997	OECD TG 203
LiCl	*Tanichthys albonubes*	Juvenile	48h	9.2-62 mg/L	1.51-10.2 mg/L	LC50	Mortality	No information	Lenntech 2007 and US EPA 2008, cited in Aral et al. 2008 (review)	No information
LiCl, dilute mineral water	*Pimephales promelas*	Larvae	7d	-	**0.57 mg/L** (measured conc.)	EC50	Growth	No information	Kszos et al. 2003	US EPA method 1000.0 (1994)
LiCl, East Fork Poplar Creek	*Pimephales promelas*	Larvae	7d	-	2.47 mg/L (measured conc.)	EC50	Growth	No information		
LiCl	*Ptychocheilus lucius*	Juvenile	96h	-	28 mg/L	LC50	Mortality	7.0-8.5 at 96h	Hamilton 1995	ASTM 1989
LiCl	*Xyrauchen texanus*	Juvenile	96h	-	53 mg/L	LC50	Mortality			
LiCl	*Gila elegans*	Juvenile	96h	-	62 mg/L	LC50	Mortality			
Lithium	*Oncorhynchus mykiss*	Juvenile	10d		**0.6 mg/L**	LOEC	Juvenile survival	No information	Kszos et al. 2003 (review)	No information
Lithium	*Pimephales promelas*	Embryo-larvae	96h		42 mg/L	LC50	Mortality		Long et al. 1998, cited in Kszos et al. 2003 (review)	
Lithium	*Morone saxalitis*	Juvenile	96h		>105.0 mg/L	LC50	Mortality		Dwyer et al. 1992, cited in Kszos et al. 2003 (review)	

Lines indicating mortality are in white and growth effects in grey.
Toxicological values below 1 mg/L are in bold.

[69] At the beginning of exposure

20.1.1.2 Long-term toxicity to fish

Data from the registration dossiers

Two studies, one following the OECD TG 210, the other the US EPA guideline OPPTS 850.1400, are reported in the registration dossier (Unnamed 2012, Long et al. 1998). They respectively tested the chronic toxicity of $LiOH*H_2O$ and LiCl on two fish species, respectively under semi-static and flow-through exposure conditions. The OECD TG 210 study, considered as Klimisch reliability 1 by the registrants, reported a **NOEC of 2.89 mg Li/L** (measured concentration) for zebrafish based on mortality at larval/juvenile stages. The other study reported a NOEC of 0.20 mg Li/L (nominal concentration) for fathead minnow (*Pimephales promelas*) based on fish length. This study was considered as Klimisch reliability 4 by the registrants on the basis that no study report was available but eMSCA did not share this view as a publication was available, and judged the study of much higher reliability.

Overall, based on the data available in the registration dossier, the lowest long-term fish NOEC is 0.2 mg Li/L (nominal concentration) for fathead minnow based on growth (length).

Data from literature search

Literature search provided two long-term studies on fish (Jing et al. 2021, Yuan et al. 2022) which are described below. However, they investigated endpoints (lipid metabolism, antioxidant defense and immune response) that may not be used for assessing chronic toxicity to fish in the frame of a regulatory assessment. Indeed, according to ECHA guidance R7b, "*chronic toxicity related to waterborne exposure refers to the potential or actual properties of a substance to cause adverse effects to aquatic organisms during exposures which are determined in relation to the life-cycle of the organism. Such chronic effects usually include a range of sublethal endpoints"* such as *"survival, growth and/or reproduction".*

Jing et al. 2021 studied the effects of lithium on oxidative damage and inflammatory response to kidneys in carp. Carps (>2 years, weight: 250 ± 50 g) were exposed to 20 mg/L lithium in water for 30d. At the end of the exposure period, lithium-exposed group showed higher lithium concentration in blood and tissue than the control group. The histological analysis showed that the intrinsic kidney structure of the lithium poisoning group was destroyed and a large amount of inflammatory cell infiltration was observed. The blood analysis showed that the ROS level in the kidney tissue and malondialdehyde level significantly increased, indicating that lithium enhanced oxidative damage. Lithium exposure significantly inhibited the antioxidant level, by decreasing superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities. Under lithium stimulation, the mRNA expression of GSK-3β and TSC2 in the kidney was inhibited but the expression of TOR increased. Pro-inflammatory factors significantly increased in the lithium -poisoning group (increase in the expression levels of TNF-α, IL-1β, and IL-6), thus stimulating inflammatory response, but the expression of the anti-inflammatory factors TGF-β and IL-10 significantly decreased. Besides, phosphorylation of p38 MAPK, JNK, and ERK was aggravated by lithium exposure in a dose-dependent manner, indicating the activation of the MAPK pathway and stimulating the regulation of the body's inflammatory response.

Yuan et al. 2022 studied the effects of dietary lithium on body weight, lipid metabolism, antioxidant defense and immune response of largemouth bass (*Micropterus salmoides*). The fish (initial body weight: 67.15 ± 0.51 g; body length: 15.81 ± 0.12 cm) were **dietary exposed** to 0, 0.01%, 0.05% and 0.1% LiCl

(measured value: 1.86, 26.82, 87.08 and 173.7 mg/kg Li diet) for 11 weeks. At the end of the exposure period, no mortality was observed. Lithium exposure induced an increase in weight gain and feed intake in a dose-independent manner. Lithium inclusion also aggravated the accumulation of hepatic lipid and serum lithium in a dose-independent manner. Gene expression analysis showed that lithium inclusion, especially overload lithium, elevated the transcription of genes related to lipid metabolism, peroxisome proliferator-activated receptor γ (PPARγ), acetyl-Co carboxylase (ACC) and fatty acid synthase (FAS). On the contrary, lithium dose-dependently inhibited the expression of fatty acid oxidation related genes, peroxisome proliferator-activated receptor α (PPARα) and acyl-coenzyme A oxidase (ACO), and lipolysis related genes, hormone sensitive lipase (HSL) and monoglyceride lipase (MGL). Besides, oxidative stress was caused by high lithium inclusion, which was partly through the inhibition of Nrf2/Keap1 pathway (nuclear factor erythroid 2-related factor 2/kelch-like ECH associated protein 1). Moreover, lithium exposure significantly depressed the hepatic lysozyme activity and increased the transcription of proinflammation factors, tumor necrosis factor-α (TNF-α), 5-lipoxygenase (5-LOX), interleukin-1β (IL-1β) and interleukin-8 (IL-8), which was suggested to be regulated by the p38 MAPK pathway.

Summary: Data from the registration dossier are synthetised in Table 46. The lowest long-term fish NOEC is 0.2 mg Li/L (nominal concentration) for fathead minnow based on growth (length).

Table 46: Long-term effects in fish

Substance	Species	Test duration	Value	Calculated value for Li ion	Endpoint	Effect parameter	pH of the test solutions	Reference	Guideline
LiOH*H2O	*Danio rerio*	34d	17.35 mg/L (measured conc.)	2.87 mg/L	NOEC	Mortality	7.15-7.84; adjustment with 1N HCl	Unnamed, 2012	OECD TG 210
LiOH*H2O	*Danio rerio*	34d	24.35 mg/L (measured conc.)	4.03 mg/L	LOEC	Mortality			
LiCl	*Pimephales promelas*	26d	8.7 mg/L (nominal conc.)	1.4 mg/L	LC50	Mortality	7.2-7.5	Long et al., 1998	EPA OPPTS 850.1400850.1400
LiCl	*Pimephales promelas*	26d	6.4 mg/L (nominal conc.)	1.0 mg/L	EC50	Mortality			
LiCl	*Pimephales promelas*	26d	1.2 mg/L (nominal conc.)	**0.2 mg/L**	NOEC	Growth			
LiCl	*Pimephales promelas*	26d	1.9 mg/L (nominal conc.)	**0.31 mg/L**	LOEC	Growth			

Toxicological values below 1 mg/L are in bold.*

20.1.2 Aquatic invertebrates

20.1.2.1 Short-term toxicity to freshwater and seawater invertebrates

Data from the registration dossiers

Three studies following the OECD TG 202 are reported in the registration dossier (Unnamed 1997a, Unnamed 1997b, Unnamed 1997c). They respectively tested the acute toxicity of Li_2CO_3, LiCl and LiOH on *Daphnia magna* under static exposure conditions. They respectively reported 48h-EC_{50} values of 6.24, 40.7 (measured concentrations) and 9.94 mg Li/L (nominal concentration, under pH adjustment) based on mobility. The registrants judged the three tests to be Klimisch reliability 1. However, the studies from Unnamed 1997a and Unnamed 1997c showed

methodological limitations that may affect the results of the tests (pH exceeding 6-9 as recommended in the OECD TG 202, low hardness and/or low substance purity). The difference in 48h-EC_{50} values could be explained by an effect of the pH due to the different lithium salts tested in both studies. In the study from 1997b with a 48h-EC_{50} value of 40.7 mg/L Li, LiCl was tested and the pH of the solution was relatively stable whereas in the study from 1997a (Unnamed 1997a), Li2CO3 was tested and the pH increased. This may be due to the dissociation of Li2CO3 into Li+ and CO_3^{2-}, this latter hydrolising to yield HCO3- and OH- leading to an increase in pH. Thus, it is questionable if the highest toxicity observed in the study from 1997a could be due to this increase in pH, rather than the presence of lithium. Besides, the study from Unnamed 1997c also showed limitations as no clear information on the test material was provided.

Overall, based on the data available in the registration dossier, only one study with no methodological limitations may be retained regarding acute toxicity of lithium to aquatic invertebrates with a 48h-EC50 of 40.7 mg Li/L (measured concentration) for *Daphnia magna*.

Data from literature search

Literature search also provided short-term data on aquatic invertebrates such as crustaceans (9 publications), molluscs (11 publications), echinoderms (10 publications), annelids from the aquatic compartment (3 publications), amphioxus (2 publications) and cnidarians (hydra) (one publication). Data are described below. Studies on crustaceans provided ecotoxicological values (EC50) that may be useful for assessing acute toxicity of lithium to aquatic invertebrates. Studies on molluscs, echinoderms and annelids provided few LC50 or EC50 values and rather investigated effect parameters (biochemical, histopathological, developmental effects) that are irrelevant for a regulatory assessment. Studies identified on amphioxus and cnidarians exclusively investigated developmental effets.

20.1.2.1.1 Crustaceans

- **Relevant publications in a regulatory purpose**

Michalaki et al., 2022 assessed the impact in acute exposures of eight individual chemicals and specifically two metals (including LiCl), four pharmaceuticals, a pesticide and a stimulant, and their composite mixture on *Daphnia magna* combining phenotypic, biochemical and metabolic markers of physiology. The test was conducted according to OECD TG 202 but older (four-day-old) daphnids were exposed and not the most sensitive neonates. The selection of this stage is explained by the authors for two reasons. This is done in order to avoid differences observed in some chemicals and their action because of the time window of the 0–24 h for collection of neonates and to increase the amount of tissue available for measuring enzyme activities. As expected, the EC values recorded were in a similar order of magnitude but higher than reported EC50 values in the literature for neonates as neonates are more sensitive. It was reported an EC50 value of 93.7 mg/L for LiCl (15.3 mg/L Li). No information on pH was reported.

Okamoto et al., 2015 studied the acute toxicity of 50 metals including lithium (LiCl) to *Daphnia magna* according to OECD TG 202. The experiment was conducted at pH 6.5-8.5. The EC50 value was 6.3 mg/L Li. It was also reported the following effect values: EC50 (*Daphnia magna*): 7.820 mg/L Li (from Khangarot and Ray 1989; Rathore 2001); EC50 (C*ypris subglobosa*): 46.850 mg/L Li (from Khangarot and Das 2009).

Nagato et al., 2013 reported a 48h-LC50 of 2.3 mg/L Li for *Daphnia magna* neonates.

Kudlak et al., 2011 determined EC50 toxicity data of selected heavy metals including lithium (LiCl) toward the freshwater crustacean *Heterocypris incongruens*. It was found a LC_{50}(6d, *Heterocypris incongruens*) of 0.904 mg/L Li / 1.182 mg/L Li (no explanation reported for these two values). No information on pH was reported. The study also reported the following values: LC_{50}(7d, *Hyalella azteca*): 0.094 mg/L Li in tap water/0.451 mg/L Li in soft water (Borgmann et al. 2005) (no explanation regarding this difference according to tap or soft water); EC_{50}(24h, lithium sulphate, *Daphnia magna*): 26.56 mg/L Li2SO4 (2.88 mg/L Li) (Lilius et al. 1995).

Lilius et al. 1994 studied the acute toxicity of 50 reference chemicals including lithium sulfate to *Daphnia magna*. The acute toxicity test in daphnids was performed according to the OECD standard protocol (1980) with a few modifications. No information on the pH of the tested solutions was reported. The EC50(24h) value for Daphnia magna was 1.79 mM for lithium sulfate (24.9 mg/L Li).

The review from Aral et al. (2008) reported the results from Lenntech (2007) and US EPA (2008) showing an EC50(24h) value of 33-197 mg/L Li2SO4 for *Daphnia magna* based on mobility. No information on the pH of the tested solutions was provided.

Khangarot et al. 2009 studied the acute toxicity of 36 metals and metalloids including Li2SO4 and 12 reference toxicants to the freshwater ostracod, *Cypris subglobosa.* The 48h-EC50 was 46.85 mg/L Li. The pH of the test water was 7.6 but no information on the pH of the tested solutions was provided.

The review from Kszos et al. 2003 already described in the section 19.1.1 on fish provided the results of a short-term toxicity test with *Ceriodaphnia dubia* (< 24h old at initiation) conducted using a method very similar to "EPA method 1002, C. dubia, survival and reproduction test" (EPA 1994). The neonates were exposed for 7d to various concentrations of '*groundwater treatment facility effluent*' (control, 3, 6, 12, 25, 50 and 100%) that was non-treated or resin-treated to remove lithium. The 25% concentration of the non-treated GWTF effluent contained 3.1 mg/L Li; the 100% concentration of the treated GWTF effluent contained 1.62 mg/L Li. The control water had an average pH of 7.9. It was observed that the removal of lithium from the effluent clearly reduced the effluent's toxicity. The NOEC for *Ceriodaphnia dubia* survival in the non-treated effluent was 3%, compared to a NOEC of 50% in the treated effluent (no corresponding lithium concentration was reported).

The study from Kszos et al. 2003 already described in the section 19.1.1 on fish evaluated the toxicity of lithium to *Ceriodaphnia dubia.* The organisms were exposed to LiCl for 7 days in dilute mineral water (DMW) (0.32 (measured), 0.93 (measured), 1.87 (measured), 3 (nominal), 4 (nominal) mg/L) or East Fork Poplar Creek water (0.36 (measured), 0.98 (measured), 1.90 (measured), 3.0 (measured), 4 (nominal) mg/L). The pH was assessed daily for the control water only; detailed values were not reported. The results showed that lithium in DMW was 5 to 10 times more toxic than in EFPC water. For lithium in DMW, the concentrations inhibiting 25% or 50% of the reproduction were 0.32 mg/L and 0.72 mg/L Li respectively. For lithium in EFPC water, these concentrations were 3.33 and >4 mg/L Li respectively. The authors hypothesized that this difference of toxicity between DMW and EFPC water is due to their content of Na, 2.8 and 17.4 mg/L respectively, as they showed that lithium was better tolerated when more Na

was present. eMSCA agrees that lithium toxicity in the environment appears to depend on the presence of other salts.

Summary: Both data on crustaceans from the registration dossier and literature are synthetised in Table 47. Acute toxicity of Li as LiCl, LiOH, Li2CO3 or Li2SO4 was studied in several crustaceans (*Daphnia magna* adults or neonates*, Heterocypris incongruens, Cypris subglobosa, Hyalella Azteca, Ceriodaphnia dubia).* **The range of EC50(48h) was between 2.3 and 41 mg/L Li based on mobility/mortality.** A lower EC50 was observed but it was related to an other endpoint than mobility and a longer exposure duration (EC50(7d) of 0.72 mg/L Li based on reproduction). A higher EC50 was also observed but no information was provided on exposure duration, so it could not be considered as the highest EC50.

Table 47: Short-term effects in crustaceans

Substance	Species	Test duration	Value	Calculated value for Li ion	Endpoint	Effect parameter	pH of the test solutions	Reference	Guideline
Li2CO3	Daphnia magna	48h	33.2 mg/L (measured conc.)	6.24 mg/L	EC50	mobility	8.5-10.2	Unnamed, 1997a	OECD TG 202
LiCl	Daphnia magna	48h	249 mg/L (measured conc.)	40.77 mg/L	EC50	mobility	Initiation: 7.1-8.6 Remainder of the test: 7.0-7.1	Unnamed, 1997b	OECD TG 202
LiOH	Daphnia magna	48h	19.1 mg/L (nominal conc.) (without pH-adjustment)	5.54 mg/L	EC50	mobility	8.9 at 4.6 mg/L to 11.2 at 100 mg/L without pH-adjustment	Unnamed, 1997c	OECD TG 202
LiOH	Daphnia magna	48h	34.3 mg/L (nominal conc.) (with pH-adjustment)	9.94 mg/L	EC50	mobility	7.9 to 8.1 at all concentrations with pH-adjustment	Unnamed, 1997c	EU Method C.2 (1992 version) OECD TG 202 (1984 version)
LiCl	Daphnia magna (adults)	48h	93.7 mg/L	15.3 mg/L	EC50	mobility	No information	Michalaki 2022	OECD TG 202
LiCl	Daphnia magna	48h		6.3 mg/L	EC50	mobility	No information	Okamoto 2015	OECD TG 202
LiCl	Daphnia magna	-		7.82 mg/L	EC50	mobility	No information	Khangarot and Ray 1989; Rathore 2001, cited in Okamoto 2015	OECD TG 202
LiCl	Cypris subglobosa	-		46.85 mg/L	EC50	-	No information	Khangarot and Das 2009, cited in Okamoto 2015	No information
LiCl	Daphnia magna	48h		2.3 mg/L	LC50	mortality	No information	Nagato 2013	No guideline followed
LiCl	Heterocypris incongruens	6d		**0.904 mg/L**	LC50	mortality	No information	Kudlak 2011	No guideline followed
LiCl	Heterocypris incongruens	6d		1.182 mg/L	LC50	mortality	No information	Kudlak 2011	No guideline followed
Li	Hyalella azteca	7d	0.094 mg/L (tap water)	**0.094 mg/L (tap water)**	LC50	mortality	No information	Borgmann 2005 cited in Kudlak 2011	No information

Li	Hyalella azteca	7d	0.451 mg/L (soft water)	**0.451 mg/L (soft water)**	LC50	mortality	No information	Borgmann 2005 cited in Kudlak 2011	No information
Li2SO4*H2O	Daphnia magna	24h	26.56 mg/L	2.88 mg/L	EC50	mobility	No information	Lilius 1995 cited in Kudlak 2011	No information
Li2SO4	Daphnia magna	24h	1.79 mM i.e. 197 mg/L	24.9 mg/L	EC50	mobility	No information	Lilius 1994	OECD standard protocol (1980) with few modifications
Li2SO4	Daphnia magna	24h	33-197 mg/L	4.2-24.9 mg/L	EC50	mobility	No information	Lenntech 2007 and US EPA 2008, cited in Aral 2008 (review)	No information
LiCl, dilute mineral water	Ceriodaphnia dubia	7d		**0.72 mg/L**	EC50	reproduction	No information	Kszos 2003	US EPA method 1002 (1994)
LiCl, East Fork Poplar Creek	Ceriodaphnia dubia	7d		>4 mg/L	EC50	reproduction		Kszos 2003	

LC50 and EC50 are indicated in roman; Lines indicating adverse effects on reproduction are in grey and mortality/mobility in white.

Toxicological values below 1 mg/L are in bold.

20.1.2.1.2 Molluscs

20.1.2.1.2.1 Studies on clams

- **Publications that may not be used in a regulatory purpose**

Barbosa et al. 2023a investigated the biochemical alterations of climate change (temperature rise and salinity changes) on the impacts of lithium in clams (*Venerupis corrugata*) collected from the Ria de Aveiro (coastal lagoon, Portugal). Clams were exposed for 14 days to 0 or 200 µg/L of added Li (form not specified), both conditions under different climate scenarios: 3 different salinities (20, 30 and 40 (no unit reported) at 17°C (control temperature); and 2 different temperatures (17 and 21°C) at salinity 30 (control salinity). Lithium was already present in the artificial seawater used in the preparation of the exposure medium. During the exposure period, after water renewal and lithium spiking, samples were collected from the aquaria for further quantification. All control treatments showed levels of lithium in water, with higher concentrations found at the highest salinity (40) (421 ± 5 µg/L) and lower levels observed at the lowest salinity (20) (166 ± 57 µg/L). In exposure treatments, at 17°C, the actual lithium concentrations were 693 ± 31, 465 ± 20 and 803 ± 28 µg/L, respectively at salinity 30, 20 and 40. At 21°C, salinity 30, lithium concentration was 556 ± 24 µg/L. During the exposure period, pH was maintained at 8.0. After 14 days of exposure, no mortality was recorded following lithium exposure. Salinity variations had a higher impact on biochemical responses of the clams than temperature increase, even when combined with lithium exposure. The combination of lithium with low salinity (20) was the most stressful treatment, provoking increased metabolism (increase in electron transport system (ETS) activity, total protein) and activation of detoxification defences (increase in carboxylesterases and glutathione S-transferases).

In another study, Barbosa et al. (2023b) studied the biochemical effects of lithium alone (0, 200, 400, 800 µg/L) on the same mollusc. Measured concentrations were respectively: 282 ± 14 (control with no Li spiking); 693 ± 31; 1096 ± 106; 1941 ± 128 µg/L. The conditions of pH was maintained at 8.0. After 14 days of exposure, no mortality was recorded. Lithium had no influence on the metabolic capacity and energy reserves of clams (electron transport system, total protein). Antioxidant and detoxification enzymes were activated, especially at 1096 and 1941 µg/L, to eliminate ROS and lithium from the cells: significant increase in glutathione peroxidase, carboxylesterases and glutathione S-transferases activities. A loss of redox balance was also observed (decrease in the ratio between reduced and oxidized glutathione (GSH/GSSG)). These different effects suggested the occurrence of oxidative stress in clams exposed to increasing Li concentrations.

20.1.2.1.2.2 Studies on mussels

- **Relevant publications in a regulatory purpose**

Fraga et al. 2022 studied the acute and sublethal effects of lithium in the same mussel species as Cunha et al. 2023. Mussels were collected in Plentzia (Basque Coast). No baseline lithium concentration was reported. The acute toxicity test showed a LC50 value of 153 mg/L Li after 9 days of exposure. In the sublethal toxicity test, mussels were exposed for 21d to 0, 0.1, 1, 10 mg/L Li. No information on the pH of the tested solutions was provided. Mussels showed lithium accumulation in soft tissues in a dose-dependent manner. Lithium exposure also induced histopathological effects in a time-dependent manner: atrophy of the digestive epithelium of the alveoli at 10 mg/L Li, higher connective tissue index and higher brown cell infiltration prevalence from 1 mg/L Li, higher prevalence of

haemocytic infiltration from 0.1 mg/L Li, degeneration of the digestive gland from 1 mg/L Li. The authors indicate that based on the acute toxicity results (LC50 of 153 mg/L Li), lithium concentrations currently found in the environment (no exposure data provided in the publication but other data reported elsewhere: 0.18 mg/L, 1-5 mg/L in Chile, 14 mg/L in the Dead Sea) would not provoke extreme effects. However, as lithium is accumulated in soft tissues, long-term exposure could still generate toxicopathic effects.

The review from Aral et al. (2008) reported the results from Lenntech (2007) and US EPA (2008) showing a LC50 (24h) value of 185-232 mg/L LiCl for zebra mussel (*Dreissena polymorpha*). No information on the pH of the tested solutions was provided.

- **Other publications that may not be used in a regulatory purpose**

Cunha et al. 2023 studied the influence of temperature on the biochemical effects of lithium and lead, alone or as a mixture, in mussels (*Mytilus galloprovincialis*). Mussels collected in the Ria de Aveiro coastal lagoon (Portugal) were exposed for 28 days to 0 or 300 µg/L Li at 17 or 21°C. For control conditions (no lithium spiking), results showed that the baseline concentration of lithium in seawater varied between 206 ± 36 µg/L at 17°C and 183 ± 9 µg/L at 21°C. After Li spiking, concentrations increased to 510 ± 26 µg/L at 17°C and 470 ± 12 µg/L at 21°C, which corresponds to an increase of roughly 300 µg/L (matching the nominal concentration). No information on the pH of the tested solutions was provided. The increased temperature did not influence the accumulation of lithium in mussels. Regarding metabolic capacity and energy reserves content, at 17°C, lithium had no effect on electron transport system (ETS) and protein content that may indicate a low-stress level, but induced a significant increase in glycogen content. At 21°C, a decrease in ETS was observed in the Li-exposed group compared to the control. Exposure to lithium activated mussels' antioxidant and detoxification capacity with significant increase in catalase at 17 and 21°C. No difference in glutathione S-transferases and carboxylesterases was observed between Li-exposed group and control. Regarding cellular damage and redox balance, Li-exposed mussels showed a significant decrease in lipid peroxidation levels at 17°C and reduced glutathione content at 17 and 21°C indicating a stress condition associated with a loss of redox equilibrium. Regarding neurotoxicity, no significant difference in AChE activity in the presence of lithium at both temperatures was observed. Lithium had no significant effect on the lysosomal membrane stability. A slight but not significant decrease in the adipogranunlar cell index, which may indicate reproduction and seasonal bioenergetic changes, was observed in Li-exposed mussels. The atrophy index, which is used as a proxy of thinning of the digestive alveoli epithelium and which can indicate general stress conditions, was significantly increased in Li-exposed group at both temperatures.

Viana et al. 2020 evaluated lithium toxicity based on the exposure of *Mytilus galloprovincialis*, assessing the biochemical changes related with mussels' metabolism, oxidative stress and neurotoxicity. The mussels were exposed to different lithium econcentrations (100, 250, 750 µg/L Li) for 28 days. Baseline lithium concentration in seawater was 203 µg/L Li. Following Li spiking, Li concentrations were 345±12, 473±39, 955±103 µg/L Li respectively for each exposure conditions. No information on the pH of the tested solutions was provided. No mortality was observed in the experiment. However, lithium leads to mussels' metabolism depression. At the highest concentrations, antioxidant and biotransformation enzymes were not activated, leading to the occurrence of lipid peroxidation and loss of redox homeostasis, with increased content in oxidized glutathione in comparison to the reduced form. Besides, the two highest lithium

concentrations induced neurotoxic effects in mussels, with a decrease in acetylcholinesterase enzyme activity.

Santos et al. 2023 assessed how different scenarios of increasing temperature may affect the response of *Mytilus galloprovincialis* to lithium. Mussels bioaccumulation levels, physiological and biochemical biomarkers were analyzed after 28 days of exposure to lithium (250 µg/L added Li) under different temperature scenarios (control - 17 °C; warming - 21 °C and simulated marine heatwave[70]). Baseline lithium concentration in seawater was 243 µg/L Li. Following Li spiking, Li concentration à 17°C was 540±4 µg/L Li. No information on the pH of the tested solutions was provided. Mussels accumulated lithium, independently of the temperature scenario. The respiration rate was higher in Li-exposed mussels than in the non-contaminated ones, with no difference among temperature scenarios. Moreover, the Li-exposed mussels under marine heatwave showed the highest metabolic rate (increase in electron transport system activity) and cellular damage (increase in lipid peroxidation levels). Lithium showed no neurotoxic effect in the mussels. Thus, it was highlighted that the combination of lithium and marine heatwave was the most stressful condition. The authors conclude that this combination induces clear negative effects in mussels that can impair the growth and reproduction of an entire population.

20.1.2.1.2.3 Studies on other mollusc species

- **Relevant publications in a regulatory purpose**

Sawasdee et al. 2010 investigated the effects of metal ions (copper, lead, lithium (0, 1, 2.5, and 3 mg Li+/L) and palladium) on the embryonic development of the ramshorn snail, *Marisa cornuarietis*. No information on the pH of the tested solutions was provided. The concentration of 2.5 mg Li+/L caused a significant delay in the formation of tentacles and eyes and a significant delay in hatching compared to the control group. The weight of the hatchlings was reduced by 3 mg Li+/L. Lithium did not cause any effect in heart rate. The LC50 value for lithium was 2.5 mg Li+/L after 14 days of exposure to Li+.

- **Other publications that may not be used in a regulatory purpose**

Marín Rodríguez et al. 2022 studied the impact of temperature on lithium biochemical effects in the gastropod *Tritia neritea*. Gastropods were exposed for 28 days to 0 or 0.08 mM Li, both conditions at ambient or high temperature (15°C or 21°C). During test periods, pH was maintained at 8.1. Regarding antioxidant and biotransformation enzymes, the catalase (CAT) activity was affected by both temperature and Li exposure, with significantly higher activity at higher temperature for control organisms and Li-exposed snails. Li exposure significantly reduced CAT activity, regardless of the temperature. The glutathione peroxidase (GPx) activity increased in controls and Li-exposed snails at 21°C, showing an interaction between temperature and lithium contamination for this biochemical parameter. Regarding biotransformation capacity, glutathione S-transferases activity was significantly increased in Li-contaminated snails in both temperatures compared to the respective controls but no significant difference was found between temperatures in Li-exposed snails. Regarding oxidative damage, Li-exposed snails showed a significant increase in lipid peroxidation when the temperature increased. Regarding metabolic capacity and energy reserves, lithium

[70] Marine heatwaves are extreme climatic events observed around the world, commonly defined as prolonged discrete anomalously warm water events that are classified by their duration, intensity, rate of evolution and spatial extent.

exposure did not affect the ETS activity but temperature rise did. Lithium decreased the glycogen and protein content at 15°C compared to the control, and this effect was enhanced at 21°C.

Sawasdee et al. 2011 studied the histopathological effects of copper and lithium in tissues of early juveniles of *Marisa cornuarietis*. Hatchlings were exposed to sublethal concentrations of lithium (0, 50, 100, 200, 1000, and 5000 µg Li+/L) for 7d. No information on the pH of the tested solutions was provided. Lithium changed the tissue structure of epidermis, hepatopancreas and gills. The following histopathological effects were observed: alterations in epithelial and mucous cells of the epidermis (at 5000 µg Li+/L, irregular apical surfaces of the epithelial cells, strong dilation of hemolymph spaces and large areas with empty mucous cells), swelling of hepatopancreatic digestive cells at 200 µg/L, alterations in the number of basophilic cells and abnormal apices of digestive cells at 1000 µg/L, irregularly shaped cilia and changes in the amount of mucus in the gills at 5000 µg/L. The most sensible organ in *M. cornuarietis* indicating Li pollution is the hepatopancreas: LOEC was 200 µg Li+/L. In epidermis, mantle and gills relevant effects occurred with higher LOEC: 1000 µg Li+/L. The following EC50 values were also reported for lithium: EC50 (epidermis) =2.5 mg/L, EC50 (digestive cells) =220.8 µg/L, EC50 (basophilic cells) =967.6 µg/L, EC50 (apex) =231.8 µg/L, EC50 (gills) =1402.5 µg/L.

The study from Kszos et al. 2003 already described in the sections 19.1.1 on fish and 19.1.2.1.1 on crustaceans evaluated the toxicity of lithium to the freshwater snail *Elimia clavaeformis*. Following exposure to 0, 0.05, 0.1, 0.15, 0.3, 1.0, 2.0, 5.0 and 10.0 mg Li/L as LiCl for 72h, lettuce consumption by *E. clavaeformis* was strongly reduced from 0.15 mg/L. Other tests were performed on *Pleurocera unicale unicale* (a close relative of *E. clavaeformis*), using a 24h test method; they showed similar results (decrease in lettuce consumption). No information on the pH of the tested solutions was provided in this study.

Summary: Data on molluscs are synthetised in Table 48. **Few mortality data were identified; the lowest LC50 value (2.5 mg Li+/L) was reported for the ramshorn snail, *Marisa cornuarietis*, at the embryonic stage** (Sawasdee et al. 2010). EC50 on histological endpoints was provided in one study with the lowest value measured for histopathological alterations in digestive cells (0.22 mg/L Li) (Sawasdee et al. 2011). Nevertheless, this effect parameter may not be used for assessing acute toxicity in the frame of a regulatory assessment.

Besides, biochemical and histopathological effects following subacute exposure to lithium were studied in several molluscs (clams, mussels, marine and freshwater snails*).* The results are not synthetised here as the investigated effect parameters may not be used in a regulatory purpose.

Table 48: Short-term effects in molluscs

Substance	Species	Test duration	Effect parameter	Endpoint	Value	Calculated value for Li ion	pH of the test solutions	Reference
Li	*Mytilus galloprovincialis*	9d	mortality	LC50	-	153 mg/L	No information	Fraga et al. 2022
Li	*Marisa cornuarietis*	14d	mortality	LC50		2.5 mg/L	No information	Sawasdee et al. 2010
LiCl	*Dreissena polymorpha*	24h	mortality	LC50	185-232 mg/L	30.3-38.0 mg/L	No information	Lenntech 2007 and US EPA 2008, cited in Aral et al. 2008
Li	*Marisa cornuarietis*	7d	histopathological effect	LOEC (lowest)		**0.2 mg/L** (swelling of hepatopancreatic digestive cells)	No information	Sawasdee et al. 2011
Li	*Marisa*	7d	histopathological	EC50		From **0.22**	No	Sawasdee et

	cornuarietis		effect			**mg/L** (digestive cells) to 2.5 mg/L (epidermis)	information	al. 2011
Li	*Mytilus galloprovincialis*	9d	histopathological effect	LOEC (lowest)	-	**0.1 mg/L** (higher prevalence of haemocytic infiltration)	No information	Fraga et al. 2022
Li	*Elimia clavaeformis*	72h	feeding consumption	LOEC	-	**0.15 mg/L**	No information	Kszos 2003

Toxicological values below 1 mg/L are in bold. Nevertheless, the effect parameters for which values below 1 mg/L are observed may not be used for acute toxicity in the frame of the regulatory assessment (histopathological effets, feeding consumption).

20.1.2.1.3 Echinoderms

- **Publications that may not be used in a regulatory purpose**

Bonaventura et al. 2022 analysed gene expression of the stress response to lithium (30 mM LiCl, i.e. 0.2 g/L Li), nickel and zinc in sea urchin embryos (*Paracentrotus lividus*). After Li exposure from 30 min hpf until 24 or 48h, the embryos were vegetalised with less ectoderm and more endomesoderm. Lithium also altered the embryonic nervous system on the basis of the embryonic localization of two neural markers.

In the study from Ghiglione et al. 1993, exposure of sea urchin (*Paracentrotus lividus*) embryos to 30 mM LiCl (0.2 g/L Li) between 30 min and 10h or 48hpf induced vegetalisation.

In the study from Kauffman et al. 2003, LiCl (20 mM (0.1 g/L Li) in *Heliocidaris erythrogramma* from the 2-cell stage through gastrulation and 20 mM in *Holopneustes purpurescens* for 24h) caused vegetalisation of treated embryos.

Kitazawa et al. 2007 investigated whether micromere-derived signal regulates larval left-right polarity during sea urchin development. In sea urchin development, the embryos keep bilateral symmetry until the six to eight-armed pluteus stage, and then begin to display left-right (LR) asymmetry by formation of the adult rudiment on only the left side of the larval body. *Hemicentrotus pulcherrimus* embryos exposed to 50 mM LiCl (0.3 g/L Li) during the 64- to 256-cell stages (from 6.5 to 8.5 hpf) became abnormal embryos developing into larvae with a rudiment on the right side. This effect was not observed in two sand dollar species, *Scaphechinus mirabilis* and *Astriclypeus manni*, exposed to various lithium concentrations (20-40 mM LiCl) at various developmental stages because embryos were vegetalised.

Kiyomoto et al. 2010 studied the effect of LiCl on the sea urchin *Hemicentrotus pulcherrimus* early development in whole embryos and isolated blastomeres. Whole embryos exposed to 30 mM LiCl (0.2 g/L Li) from 3hpf to 24hpf developed vegetalised exogastrulae.

In the study from Logan et al. 1999 examining whether β-catenin is involved in cell fate specification during sea urchin embryogenesis, *Lytechinus variegatus* embryos incubated from 30 min postfertilization to the 7th cleavage stage in 30-50 mM LiCl (0.2-0.3 g/L Li) in artificial seawater were vegetalised.

Nocente-MacGrath et al. 1991 examined the effects of various concentrations of LiCl (15, 30 and 60 mM, i.e. 0.1, 0.2 and 0.4 g/L Li) on a lineage-specific protein expressed during embryogenesis in the sea urchin *Strongylocentrotus purpuratus*.

Embryos were exposed between 30 min and 16 hpf. Embryos treated with 15 mM LiCl showed everted guts due to exogastrulation. These embryos were nearly normal with respect to the proportions of the primary tissue layers. Embryos exposed to 30 mM LiCl were vegetalized. Embryos exposed to 60 mM LiCl underwent cleavage which resulted in relatively normal blastulae, but they became increasingly disorganized as morphogenesis proceeded. There was a graded response to increasing LiCl concentrations characterized by an increase in endoderm-like tissue and a concurrent decrease in ectoderm.

Ruocco et al. 2016 described the morphological and molecular effects of LiCl in *Paracentrotus lividus* embryos. LiCl was added to the eggs 10 minutes before fertilization or 10 minutes post-fertilization at the following concentrations for 48h: 1, 2, 3, 4, 5, 10 and 80 mM LiCl (i.e. between 6.9 mg/L Li and 0.5 g/L Li). Morphological analysis at the pluteus stage (48 hpf) revealed that the different concentrations tested of LiCl induced the same malformations, which mainly affected the arms, spicules and apex, in comparison with control embryos.

The effects of LiCl were stronger when added before fertilization. At 1 mM LiCl, an increase of about 30% malformed plutei in comparison with control embryos was observed. From 1 mM to 10 mM, this percentage of abnormal plutei significantly increased (from 30% to 80%). When LiCl was added after fertilization, a significant increase in the percentage of abnormal plutei was only detected at 5 and 10 mM (30 and 80%). When extending the times of exposure to 5 and 10 mM to 1 week post fertilisation, the embryos showed spicules that were not-joined or crossed at the tip. In fact, the general body plan was definitely compromised and the entire body of the embryos was malformed. Several embryos had the pyramid shape and poorly-retracted and degraded arms. When 80 mM LiCl was added before fertilization, embryonic development was blocked at the blastula stage before the hatching. At 48 hpf 80 mM lithium blocked embryos then died. Recovery experiments were also performed and results showed that the embryos were able to recover. When washed, they were able to grow up to the pluteus stage even if some of them were malformed. The number of malformed embryos increased with the time of exposure to lithium.

In the study from Russo et al. (2018) Paracentrotus lividus sea urchin embryos were exposed to 30 mM LiCl (0.2 g/L Li) 30 min after fertilisation and development was monitored at 24, 48 and 72hpf. Lithium exposure produced homogeneous populations of abnormal embryos after cleavage, approximately from the blastula stage (12 hpf). At 24 hpf, they showed a mesenchyme blastula shape with a thickened vegetal plate and many cells spread inside the blastocoelic cavity. At 48 hpf, embryos was characterized by a sphere with many pigment cells and an endoderm-derived everted gut. At 72 hpf, they maintained the exogastrula morphology already observed at 48hpf with a prominent everted gut but the sphere with pigment cells was larger than at 48hpf and contained some spicules.

Kominami et al. 1984 aimed at demonstrating interactions among blastomeres during cleavage stages, with respect to the determination of presumptive mesendodermal cells in whole embryos of starfish *Asterina pectinifera*. The starfish embryos were exposed to 30 mM LiCl (0.2 g/L Li) during the period from 7 to 10h of development. No immediate effect of LiCl exposure was apparent at the end of treatment but at the early-to-middle-gastrula stage, treated embryos formed archenterons (mesendoderm) that were longer and wider (30% expansion of the volume of this tissue). The length of the whole embryo along the animal-vegetal axis became slightly shorter. Other developmental processes in treated embryos seem to be unaffected. Due to the enlargement of the archenteron at the early-to-middle-gastrula stage, Li-exposed embryos differentiated larger digestive tracts in the oesophagus and anterior stomach regions. Pulse treatment with LiCl showed that the effect of LiCl was limited to the period from 7 to 10 h of development.

Summary: Only one study reported mortality of sea urchin embryos at 80 mM LiCl (0.55 g/L Li) (Ruocco et al. 2016). The other studies identified on echinoderms mostly investigated developmental effects of lithium at embryonic stage. As mentioned before in the section on fish data, these effects may not be used for assessing acute toxicity in the frame of a regulatory assessment. As in xenopus embryos (data described in section 19.1.6), **lithium (from 20 mM LiCl, i.e. 0.1 g/L Li) caused vegetalisation of sea urchin embryos** (Russo et al. 2018, Ruocco et al. 2016, Nocente-MacGrath et al. 1991, Logan et al. 1999, Kiyomoto et al. 2010, Kitazawa et al. 2007, Kauffman et al. 2003, Ghiglione et al. 1993, Bonaventura et al. 2022). **Developmental abnormalities were also observed in starfish exposed to 30 mM LiCl (0.2 g/L Li)** (Kominami et al. 1984).

20.1.2.1.4 Annelids from aquatic compartment

The review from Aral et al. (2008) (cited in MELCC 2022) reported the results from Lenntech 2007 and US EPA 2008 indicating an EC50 (24h-96h) value of 9.3-44.8 mg/L Li2SO4 (corresponding to 1.17-5.66 mg/L Li) for *Tubifex tubifex* based on mobility.

Okamoto et al., 2015 and Khangarot et al. 2009 reported an EC50 of 9.3 mg/L Li for *Tibufex tubifex* based on mobility (extracted from the study of Khangarot et al. 1991).

Ghribi et al. 2023 investigated the potential toxic effects of LiCl on the redox status, fatty acid composition, and histological aspects of the marine ragworm *Perinereis cultrifera*. The worms were exposed to 0, 20, 40 or 80 mg /L LiCl for 48h. Lithium accumulation was observed in LiCl-exposed worms in the dose-dependent manner. Lithium induced oxidative stress. The enzymatic and non-enzymatic antioxidants were also activated by lithium treatment. The biochemical responses were corroborated by the histopathological observations showing hyperplasia and loss of the intestine structure in Li-treated worms. Besides, LiCl exposure induced a modulation of the fatty acid composition in response to redox status disruption.

Summary: Four studies described the effects of lithium on annelid species (*Perinereis cultrifera, Tubifex tubifex*) (Ghribi et al. 2023, Lenntech 2007 and US EPA 2008 cited in Aral et al. 2008, Okamoto et al. 2015, Khangarot et al. 2009). Ghribi et al. 2023 showed lithium accumulation and activation of biomarkers indicating oxidative stress in the worms. Lithium also induced histopathological changes. Nevertheless, these effect parameters may not be used in a regulatory assessment.

The lowest **EC50 value reported was 1.17 mg/L Li for Tubifex tubifex** (Lenntech 2007, US EPA 2008, cited in Aral et al. 2008). Short-term data are synthetised in Table 49.

Table 49: Short-term effects in annelids

Substance	Species	Test duration	Effect parameter	Endpoint	Value	Calculated value for Li ion	Reference
Li2SO4	*Tubifex tubifex*	24-96h	mobility	EC50	9.3-44.8 mg/L	1.17-5.66 mg/L	Lenntech (2007) and US EPA (2008), cited in Aral et al. 2008
LiCl	*Tubifex tubifex*	-	mobility	EC50		9.3 mg/L	Khangarot (1991), cited in Okamoto 2015; Khangarot et al. 2009

20.1.2.1.5 Amphioxus

Holland et al. 2005 investigated the roles of Wnt/β-catenin in amphioxus (*Branchiostoma floridae*) development by upregulating *Wnt/β-catenin* signalling with Li^+. Amphioxus embryos were exposed to Li+ for varying durations during cleavage and blastula stages. When Li+ was applied before the late blastula stage, no detectable effect on axial patterning was observed. However, when it was applied at high concentrations (400 mM Li+, i.e. 2.8 g/L Li) at the late blastula stage, the embryos were markedly posteriorised and lacked a neural plate.

Yoshida et al. 1998 investigated the effects of high concentration of lithium chloride (20-40 mM) (0.14-0.16 g/L Li) on ascidian development. The effects of lithium treatment in the ascidian embryos (*Ciona savignyi*) were: reduction of the larval tail, anterior translocation of the descendants of the A4.1 blastomere of the tail, and change of cell fate of the A4.1-derived notochord to endoderm cells in the trunk.

Summary: Developmental defects in amphioxus and ascidian embryos were caused by lithium exposure, respectively at 2.8 and 0.14-0.16 g/L Li (Holland et al. 2005, Yoshida et al. 1998). Nevertheless, the developmental effects are usually not used in the regulatory assessment.

20.1.2.1.6 Cnidarians

Johnson et al. 1982 investigated the developmental effects of lithium in *Hydra attenuata* (adults and embryos). Among the findings, it was observed that embryos exposed to ≥ 50 mg/L LiCl were disrupted and those exposed to ≤ 40 mg/L were not affected. The embryos had maintained the hollow laminar form achieved by 18h and tentacles had not elongated. Moreover, hypostomes and body axis formation had not occurred. In an additional experiment where adults were exposed from 20 to 90 mg/L LiCl, 70 mg/L appeared toxic but this result was not confirmed in another experiment determining the adult effective dose to be 90 mg/L.

Summary: Lithium exposure (8 mg/L Li) **caused developmental effects in hydras (Johnson et al. 1982).** Nevertheless, the developmental effects are usually not used in the regulatory assessment.

20.1.2.2 Long-term toxicity to aquatic invertebrates

Two studies following the OECD TG 211 are reported in the registration dossier (Unnamed 2012, Unnamed 2010). They respectively tested the chronic toxicity of Li and $LiOH*H_2O$ on *Daphnia magna* under semi-static exposure conditions. The study from Unnamed 2012, considered as Klimisch reliability 2 by the registrants, reported a NOEC of 1.7 mg Li/L (nominal concentration) based on mortality. However, this study cannot be considered reliable as the test medium was Elendt which is not recommended for testing substances containing metals (Elendt medium especially contains LiCl and EDTA). The other study (Unnamed 2010) reported a NOEC of 0.66 mg Li/L (nominal concentration) based on reproduction. The pH and dissolved oxygen concentration were not reported for this study in ECHA disseminated website. Nevertheless, from the acute toxicity data on aquatic invertebrates, especially the study from Unnamed (1997c) in which LiOH was tested and pH varied and needed to be adjusted, it may be assumed that pH may have varied in the long-term study from Unnamed (2010). Thus, without certainty in the potential pH adjustment in the latter study, the NOEC of 0.66 mg/L may not be retained as it is not possible to known if the observed effects were due to lithium toxicity or a pH effect.

Overall, all data available in the registration dossier show limitations. Thus, no long-term NOEC may be retained for *Daphnia magna*.

Literature search also provided long-term data on crustaceans. Data are described below.

Martins et al., 2022 studied the long-term effects of relatively low doses of lithium (control, 0.02, 0.04 and 0.08 mg/L Li as LiCl) and lithium-microplastic mixtures on *Daphnia magna* according to OECD TG 211. These concentrations were selected by the authors based on previous studies from Kszos et al. (2003) and Nagato et al. (2013). They also investigated the effects of these chemical stresses (same concentrations tested) combined with high light intensity (26000 lx) or warmer temperature (25°C) (Martins et al. 2023). In the study from Martins et al. 2022, the pH means of the tested solutions ranged from 8.36 to 8.44. In the study from Martins et al. 2023, the pH was also in accordance with OECD TG 211. After 21d of exposure, parental and juvenile mortality were observed as well as decreased somatic growth and reproduction. Detailed effect values are reported below (Table 50). Only the experimental conditions with lithium and control temperature (20°C) and light intensity may be used in the regulatory assessment. Thus, the other experimental conditions with increased temperature or light intensity are not detailed but only reported in Table 50 as additional informations.

		Growth	1st brood	Brood number	Total offspring	Living offspring	Dead offspring	Population growth rate	Reduction of living offspring
LiCl, 20°C, control light intensity (10830 lx) (Martins et al. 2022)	NOEC	0.04	0.04	0.04	0.02	0.02	0.02	0.04	-
	LOEC	0.08	0.08	0.08	0.04	0.04	0.04	0.08	-
	EC50 (SD)	-	-	-	-	-	-	-	0.039 (0.038-0.040)
LiCl, 25°C, control light intensity (10830 lx) (Martins et al. 2023)	NOEC	0.05	0.03	0.05	0.03	0.03	0.03	0.03	-
	LOEC	0.1	0.05	0.1	0.05	0.05	0.05	0.05	-
	EC50 (SD)	-	-	-	-	-	-	-	0.032 (0.031-0.032)
LiCl, 20°C, high light intensity (26000 lx) (Martins et al. 2023)	NOEC	0.04	0.02	0.02	0.02	0.02	0.02	0.02	-
	LOEC	0.08	0.04	0.04	0.04	0.04	0.04	0.04	-
	EC50 (SD)	-	-	-	-	-	-	-	0.029 (0.028-0.031)

Table 50: Effect values (NOEC, LOEC, EC50) in mg/L for somatic growth, first brood, brood number, total offspring, living and dead offspring, population growth rate and reduction of living offspring for different bioassays performed at 20°C or 25°C and control light intensity (10830 lx), 20°C and high light intensity (26000 lx). Data extracted from Martins et al. 2022, 2023.

At 20°C/control light intensity, 0.04 and 0.08 mg/L Li induced parental mortality (10% and 20% resp.). At 0.04 mg/L, Li caused offspring mortality (31%), significantly decreased the total offspring number by 32% and the living offspring number by 53%. At 0.08 mg/L, Li significantly decreased the somatic growth by 40%, increased the number of days until the first brood release by 1.3 fold, and reduced the brood number by 20%, the total offspring number by 78%, the living offspring number by 93% and the population growth rate by 67%.

Compared to moderate conditions (LiCl, 20°C, control light intensity), the medium and highest concentrations of lithium (0.04 and 0.08 mg/L) combined with high light intensity or warmer temperature caused greater parental mortality. Regarding the effect on *D. magna* reproduction (living offspring), the exposure to LiCl at high light intensity or warmer temperature moved the toxicity curves towards lower concentrations. At warmer temperature, the somatic growth was significantly reduced under exposure to the highest concentration of Li (0.08 mg/L) in comparison to the control group (20°C, control light intensity). Regarding the effect on population growth rate, at high light intensity, it was lower in all Li treatments. At warmer temperature, it was significantly higher in the control group and in the lowest concentration of Li, and the population growth rate was significantly reduced at the medium concentrations of Li (0.04 mg/L). As the individual effects of lithium and high light intensity on *D. magna* population growth rate were both negative, as well as their combined effects, the two stressors act synergistically in the two exposure scenarios. Warmer temperature always acted synergistically with lithium.

To sum up the results from Martins et al. 2022, 2023, lithium caused parental and juvenile mortality, significantly decreased the somatic growth and the reproductive success, leading to population fitness reduction in *D. magna*. In control experimental conditions (20°C, control light intensity) the lowest NOEC was 0.02 mg/L Li based on effects on living offspring. The decline in the population growth rate increased at warmer temperature (25°C) or high light intensity (26000 lx). One of these conditions combined with the highest concentration of lithium (0.08 mg/L) caused population extinction.

Okamoto et al., 2021 studied the chronic toxicity of 50 metals including LiCl to *Ceriodaphnia dubia* according to EPS 1/RM/21 (Environment Canada, 2007). The following concentrations were tested for a duration of 6-7d depending on the time required to produce the third brood: 3.13, 6.25, 12.5, 25, 50 mg/L (nominal concentrations) (from 0.5 to 8 mg/L Li) corresponding to 1, 2.1, 3.6, 7, 13 mg/L as measured concentrations. No information was provided by the authors in order to explain these measured concentrations. No information on the pH of the tested solutions was provided. It was reported the following effect value based on reproduction: NOEC (mg/L) = 3.6 (0.59 mg/L Li); IC[71]10 (mg/L) = 5.6 (0.92 mg/L Li); IC20 (mg/L) = 6.5 (1.06 mg/L Li); IC50 (mg/L) = 8.1 (1.33 mg/L Li).

Bozich et al., 2017 studied the chronic toxicity (21d) of lithium nickel manganese cobalt oxide (NMC), lithium cobalt oxide (LCO) and respective metal ions (Ni, Co, Li (nominal concentrations: 0.5, 1, 2.5, 5, 10 mg Li/L)) on *Daphnia magna* in static exposure conditions. The chronic toxicity assay with lithium exposure showed that only the highest measured concentration of lithium (10 mg/L) leached from 25 mg/L of NMC caused significant impairments to daphnid survival and to reproduction. At 2.5 mg/L Li, daphnids exhibited a 97.8% reduction in reproduction. At 2.5, 5.0 and 10 mg/L, 100% reduction in daphnid survival was observed at day 21. No impact on daphnid body size was observed. Due to the absence of dose-response, the results of this study were not taken into account.

[71] Inhibition concentration

Zhao et al., 2017 assessed the acute and chronic toxicity of lithium to *Daphniopsis tibetana.* The authors reported that 5-10 mg/L Li could accelerate the growth and reproduction of *D. tibetana*. Due to methodological limits (no unexposed group as the control group comprised D. tibetana reared at a safe concentration defined to be 69.3 mg/L Li, lack of clarity regarding the concentrations tested in the acute test), the results of this study were not taken into account.

Summary: Long-term data on aquatic invertebrates from the registration dossiers and literature are synthetised in Table 51. **Chronic toxicity of lithium as LiCl was studied in several arthropods (*Daphnia magna, Ceriodaphnia dubia, Daphniopsis tibetana).* The lowest NOEC was 0.02 mg/L (nominal) Li for Daphnia magna based on living offspring.**

Table 51: Long-term effects in aquatic invertebrates

Substance	Species	Test duration	Value	Calculated value for Li ion	Endpoint	Effect parameter	pH of the tested solutions	Reference	Guideline
Li	*Daphnia magna*	21d	1.7 mg/L (nominal conc.)	-	NOEC	mortality	7.06-8.45 pH adjustment with 1N HCl	Unnamed, 2012	OECD TG 211 (2008 version)
LiOH*H2O	*Daphnia magna*	21d	4 mg/L (nominal conc.)	**0.66 mg/L**	NOEC	reproduction	No information but expected to increase	Unnamed, 2010	OECD TG 211
LiCl, 20°C, control light intensity	*Daphnia magna*	21d	-	**0.02 mg/L (nominal conc.)**	NOEC	reduction of living offspring	8.36-8.44	Martins 2022	OECD TG 211
LiCl, 20°C, control light intensity	*Daphnia magna*	21d	-	**0.04 mg/L (nominal conc.)**	NOEC	population growth rate	8.36-8.44	Martins 2022	OECD TG 211
LiCl	*Ceriodaphnia dubia*	6-7d	3.6 mg/L (measured conc.)	**0.59 mg/L**	NOEC	reproduction	No information	Okamoto 2021	EPS 1/RM/21

Toxicological values below 1 mg/L are in bold.*

20.1.3 Algae and aquatic plants

Four studies following the OECD TG 201 are reported in the registration dossier (Unnamed 2010a, Unnamed 2010b, Unnamed 2010c, Unnamed 2004). They respectively tested the toxicity of Li_2CO_3, $LiOH*H_2O$, LiCl and LiOH on two algae species (*Desmodesmus subspicatus* and *Raphidocelis subcapitata*) under static exposure conditions. The registrants judged the tests to be Klimisch reliability 1 or 2. The studies reported 72h-EC_{50} between 1.67 and > 75.15 mg Li/L based on growth rate, and NOEC between 0.20 and 9.39 mg Li/L based on growth rate.

Literature search also provided data on algae. They are described below and in Table 52.

Karlander et al. 1972, cited in Liang et al. 2020, studied absorption and toxicity of beryllium and lithium in *Chlorella vannielii Shihira and Krauss.* Lithium concentrations were 0, 5, 10, 25, 50, 100, 1000 mg/L. Under optimal growth conditions of light, CO2 and temperature, concentrations up to 100 mg/L of lithium were not inhibitory to growth of *Chlorella*. At 1000 mg/L lithium, the autotrophic growth rate was reduced to 52% of the control.

Mendes et al. 2013 reported a median inhibitory concentration (IC50) of 96 $\pm$ 1 mg/L Li (13.8 $\pm$ 0.1 mM) for the seaweed *Gracilaria domingensis* (Gracilariales, Rhodophyta).

Overall, based on the data available in the registration dossier, the lowest acute 72h-EC50 is 1.67 mg Li/L (nominal concentration) and the lowest long-term NOEC is 0.20 mg Li/L for *Raphidocelis subcapitata* based on growth rate. Two studies from literature search showed much higher toxicological values.

Table 52: Effects in algae and aquatic plants

Substance	Species	Test duration	Value	Calculated value for Li ion	Endpoint	Effect parameter	Reference
Li2CO3	*Desmodesmus subspicatus*	72h	> 400 mg/L (nominal conc.)	> 75.15 mg/L	EC50	growth rate	Unnamed, 2010
Li2CO3	*Desmodesmus subspicatus*	72h	90 mg/L (nominal conc.)	16.91 mg/L	EC10	growth rate	Unnamed, 2010
Li2CO3	*Desmodesmus subspicatus*	72h	50 mg/L (nominal conc.)	9.39 mg/L	NOEC	growth rate	Unnamed, 2010
LiOH*H2O	*Raphidocelis subcapitata*	72h	153.44 mg/L (nominal conc.)	25.39 mg/L	EC50	growth rate	Unnamed, 2010
LiOH*H2O	*Raphidocelis subcapitata*	72h	10 mg/L (nominal conc.)	1.65 mg/L	NOEC	biomass and growth rate	Unnamed, 2010
LiCl	*Desmodesmus subspicatus*	72h	> 400 mg/L (nominal conc.)	> 65.4 mg/L	EC50	growth rate	Unnamed, 2010
LiCl	*Desmodesmus subspicatus*	72h	80 mg/L (nominal conc.)	13.1 mg/L	EC10	growth rate	Unnamed, 2010
LiCl	*Desmodesmus subspicatus*	72h	25 mg/L (nominal conc.)	4.09 mg/L	NOEC	growth rate	Unnamed, 2010
LiOH	*Raphidocelis subcapitata*	72h	5.76 mg/L (nominal conc.)	1.67 mg/L	EC50	growth rate	Unnamed, 2004
LiOH	*Raphidocelis subcapitata*	72h	1.39 mg/L (nominal conc.)	0.40 mg/L	EC10	growth rate	Unnamed, 2004
LiOH	*Raphidocelis subcapitata*	72h	0.68 mg/L (nominal conc.)	0.20 mg/L	NOEC	growth rate	Unnamed, 2004
LiOH	*Raphidocelis subcapitata*	72h	1.5 mg/L (nominal conc.)	0.43 mg/L	LOEC	growth rate	Unnamed, 2004
Li	*Gracilaria domingensis*	?	96 mg/L		IC50	growth rate	Mendes et al. 2013

20.1.4 Sediment organisms

In the registration dossier, a data waiving was performed for Li2CO3 and LiOH. The reason provided in the dossiers is that the chemical safety assessment does not indicate the need to investigate further the effects of the substances and/or relevant degradation products on sediment organisms. In addition, the registrant claims also that according to their physico-chemical properties, lithium carbonate and lithium hydroxide are not expected to be distributed into sediment and the ad-/desorption behaviour does not indicate that lithium will be present in the sediment. The log Kd value for sediment was found to be 2.72, i.e. adsorption potential to sediment can be regarded as neglible. Thus, concentration and consequently effects in sediment are negligible. Additionally, carbonate and hydroxide naturally occur in water and are natural components of soil minerals. Therefore, no testing on sediment toxicity is required. Regarding LiCl, a sediment-water chironomid life-cycle toxicity test using spiked water or spiked sediment (OECD TG 233) is proposed by the registrants as testing proposal (TPE). No data is available for Li.

20.1.5 Microbiological activity in sewage treatment systems

A study following the OECD TG 209 is reported in the registration dossier (Unnamed 2004). It tested the effects of LiOH on microorganisms from activated sludge by measuring their respiration rate. The study reported a 3h-EC50 of 52.4 mg Li/L (nominal concentration) based on respiration rate.

20.1.6 Other aquatic organisms

No data are available for Li2CO3, LiCl, LiOH and Li in the registration dossier. But the Literature search provided data on several aquatic organisms.

20.1.6.1 Amphibians

20.1.6.1.1 Xenopus embryos

Boga Pekmezekmek et al., 2020 studied the effects of 0.02 g/L LiCl (3.3 mg/L Li) (relatively high concentration) on the development of *Xenopus laevis* embryos by using the FETAX (frog embryo teratogenesis assay of Xenopus) test. Embryos that were exposed to LiCl for 96h between the midblastula (stage 8) and early gastrula (stage 11) showed developmental abnormalities (25%) (microcephaly, microphthalmia, cyclopia, gut and tail malformation, bullae), decrease in length, hyperpolarization, mortality (75%). Thus, LC50 is below 3.3 mg/L Li.

Monteiro et al., 2018 studied the transcriptomes of ventralized or dorsalized *Xenopus tropicalis* embryos exposed to UV or LiCl treatment. Exposure to 0.3M LiCl (2 g/L Li) (very high concentration) for 5 min caused dorsalisation of the 32-cell embryos. Hyperdorsalisation caused blastopore lip formation to be initiated on time but circumferentially. At the tailbud stage, hyperdorsalised embryos consisted of radially symmetric, extended anterior structures such as a circular cement gland at the expense of any posterior structures such as trunk and tail.

Klein et al., 1996 studied the molecular mechanism for the effect of lithium on xenopus embryos development. The xenopus species was not reported. Stage-30 embryos treated with 0.3 M LiCl (2 g/L Li) (very high concentration) at the 32-cell stage showed profound dorsalization with concentric cement gland, expanded anterior neural structures, and absent ventral and posterior structures.

Lettice et al., 1993 studied the timing and transmission characteristics of dorsalization in explants of *Xenopus laevis*. It could be seen that explants that are treated with 0.1 M LiCl (0.7 g/L Li) (very high concentration) at stage 7 form large blocks of muscle, and also notochord, while those treated at stage 9 will still give rise to a lot of muscle, but are never observed to contain notochord. When the treatment is conducted later than this, i.e. at gastrula stages, there is no discernible effect, with explants giving rise to ventral tissues such as mesenchyme, mesothelium, blood and, occasionally, wisps of muscle. Pronephric tubules are never observed in lithium-treated explants. These results show that lithium has a much stronger dorsalizing effect when applied to blastula stages than at any point during gastrulation.

Klein et al., 1991 studied the mechanism by which lithium influences *Xenopus laevis* dorsal pattern formation. Embryos treated before the 32-cell stage or after the 512-cell stage were completely normal. The effects of the treatment (0.3 M LiCl) (2 g/L Li) (very high concentration) were maximal at the 64 and 128-cell stage.

Klein et al., 1989 studied the distribution of the progeny of blastomeres of early cleavage stage *Xenopus laevis* embryos exposed to 0.3 M LiCl (2 g/L Li) (very high concentration) for 6 min interval during the 32- to 128-cell stage. The majority (up to 90%) of the exposed embryos were head-only embryos with normal faces and heads. Thus, LiCl treatment reversed the embryo's polarity so that the head develops in the region that normally produces posterior structures, via changes in cell fate of dorsal blastomeres from posterior to anterior. These changes in cell fate result from alterations in the pattern of gastrulation.

Nagajski et al., 1989 studied the correlation between cell-to-cell communication through gap junctions at the 32-cell stage and the subsequent patterning of the embryonic axis by disturbing the embryonic axis formation with UV irradiation or LiCl. As expected, embryos treated with 0.1 M LiCl (0.7 g/L Li) (very high concentration) between the 2-cell and 32-cell stage showed ventral axial deficiencies.

Stanisstreet et al., 1974 compared the effects of several substances including lithium on Xenopus embryos. Exposure to 0.15 M LiCl (1.04 g/L Li) (very high concentration) caused arrested cleavage and adnormal pigment distribution; exposure to 0.025 M LiCl produced very few abnormalities. Using 0.1 M LiCl, 82% of embryos exposed at early cleavage stages (stage 2-4) were vegetalized, and 69% of embryos exposed at mid-cleavage (stage 8-9) to early blastula stages (stage 8-9) were also vegetalized. By the late blastula stage (stage 9-$9^{1/2}$), embryos were not affected by a 3h exposure to lithium. Thus earlier embryos are more susceptible to lithium than later embryos. Continuous exposure to lithium was found to produce disaggregation of the embryos.

Cooke et al., 1988 carried out an anatomical study of Xenopus larval and gastrula stages resulting from treatment of synchronous early blastulae (64-128-cell stage) for brief periods with LiCl. Exposure to 0.125 M LiCl (0.9 g/L Li) (very high concentration) for 45 min or 0.25 M LiCl for 9-12 min during the early blastula caused dorsalisation of xenopus embryos.

Summary: Only one study reported mortality of xenopus embryos with LC50 < 3.3 mg/L Li (Boga Pekmezekmek et al. 2020). The other studies identified on xenopus mostly investigated developmental effects of lithium at embryonic stage at very high concentrations. As mentioned before in the section on fish and aquatic invertebrates' data, these effects may not be used for assessing acute toxicity in the frame of a regulatory assessment. **Lithium caused developmental abnormalities in xenopus embryos, characterised by a dorsalisation of the animals** (Boga Pekmezekmek et al. 2020, Monteiro et al. 2018, Klein et al. 1996, 1991, 1989, Lettice et al. 1993, Nagajski et al. 1989, Stanisstreet et al. 1974; Cooke et al. 1988)**.**

20.1.6.1.2 American bullfrog

Pinto-Vidal et al. 2021a studied the effects of lithium and selenium on American bullfrog (*Lithobates catesbeianus*) tadpoles at the Gosner's stage 25 (premetamorphic stage) according to the OECD Test 231 (amphibian metamorphosis assay). Tadpoles were exposed to 0 or 2.5 mg/L Li as LiCl (n = 20 animals per group) for 21d with a static renewal system. The following biomarkers of larval development were investigated: total wet weight, snout-vent-length, hind-limb-length, activity level, histologic evaluation of the thyroid gland and mortality rate. At the end of the exposure period, Li-exposed tadpoles showed no effect on snout-vent-length, hind-limb-length and total wet weight when compared to the control group. In the Li-exposed group, 2 dead animals (10%) were observed (at day 19 and 20) but there was no statistical significance. These animals expressed different pattern of coloration and loss of the black dots in the back; they also presented feeding cessation and abnormal swimming patterns, evolving to death few days after. At day 7 and 21, Li-exposed animals were lethargic, compared to the control group ($P < 0.001$). They had a lower activity level ($P < 0.001$) than the controls. Regarding the histopathology of the thyroid gland which was analysed on day 21, and in comparison to the controls, Li-exposed animals showed a reduction of 26% ($P < 0.05$) in the total gland area, a reduction of 55% ($P < 0.05$) in the total follicular area of the gland, a reduction of 48% ($P < 0.05$) in the number of

follicles. These effects on the thyroid gland are signs of up-regulation and thus acceleration of the metamorphic process.

In another study, the same authors assessed the metabolic, immunologic and histopathologic alterations in liver samples of American bullfrog (*Lithobates catesbeianus*), at the premetamorphic stage, through biomarkers indicative of general energetic status, i.e., glucose, lipid and protein metabolism (Pinto-Vidal et al. 2021b). The same experimental conditions as Pinto-Vidal et al. 2021a were applied. The Li-exposed animals showed an increase in the glucose mobilization in comparison to the controls on day 7, which was correlated with reduced glycogen, but not on day 21. A statistically significant increase in the triglycerides' mobilization was observed on both days; this was correlated with a decreased storage of lipids. Exposure to LiCl did not cause any effect on the total soluble protein. The histopathological analysis showed that the Li-group presented microgoticular steatosis (on d7 and 21), increase presence of melanomacrophage centres (MMCs) (on d7), rarefaction of the cytoplasm and disturbed architecture of the sinusoids (on d7 and 21), which is indicative of hepatotoxicity.

Summary: Lithium (2.5 mg/L LiCl) caused up-regulating effects on the thyroid gland of premetamorphic American bullfrog which were the signs of an acceleration of the metamorphic process. Hepatotoxicity was also observed (Pinto-Vidal et al. 2021a, 2021b, 2022).

20.2 Terrestrial compartment

20.2.1 Toxicity to soil macro-organisms

In the registration dossier, a data waiving for Li2CO3, LiOH and Li was performed. The reason provided in the dossiers is that according to their physico-chemical properties, lithium carbonate, LiOH and lithium ions are not expected to be distributed into soil and the ad-/desorption behaviour does not indicate that lithium will be present in the soil. The log Kd value for soil was found to be 2.57, i.e. adsorption potential to soil can be regarded as negligible. Thus, concentration and consequently effects in soil are expected to be low. Additionally, carbonate and hydroxides naturally occur in water and are natural components of soil minerals. Therefore, no further testing is needed for the soil compartment. Regarding LiCl, an Earthworm Reproduction Test (OECD TG 222) was submitted by the registrant as testing proposal.

Literature search provided short-term data on nematodes (7 publications), earthworms (2 publications) and insects and arachnids (10 publications). Data are described below and synthetised in Tables 53 and 54 respectively for nematodes and earthworms.

20.2.1.1 Nematodes

Hunt et al. 2018 used lithium organometal as a control developmental toxin to evaluate the ability of a novel worm Development and Activity test (aqueous exposure) using soil nematode *Caenorhabditis elegans* to distinguish a developmental toxin from developmental neurotixicity. *C. elegans* larval growth was first assessed by exposing the nematode to 0, 0.25, 0.5, 1, 2 mg/mL of lithium acetate dihydrate (LiOAc) (from 17 to 140 mg/L Li). Significant reductions in larval growth were detected at 0.5 mg/mL LiOAc. The worm larval Development and Activity test was then conducted by exposing the worms to 0, 25, 50, 100, 150, 200, 250 µg/mL LiOAc. Significant delays in *C. elegans* development were detected

at 150 µg/mL LiOAc. Hyperactivity was not detected but hypoactivity was at 150 µg/mL LiOAc.

Inokuchi et al. 2015 (cited in MELCC 2022) studied the effects of lithium on growth, maturation, reproduction and gene expression in *C. elegans*. In a lethal toxicity test (aqueous exposure), mixed-stage (L1-L4) worm populations dominated by L1 larvae were exposed for 24h to 0 (KCl), 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10 mM LiCl (i.e. 1 to 69 mg/L Li) or 0 (K2CO3), 1.562, 3.125, 6.25, 12.5, 25, 50 and 100 mM Li2CO3 (i.e. 22 to 1388 mg/L Li). LiCl exposure did not cause mortality. However, dose-dependent mortality was observed with Li2CO3. The corresponding LC50 value was 7.5 mM Li2CO3 (104 mg/L Li). In a growth and maturation test exposing L1 larvae to the same LiCl concentrations or 0 (K2CO3), 0.047, 0.094, 0.188, 0.375, 0.75, 1.5 and 3 mM Li2CO3 for 55h, LiCl and Li2CO3 decreased growth and maturation of the nematodes. The effect values were the following: NOEC (LiCl, growth): 0.625 mM (4.3 mg/L Li); NOEC (Li2CO3, growth): 0.375 mM (5.2 mg/L Li); NOEC (LiCl, maturation): 0.156 mM (1.1 mg/L Li); NOEC (Li2CO3, maturation): 0.375 mM (5.2 mg/L Li). For the reproduction test, the nematodes used for the growth and maturation test were exposed to the same previous concentrations until egg production ceased. LiCl and Li2CO3 decreased the nematode's reproduction: NOEC (LiCl, reproduction): 5 mM (35 mg/L Li); NOEC (Li2CO3, reproduction): 1.5 mM (21 mg/L Li). Among approximately 300 unique genes, LiCl and Li2CO3 downregulated the expression of metabolic genes.

Jaworska et al. 1996 studied the effect of 16 metal ions in aquous exposure including Li+ on the enthomopathogenic nematode *Steinernema carpocapsae*. In a lethality test where the nematodes were exposed to 5-50 mg/L Li2SO4.H2O (0.54-5.4 mg/L Li) for 96h, no mortality was observed. Thus, NOEC value is bigger than 5.4 mg/L Li so does the EC50 that is above the NOEC.

Jaworska et al. 1999 studied the effect of lithium cation on entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* mortality, pathogenicity, and productivity. In a lethality test exposing the nematodes (aqueous exposure) to 0, 5, 10, 20, 30 and 50 mg/L Li2SO4 (from 0.6 to 6 mg/L Li) for 5d at 5 or 25°C, low mortality was observed for the both species. For *Steinernema carpocapsae*, the average percentage of mortality considering all concentrations was 10.9% and 6.6% after 5d, respectively at 5 and 25°C. For *Heterorhabditis bacteriophora*, the corresponding values were 5.9 and 9.5%.

Meisel et al. 2016 studied the molecular mechanisms underlying the action of lithium on the nervous system in *C. elegans*. It was shown that acute exposure to 15 mM LiCl (0.1 g/L Li) decreased the expression of all ASJ-specific genes, reduced the cytoplasmic volume of the ASJ neurons and dauer exit[72] behaviour was inhibited.

Tam et al. 2014 investigated the effects of upregulating autophagy using lithium in *C. elegans*. It was found that lithium exposure (10 mM LiCl, i.e. 69 mg/L Li) increased both the lifespan and healthspan of the nematode without any significant change in the mortality rate and oxidative protein damages. The increase in healthspan was accompanied by improvements in mitochondrial energetics, evidenced by an increase in the ATP levels. In contrast, mitochondrial DNA copy number decreased faster with age under lithium.

[72] Under unfavorable conditions, larvae enter the non-feeding and non-reproductive stress-resistant dauer stage and adapt their behavior to cope with the harsh new environment.

Tatara et al. 1998 (cited in MELCC 2022) used ion characteristics to predict relative toxicity of mono-, di- and trivalent metal ions in *C. elegans*. After 24h exposure to Li+, the LC50 was 215±13 mM (i.e. 1.5 g/L Li).

Summary: Several studies investigated the effects of lithium on various nematode species (the soil nematode *Caenorhabditis elegans,* and two entomopathogenic nematodes *Steinernema carpocapsae and Heterorhabditis bacteriophora*). Developmental effects were observed in C. elegans: reduction in larval growth with NOEC values at 0.625 mM LiCl (4.3 mg/L Li) and 0.375 mM Li2CO3 (5.2 mg/L Li) (Inokuchi et al. 2015), developmental delay from 150 µg/mL LiOAc (10 mg/L Li) (NOEC: 100 µg/mL (6.8 mg/L Li)) (Hunt et al. 2018) as well as decreased reproduction capacity with NOEC values at 5 mM LiCl (35 mg/L Li) and 1.5 mM Li2CO3 (21 mg/L Li).

While mortality was observed in *C. elegans* following lithium exposure (Inokuchi et al. 2015 (LC50: 7.5 mM Li2CO3 (104 mg/L Li)), Tatara et al. 1998 (LC50: 215 mM Li+ i.e. 1.5 g/L Li)), no or low mortality (below 10%) was found in *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* (Jaworska et al. 1996, 1999 (5-50 mg/L Li2SO4, i.e. 0.6-6 mg/L Li)). One study showed that lithium (10 mM LiCl, i.e. 69 mg/L Li) was able to increase the lifespan and healthspan of *C. elegans* (Tam et al. 2014).

Table 53: Short-term effects in nematodes

Substance	Species	Test duration	Effect parameter	Endpoint	Value	Calculated value for Li ion	Reference
Li2CO3	*C. elegans*	24h	mortality	LC50	7.5 mM	104 mg/L	Inokuchi et al. 2015
Li	*C. elegans*	24h	mortality	LC50	-	215 mM <=> 1492 mg/L	Tatara et al. 1998
LiOAc	*C. elegans*	3d	growth	NOEC	100 µm/mL	6.8 mg/L	Hunt et al. 2018
LiCl	*C. elegans*	55h	growth	NOEC	0.625 mM	4.3 mg/L	Inokuchi et al. 2015
Li2CO3	*C. elegans*	55h	growth	NOEC	0.375 mM	5.2 mg/L	Inokuchi et al. 2015
LiCl	*C. elegans*	55h	maturation	NOEC	0.156 mM	1.1 mg/L	Inokuchi et al. 2015
Li2CO3	*C. elegans*	55h	maturation	NOEC	0.375 mM	5.2 mg/L	Inokuchi et al. 2015
LiCl	*C. elegans*	until egg production ceases	reproduction	NOEC	5 mM	35 mg/L	Inokuchi et al. 2015
Li2CO3	*C. elegans*	until egg production ceases	reproduction	NOEC	1.5 mM	21 mg/L	Inokuchi et al. 2015

20.2.1.2 Earthworms

Xu et al. 2023 assessed the ecotoxicological effects of lithium on earthworm *Eisenia fetida*. After 7d of exposure to 0, 10, 20, 40, 80, 160, 320, 640, 960, and 1280 mg/kg Li2SO4.H20, the mortality was dose-dependent and the LC50 was 94.95 mg/kg. The bioaccumulation factor based on measured exogenous lithium content respectively was 0.79, 1.01, 1.57, and 1.27 with the increasing lithium levels from 10 to 80 mg/kg, suggesting that lithium accumulation was averagely 1.16-fold to the exogenous lithium content in soil. The accumulated lithium in exposed earthworms was mainly in cytosol fraction, then in debris and granule fractions. Then, lithium stress stimulated the activity of superoxide dismutase, peroxidase, catalase, acetylcholinesterase and glutathione S-transferase as well as the content of 8-hydroxy-2-deoxyguanosine and metallothionein, indicating the generation of oxidative damage. On the contrary, the content of reactive oxygen species and malondialdehyde decreased. Finally, lithium induced histopathological changes such as the degeneration of the seminal vesicles, indicating the decline of

earthworm's reproductive capacity, and muscle hyperplasia, as well as high or extreme nuclear DNA damage.

The review from Aral et al. (2008) (cited in MELCC 2022) reported the results from Lenntech 2007 and US EPA 2008 indicating mortality at ~70 mg LiCl/kg soil for *E. fetida* following 7-week exposure to LiCl.

Summary: Two studies described the effects of lithium on Eisenia fetida (Xu et al. 2023, Lenntech 2007 and US EPA 2008 cited in Aral et al. 2008). Xu et al. 2023 and US EPA 2008 showed mortality of *E. fetida* following Li exposure (LC50: 95 mg/kg, mortality at ~70 mg/kg, resp.). It also showed lithium accumulation and activation of biomarkers indicating oxidative stress in the worms. Lithium also induced histopathological changes.

Table 54: Short-term effects in earthworms

Substance	Species	Test duration	Effect parameter	Endpoint	Value	Calculated value for Li ion	Reference
Li2SO4.H20	*Eisenia fetida*	7d	mortality	LC50	94.95 mg/kg	10.3 mg/kg	Xu et al. 2023
LiCl	*Eisenia fetida*	7d	mortality	-	70 mg/kg	11.5 mg/kg	Aral et al. 2008

20.2.1.3 Insects and arachnids

Rein et al. 2022 studied the effects of different concentrations of LiCl on the mortality of honey bee larval stages, using both feeding of artificially reared larvae and field-realistic applications in free flying colonies. In a first approach, the artificially reared larvae were fed with 0, 1, 1.5 and 2 mM LiCl for 6d. In a second approach, the larvae were fed for 3d with 0 or 2 mM LiCl from 1st to 3rd larval instar or from 3rd to 5th larval instar. In the first approach, the survival of the Li-exposed larvae was significantly reduced from 1 mM. The second approach led to a significant reduction in the survival rates in both treated groups but the **younger larvae were more susceptible to LiCl compared to the larvae treated from 3rd to 5th larval instar**. The authors also assessed the brood development in free flying colonies following LiCl exposure to 0, 10, 17.5 and 25 mM for 8d. The concentration of 10 mM was well tolerated. However, a chronic exposure to 25 mM induced brood mortalities between 60 and 90%. Shorter feeding periods of 2 or 4 days reduced the brood damages significantly. Besides, it was frequently observed that the pupae were malformed with an incomplete development of head, thorax and abdomen while most of the larval instars seemed normally developed. The authors suggested that this might indicate that lithium disturbed the metamorphosis of the honey bee larva which is supported by the high mortality and brood removal rates of pre-pupal and pupal stages.

Sevin et al. 2022 studied the long-term *in vitro* and *in vivo* bee toxicities, short-term motor toxicity to bees and long-term anti-Varroa field efficacy of several lithium salts (lithium citrate, lithium lactate and lithium formate). In the *in vitro* chronic-toxicity assay where the honey bees were orally exposed to 0, 10, 25 and 50 mM Li salts until they all died (36d), all lithium salts reduced the lifespan of the bees. **Lithium citrate was the most toxic with the lowest LT50 value and maximum life expectancy at 25 and 50 mM, followed by lithium lactate and lithium formate, which did not significantly differ**. In ters of acute locomotor toxicity to bees, all of the lithium salts were well tolerated and no acute locomotor toxicity was observed. In the bee mortality field study where the bees were exposed to 25 mM lithium formate or lactate for 6 weeks, no significant effect on mortality was observed. In the long-term anti-Varroa field efficacy evaluation with the same exposure conditions as the field bee toxicity study, lithium formate exhibited

acaricide effects towards Varroa, which were significantly different from those observed for lithium lactate and the negative control.

Kurbalija et al. 2021 studied the influence of lithium on the whole-organism metabolic rate in *Drosophila subobscura*. It was observed that survival reduced with increasing concentrations of lithium sulfate and the developmental period was extended. The LD50 value was 0.0006113 mg/ml. Within a narrow dose range, lithium influenced the whole-organism metabolic rate which switched from lower to higher than baseline. Moreover, lithium exposure influenced metabolism differently based on mtDNA haplotypes and sex.

As lithium is an emerging varroa control substance, Prešern et al. 2020 investigated its accumulation in honey, bee bread, brood and adults along with the mortality of bees. Workers fed once per os with 10 µL of 25 mM LiCl in sucrose solution or having the same solution available ad libitum showed increased lithium concentrations in head, thorax and abdomen. Following a 3d treatment of honeybee colonies with 25 mM LiCl in 1L/day sucrose solution, increased lithium concentrations were observed in 5d-old larvae, honey and bee bread (up to 45.0, 1.2 and 47.0 mg/kg, respectively). Lithium concentrations peaked three days post-treatment in both larvae and honey and increased worker mortality was observed.

Hurst et al. 2014 used the honeybee *Apis mellifera* to investigate how intoxication produced by injection or ingestion with three toxins with different pharmacological modes of action (quinine, amygdalin and lithium chloride) affected behaviour. The injection or ingestion of 0.1 or 1 mM LiCl significantly induced less walking in bees and more time spent stopped. They also showed more time spent grooming (non significant). Injection with LiCl was more likely to cause a failure to right whereas ingestion did not. Neither injection nor ingestion of LiCl affected abdomen dragging.

Kasuya et al. 2009 used *Drosophila melanogaster* genetics to investigate functional roles of the Lithium-inducible solute carrier 6 transporter (List) gene, CG15088, in the lithium-responsive neurobiological process. An RT-PCR analysis showed upregulation of List in head and body of wild-type virgin female flies (0-24h old) fed with 50 mM LiCl for 24h. A reactive climbing assay showed that 50 mM LiCl had little effect on the climbing behaviour of the flies. In terms of survival, it took 39.3 and 65.0 days for drosophila to reach 50% and 100% mortality, respectively. Besides, 0.68 +/- 0.21 mM Li was measured in wild-type flies treated with 50 mM LiCl.

Dokucu et al. 2005 measured Drosophila circadian locomotor activity in response to lithium and valproate exposure. First, for determining the appropriate doses for the behavioural analysis, the flies were exposed to 3-300 mM LiCl in the nutrition media for 8d. The lethality following lithium exposure up to 30 mM in the food was quite low until the end of the experiment. Flies receiving lower concentrations of lithium continued to live for at least another 10 days. At 8d, 100% of lethality was observed at 300 mM. In the locomotor behaviour assay, the flies were exposed to 0.03-300 mM LiCl in food. Regarding the circadian behaviour, lithium induced a significant increase in the period of the free-running locomotor rhythms of the fly. Regarding the behavioural rhythmicity, Li increased the number of arrhythmic animals in prolonged constant darkness as determined by lack of significant rhythmic locomotor activity.

Matsagas et al. 2009 proposed a new type of assay for the long-term effects of candidate medications. The assay was performed in order to study the chronic side-effects of four central nervous system agents (caffeine, theobromine, lithium carbonate and valproic acid) on *Drosophila melanogaster.* Flies exposed to lithium carbonate received 0, 0.026, 0.26 and 2.6 mg/gm yeast. At the two lowest doses of lithium, a slight elevation in mean longevity was observed compared to the

control. However, lithium caused a drastic decrease in female fecundity. Regarding male mating, lithium significantly lowered the mating success of males.

McPhee et al. 1957 reported that fully active 21-day old clothes-moth larvae (*Tineola bisselliella Humm*.) all died in 6-7 days when allowed to feed on fabrics containing 0.2 gm.-ion of lithium (lithium sulfate, lithium chloride and lithium citrate) per 10^4 gm. wool (0.1-0.5% by weight of their salts).

Solti et al. 2022 assessed the acaricidal activity of LiCl against the two-spotted spider mite *Tetranychus urticae*. It was reported three different concentrations that bear 100% mortality: 5.52 M, 2.76 M, and 1.38 M.

Summary: Several studies showed decreased survival following lithium exposure in honeybees (Rein et al. 2022 – from 1 mM LiCl (6.9 mg/L Li), Sevin et al. 2022 – LT50: 25 mM, Prešern et al. 2020), Drosophila (Kurbalija et al. 2021 – LD50: 6.10-4 mg/mL Li2SO4 (77 µg/L Li), Dokucu et al. 2005 – 100% mortality at 300 mM LiCl (2 g/L Li)), clothes-moth larvae (McPhee et al. 1957 – 0.2 gm.-ion Li) and the two-spotted spider mite *Tetranychus urticae* (Solti et al. 2022). While **no acute locomotor toxicity was observed in Li-exposed bees** in the study from Sevin et al. 2022 (up to 50 mM Li salt), Hurst et al. (2014) showed **behavioural effects in Li-exposed bees from 0.1 mM LiCl (0.7 mg/L Li) such as less walking, more time spent stopped, more time spent grooming**. In Drosophila, while lithium exposure (50 mM LiCl, i.e. 0.3 g/L Li) caused little effect on the climbing behaviour of the flies in the study from Kasuya et al. (2009), it lenghtened the period of circadian rhythms and increased arrhythmicity. Finally, **decreased female fecundity and mating success were observed in Drosophila** (Matsagas et al. 2009 - from 0.026 mg/gm yeast) and the whole-organism metabolic rate was affected by Li exposure (Kurbalija et al. 2021).

20.2.2 Toxicity to terrestrial plants

In the registration dossier, a data waiving for Li2CO3, LiOH and Li was performed. According to its physico-chemical properties, Li2CO3, LiOH and lithium ions are not expected to be distributed into soil and the ad-/desorption behaviour does not indicate that lithium will be present in the soil. The log Kd value for soil was found to be 2.57, i.e. adsorption potential to soil is expected to be low (refer to IUCLID section 5.4). Additionally, carbonate and hydroxide naturally occur in water and are natural components of soil minerals. Therefore, no further testing is needed for the soil compartment according to the registrant. An existing study with terrestrial plants (maize; Zea mays L., family: Poaceae) further shows that lithium exposure does not lead to any significant toxicity to these plants. In this study an LC50 of 140 mg/L was determined for treatment period of 2 months (rf. to IUCLID section 6.6 additional ecotoxicological information). This study can be regarded as worst case as the plants were treated under hydroponic conditions. Regarding LiCl, a Seedling Emergence and Seedling Growth Test (OECD TG 208) was proposed by the registrant as testing proposal.

MELCC (2022) reported that the studies assessing the toxicity of lithium in plants have demonstrated that **at low concentrations, lithium can stimulate the growth and increase yields of cultivated plants. Adverse effects are observed mainly at high concentrations**. These effects include the appearance of necrosis, as well as a reduction in germination rate, biomass and growth. Additionally, lithium interferes with the absorption and translocation of other biologically important elements, such as magnesium, sodium, potassium and calcium, which can cause deficiencies. Lithium tolerance in certain plant species is also closely linked to their sodium tolerance. Therefore, species known to be easily affected by low amounts of sodium (e.g. deciduous trees, citrus fruits, avocados,

kidney beans) are more sensitive to the effects of lithium, while other sodium tolerant crops would be relatively tolerant to this metal (MELCC 2022).

The complementary literature search provided other data on several plant species. Data are described below. Some publications were already addressed in the report from MELCC (Jiang et al. 2014, 2018a, 2018b, Duff et al. 2014, McStay et al. 1980, Bingham et al. 1964, Shahzad et al. 2016).

Afzal et al. 2023 investigated the bioaccumulation potential of lithium by quinoa (*Chenopodium quinoa*) and its effects on growth and physiology. Quinoa was exposed to various concentrations of lithium (0, 2, 4, 8 and 16 mM, i.e. 0, 0.01, 0.03, 0.06 and 0.1 g/L Li) at germination as well as seedling stages. From 0 to 8 mM, lithium increased seed germination, biomass and grain yield. At higher level (16 mM), lithium was detrimental to quinoa growth by decreasing seed germination.

Shakoor et al. 2023 conducted a meta-analysis of the existing literature investigating the impact of lithium sources and levels on plants species. It was reported that **toxic effects of lithium exposure in plants varies as a function of medium and more negative responses are reported in hydroponic media as compared to soil and foliar application**. Moreover, **toxic effects of lithium vary with lithium source materials**. LiCl more negatively affected plant development parameters such as plant germination (n = 48) and root biomass (n = 57) and recorded highly uptake in plants (n = 78), while LiNO3 had more negative effects on shoot biomass. Lithium at concentration < 50 mg/L significantly influenced the plant physiological indicators including plant germination and root biomass, while 50–500 mg/L Li influenced the biochemical parameters. **The dose-response relationship (EC50) ranges regarding the exposure medium of lithium sources in plant species were 24.6–196.7 ppm**. The uptake potential of lithium is dose-dependent and their translocation/bioaccumulation remains unknown.

Török et al. 2022 investigated the lithium uptake and changes induced by lithium exposure in the major and trace element contents, photosynthetic pigments, antioxidant activity and elemental composition of *Salvinia natans*. The results showed that *Salvinia natans* grown in Li-enriched nutrient solutions was able to accumulate lithium. The lithium uptake increased with the increase in the lithium concentration in the growth medium, up to 20 mg-Li/L, and remained more or less constant at higher concentrations. This could be a consequence of the growth-stimulating effect of low lithium concentrations and the toxic effects of high lithium contents. Exposure to a high lithium content decreased potassium and iron; however, it did not change the sodium, calcium, and magnesium concentrations. Exposure to lithium also led to changes in the photosynthetic pigment content (decrease in chlorophyll a, b and carotenoids content), while their total antioxidant activity did not change substantially.

Bakhat et al. 2020 investigated spinach plant's potential lithium uptake, its effect on physiological and biochemical attributes, and its antioxidant enzymatic response to elevated lithium levels. Spinach was exposed to various concentrations of lithium (0, 20, 40, 60, 80 mg Li/kg soil). Lithium accumulation in root and shoots of spinach increased with lithium concentrations from 0 to 80 mg Li/kg soil. At 20 mg/kg, the plant growth was enhanced but at higher concentrations from 40 to 80 mg/kg, it decreased. Higher concentrations of lithium in soil interfered with potassium and calcium uptake in plants. Regarding the pigment contents, a decrease in chlorophyll a and b content was observed at 20 mg/kg soil but an increase was observed from 40 mg/kg. Plants showed a little increase in generation of H2O2 and lipid peroxidation. Moreover, significant increase was observed in the activities of antioxidant enzymes (SOD, CAT, APX) at all applied levels of lithium.

Jiang et al. 2014 investigated Li tolerance and accumulation potential of *A. venetum* in the field and in greenhouse cultivation. The field experiment showed higher lithium concentrations in leaves of *A. venetum* than in leaves of main accompanying plants. In the pot experiment, plants were exposed to 0, 50, 200 and 400 mg Li/kg for 4 weeks. At 50 mg/kg, the plants did not show obvious symptom of phytotoxicity. At 200 and 400 mg/kg, the dry weight significantly decreased. *A. venetum* could accumulate >1800 mg/kg Li in leaf tissues and survived under 400 mg/kg Li supply. The bioconcentration factor and translocation factor values were greater than 1.0. Thus, *A. venetum* has the characteristics of a Li-accumulator.

Jiang et al. 2018a studied the effects of two lithium salts (LiCl and Li2CO3) on seed germination of *Apocynum venetum*. For the germination tests, seeds were exposed to 0 (distilled water control), 1, 10, 25, 50, 100, 200, 300 and 400 mmol/L LiCl (6.9 mg/L – 2.8 g/L Li) or 0 (distilled water control), 1, 10, 25, 50 and 100 mmol/L Li2CO3 (14 mg/L – 1.4 g/L Li) for 15d at 25°C. The germination percentages decreased when the concentrations of LiCl or Li2CO3 increased. At 1-50 mmol/L LiCl, *A. venetum* seeds achieved high germination percentage (> 82%). At 200 mmol/L LiCl, it reached 53%, suggesting a high level of Li tolerance. The simulated critical value (when germination percentage is 50%) was 196 mmol/L LiCl. When the seeds were exposed to Li2CO3, the decreasing trends of germination percentage were similar as the trends affected by LiCl. However, the changes were much more dramatic with a drop from 91 to 39% when the concentrations of Li2CO3 changed from 0 to 50 mmol/L. The simulated critical value is 36 mmol/L Li2CO3. For studying physiological parameters, seeds were exposed to 0 (distilled water control), 25 and 300 mmol/L LiCl or 0 (distilled water control), 10 and 100 mmol/L Li2CO3. Under 25 mmol/L LiCl and 10 mmol/L Li2CO3, α-amylase activity (indicator of catalytic efficiency of hydrolysis of starch into sugars) of seeds did not show significant difference when compared to control. But it was affected at higher concentrations, especially at medium and late germination stages. Contents of MDA, soluble sugar and proline under high lithium salt treatments were significantly higher, indicating that seed and seedling of *A. venetum* strengthened their adaptability to the lithium salt stress by increasing the contents of organic solutes. Thus, the study showed that *A. venetum* seeds are tolerant to lithium salts during germination, especially LiCl.

Jiang et al. 2018b investigated germination, growth and physiological responses of *A. pictum* to different levels of LiCl (0 (distilled water control), 25, 50, 100, 150, 200, 300, and 400 mmol/L). The results showed that germination was not significantly affected by low lithium concentration (0-100 mmol/L) (>80%). When LiCl increased from 100 to 400 mmol/L, both germination percentage and index decreased gradually. The simulated critical value was 235 mmol/L. *A. pictum* could accumulate >1,800 mg/kg Li in leaves and still survived under 400 mg kg-1 Li supply. The high lithium tolerance of *A. pictum* during germination and growth stage was also reflected by activity of alpha-amylase and contents of soluble sugar (increase) and proline (increase) under different lithium treatments. Pigment contents (chlorophyll a, b and cartenioid) were not significantly affected by 50 mg/kg additional lithium. However, at 200 and 400 mg/kg Li, they were significant reduced when compared with control. The bioconcentration factors (BCF) (except control) and translocation factors (TF) were higher than 1.0. High tolerance and accumulation of lithium indicated that *A. pictum* is Li-accumulator.

Schlosser et al. 2016 performed a growth experiment with various crop species (buckwheat (*Fagopyrum esculentum L.*), corn (*Zea mays L.),* garden bean (*Phaseolus vulgaris L.),* spelt wheat (*Triticum spelta L.*) and ribwort plantain (*Plantago media L.))* using different lithium salts (LiCl and Li2CO3) and concentrations (2000, 1000, 500, 200, 100, 20 and 0 ppm Li representing 800, 400, 200, 80, 40, 8 and 0 mg/kg soil). Since spelt and ribwort plantain did not

germinate readily, only the results from buckwheat, corn and garden bean were evaluated in the study. At > 80ppm, a clear reduction in shoot length was observed in both lithium salts. Shoot dry mass decreased with increasing soil lithium levels, while the lithium concentrations in leaves are related to soil lithium levels. Corn leaves exposed to a soil concentration of 400 ppm showed lithium accumulation by a factor of 10 in Li2CO3 exposed soil and an even higher enrichment if the lithium was supplied as LiCl. Besides, calcium, magnesium and potassium levels decreased sharply in corn leaves from plants that were grown on lithium-treated soils, while the metal levels iron, nickel and cobalt were unaffected. Based on the response parameter shoot mass, an EC50 of 118 ppm was derived for LiCl in corn, while lower effective doses of 55 and 47 ppm were derived for garden bean and buckwheat, respectively. EC50 values for Li2CO3 were higher in corn (171 ppm) and bean (71 ppm) but lower in buckwheat (16 ppm).

Duff et al. 2014 studied short- and long-term effects of Li+ exposure in Arabidopsis with Li+ uptake studies and measured shoot mRNA transcript abundance levels in treated and control plants. Hydroponic plants exposed to 1 mM LiCl for 7d showed chlorosis and shrivelling, which were most evident in the older leaves; root tissues were severely compromised and much of the root tissue biomass was lost in comparison with the controls. When plants were hydroponically exposed to 1 mM LiCl for 1d or were soil-grown with 0.05 mM LiCl for 4 weeks, they did not show any symptom of stress. Stress, pathogen-response and arabinogalactan protein genes were typically more up-regulated in older (chronic, low level) Li-treatment plants and in the much younger plants from acute high-level exposures. The gene regulation behaviour of high-level Li+ resembled prior studies due to its influence on: inositol synthesis, 1-aminocyclopropane-1-carboxylate synthases and membrane ion transport. In contrast, chronically-exposed plants had gene regulation responses that were indicative of pathogen, cold, and heavy-metal stress, cell wall degradation, ethylene production, signal transduction, and calcium-release modulation. Acute Li+ exposure phenocopies magnesium-deficiency symptoms and is associated with elevated expression of stress response genes that could lead to consumption of metabolic and transcriptional energy reserves and the dedication of more resources to cell development. In contrast, chronic Li+ exposure increases expression signal transduction genes.

Kopittke et al. 2011 studied the toxicity of metals including lithium to roots of cowpea. Based on reduced growth, the EC50 for Li was 6400 µM.McStay et al. 1980 studied the toxicity of lithium on *Phaseolus vulgaris L.*. The plants were exposed to 0, 4, 8, and 12 ppm Li as LiNO3. lithium exposure produced toxic symptoms such as chlorosis at all concentrations, necrosis at 8 and 12 ppm, abnormal leaf development at 12 ppm. Root and stem fresh weight decreased as lithium concentrations increased. Dry weights of roots, stems and leaves were negatively correlated with lithium treatments. The physiological age of the plants was not significantly affected. At > 4 ppm Li, stomatal diffusive resistance increased temporarily, indicating partial stomatal closure. The authors concluded that lithium interferes with biomass accumulation and plant water relations.

Sneva 1979 studied lithium toxicity in seedlings of created wheatgrass (*Aoropyron desertorum*), Sherman bluegrass (*Poa ampla*) and Whitmar wheatgrass (*Agropyron inerme*). In a first study, plants were exposed to 1.63, 16.3, and 326 ppm Li as LiCl. At the highest concentration, 10 days after transplanting, all seedlings were dead. After 24d, seedlings of Sherman bluegrass growing in 16.3 ppm were all dead. At test termination (48d), crested wheatgrass showed evidence of leaf damage at the 16.3 ppm but grew a new leaf at levels of 1.6 and 16.3 ppm. Whitmar wheatgrass leaf tips were yellowed at 1.6 ppm and entire leaf yellowing occurred at 16.3 ppm Li. Sherman bluegrass showed leaf tip injury from 1.6 ppm Li even though it grew 2 new leaves. Another trial was performed with lithium levels

of 0, 2.5, 5, 15 and 60 ppm. Yields of roots and shoots were unaffected at 2.5 and 5 ppm but were significantly depressed at 15 and 60 ppm. At these two highest concentrations, 19d after transplanting, seedlings were either dead, had yellow leaves or brown leaf tips. After 45d, all seedlings growing at 60 ppm were dead. At 15 ppm, one each of crested wheatgrass and Sherman bluegrass was dead. At 5 ppm, one seedling each of Sherman bluegrass and Whitmar wheatgrass was dead. Species tolerancy to lithium appeared to be: crested wheatgrass > Sherman bluegrass > Whitmar wheatgrass.

Sulochana 1952 studied the effects of micro-elements including lithium on the cotton plant development and disease incidence. Following lithium exposure at 50, 100, 200 and 400 ppm, no effect on germination was observed. Regarding plant growth, from 50 to 400 ppm, lithium induced slight to very severe toxicity symptoms. Pre-soaking of cotton seeds in micro-element solution had no latter impact on germination and growth of cotton seeds. No pre-emergence of fungi was observed when cotton seeds were grown in micro-element amended soils.

The report from the Ministry of Environment of Québec described the results from Bingham et al. 1964. It was demonstrated that < 10 mg/kg Li in soil caused 25% of growth depression in avocado, sour orange and soybean. Around 10 mg/kg caused the same effect to grape, tomato and red kidney bean. Maize, grass, red beet and cotton were more tolerant to lithium exposure; the EC50 values regarding growth decrease were higher than 20 mg/kg.

In the mini-review article from Tanveer et al. (2019), it was reported the results from several studies showing positive or negative effects on lithium plant growth. At low doses, lithium showed positive effects such as significant increase in root growth and proliferation in lettuce at 2.5 mg/dm^3 in nutrient solution (Kalinowska et al. 2013), significantly increased shoot biomass at 5 mg/dm^3 in maize and sunflower (Hawrylak-Nowak et al. 2012), significantly increased crop yield in maize at 16 mg/dm^3 (Antonkiewicz et al. 2016). On the contrary, higher lithium concentrations could cause deleterious effects: decrease in shoot length, decline of circumnutation in sunflower at 60 and 80 mM Li (Stolarz et al. 2015), reduction in root growth and proliferation in lettuce at 50 or 100 mg/dm^3 (Kalinowska et al. 2013), significantly reduced shoot biomass in maize and sunflower at 50 mg/dm^3 (Hawrylak-Nowak et al. 2012), significantly decreased crop yield in maize at 128 and 265 mg/dm^3 (Antonkiewicz et al. 2016).

In addition, the review from Shahzad et al. (2016) reported the results from other studies regarding the effects of lithium on plant growth and development: tolerance against lithium toxicity in plants of Asteraceae and Solanaceae families (Schrauzer (2002) and Kabata-Pendias and Mukherjee (2007)), sensitivity to lithium stress in citrus plants (Aral and Vecchio-Sadus (2008)), altered rhythmic movement of petals (Birch, 1991), disrupted pollen development (Zonia and Tupy, 1995), altered root gravitropic growth of maize roots (Mulkey (2005)), altered cold-induced de-phosphorylation of microtubules in mesophyll cells of spinach (Bartolo and Carter 1992), damage of root tips and chlorotic and necrotic spots on leaves in corn (Kabata-Pendias and Mukherjee, 2007), decrease in the yield of oat exposed to 25 mg Li/kg, and maize and spinach, when exposed to 40 mg Li/kg cultivated in the soil (Jurkowska et al. 1998), affected circumnutation in *Phaseolus* and *Helianthus* (Zachariassen and Johnsson 1988; Millet and Badot 1996), affected movement of *Cassia fasciculata* leaves (Gaillochet 1981), and *Desmodium motorium* lateral leaflets (Weber et al. 1992), changes to the graviresponsiveness of roots in *Pisum sativum* (Belyavskaya, 2001), no toxic effect of lithium up to 200 mg Li/dm3 on seed germination and growth of *Brassica carrinata* but abrupt decline in growth at higher concentrations (Li et al. 2009).

Besides, the review from Shahzad et al. (2016) reported that lithium induces effects on photosynthetic pigments, carbon assimilation, induces oxidative damage and has effects on nucleic acids and enzymes. A decrease in the photosynthetic pigments content was observed in sunflower (decrease in carotenoid content at 25 mg Li/dm^3), maize (decrease in chlorophyll a, b and carotenoid contents at 50 mg/dm3 Li) (Hawrylak-Nowak et al. 2012) and *Brassica carinata* (depressed chlorophyll content) (Li et al. 2009). Lithium can also cause oxidative damage via inducing the production of reactive oxygen species. Hawrylak-Nowak et al. (2012) reported increased levels of melanoaldehyde contents in different plant tissues (leaves and roots of sunflower and maize) under 50 mg Li/dm^3. Lithium can also be detrimental to nucleic acids that play a crucial role in plant survival. Although these effects have not been extensively studied, some old publications reported alteration in conformation of DNA (Weeks 1956), interruption of protein and nucleic acid metabolism (Vlasyuk et al. 1979), and alteration of the transcription and translation process (Vlasyuk and Kuz'menko 1975; Vlasyuk et al. 1975a, b). It was also reported that lithium interferes with uptake and translocation of some biologically important elements.

The review from Bolan et al. 2021 also reported the following effects: development of necrotic spots due to ethylene accumulation (Naranjo et al. 2003), reduced enzyme activities during pollen development (Gumber et al. 1984), altered gravitrophism in plant roots (Mulkey 2007), altered plant metabolism in different organelles (e.g., mitochondria, peroxisomes, and chloroplasts) (Qiao et al. 2018).

Summary: Lithium showed phytotoxicity in various plant species as described earlier. The effects may vary among the species, with some that are more tolerant to lithium exposure than others. It varies also depending on the source of Li and the medium. Several studies similarly showed that lithium at low concentration stimulates plant growth or has no effect, whereas at higher concentrations it inhibits plant growth. Several studies confirmed that lithium affects the photosynthetic pigments (at 25 mg Li/dm3 in sunflower for example), may interfere with the uptake of mineral elements and causes oxidative damage (at 50 mg Li/dm3 in sunflower and maize) as indicated by the review from Shahzad et al. (2016).

20.2.3 Toxicity to soil micro-organisms

A data waiving for Li2CO3, LiCl, LiOH and Li was performed in the registration dossier. The reason provided in the dossiers is that according to their physico-chemical properties, Li2CO3, LiCl, LiOH and lithium ions are not expected to be distributed into soil and the ad-/desorption behaviour does not indicate that lithium will be present in the soil. The log Kd value for soil was found to be 2.57, i.e. adsorption potential to soil can be regarded as negligible. Thus, concentration and consequently effects in sediment are negligible. Additionally, carbonate, chloride and hydroxide naturally occur in water and are natural components of soil minerals. Therefore, no further testing is needed for the soil compartment.

The report from MELCC (2022) indicated that available data show tolerance of micro-organisms to lithium, in laboratory conditions or *in situ*.

The complementary literature search provided other data on some micro-organisms. They are described below.

20.2.3.1 Bacteria

In the review from Liang et al. (2020), it was reported the results from Mota et al. (2015) showing cell death in *Cyanothece sp.* with 70 mg/L Li.

Sommaruga 2001 studied the effect of LiCl (5 µg Li+/L or 400 mg Li+/L, 6h-exposure) on heterotrophic bacterial activity of natural bacterial community of Alpin Lake. After 6h, the bacterial activity was significantly reduced in the treatment receiving the highest Li+ concentration. No effect was found at the final concentration of 5 µg Li+/L. The critical concentration (e.g., 50% reduction in bacterial activity) of LiCl was above the highest concentration tested.

20.2.3.2 Fungi

The review from Aral et al. (2008) reported the results from Morris (1958) showing that the yeast *Saccharomyces cerevisiae* was shown to take up limited amounts of lithium, and growth inhibition occurred at high levels (115–400 ppm).

Markina-Iñarrairaegui et al. 2020 analysed the roles of sodium-extrusion family (ENA) transporters EnaA, EnaB and EnaC in the response to various stress conditions (alkalinity and high salt concentrations) in the filamentous fungus *Aspergillus nidulans*. The authors tested mutant combinations with genetic construction. The results that were obtained on wild-type strain showed that 0.3 M Li+ impaired the growth of the fungus. Moreover, when alkaline pH 8 and 0.3 M Li+ were combined, growth was more largely impaired.

Mendes et al. 2010a described the development of a procedure to assess the acute toxicity of metal cations (Na+, K+, Li+ (as LiCl), Ca2+,Mg2+, Co2+, Zn2+, Ni2+, Mn2+, Cd2+ and Cu2+) to the bioluminescent basidiomycete fungus *Gerronema viridilucens*. The EC50 for Li+ was 7.6 +/- 0.6 g/L. Regarding the other metal cations, Li+ was ranked as follows in the following metal toxicity series: Cd2+ > Cu2+ > Mn2+ ≈ Ni2+ ≈ Co2+ > Zn2+ > Mg2+ > Li+ > K+ ≈ Na+ > Ca2+.

Summary: Few studies investigated the effects of lithium on micro-organisms (n = 5). Cell death at 70 mg/L Li (Liang et al. (2020)) or reduced activity at 400 mg/L Li (Sommaruga 2001) were observed in bacteria. Li inhibited the growth of various fungus species (Aral et al. (2008) – 115-400 ppm i.e. 0.1-0.4 g/L Li, Markina-Iñarrairaegui et al. 2020 – 0.3 M Li+ i.e. 2 g/L Li, Mendes et al. 2010a – 7.6 g/L Li). These results moderate the conclusions of the report from MELCC (2022) indicating tolerance of micro-organisms to much higher lithium concentrations (e.g. 63.6 g/kg LiCl).

20.2.4 Toxicity to other terrestrial organisms

No data is available for Li2CO3, LiCl, LiOH and Li in the registration dossier.

20.3 Atmospheric compartment

No data is available for Li2CO3, LiCl, LiOH and Li in the registration dossier.

20.4 Toxicity to birds

A data waiving for Li2CO3, LiCl, LiOH was performed. The reason provided in the dossiers is that according to their physico-chemical properties, Li2CO3, LiOH and LiCl are not expected to be distributed into soil and the ad-/desorption behaviour does not indicate that lithium will be present in the soil. The log Kd value for soil was found to be 2.57, i.e. adsorption potential to soil can be regarded as negligible. According to the available information, an input via sludge application on agricultural soil is considered to be negligible, as Li2CO3 and LiOH do not adsorb to the sewage sludge to a significant extent. It can be concluded, that the risk for birds / the terrestrial compartment is low and no further information and/or testing is needed.No data was available for Li in the registration dossier.

The report from MELCC (2022) described acute toxicity studies in chicks showing a decrease in body weight, shorter legs, mortality, slowed growth and lower survival rate.

The complementary literature search provided other data on birds. They are described below.

Bai et al. 2017 evaluated the morphological, biochemical and molecular changes in adipose tissue of chickens after dietary overload lithium treatment. One-day-old male chicks were fed with the basal diet added with 0 (control) or 100 mg lithium/kg diet from lithium chloride for 35 days. Dietary overload lithium decreased the adipogenesis in abdominal adipose tissue of chicken, which was accompanied by depressing transcriptional expression of adipogenesis-associated factors.

The review from Kakhki and Ahmadi-Soleimani (2022) reported some experimental data on lithium salts, including data on chicken (Ikonomov et al. 2000) showing that lithium caused delayed heart rate and slower growth of the embryos. The mentioned effect on the heart rate has been observed within the concentration range of 0.45 and 0.6 mM, whereas lower lithium concentrations had negligible effect. In contrast, higher concentrations of lithium (0.75 and 1.0 mM) caused slower growth of the embryos and lowered the survival rate, compared to the saline-treated subjects.

In the study from Shaikh Qureshi et al. 2014, the toxic effects of Li2CO3 in chick embryonic cardiomyocyte micromass system and embryonic stem cell derived cardiomyocyte were evaluated. In chick embryonic cardiomyocyte micromass system, Li2CO3 (50-2000 µM) showed no significant inhibitory signs on cell viability and total cellular protein content. The cardiomyocyte contractile activity remained unaffected at low doses tested. At higher doses (1200–2000 µM), some decrease in contractile activity was observed but this effect was not statistically significant. The drug exposure for a short period (48h) and chronic exposure (144h) did not affect the values to a great extent. The cardiomyocytes in culture looked healthy, well attached and formed large beating foci compared to control.

Rasooli et al. 2018 evaluated the effects of experimental lithium consumption in chickens. Following exposure to 200 ppm Li2CO3 in water for 20 days, the chickens were lethargic and drowsy and some started to perish about 10-12 days after receiving lithium and after necropsy, hydropericard lesions were seen. Degenerative and necrotic changes were observed in the cell lining in the tubules of the kidneys, with proteinaceous casts and cellular debris in some of them. The epithelium of proximal convoluted tubules was vacuolated and showed necrotic and sloughing changes in some sections. Some of the necrotic tubules also showed mineralisation of the basement membrane, epithelial cells and lumen. The

glomeruli showed hyperemia, glomerular atrophy and dilatation of the urinary spaces. The blood vessels were hyperemic and foci of hemorrhages were observed in the interstitial tissue of these organs. The hepatocytes showed different extents of fatty infiltration and in some instances focal necrosis. The portal area showed chronic portal hepatitis and were infiltrated with lymphocytes, plasma cells and macrophages. The blood vessels were hyperemic and multifocal or diffused hemorrhages were seen in the parenchyma of the liver. The white matter of cerebrum showed cellular vacuolation and spongiosis. The submucosa and lamina propria of different parts of the gastrointestinal tract were infiltrated by lymphocytes, plasma cells and macrophages. Hyperemia and hemorrhages were other consistent changes in the submucosa and lamina propria of intestine.

Summary: The report from MELCC (2022) described acute toxicity studies in chicks showing a decrease in body weight, shorter legs, mortality, slowed growth and lower survival rate. Besides, three *in vivo* studies and one *in vitro* study found in literature investigated the effects of lithium in chickens. It was observed a decrease in the adipogenesis in the abdominal adipose tissue (Bai et al. 2017 – 100 mg Li/kg diet), delayed heart rate with 0.45 and 0.6 mM Li salts and slower growth of chicken embryos exposed to 0.75 and 1.0 mM (Ikonomov et al. 2000 cited in the review from Kakhki and Ahmadi-Soleimani (2022)) while no significant cardiotoxic effect was observed *in vitro* in a chick embryonic cardiomyocyte micromass system (Shaikh Qureshi et al. 2014 – 50-2000 µM Li2CO3). Li-exposed chickens (200 ppm Li2CO3) also showed various deleterious effects in kidneys, liver, gastrointestinal tract and intestine (Rasooli et al. 2018).

20.5 Mammalian wildlife

No data are available for Li2CO3, LiCl, LiOH and Li in the registration dossier. Literature search provided data on some mammal species. They are described below.

In cattle exposed by gavage to 250, 500 or 700 mg LiCl/kg bw, the lowest dose did not cause any mortality. However, at the highest concentration, all the animals died within 4 to 7 days. At the intermediate concentration (500 mg/kg), 75% of the animals died within 7 to 11 days (Johnson et al. 1980, cited in MELCC 2022).

Kelley et al. 1978 studied lithium toxicity in pregnant swine fed once daily with a ration supplemented with 3000 mg Li2CO3/kg during the final 80 days of gestation. It was observed body weight loss, no completed pregnancy for all females, fewer live piglets, more mummies and stillbirths, lighter litters and fewer survival of the liveborn piglets.

Lanoir and Landennois 1977 studied the possible effects of lithium on the sleep-waking cycle in the cat exposed to 30, 50 and 90 mg/kg/day. Adverses effects on sleep parameters were recorded at low doses with morphological changes with a reshaping of sleep-waking phases. At high doses, the effect were more pronounced and occurred faster.

Pohl et al. 1987 tested the influence of lithium on the hibernation behaviour in Turkish hamsters *(Mesocricetus brandti*). Exposure to > 100 µg LiCl/g bw via drinking water at the beginning of the hibernation season delayed or suppressed daily torpor or hibernation.

Romera et al. 2000 investigated the effect of lithium chloride exposure (1 mmol/kg bw i.p./d for 35 days) on the testes and sperm of viscacha (*Lagostomus maximus maximus*), a nocturnal rodent found only in the pampas of Argentina. The histological study showed that the seminiferous tubules had a reduced diameter, epithelial disorganization and a decreased number of germ cells, mostly round and elongate spermatids, in comparison with control animals. The sperm number decreased significantly after lithium exposure. On the contrary, no significant difference in the sperm number between experimental and control rats was observed. Besides, the viscacha sperm motility was either erratic, slow or null and the sperm viability decreased.

Summary: Some studies investigated the effects of lithium in several mammalian species (cattle, swine, cat, Turkish hamster, viscacha). Li oral exposure could cause mortality in cattle (Johnson et al. 1980 – from 500 mg/kg LiCl), adverse effects on swine offspring exposed to 3000 mg Li2CO3/kg during gestation (Kelley et al. 1978), on sleep parameters in cat from 30 mg/kg/d (Lanoir and Landennois 1977) and delayed or suppressed daily torpor or hibernation in Turkish hamsters exposed to > 100 µg/g LiCl. Viscacha exposed to 1 mmol/kg/d Li via intraperitoneal injection showed deleterious effects on testes and sperm (Romera et al. 2000).

21 Detailed information from registration dossiers

21.1 Fish

Table 55: Short-term effects on fish

Method	Species	Test material	Results	Remarks	Reference
OECD TG 203 (Fish, Acute Toxicity Test) (1992 version) EPA OTS 797.1400 (Fish Acute Toxicity Test) GLP Static Freshwater Exposure duration: 96h Nominal concentrations : 0, 5.0, 10, 20, 40 and 80 mg/L Measured concentrations: 0.0, 4.99, 10.0, 19.1 38.9 and 77.7 mg/L Mean length: 40 +/- 4.4 mm Mean weight: 0.77 +/- 0.28 g Hardness: c(CaCO3)= 74 mg/L Temperature: 11.0–12.5°C pH: affected by the presence of Li2CO3; the pH increased as the test substance concentrations increased; 8.7–10.4 at test initiation and 6.7–9.8 for the remainder of the test Dissolved oxygen: 11.7-12.2 mg/L (> 100 % of saturation)	Rainbow trout (*Oncorhynchus mykiss)*	Li2CO3 Purity: 99.2%	LC50 (96h): 30.3 mg/L (95% CI: 19.1–38.9 mg/L) (meas. (geom. mean)) (calculated LC50 for lithium ion : 5.69 mg/L) NOEC (96h): 19.1 mg/L (meas. (geom. mean)) (calculated NOEC for lithium ion : 3.59 mg/L) Based on mortality	Klimisch score in registration dossier: 1 Limitations: unstable pH and exceeding 6.0-8.5 as recommended in the OECD TG 203	Unnamed, 1996 Included in ECHA disseminated website for Li2CO3

OECD TG 203 (Fish, Acute Toxicity Test) (1992 version) GLP Static Freshwater Exposure duration: 96h Nominal concentrations : 0, 62.5, 125, 250, 500 and 1000 mg/L Measured concentrations: 0.0, 59.4, 188, 249, 516 and 1021 mg/L Mean length: 27 +/- 1.6 mm Mean weight: 0.2 +/- 0.05 g Hardness: c(CaCO3)= 56 mg/L Temperature: 11.9–13.4°C pH: affected by the presence of LiCl; the pH increased as the test substance concentrations increased; 7.5–8.7 at test initiation and 7.0–7.5 for the remainder of the test Dissolved oxygen: 10.6-11.0 mg/L (98-102% of saturation) at test initiation and ≥ 6.6 mg/L (≥ 61 % of saturation) for the remainder of the test	Rainbow trout (*Oncorhynchus mykiss)*	LiCl No information on purity	LC50 (96h): 158 mg/L (95% CI: 118–249 mg/L) (meas. (geom. mean)) (calculated LC50 for lithium ion : 25.9 mg/L) NOEC (96h): 59.4 mg/L (meas. (geom. mean)) (calculated NOEC for lithium ion : 9.73 mg/L) Based on mortality	Klimisch score in registration dossier: 1	Unnamed, 1997 Included in ECHA disseminated website for LiCl
OECD TG 203 (Fish, Acute Toxicity Test) GLP Static Freshwater Exposure duration: 96h Nominal concentrations : 0, 12.5, 25.0, 50.0, 100 and 200 mg/L Stable concentrations throughout the exposure period (recoveries ranged from 92.6 to 96.2% of the nominal concentrations at study start and from 98.8 to 101% at the end) Temperature: 21.6–22.3°C pH: 10.7 at the beginning of the test; no other information for the remainder of the test Dissolved oxygen: 96% at the beginning of the test	Zebrafish (*Danio rerio)*	Lithium hydroxide monohydrate (LiOH*H2O) Purity: 101.3%	LC50 (96h): 109 mg/L (nominal) (calculated LC50 for lithium ion : 18.0 mg/L) LC10 (96h): 90 mg/L (nominal) (calculated LC10 for lithium ion : 14.9 mg/L) NOEC (96h): 25 mg/L (nominal) (calculated NOEC for lithium ion : 4.14 mg/L) LOEC (96h): 50 mg/L (nominal) (calculated LOEC for lithium ion : 8.27 mg/L) Based on mortality	Klimisch score in registration dossier: 1 Limitations: pH exceeding 6.0-8.5 as recommended in the OECD TG 203; no information on hardness	Unnamed, 2010 Included in ECHA disseminated website for LiOH and Li

Table 56: Long-term effects on fish

Method	Species	Test material	Results	Remarks	Reference
OECD TG 210 (Fish, Early-Life Stage Toxicity Test) (1992 version) GLP Semi-static Freshwater Exposure duration: 30d after hatching (total experiment duration: 34d) Nominal concentrations : 0, 1.8, 3.0, 7.2, 15, 21.1 mg/L Mean measured concentrations: 0, 2.43, 3.82, 8.60, 17.35, 24.35 mg/L Hardness: 178-237 mg CaCO3/L Temperature: 24.4–25.6°C pH: 7.15-7.84; pH adjustment with 1N HCl Dissolved oxygen: 61.2-97.2%	Zebrafish *(Danio rerio)*	Lithium hydroxide monohydrate (LiOH*H2O)	LOEC (34d): 24.35 mg/L (meas. (arithm. mean)) NOEC (34d): 17.35 mg/L (meas. (arithm. mean)) Based on mortality at larval/juvenile stages but not at the embryonic stage (calculated LOEC and NOEC resp. for lithium ion: 4.03 and 2.87 mg/L) Some deformations or abnormal behaviors recorded but the nature and frequency of these abnormalities in the test item treated groups did not differ from the control.	Klimisch score in registration dossier: 1	Unnamed, 2012 Included in ECHA disseminated website for Li2CO3, LiCl, LiOH, Li
EPA OPPTS 850.1400 (Fish Early-life Stage Toxicity Test) GLP: no Flow-through Freshwater Exposure duration: 22-23d after hatching (total experiment duration: 26d) Nominal concentrations : 0, 1.2, 1.9, 3.2, 5.4, 9.0 and 15.0 mg/L Hardness: 56-70 mg CaCO3/L Temperature: 24.4–25.0°C pH: 7.2-7.5 Dissolved oxygen: 6.8-8.6 mg/L (>84% of saturation)	Fathead minnow *(Pimephales promelas)*	LiCl No information on purity	LC50 (26d): 8.7 mg/L (95% CI: 5.4–15.0 mg/L) (nominal) EC50 (26d) : 6.4 mg/L (95% CI: 5.68–7.36 mg/L) (nominal) Based on mortality NOEC (26d) : 1.2 mg/L (nominal) LOEC (26d): 1.9 mg/L (nominal) Based on length (calculated LC50, EC50, NOEC and LOEC resp. for lithium ion: 1.4, 1.0, 0.20 and 0.31 mg/L)	Klimisch score in registration dossier: 4 due to no study report available Klimisch score from eMSCA: 2 Limitations: chemical analysis not conducted	Long et al. 1998 Included in ECHA disseminated website for LiCl

21.2 Aquatic invertebrates

Table 57: Short-term effects on aquatic invertebrates

Method	Species	Test material	Results	Remarks	Reference
OECD TG 202 (Daphnia sp. Acute Immobilisation Test) (1984 version) EPA OTS 797.1300 (Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids) GLP Static Freshwater Exposure duration: 48h Nominal concentrations : 0, 5.0, 10, 20, 40 and 80 mg/L Measured concentrations: 0.0, 4.76, 9.84, 20.0, 43.7 and 82.8 mg/L Hardness: c(CaCO3)= 56 mg/L Temperature: 19.0–20.3°C pH: 8.5–10.2 Dissolved oxygen: 7.2–8.9 mg/L (79 to 98% of saturation)	Water flea (*Daphnia magna)*	Li2CO3 Purity: 99.2%	EC50 (48h): 33.2 mg/L (95% CI: 20.0-43.7 mg/L) (meas. (geom. mean)) (calculated EC50 for lithium ion: 6.24 mg/L) NOEC (48h): 20.0 mg/L (meas. (geom. mean)) (calculated NOEC for lithium ion: 3.76 mg/L) Based on mobility	Klimisch score in registration dossier: 1 Limitations: pH exceeding 6-9 as recommended in the OECD TG 202; no information on pH variation according to the concentrations; low hardness	Unnamed, 1997a Included in ECHA disseminated website for Li2CO3
OECD TG 202 (Daphnia sp. Acute Immobilisation Test) (1984 version) GLP Static Freshwater Exposure duration: 48h Nominal concentrations : 0, 64.1, 124, 249, 501, 992 mg/L Measured concentrations: 0.0, 63.4, 123, 249, 501, 978 mg/L Hardness: c(CaCO3)= 82 mg/L Temperature: 19.4–20.8°C pH: 7.1–8.6 at test initiation; 7.0-7.1 for the remainder of the test Dissolved oxygen: 9.3 mg/L (100% of saturation) at test initiation; 8.4-8.9 mg/L (94-98% of saturation) for the remainder of the test	Water flea (*Daphnia magna)*	LiCl No information on purity	EC50 (48h): 249 mg/L (95% CI: 197-315 mg/L) (meas. (geom. mean)) (calculated EC50 for lithium ion: 40.72 mg/L) NOEC (48h): 63.4 mg/L (meas. (geom. mean)) (calculated NOEC for lithium ion: 10.38 mg/L) Based on mobility	Klimisch score in registration dossier: 1 Limitations: low hardness according to current OECD TG 202 but this limitation does not apply according to the former OECD TG 202 from 1984	Unnamed, 1997b Included in ECHA disseminated website for LiCl

EU Method C.2 (Acute Toxicity for Daphnia) (1992 version) OECD TG 202 (Daphnia sp. Acute Immobilisation Test) (1984 version) GLP Static Freshwater Exposure duration: 48h Nominal concentrations : 0, 4.6, 10, 21, 46, 100 mg/L Measured concentrations: 19.64; 43.90; 99.20 mg/L with pH adjustment; 9.58, 19.86, 44.80 without pH adjustment Stable concentrations throughout the exposure period (recoveries ranged from 93.5 to 103.2% of the nominal concentrations) Hardness: c(CaCO3)= 250 mg/L Temperature: 20°C pH:8.9 at 4.6 mg/L to 11.2 at 100 mg/L without pH-adjustment; 7.9 to 8.1 at all concentrations with pH-adjustment; Dissolved oxygen: 8.5-8.9 mg/L without pH-adjustment; 8.4-8.8 mg/L with pH-adjustment	Water flea (*Daphnia magna)*	No clear information on test material: it is reported LiOH (purity: 55.2%) but the Lead registrant indicates that the test material would rather be lithium hydroxide monohydrate	Without pH-adjustment: EC50 (48h): 19.1 mg/L (95% CI: 15.1-24.1 mg/L) (nominal) (calculated EC50 for lithium ion: 5.54 mg/L) NOEC (48h): 10 mg/L (nominal) With pH-adjustment: EC50 (48h): 34.3 mg/L (95% CI: 29.6-39.7 mg/L) (nominal) (calculated EC50 for lithium ion: 9.94 mg/L) NOEC (48h): 21 mg/L (nominal) Based on mobility	Klimisch score in registration dossier: 1 Limitations: low substance purity, the CSR of the Lead registrant indicates that the test material is lithium hydroxide monohydrate and not LiOH anhydrous	Unnamed, 1997c Included in ECHA disseminated website for LiOH and Li

Table 58: Long-term effects on aquatic invertebrates

Method	Species	Test material	Results	Remarks	Reference
OECD TG 211 (Daphnia magna Reproduction Test) (2008 version) EU Method C.20 (Daphnia magna Reproduction Test) (2008 version) EPA OPPTS 850.1300 (Daphnid Chronic Toxicity Test) (1996 version) GLP Semi-static Freshwater Exposure duration: 21d Nominal concentrations : 0, 0.50, 0.75, 1.13, 1.70, 2.53, 3.80 and 5.70 mg/L Measured concentrations (lowest and highest) are within ± 20% of the nominal concentration values Test medium: Elendt M4 Medium Hardness: >140 mg CaCO3/L Temperature: 19.8–22.0°C pH: 7.06-8.45; pH adjustment with 1N HCl Dissolved oxygen: > 3 mg/L (lowest value: 6.27 mg/L)	Water flea (*Daphnia magna)*	Lithium	LOEC (21d): 2.53 mg/L (nominal) NOEC (21d): 1.7 mg/L (nominal) EC50 (21d): >1.7 mg/L (nominal) Based on mortality	Klimisch score in registration dossier: 2 Limitations: Elendt medium are not recommended for testing substances containing metals	Unnamed, 2012 Included in ECHA disseminated website for Li2CO3, LiCl and Li
OECD TG 211 (Daphnia magna Reproduction Test) GLP Semi-static Freshwater Exposure duration: 21d Nominal concentrations : 0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg/L Stable concentrations throughout the exposure period (recoveries ranged from 95.5 to 102% of the nominal concentrations) Hardness: 246 mg CaCO3/L Temperature: 19.7–21.9°C	Water flea (*Daphnia magna)*	Lithium hydroxide monohydrate (LiOH*H2O)	LOEC (21d): 8 mg/L (nominal) (calculated LOEC for lithium ion: 1.32 mg/L) NOEC (21d): 4 mg/L (nominal) (calculated NOEC for lithium ion: 0.66 mg/L) Based on reproduction	Klimisch score in registration dossier: 1 Limitations: no information on pH and dissolved oxygen	Unnamed, 2010 Included in ECHA disseminated website for LiCl and LiOH

21.3 Algae and aquatic plants

Table 59: Effects on algae and other aquatic plants

Method	Species	Test material	Results	Remarks	Reference
OECD TG 201 (Alga, Growth Inhibition Test) (2006 version) GLP Static Freshwater Exposure duration: 72h Nominal concentrations : 0, 25.0, 50.0, 100, 200 and 400 mg/L Stable concentrations throughout the exposure period (recoveries ranged from 95.9 to 103 % of the nominal concentrations at 72h) Temperature: 24.2–24.7°C pH: significant effect of the test material on the pH; 8.0–10.9 at 0h; 9.6–10.6 at 72h	*Desmodesmus subspicatus*	Li2CO3 Purity: 99.6%	EC50 (72h): > 400 mg/L (nominal) (calculated EC50 for lithium ion: > 75.15 mg/L) EC10 (72h) : 90 mg/L (nominal) (calculated EC10 for lithium ion: 16.91 mg/L) NOEC (72h): 50 mg/L (nominal) (calculated NOEC for lithium ion: 9.39 mg/L) LOEC (72h): 100 mg/L (nominal) (calculated LOEC for lithium ion: 18.79 mg/L) Based on growth rate	Klimisch score in registration dossier: 1 Limitations: variation of pH by more than 1.5 units at the start of the exposure period; high pH at the end of the test	Unnamed, 2010 Included in ECHA disseminated website for Li2CO3 and LiCl
OECD TG 201 (Alga, Growth Inhibition Test) (2006 version) EU Method C.3 (Algal Inhibition test) (2009 version) GLP Static Freshwater Exposure duration: 72h Nominal concentrations : 0, 5, 10, 20, 40, 80 and 160 mg/L Stable concentrations throughout the exposure period (recoveries ranged from 96 to 102 % of the nominal concentrations at test initiation and from 94 to 101% at the end of the test) Temperature: 22.8-23.1°C pH adjustment to ~7.83 with 1N HCl; 7.80-7.83 at test start; 8.08-9.92 at test end Positive control: potassium dichromate	*Raphidocelis subcapitata*	Lithium hydroxide monohydrate (LiOH*H2O) Purity: 100%	EC50 (72h): 153.44 mg/L (95% CI: 120.05–196.12 mg/L) (nominal) (calculated EC50 for lithium ion: 25.38 mg/L) Based on growth rate NOEC (72h): 10 mg/L (nominal) (calculated NOEC for lithium ion: 1.65 mg/L) LOEC (72h): 20 mg/L (nominal) (calculated LOEC for lithium ion: 3.31 mg/L) Based on biomass and growth rate	Klimisch score in registration dossier: 1 Limitations: variation of pH by more than 1.5 units	Unnamed, 2010 Included in ECHA disseminated website for LiCl, LiOH and Li

OECD TG 201 (Alga, Growth Inhibition Test) (2006 version) GLP Static Freshwater Exposure duration: 72h Nominal concentrations : 0, 25.0, 50.0, 100, 200, and 400 mg/L Stable concentrations throughout the exposure period (recoveries ranged from 91.9 to 98.2 % of the nominal concentrations at test initiation and from 92.4 to 97.8% at the end of the test) Temperature: 24.2-24.7°C pH: 7.7-8.0 at 0h; 9.6-10.5 at 72h No positive control	*Desmodesmus subspicatus*	LiCl No information on purity	EC50 (72h): > 400 mg/L (nominal) (calculated EC50 for lithium ion: > 65.5 mg/L) EC10 (72h) : 80 mg/L (nominal) (calculated EC10 for lithium ion: 13.1 mg/L) NOEC (72h): 25 mg/L (nominal) (calculated NOEC for lithium ion: 4.09 mg/L) LOEC (72h): 50 mg/L (nominal) (calculated LOEC for lithium ion: 8.18 mg/L) Based on growth rate	Klimisch score in registration dossier: 1 Limitations: non information on substance purity; variation of pH by more than 1.5 units	Unnamed, 2010 Included in ECHA disseminated website for LiCl
EU Method C.3 (Algal Inhibition test) (1992 version) OECD TG 201 (Alga, Growth Inhibition Test) GLP Static Freshwater Exposure duration: 72h Nominal concentrations : 0, 0.31; 0.68; 1.5; 3.3; 7.3 and 16.0 mg/L Stable concentrations throughout the exposure period (recoveries ranged from 105 to 125% of the nominal concentrations during the test period) Temperature: 23.2-23.6°C pH: the test material caused marked changes in the pH: 8.77-10.09 at test start; 8.88-9.72 at test end Positive control: potassium dichromate	*Raphidocelis subcapitata*	LiOH Purity: 99.6%	EC50 (72h): 5.76 mg/L (95% CI: 4.94–6.71 mg/L) (nominal) (calculated EC50 for lithium ion: 1.67 mg/L) EC10 (72h) : 1.39 mg/L (95% CI: 1.11–1.73 mg/L) (nominal) (calculated EC10 for lithium ion: 0.40 mg/L) NOEC (72h): 0.68 mg/L (nominal) (calculated NOEC for lithium ion: 0.20 mg/L) LOEC (72h): 1.5 mg/L (nominal) (calculated LOEC for lithium ion: 0.43 mg/L) Based on growth rate	Klimisch score in registration dossier: 2	Unnamed, 2004 Included in ECHA disseminated website for LiOH

21.4 Sediment organisms

Data waiving for Li2CO3 and LiOH. TPE for LiCl. No data available for Li.

21.5 Other aquatic organisms

No data available for Li2CO3, LiCl, LiOH. No data available for Li.

21.6 Terrestrial compartment

21.6.1 Toxicity to soil macro-organisms

Data waiving for Li2CO3, LiOH and Li. TPE for LiCl.

21.6.2 Toxicity to terrestrial plants

Data waiving for Li2CO3, LiOH and Li. TPE for LiCl.

21.6.3 Toxicity to soil micro-organisms

Data waiving for Li2CO3, LiCl, LiOH and Li.

21.6.4 Toxicity to other terrestrial organisms

No data available for Li2CO3, LiCl, LiOH and Li.

21.7 Atmospheric compartment

No data available for Li2CO3, LiCl, LiOH and Li.

21.8 Microbiological activity in sewage treatment systems

Method	Species	Test material	Results	Remarks	Reference
EU Method C.11 (Biodegradation: Activated Sludge Respiration Inhibition Test) (1988 version) OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test) (1984 version) EPA OPPTS 850.6800 (Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals) (1996 version) GLP Static Freshwater Exposure duration: 3h Nominal concentrations : 0, 10, 32, 100, 320, 1000 mg/L Temperature: 19.3–20.3°C (during the incubation) and 19.1-20.5°C (during oxygen measurement) pH of the activated sludge inoculum: 7.88 Dissolved oxygen: not below 2.5 mg O2/L (during the incubation) and at least 6.5 mg O2/L just before measurement of the respiration rates Positive control: 3,5-Dichlorophenol	Activated sludge	LiOH Purity : 99.6 %	EC10 (3h): 79.2 mg/L (nominal) (calculated EC10 for lithium ion: 22.95 mg/L) EC20 (3h): 114.3 mg/L (95% CI: 98.9-132.0 mg/L) (nominal) (calculated EC20 for lithium ion: 33.13 mg/L) EC50 (3h): 180.8 mg/L (95% CI: 160.5-203.7 mg/L) (nominal) (calculated EC50 for lithium ion: 52.40 mg/L) EC80 (3h): 286.1 mg/L (95% CI: 248.5-329.5 mg/L) (nominal) (calculated EC80 for lithium ion: 82.92 mg/L) Based on respiration rate	Klimisch score in registration dossier: 2	Unnamed, 2004 Included in ECHA disseminated website for Li2CO3, LiCl, LiOH and Li

21.9 Toxicity to birds

Data waiving for Li2CO3, LiCl, LiOH. No data available for Li

21.10 Mammalian wildlife

No data available for Li2CO3, LiCl, LiOH. No data available for Li

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Annex 4: Human health risk assessments reported in literature

Table 60: Summary of human health risk assessments related to lithium in water (the colours of the last colomn are corresponding to no risk in green, variable risk (no risk/risk) in orange and risk in red)

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
Iqbal et al. (2012a)	Assessment of the water quality of Khanpur Lake and comparison of the observed levels of physicochemical parameters and selected metals (Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Pb, Sr and Zn) with the corresponding WHO and other guideline values. Non-carcinogenic health risk assessment regarding exposure to the selected metals via oral ingestion and dermal absorption	Pakistan	Freshwater from Khanpur Lake	50 surface water samples collected in triplicate from Khanpur Lake in May 2009 Li concentrations in freshwater: 0.001-0.055 mg/L (min-max); mean: 0.016 mg/L Broad spread Believed to be contributed by natural sources like rock weathering and soil erosion Background value: 0.003 mg/L (Shah 2011)	US EPA Risk Assessment Guidance for Superfund methodology (US EPA 1989)	$RfD_{ingestion}$(Li): 2 µg/kg/d RfD_{dermal}(Li): 1 µg/kg/d (US EPA 2004)	Exposure dose through ingestion of water (Li): 0.487 µg/kg/d Exposure dose through dermal absorption (Li): $9.5x10^{-4}$ µg/kg/d $HQ_{ingestion}$(Li): 0.244 < 1 HQ_{dermal}(Li): $9.5x10^{-4}$ < 1
Bai et al. (2022)	1) Determine the occurrence of heavy metals in well water 2) Use the method recommended by the US EPA to assess health risks for adults and children 3) Identify the main sources of heavy metals by using principal component analysis (PCA)	Northern China 3 areas: Inner Mongolia (NMG), Heilongjiang (HLJ), and the suburbs of Beijing (BJ)	Drinking water from self-supplied wells	156 water samples collected from May to August 2017 from self-supplied wells in the 3 areas NMG, HLJ and BJ The average (and maximum) concentrations of Li were 9.80 µg/L (71.33 µg/L), 4.07 µg/L (7.18 µg/L), and 36.39 µg/L (191.50 µg/L) in HLJ, BJ, and NMG, respectively.	US EPA. Risk Assessment Guidance for Superfund Volume I (US EPA 2004)	No limits available for Li in either the Standards for Drinking Water of China or WHO standards RfD(Li): 0.02 mg/kg/d (Wu et al. 2009)	HQ(Li) < 1, indicating non-significant non-carcinogenic risks

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
Lu et al. (2022)	1) Determine the concentrations of trace elements in public drinking water and their spatial distribution in China 2) Evaluate the health risks of trace elements in public drinking water 3) Calculate the contributions of trace element intakes from drinking water to their daily recommended nutrient intakes and analyze the spatial distributions of nutrient-based scores of trace elements	China (southern China (SC), northern China (NC), northwest China (NWC) and Qinghai-Tibetan Plateau (QT)	Public drinking water	314 public drinking water samples collected from 314 prefecture-level cities in China from December 2019 to April 2020 Li average (min-max) concentrations: 0.0095 (0-0.4604) mg/L. Li concentrations slightly higher in China than in Italy (0.0095 vs. 0.0051 mg/L). Significant differences in Li concentrations in different regions: 0.0063 mg/L in SC, 0.0089 mg/L in NC, 0.0268 mg/L in NWC and 0.0129 mg/L in QT.	Equations for risk assessment from Dippong et al., 2020; Peng et al., 2021	RfD(Li): 0.02 mg/kg/d (oral); 0.01 mg/kg/d (dermal) (Wu et al. (2009)	Mean daily intake of Li through drinking water for children and adults: 0.0116 and 0.0185 mg resp. One water sample each in SC and NC with Hazard Index of Li > 1; two water samples in NWC with HI of Li > 1 HI_{max} of Li was 6.54 in SC, 1.19 in NC and 8.25 in NWC.
Chai et al. 2021	Investigate spatial-temporal changes, assess the water quality, and evaluate the health risk	China	Fen River	115 samples from the Fen River collected in May and September 2019 Li concentration (min-max) in the Fen River: 0.61-69.71 µg/L (dry season); 0.69-90.13 µg/L (wet season) Average concentrations: 14.80 µg/L (dry season); 19.45 µg/L (wet season) Li is dominantly from anthropogenic inputs	-	No national standards for drinking purposes for Li RfD(Li): 0.02 mg/kg/d (oral); 0.01 mg/kg/d (dermal) (Wu et al., 2009)	Dry season: $HQ_{ingestion}$(Li): $2.81x10^{-4}$ (adult); $2.92x10^{-4}$ (children) < 1 HQ_{dermal}(Li): $2.10x10^{-1}$ (adult); $4.31x10^{-1}$ (children) < 1 Wet season: $HQ_{ingestion}$(Li): $4.06x10^{-4}$ (adult); $4.22x10^{-4}$ (children) < 1 HQ_{dermal}(Li): $3.03x10^{-1}$ (adult); $6.22x10^{-1}$ (children) < 1 (supplementary data)
Sadeghi et al. (2021)	1) Determine the chemical properties and level of heavy metals in different water sources 2) Assess the risk factor, cancer and non-cancerous effects, as well as adverse threshold for	Iran, Gonbad-e-Kavus	Well water from Fajr village Drinking water from villages Water from Gorgan River	- 1 sample collected from well water located in Fajr village - 3 samples of drinking water from 3 villages (Fajr, Aq Abad, Soltan Ali) - 3 samples of Gorgan River	US EPA guidelines for exposure assessment, (US EPA 1992)	RfD(Li): 2 µg/kg/d (no reference cited)	Male/female $HQ_{ingestion}$(Li): < 1 for 8/9 samples and > 1 for 1/9 sample Children $HQ_{ingestion}$(Li) > 1 for all water samples

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
	men, women, and children.			Mean [Li]water: 30.29±23.52 µg/L			
Chen et al. (2020)	The non-carcinogenic risks exposure to multiple metal(loid)s in the groundwater of Hetao Plain were calculated to assess the drinking water safety for the local people, and the specific health risks of metal(loid) species were compared to further discuss the feasibility of drinking water standards.	China	Shallow groundwater of the Hetao Plain	66 shallow groundwater samples collected from private wells in October 2017 Li maximum (and average) concentrations: $1.79.10^3$ (92.4) µg/L min: 16.5 µg/L	US EPA 1989, 2004	RfD(Li): 0.002 mg/kg/d (oral); 0.002 mg/kg/d (dermal) (US EPA 2019, 2018)	For children: - $HQ_{oral}(Li) > 1$ The mean HQ_{oral} value of Li was slightly greater than 1, suggesting a smaller non-carcinogenic risk compared with As. - $HQ_{dermal}(Li) < 1$ - Total HQ(Li) : 1.95 ($8.76.10^{-1}$) (mean (median))
Al-Khatib et al. (2019)	Evaluate the heavy metal levels in rainwater harvesting cisterns in the Yatta area, assess the human health risk and identify the potential sources of contamination	Palestine	Rainwater from rain-fed cisterns, Yatta area	74 harvested rainwater samples of rain-fed cisterns collected from different localities in the Yatta area in January and February 2016 Lithium was detected in almost all the samples and the highest mean concentration was 6.59±6.22 µg/L (Khallet Salih)	US EPA 1989, 2004	RfD = 0.2 µg/kg/d (US EPA 2016) Suggested standard not yet adopted but already applied by WHO: 5000 µg/L	Range of chronic daily intake of Li: Adult: 0.13-0.18 µg/kg/j Children: 0.13-0.20 µg/kg/j HI(Li) < 1 for adult and children, except for children in Khallet Salih (HI = 1.0) Carcinogenic risk for Li are between 10^{-6} and 10^{-4}, except for adult in Khallet Salih (10^{-3})
Elumalai et al. (2017)	Quantify the heavy metals in groundwater and evaluate the impact of human-induced activities on groundwater in South Africa using multivariate statistical analysis and human exposure risk analysis through the drinking water pathway	South Africa, Empangeni and Richards Bay	Groundwater from wells and surface water from river or dam	- Groundwater samples collected in Sept. 2015 from 35 wells - 3 surface water samples from the Mhlathuze River - 1 sample from the Redding Dam [Li]water: ~0.5 mg/L (mean)	US EPA 1989	Lithium does not have any drinking water health-based guidelines or standard limits proposed by various organisations	Intake (Li) < 1 mg/kg/d for infant, children and adult (in the order of 10-2 or 10-3 mg/kg/d) HQ (mean (min-max)): Infant: 0.8 (0.6-0.9) Children: 1.4 (1.2-1.6) > 1 Adult: 0.9 (0.8-1.0) => Human exposure dose through the drinking water pathway indicated moderate risk due to lithium (lithium is at the border of

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
				Li may have been from mining and associated activities		RfD(Li): 0.02 mg/kg/d (Virginia Department of Health 2017)	high risk level based on maximum value of 1.6 for children, but is potential risk to infant and adults)
Wu et al. (2009)	Analysis of the levels of 19 metals including Li Health risk assessment	China	Surface water from river	Surface water collected from 6 regions along Yangtze River in Nanjing Section in January 2007 [Li]river: 13.1-15.6 µg/L; mean: 14.1 µg/L	US EPA 1989	RfDing: 20 µg/kg/d RfDderm: 10 µg/kg/d (US EPA 2006)	$HQ_{ingestion}$(Li): $1.09x10^{-2}$ < 1 HQ_{dermal}(Li): $3.35x10^{-8}$ < 1
Benson et al. (2017)	Apply health screening values to the contaminants detected in treated drinking water to assess the potential of the detected contaminants to pose a human health risk from long-term exposure.	USA	Source water and treated drinking water	Source water and treated drinking water samples from 25 drinking water treatment plants (DWTPs) collected in 2010-2012 The concentrations of Li found in treated water from several DWTPs are within the range of the concentrations of Li observed in studies showing a human health effect of lithium (mean lithium level: 0.0113 mg/L (Kapusta et al. 2011))	US EPA	-	Drinking water equivalent level(Li)=66.67 mg/L Calculated Margin of Exposure(Li)=1600 The screening MoE for lithium was set at 1000, equal to the total UF used in the assessment. None of the calculated MOEs were less than the screening value. This result suggest that exposure to lithium from drinking water is not likely to pose a public health concern.
Vetrimurugan et al. (2017)	Assess the health risk to humans due to drinking groundwater containing heavy metals with the help of heavy metal pollution index (HPI) and statistical tools	India	Groundwater from the Cauvery river basin	Groundwater samples collected from 40 locations in the Cauvery river basin in January 2015 Li concentrations: min: 0.27 mg/L max: 1.42 mg/L mean: 0.95±0.53 mg/L	US EPA 1989	RfD(Li): 0.02 mg/kg/d (US EPA 2016) No desirable or permissible limit proposed by Bureau of Indian Standards for Li	Potential risks due to lithium HQ / Adult / Children / Infant Min / 0.7 / 1.1 / 0.5 Max / 3.7 / 5.7 / 2.6 Mean / 2.5 / 3.8 / 1.7 Median / 3.6 / 5.6 / 2.5 SD / 1.4 / 2.1 / 0.9

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
						WHO, US EPA and Health Canada also did not propose limits to Li and hence this metal could not be included in checking the groundwater suitability for drinking	

Table 61: Summary of human health risk assessments related to lithium in diet

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
Abbas et al. (2023)	(1) Compare heavy metals and lithium concentrations in crayfish tissues, sediment, and water from El-Qanatir and El-Rahawi stations; (2) Determine the bioaccumulation and biosedimentation factors of heavy metals and lithium; (3) Evaluate the possible human health hazards associated with ingestion of crayfish muscle.	Egypt	Crayfish (*Procambarus clarkii*)	Sediment and water samples collected from 2 locations (El-Qanatir station and El-Rahawi drain), 50 crayfish collected in 2021 - Mean [Li]water (n=5): 2.30±0.29 µg/L (El-Qanatir); 7.66±0.66 µg/L (El-Rahawi) - Mean [Li]sediment (n=5): 28.06±1.33 µg/g dw (El-Qanatir); 87.28±2.52 µg/g dw (El-Rahawi) - Mean [Li]crayfish (n=5): exoskeleton (4.87±0.17 µg/g dw in El-Rahawi drain) > gills > hepatopancreas >	US EPA 2011	RfD(Li): 20 µg/kg/d (US EPA 2011)	EDI < RfD THQ < 1

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
				muscles (1.22±0.01 µg/g dw in El-Rahawi drain)			
Frydrych et al. (2023)	Identify and evaluate the toxicological risk to humans of novel and traditional elemental impurities (Ag, Au, Co, Cr, Cs, Li, Mo, Se, and Sr) in green tea infusions (n = 12) accessible in the Polish market	Poland	Green tea	Green tea samples (n=12) purchased from June to Sept. 2022 in Poland [Li]tea infusion: 0.205-2.667 µg/L (min-max); mean: 1.340 µg/L	Estimation of weekly intake g/L infusion/week) based on weekly tea consumption Comparison to the provisional tolerable weekly intake	Permitted daily exposure (Li): 560 µg/d (ICH Q3D guideline (R1) No provisional tolerable weekly intake established for Li	Estimated daily exposure to Li: 0.0185-0.7170 µg/day for the consumption of 250 mL of green tea infusion per day < 560 µg/d => no potential issue with Li exposure after green tea consumption
Ahmadi et al. (2022)	Highlight trace elements content including Al, As, Cr, Hg, Li, Ni, Pb, Se, Sr, V (potentially toxic) and Co, Cu, Fe, Mo, and Zn (essential elements) in the muscle and liver tissues of selected commercially important marine species Health risk assessment	Qeshm island, Persian Gulf, Iran	Marine fish species including tuna longtail, mangrove red snapper and Klunzinger's mullet and a species of prawn (green tiger)	368 individual samples (16 composite samples) from 3 consumable marine fish species including tuna longtail, mangrove red snapper and Klunzinger's mullet and a species of prawn (green tiger), collected from various fishery zones in Jan. 2019 Li concentration: 0.04-1.46 mg/kg muscle ww	US EPA 2002, 2010	RfD(Li): 0.002 mg/kg/d (US EPA 2015)	THQ(Li) < 1 for adult and children (lowest THQ: 1.02x10-2 for the consumption of prawn by adult; highest THQ: 9.13x10-1 for the consumption of Klunzinger's mullet by children)
Ahmadpour et al. (2022)	1) Quantify levels of TEs in paddy soils and rice during the June and October rice-growing seasons 2) Assess the potential risks posed by	Iran (Amol, Babolsar, Mahmudabad, and Fereydunkenar counties,	Rice and soil	480 paired rice and soil samples collected in June and Oct. 2020 [Li]rice and paddy soils: 56±27 mg/kg (June	-	Tolerable daily intake (Li): 0.002 mg/kg/d (JECFA; USEPA, no date cited)	THQ(Li) < 1 (0.37 (June 2020); 0.34 (Oct. 2020))

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
	elements in paddy soils and rice grains to human consumers	Mazandaran province)		2020); 56±21 mg/kg (Oct. 2020) -> 2 times higher than Li in upper crust (24 mg/kg) [Li]rice grains: 0.47±0.24mg/kg (June 2020); 0.44±0.28 mg/kg (Oct. 2020)			
Milan et al. (2022)	Evaluation of the health risks of the novel elemental impurities (Ag, Au, Co, Cs, Li, Mo, Se, Sr, and V) in mint tea infusions available in Poland	Poland	Mint tea	Samples of mint tea (n=17) purchased in Poland in 5 cities from June to Sept. 2021 [Li]tea: 0.904-9.641 µg/L (mean: 3.353 µg/L)	Estimation of weekly intake µg/L infusion/week) based on weekly tea consumption Comparison to the provisional tolerable weekly intake	Permitted daily exposure (Li): 560 µg/d (ICH Q3D	Estimated daily exposure to Li: 0.226–2.41 µg/day for the consumption of 250 mL of mint tea infusion per day < 560 µg/d => no potential issue with Li exposure after green tea consumption
Freire et al. (2023)	Determine the concentrations of Al, Sb, and Li in milk samples, identifying sociodemographic, lifestyle, and nutritional factors associated with the concentrations found	Spain	Breast milk	242 pooled breast milk samples collected from 83 donor mothers at different times post-partum in 2015-2018 Recruitment of the donors at the Regional Milk Bank of the Virgen de las Nieves University Hospital, Granada Li detected in 79% of samples at median concentration of 0.58 µg/L; P25-P75=0.25-1.48 µg/L; P95=15.77 µg/L; max=1333 µg/L)	-	TDI(Li): 20 µg/kg/d (adult) (Naeem 2021)	Baby daily oral intake of Li via donated breast milk: 0.08 µg/kg/day (median), rising to 2.10 µg/kg/day (P95) HQ% Li = EDI(median)/TDI x 100 = 0.40% EDI(Li) did not exceed TDI(Li) but this TDI was set for adults, and infants may experience adverse health effects at much lower concentrations. Although Li concentrations were low, comparable to those reported for other lactating women from the general population, its potential to cause adverse health effects in term and preterm infants is uncertain.

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
Otachi et al. (2015)	Investigate the distribution of trace elements in various compartments of the lake (sediment, water and fish tissues) to further the understanding of their distribution and bioaccumulation properties, as well as to evaluate potential health risks for fish consumers in the area.	Kenya	Fish tissue	34 specimens of the elongate tigerfish H. forshalii [Li]fish muscle: 206+/-87.4 mg/kg dw [Li]fish liver: < 0.75 mg/kg dw [Li]water: <0.01 mg/L (LOD) [Li]sediment: <0.2 mg/kg dw (LOD)	US EPA 2012	RfD(Li): 0.002 mg/kg/d (US EPA 2012)	Target HQ(Li): 138.7 => the consumption of these fish poses a health risk to humans in the area.
Perošević et al. (2018)	Determine Al, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sr, Zn, and Hg in soft tissues of mussels Mytilus galloprovincialis collected in Boka Kotorska Bay, the Adriatic coast, and to evaluate possibilities of their harmful effects on human health.	Montenegro	Mussel	Sampling of mussels during one year in 4 different seasons in 2015, at 3 sites in the semi-enclosed Boka Kotorska, Adriatic Sea (Institute of Marine Biology, Cogimar (fish and mussel farm near Orahovac), Žanjice beach) 2kg of mussels at each sampling site Li concentrations in Boka Kotorska: 0.27-1.14 mg/kg ww Average: 0.50±0.25 mg/kg ww	US EPA 1989	RfD(Li): 0.002 mg/kg/d (US EPA 2017)	THQ for average Li concentration: 0.061 THQ for high Li concentration: 0.123
Filippini et al (2020)	Estimate the dietary intake of trace elements (including lithium) in a Northern Italy population, in order to assess their exposure level through diet and evaluate whether these levels	Italy	Diet	Highest lithium content found in fish (especially crustaceans and molluscs) (10.49-38.80 µg/kg), legumes, cereal products (all but rice), and potatoes, with also high concentration in dry fruits, leafy vegetables and cabbage, sweet	US EPA	p-RfD(Li): 0.002 mg/kg/d	Dietary daily intake(Li): 0.258 µg/kg/d THQ(Li):0.130

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
	are safe for human health			confectionery not chocolate, red wine, and fresh cheese (Supplemental Table S2).			
Rafiq et al. (2019)	Measure the concentrations of selected essential and toxic trace metals in most commonly available citrus fruits in Pakistan and to assess their health benefits in terms of antioxidant capacity	Pakistan	Citrus fruits	Fresh citrus fruits purchased from local markets in Pakistan from Sept. 2012 to March 2013 (n=54) Average concentration of Li was found to be lowest among all metals and in the blood orange. Highest Li concentration was found in mandarin (0.104 mg/kg).	US EPA 2006	Dietary reference intakes of the elements were taken as RfD (FNB 2004)	Health risk index(Li) < 1 for all citrus fruits THQ(Li) = 3.20×10^{-5}
Sofoulaki et al. 2019	Assess the public health risks and benefits deriving from the total metal content of two of the most widely consumed fish species in Mediterranean countries: anchovy and sardine	Greece	Sardine, anchovy	90 samples of sardine and 90 samples of anchovy collected from 6 coastal areas in Greece Li levels in sardine: from ~0.07 to ~0.3 µg/g ww of fish Li levels in anchovy: from ~0.1 to ~0,32 µg/g ww of fish	-	RfD(Li): 2 µg/kg bw/d (US EPA 2017)	Estimated daily intake(Li): $0.80\text{-}2.68 \times 10^{-1}$ µg/kg bw/d (sardine); $1.06\text{-}2.77 \times 10^{-1}$ µg/kg bw/d (anchovy) HQ(Li): $0.40\text{-}1.34 \times 10^{-1}$ (sardine); $0.53\text{-}1.38 \times 10^{-1}$ (anchovy)
Kalantzi et al. 2013	Investigate whether sediment geochemistry plays an important role in the bioavailability of a range of elements, making them less available to farmed fish but more available to deposit-feeding wild fish that inhabit the	Greece	Farmed seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus auratus*)	Fish collected from 2 different sites in the Aegean Sea and 2 in the Ionian Sea (n=73)	-	RfD(Li): 2 µg/kg bw/d	EDI (Li) < RfD

References	Objective	Country	Media	Exposure assessment	Risk assessment method	Reference value	Results (daily intake, estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI) or target hazard quotient (THQ))
	environment surrounding the fish cages. + Human health risk assessment						
Saleem et al. 2014	Assess surface water quality from Mangla Lake and to compare the measured levels of the studied parameters with national and international water quality guideline values + Human health risk assessment	Pakistan	Surface water from Mangla Lake	Triplicate surface water samples (n = 150) collected in each season [Li] range: <0.01-0.03 mg/L; median: 0.01 mg/L (summer); <0.01-0.02 mg/L; median: 0.01 mg/L (winter)	US EPA	RfD(Li): 2 µg/kg bw/d	HQing and HQderm for adults and children (summer and winter results) < 1

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