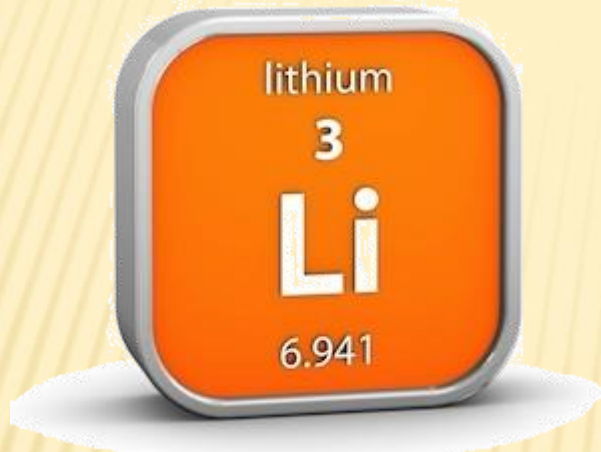


LITHIUM



Treating Bees for
Varroa Mites Using Lithium -
an Accidental Discovery!

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PRESENTATION DOWNLOAD

This presentation is available as
a pdf download to the public.
Please give credit if you use it.
It is a wonderful resource for
you and your fellow beekeepers.

Short URL:

<https://tinyurl.com/yarsme5d>



INTRODUCTION

- ✘ According to the USDA, 42% of commercial hives in the U.S. were infested in summer 2017, and 40% of beekeepers said the parasite seriously harmed their colonies. By comparison, only 13% reported harm from pesticides. (Data has been self-reported by beekeepers.)
- ✘ Varroa mites have become the world's most destructive bee pests. Beekeepers have a limited arsenal when it come to treating for mites. Currently infested beehives are either treated with aggressive organic acids such as formic acid or chemical miticides that cause residue and resistance problems.
- ✘ The agent lithium chloride, however, has a completely different mechanism of action. It is easy to use and, according to the researchers, there are no dangerous side effects for bees, beekeepers, or consumers.
- ✘ "Lithium chloride can be used to feed bees in sugar water. In our experiments, even small amounts of saline solution were sufficient to kill the mites sitting on the bees within a few days - without side effects for the bees," explains Peter Rosenkranz, who heads the Bee Research Institute "Landesanstalt für Bienenkunde" in Hohenheim.

LITHIUM HISTORY

- ✗ **Johan August Arfwedson** (January 12, 1792 – October 28, 1841) was a Swedish chemist who discovered the chemical element lithium in 1817 by isolating it as a salt.
- ✗ In 1818, **Christian Gmelin** was the first to observe that lithium salts give a bright red color to flame.
- ✗ In 1855, larger quantities of lithium were produced through the electrolysis of lithium chloride by **Robert Bunsen** and **Augustus Matthiessen**. The discovery of this procedure led to commercial production of lithium in 1923 by the German company **Metallgesellschaft AG**, which performed an electrolysis of a liquid mixture of lithium chloride and potassium chloride.



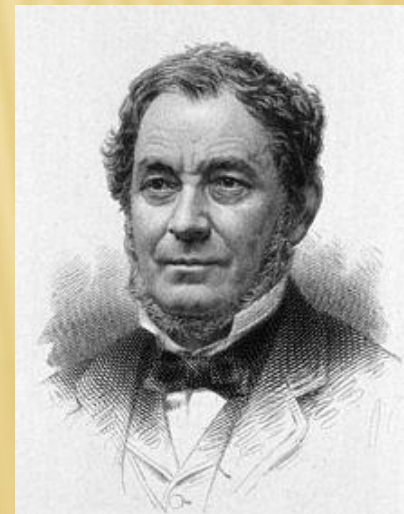
Reskammraten Arfwedson.

Lithographiskt tryck, gjort i Stockholm af Fehn & Möller.

Johan August
Arfwedson



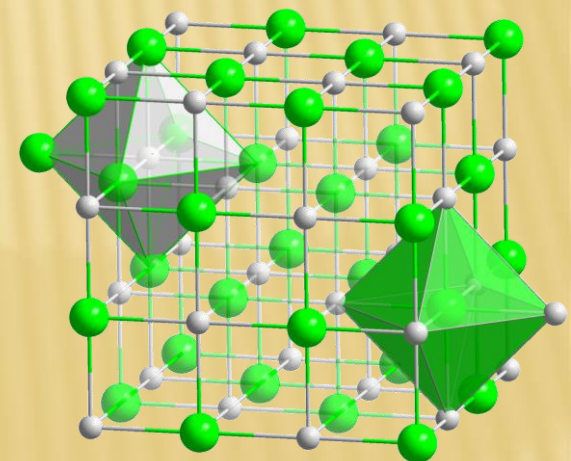
Christian Gmelin



Robert Bunsen

WHAT IS LITHIUM CHLORIDE

- It is a salt found naturally. Lithium carbonate or bicarbonate is the soda version of the salt. It is found in many mineral springs.
- It is used for lithium batteries and in welding of aluminum. It is widely available.
- In biochemical applications it can be used to precipitate RNA from cellular extracts.
- Lithium chloride is also used as a flame colorant to produce dark red flames. Love fireworks?



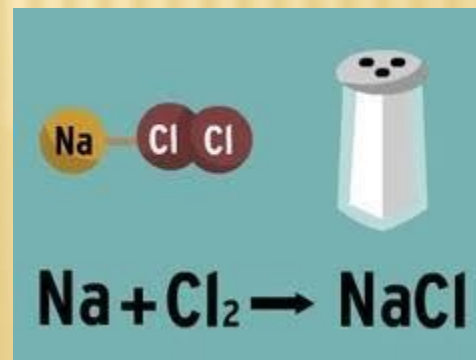
SALTS

1	2											3	4	5	6	7	8
Li ⁺	Be ²⁺													N ³⁻	O ²⁻	F ⁻	
Na ⁺	Mg ²⁺											Al ³⁺			S ²⁻	Cl ⁻	
K ⁺	Ca ²⁺											Ga ³⁺			Se ²⁻	Br ⁻	
Rb ⁺	Sr ²⁺											In ³⁺			Te ²⁻	I ⁻	
Cs ⁺	Ba ²⁺																

Transition metals form cations with various charges

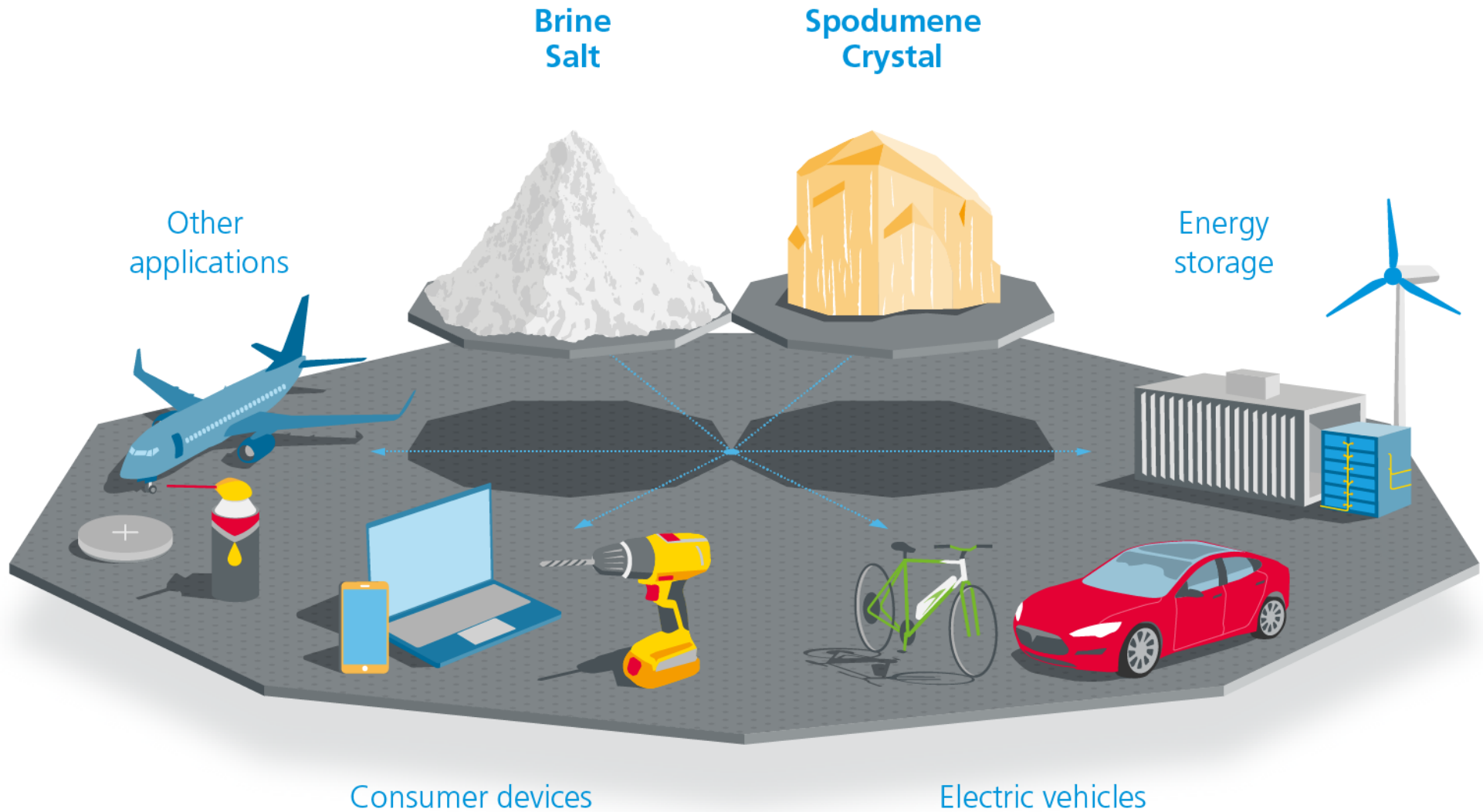
Lithium Chloride
Sodium Chloride
Potassium Chloride
Rubidium Chloride
Caesium Chloride

Beryllium Chloride
Magnesium Chloride
Calcium Chloride
Strontium Chloride
Barium Chloride

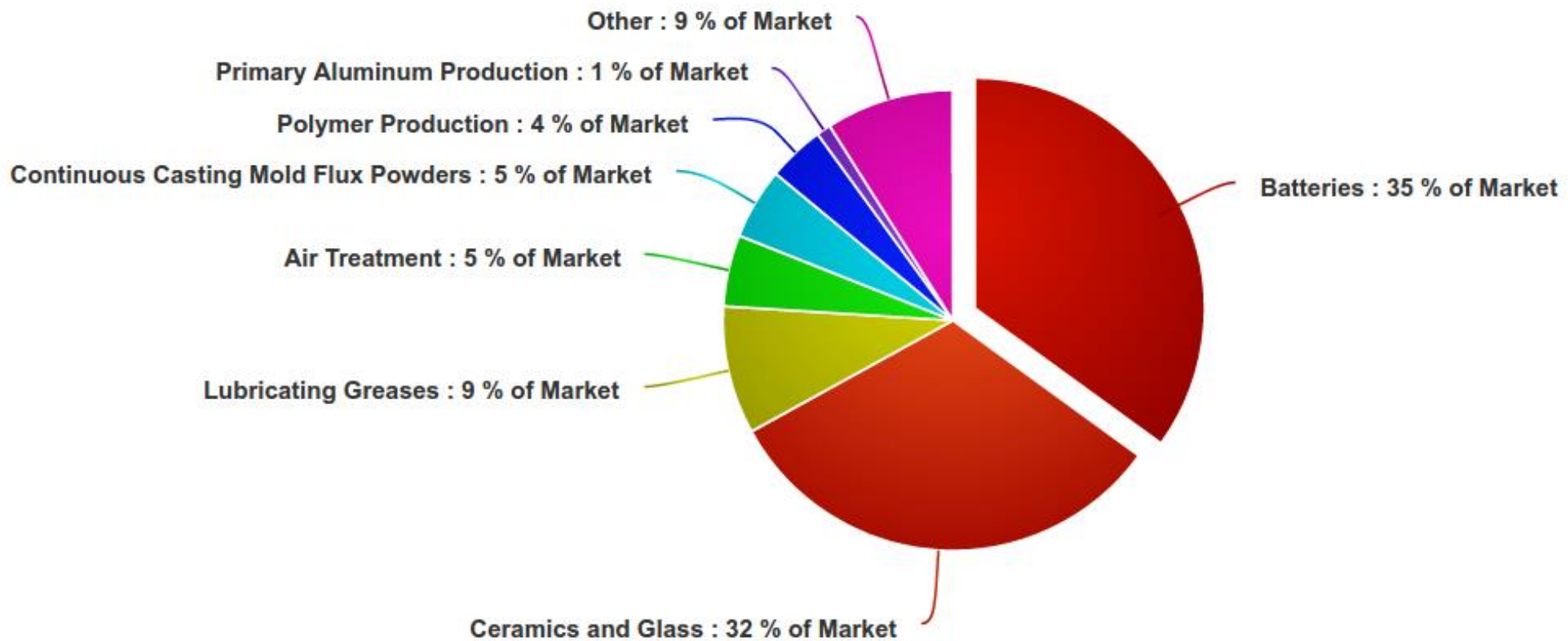


Sodium Chloride
=
Table Salt

SOURCES OF LITHIUM AND ITS APPLICATION



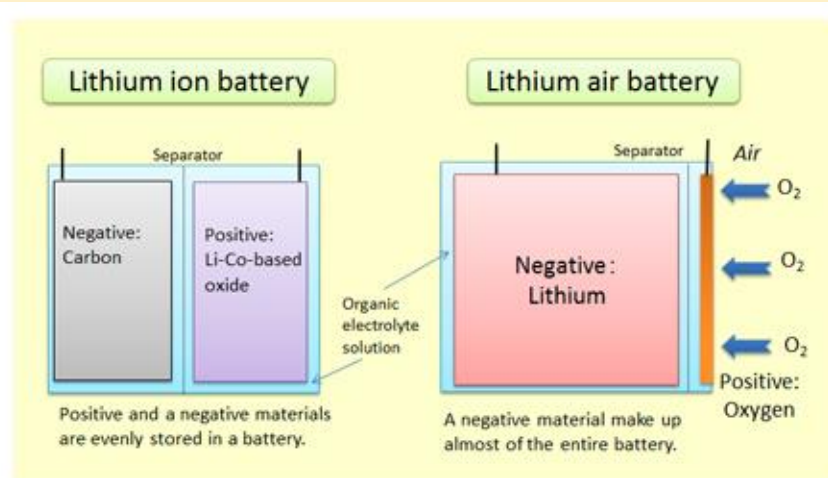
LITHIUM BY END USE (USGS, 2016)



Main Uses of Lithium

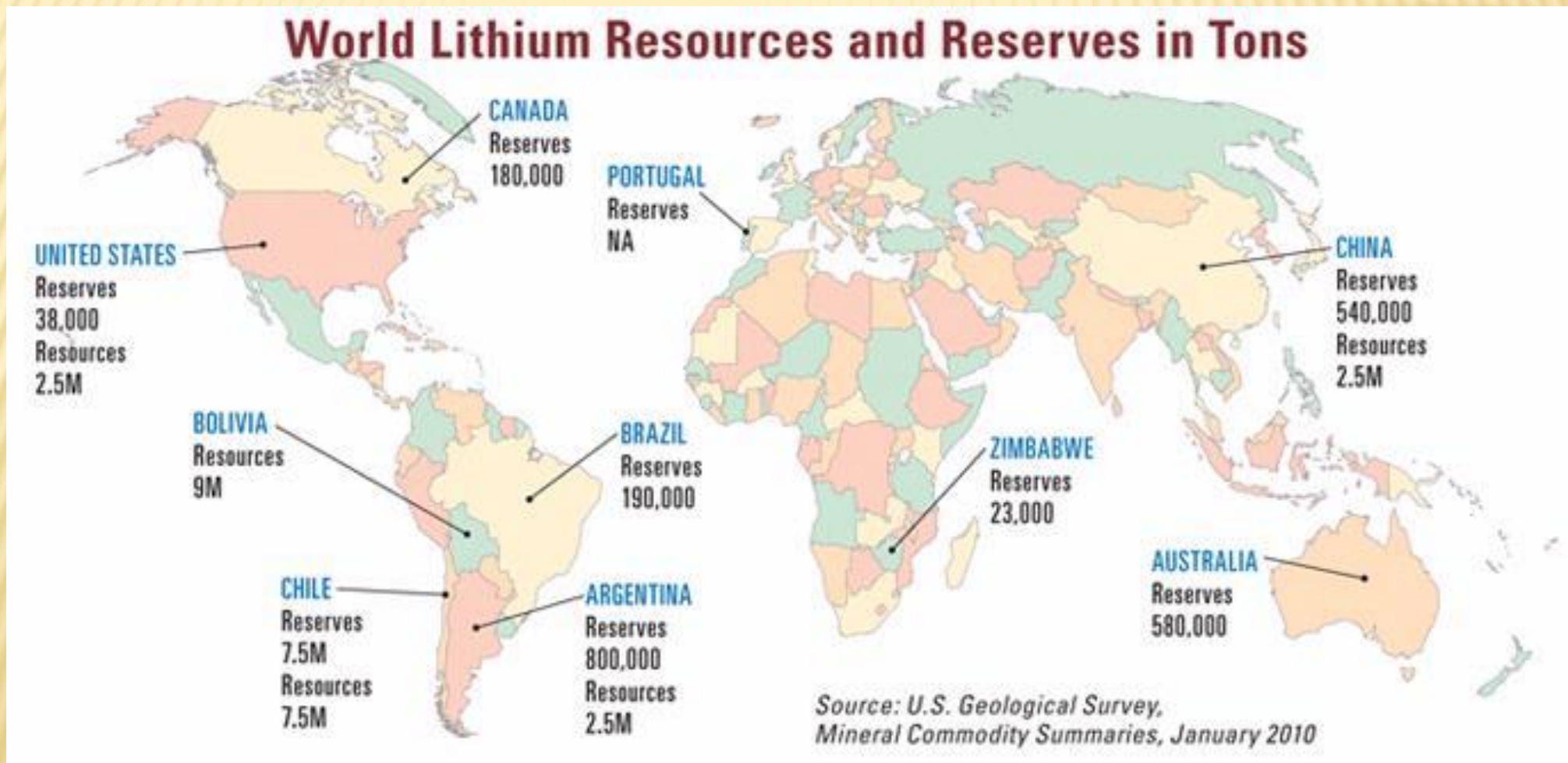
- | | |
|---------------------|------------------------------|
| ❖ Batteries | ❖ Ceramic enamels and glazes |
| ❖ Ceramic glass | ❖ Cement and adhesives |
| ❖ Lubricant greases | ❖ Aluminum |
| ❖ Air conditioning | ❖ Dyes |

OTHER USES

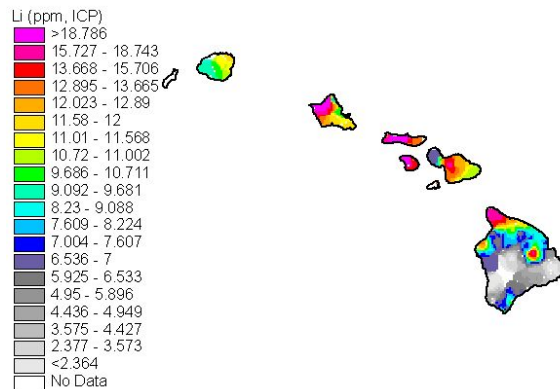
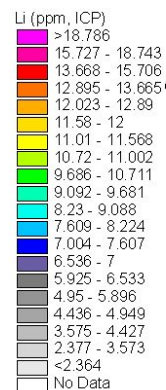
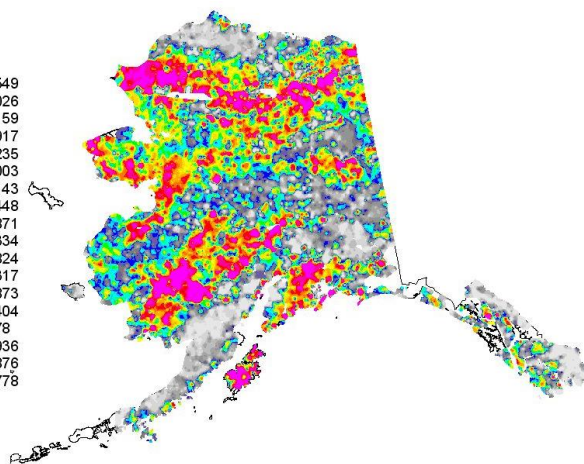
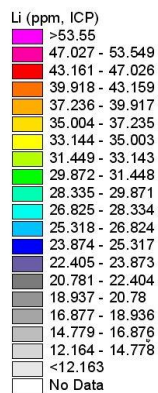
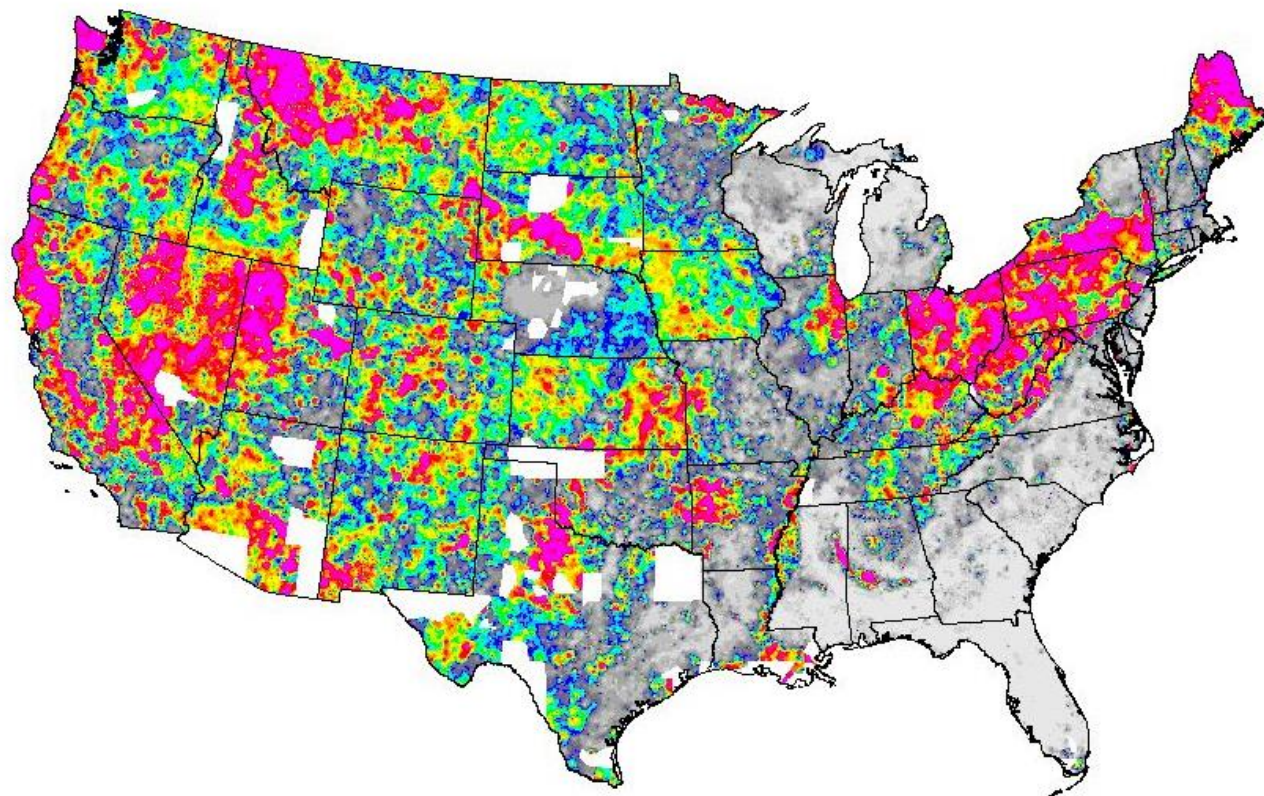
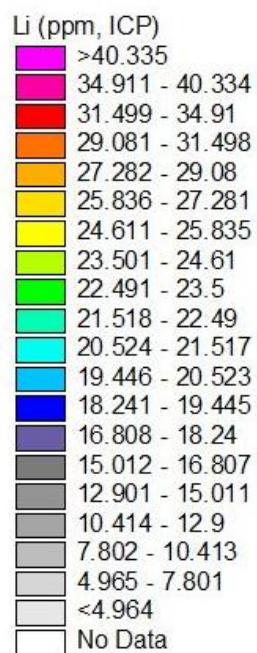


- Lithium salts affect the central nervous system in a variety of ways. While the citrate, carbonate, and orotate salts are currently used to treat bipolar disorder, other lithium salts including the chloride were used in the past. For a short time in the 1940s lithium chloride was manufactured as a salt substitute, but this was prohibited after the toxic effects of the compound were recognized.

LITHIUM RESERVE BASE



LITHIUM RESOURCES



<https://mrdata.usgs.gov/geochem/method.php?element=Li>

EXTRACTION OF LITHIUM



In Bolivia, lithium chloride is one of the components present in large deposits of rock salt. The salt is dissolved by pumping water into the ground, and the salt solution percolates to the surface. The water is allowed to evaporate naturally, leaving behind lithium chloride salt.

Lithium metal is extracted by electrolysis of lithium chloride.

LITHIUM SALT EXTRACTION



ASHLAND – OREGON

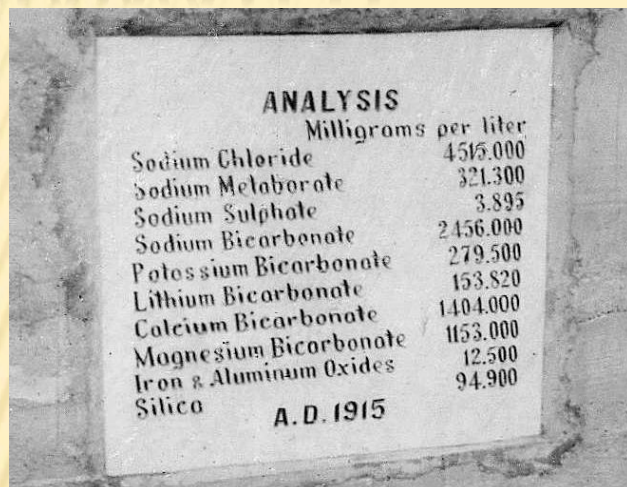
One among a long list of springs in Oregon.

**Public Fountain Bubbles Up
Foul-Tasting Mineral Brew
Go ahead, Have a Sip**

<https://tinyurl.com/ya3x6kzu>



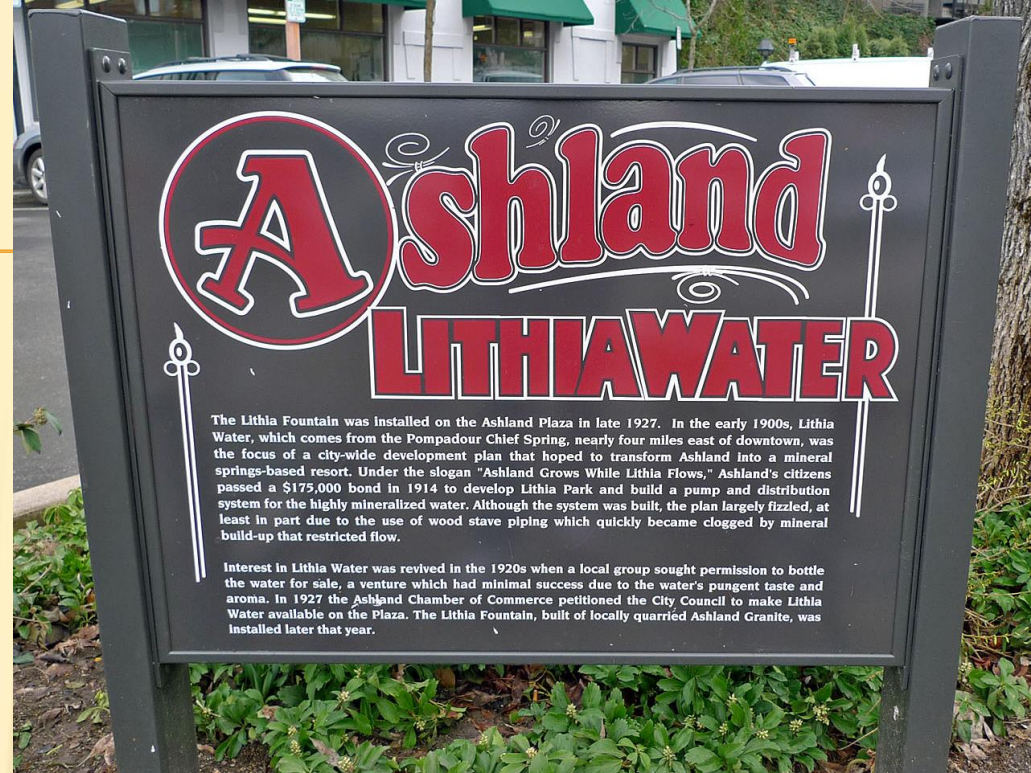
ASHLAND LITHIAWATER



ANALYSIS
Milligrams per liter

Sodium Chloride	4515.000
Sodium Metaborate	321.300
Sodium Sulphate	3.895
Sodium Bicarbonate	2456.000
Potassium Bicarbonate	279.500
Lithium Bicarbonate	153.820
Calcium Bicarbonate	1404.000
Magnesium Bicarbonate	1153.000
Iron & Aluminum Oxides	12.500
Silica	94.900

A. D. 1915



The Lithia Fountain was installed on the Ashland Plaza in late 1927. In the early 1900s, Lithia Water, which comes from the Pompadour Chief Spring, nearly four miles east of downtown, was the focus of a city-wide development plan that hoped to transform Ashland into a mineral springs-based resort. Under the slogan "Ashland Grows While Lithia Flows," Ashland's citizens passed a \$175,000 bond in 1914 to develop Lithia Park and build a pump and distribution system for the highly mineralized water. Although the system was built, the plan largely fizzled, at least in part due to the use of wood stave piping which quickly became clogged by mineral build-up that restricted flow.

Interest in Lithia Water was revived in the 1920s when a local group sought permission to bottle the water for sale, a venture which had minimal success due to the water's pungent taste and aroma. In 1927 the Ashland Chamber of Commerce petitioned the City Council to make Lithia Water available on the Plaza. The Lithia Fountain, built of locally quarried Ashland Granite, was installed later that year.

7 UP



- ✗ Charles Leiper Grigg, who launched his St. Louis-based company The Howdy Corporation, invented a formula for a lemon-lime soft drink in 1920.
- ✗ The product, originally named "Bib-Label Lithiated Lemon-Lime Soda", was launched two weeks before the Wall Street Crash of 1929. It contained the mood stabilizer lithium citrate; and was one of a number of patent medicine products popular in the late-19th and early-20th centuries.
- ✗ Its name was soon changed to 7 Up.
- ✗ All American beverage makers were forced to remove lithium in 1948. Despite the 1948 ban, in 1950 the *Painesville Telegraph* still carried an advertisement for a lithiated lemon beverage.

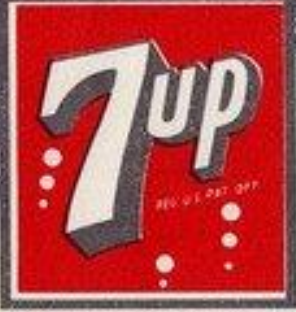


Why we have the youngest customers in the business

This young man is 11 months old—and he isn't our youngest customer by any means.
For 7-Up is as pure, so wholesome, you can even give it to babies and feel good about it. Look at the back of a 7-Up bottle. Notice that all our ingredients are listed. (That isn't required of soft drinks, you know—but we're proud to do it and we think you're pleased that we do.)
By the way, Mom, when it comes to toddlers—if they like to be coaxed to drink their milk, try this: Add 7-Up to the milk in equal parts, pouring the 7-Up gently into the milk. It's a wholesome combination—and it works! Make 7-Up your family drink. You like it... it likes you!

Nothing does it like Seven-Up!





Seven-Up Settles the Stomach
For Hospital or home use.

LITHIATED LEMON SODA

The added citrates neutralize free acid. The sugar is inverted... burns clean. 7-Up is more than a mixer... It blends out the harsh features... Dispels hangovers... takes the "ouch" out of grouch.

Slenderizing

PRINTED IN U. S. A.

PROVEN MEDICINAL USES BY THE MEDICAL WORLD

- ✘ Mental illnesses, including bipolar disorder, depression, and schizophrenia; for eating disorders, including anorexia and bulimia; and for blood disorders, including anemia and low white-cell count (neutropenia).
- ✘ Lithium is also used for headache, alcoholism, epilepsy, diabetes, liver disease, kidney disorders, arthritis, a skin condition called seborrhea, and overactive thyroid.
- ✘ Other uses include treatment of asthma, Huntington's disease, Graves' disease, herpes simplex, a movement disorder called tardive dyskinesia, Tourette's syndrome, cyclical vomiting, Meniere's disease, a tingling or "crawling" sensation in the skin, and aggressive behavior in people with attention deficit-hyperactivity disorder (ADHD).
- ✘ **Do not use without medical consent or prescription from your doctor. Usage must be monitored with lab work.**

HOMEOPATHIC HEALTH BENEFITS

- ✗ Promotes Normal Brain Health
- ✗ Supports Bone Health
- ✗ Promotes Focus and Attention
- ✗ Therapeutic Support for Tourette Syndrome
- ✗ Supports Immune Health
- ✗ Encourages Longevity
- ✗ Helps Balance Obsessive-Compulsive Behaviors



- ✗ Be careful - lithium can have permanent and dangerous side effects.
- ✗ Read WebMD's Information Page: www.webmd.com/vitamins-supplements/ingredientmono-1065-lithium.aspx?activeingredientid=1065

TOXICOLOGY

- ✗ Lithium can cause nausea, diarrhea, dizziness, muscle weakness, fatigue, and a dazed feeling.
- ✗ These unwanted side effects often improve with continued use. (Body gets used to lithium.)
- ✗ Fine tremor, frequent urination, and thirst can occur and may persist with continued use.
- ✗ Weight gain and swelling from excess fluid can also occur. This can put stress onto the heart.
- ✗ Lithium can also cause or make skin disorders such as acne, psoriasis, and rashes worse.
- ✗ The amount of lithium in the body must be carefully controlled and is checked by blood tests.

WARNING

- ✘ **Pregnancy and breast-feeding:** Lithium can poison a developing baby (fetus) and can increase the risk of birth defects, including heart problems. However, when the benefits of giving lithium to the mother outweigh the risks to the fetus, lithium may be given by a healthcare provider, as long as there is careful monitoring.
- ✘ Lithium treatment is **UNSAFE** in women who are breast-feeding. Lithium can enter breast milk and cause unwanted side effects in a nursing infant.
- ✘ **Heart disease:** Lithium may cause irregular heart rhythms. This may be a problem, especially for people who have heart disease.
- ✘ **Kidney disease:** Lithium is removed from the body by the kidneys. In people with kidney disease, the amount of lithium that is given might need to be reduced.
- ✘ **Surgery:** Lithium might change levels of serotonin, a chemical that affects the central nervous system. There is some concern that lithium might interfere with surgical procedures that often involve anesthesia and other drugs that affect the central nervous system. Lithium use should be stopped, with the approval of a healthcare provider, at least two weeks before a scheduled surgery.
- ✘ **Thyroid disease:** Lithium might make thyroid problems worse.

FIRST AID

- ✖ **EYES:** First check the victim for contact lenses and remove if present. Flush victim's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center. Do not put any ointments, oils, or medication in the victim's eyes without specific instructions from a physician. If symptoms (such as redness or irritation) develop, immediately transport the victim to a hospital.
- ✖ **SKIN:** IMMEDIATELY flood affected skin with water while removing and isolating all contaminated clothing. Gently wash all affected skin areas thoroughly with soap and water. If symptoms such as redness or irritation develop, IMMEDIATELY call a physician and be prepared to transport the victim to a hospital for treatment.
- ✖ **INHALATION:** IMMEDIATELY leave the contaminated area; take deep breaths of fresh air. If symptoms (such as wheezing, coughing, shortness of breath, or burning in the mouth, throat, or chest) develop, call a physician and be prepared to transport the victim to a hospital. Provide proper respiratory protection to rescuers entering an unknown atmosphere. Whenever possible, Self-Contained Breathing Apparatus (SCBA) should be used; if not available, use a level of protection greater than or equal to that advised under Protective Clothing.
- ✖ **INGESTION:** DO NOT INDUCE VOMITING. If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and IMMEDIATELY call a hospital or poison control center. Be prepared to transport the victim to a hospital if advised by a physician. If the victim is convulsing or unconscious, do not give anything by mouth, ensure that the victim's airway is open and lay the victim on his/her side with the head lower than the body. DO NOT INDUCE VOMITING. IMMEDIATELY transport the victim to a hospital. (NTP, 1992)
- ✖ Source: National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD20894, USA

GHS HAZARD STATEMENTS

- ✘ **H302: Harmful if swallowed**
[Warning Acute toxicity, oral]
- ✘ **H315: Causes skin irritation**
[Warning Skin corrosion/irritation]
- ✘ **H319: Causes serious eye irritation**
[Warning Serious eye damage/eye irritation]
- ✘ **H335 (16.72%): May cause respiratory irritation**
[Warning Specific target organ toxicity, single exposure; Respiratory tract irritation]
- ✘ **H361: Suspected of damaging fertility or the unborn child**
[Warning Reproductive toxicity]
- ✘ **H371: May cause damage to organs**
[Warning Specific target organ toxicity, single exposure]
- ✘ **H373: Causes damage to organs through prolonged or repeated exposure**
[Warning Specific target organ toxicity, repeated exposure]
- ✘ **H402: Harmful to aquatic life**
[Hazardous to the aquatic environment, acute hazard]
- ✘ **H412: Harmful to aquatic life with long lasting effects**
[Hazardous to the aquatic environment, long-term hazard]

- ✘ GHS stands for the **Globally Harmonized System** of Classification and Labelling of Chemicals. GHS defines and classifies the hazards of chemical products, and communicates health and safety information on labels and safety data sheets).



NOW TO THE BEE RESEARCH



THE GERMAN TEAM



Left to right: Prof. Dr. Lothar Frey, Forschungsförderung, **PD Dr. Peter Rosenkranz, Universität Hohenheim**, Staatssekretär Füracker, **Dr. Bettina Ziegelmann, Universität Hohenheim**, **Dr. Stefan Hanus, siTOOLS Biotech GmbH**, **Dr. Stefan Berg, Bayerische Landesanstalt für Weinbau und Gartenbau**, Dorothea Leonhardt, Forschungsförderung (Bild: Forschungsförderung/Rölz)

VARROA



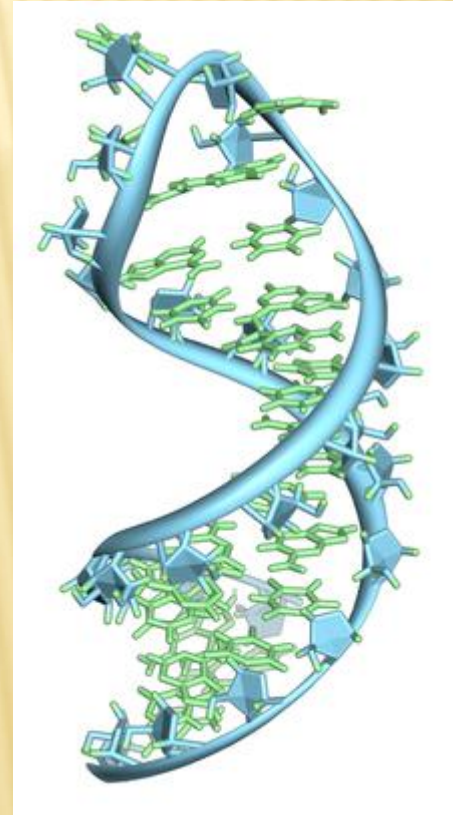
Researchers at Hohenheim University have a new remedy against the Varroa mite: the chemical lithium chloride. It seems highly effective and is easy to dispense.
Copyright: Universität Hohenheim / Bettina Ziegelmann

VARROA RESISTANCE AND TOLERANCE

- ✘ Beekeepers provide an environment for Varroa to flourish. High colony densities, large colonies, the prevention of swarming, and periodical mite control have created mite resistance and a rather unbalanced relationship between bee and mite. Sadly, the mite has the upper hand.
- ✘ Feral bees and Varroa resistant stock have been shown not to be the solution. They fair better in small nests with frequent swarming.
- ✘ We have seen increased viral activity due to mites.
- ✘ Bees do not have an immune system to teach them about viruses. They do not become immune like we do.
- ✘ RNA technology opens a window to give them immunity towards viruses. We are in the early stages to do just that.

RNA INTERFERENCE – WHAT IS IT?

- ✗ Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation, and expression of genes.
- ✗ RNA has been discovered in the 1960s. Utilizing RNA methods is relatively new, but it opens avenues to treat illnesses and perhaps ways to replace pesticides with a targeted approach.



MILLIMOLAR – WHAT IS IT?

- ✗ Having a concentration of one thousandth of a mole per liter.
- ✗ mM stands for milli-molar
m=mil (thousand) M=Molar (mole per liter)
- ✗ Based on molecular weight every element and compound has it's own molecular weight (mass).
- ✗ Molar mass of Lithium chloride LiCl = 42.394 g/Mol
- ✗ Equates to: 1 mM = 42.39 mg/L
- ✗ The experiments will use multiples of mM.
- ✗ Below a table to show how it calculates into mixes.
- ✗ en.wikipedia.org/wiki/Molar_concentration

Agent	Lithium chloride LiCl	Lithium citrate $\text{Li}_3\text{C}_6\text{H}_5\text{O}_7$
Molecular Weight (g)	42.39 g/Mol or 42.39mg/L	209.92 g/Mol
25mMol	1059.75 mg +1 liter (1000ml) sugar syrup	5248 mg +1 liter (1000ml) sugar syrup
4mMol	169.56 mg +1 liter (1000ml) sugar syrup	839.68 mg +1 liter (1000ml) sugar syrup

RNA TEST STUDY IN ISRAEL REVEALED...

- ✘ Bees that were fed dsRNA (double-stranded) with a sequence the same as that of the Israeli acute paralysis virus (IAPV), were protected from the viral disease. This opened the passage to further research.
- ✘ Next, dsRNA ingested by bees was transferred to the *Varroa* mite via feeding on a bee. This cross-species, reciprocal exchange of dsRNA between bee and *Varroa* caused targeted gene silencing in the mite. Resulting in an over 60% decrease in the mite population. Thus, transfer of gene-silencing-triggering molecules between this invertebrate host and its ectoparasite could lead to a conceptually novel approach to *Varroa* control.

- ✘ Bidirectional Transfer of RNAi between Honey Bee and *Varroa* destructor: *Varroa* Gene Silencing Reduces *Varroa* Population; Yael Garbian

THE ACCIDENTAL DISCOVERY

- ✘ German researchers were experimenting with RNA technology to find ways to kill Varroa via digesting hemolymph on their bee host.
- ✘ Bees were fed sugar solution containing RNA.
- ✘ Mites infesting these bees rapidly died, but so did mites in a control group given another RNA that should have been ineffective.
- ✘ The astonishing results prompted the researchers to suspect that the lithium chloride used to produce the RNA – and thus present in the sugar water – was actually killing the parasites.
- ✘ A battery of subsequent examinations confirmed their hypothesis.

EXPERIMENT 1 – SIRNA TEST

siRNA (small interfering) approach to fight Varroa infestation

- ✘ Bees were fed sugar syrup with dsRNA of potential essential Varroa genes. Each cage had 100 bees and 30 mites.
- ✘ A control group of bees were fed sugar syrup with dsRNA from the *Drosophila* (small fruit fly). This homologous gene does not exist in Varroa or honey bees. Thus, no ill effect in either insect is expected.

Agent	Mite Loss Day 1	Mite Loss Day 2	Mite Loss Day 3	Mite Loss Day 4	Bee Loss after 6 days
Mite specific gene	18	68	5		30%
Non specific gene	10	64	13	2	30%
Sucrose solution	0	0	0	0	0%

How could that be?

There was something else killing the mites.
It was not the sugar syrup.
Further testing was needed...



EXPERIMENT 2 – LITHIUM CHLORIDE

Identification of lithium chloride as a miticide against Varroa

- ✖ Lithium chloride was used in the production process of dsRNA at a concentration of 7,500mM.
- ✖ 2 washes with 70% ethanol reduced it down to 25mM.
- ✖ Cages with 50 bees and 20 mites each were setup.

Agent	Mite Death 24 hours	Mite Death 48 hours	Mite Death 72 hours
Sucrose with dsRNA	1	4	15
25mM LiCl	4	15	20
Sucrose Solution	0	0	0

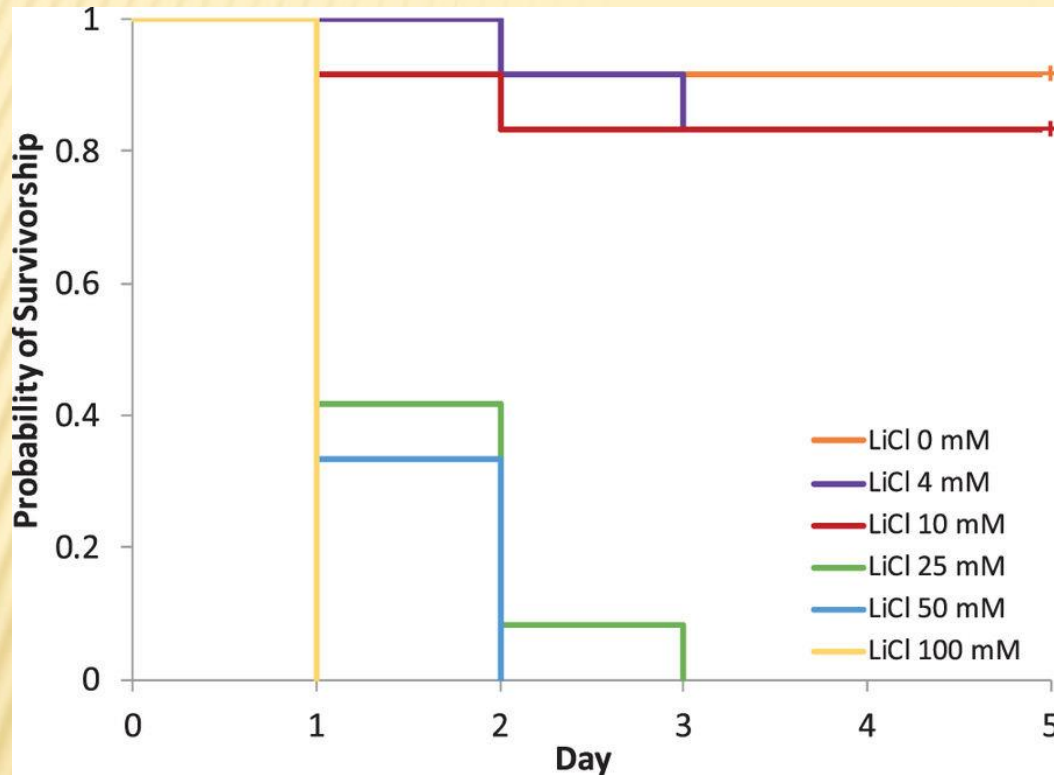
EXPERIMENT 3 – LiCl DOSE RESPONSE

Dose response relation of lithium chloride as miticide against Varroa

- ✗ 3 Cages each with **50 bees and 25 mites** were used.
- ✗ Lithium chloride was added to 70% sucrose solution in various concentrations. 16µM, 80µM, 400µM, 2mM, 10mM, 50mM, and 250mM. Plain sucrose was used as a control.
- ✗ The solution was feed until all mites were dead, and continued afterward with sucrose solution only to monitor bee response for a total of 2 weeks.

Agent	Mites	Bees
250mM LiCl	100% kill within 48 hours	27 bees were lost, average 3 per day, indicating toxic effect on bees even after treatment was terminated and bees were fed sucrose.
100mM LiCl	100% kill within 48 hours	Continuous loss of bees showed toxicity
50mM LiCl	100% kill within 48 hours	20 bees total over the live span of the test
25mM LiCl (single dose only)	100% kill within 72 hours	No negative impact on bee health could be observed.
10mM LiCl	100% kill over the test span day 1=3, day 2=10, day 3=7, day 4=3	Improved survival with 34 bees surviving
2mM LiCl	Failed to kill all mites over the full span of the test. 11 mites survived.	42 bees survived
Sucrose	0%	0%

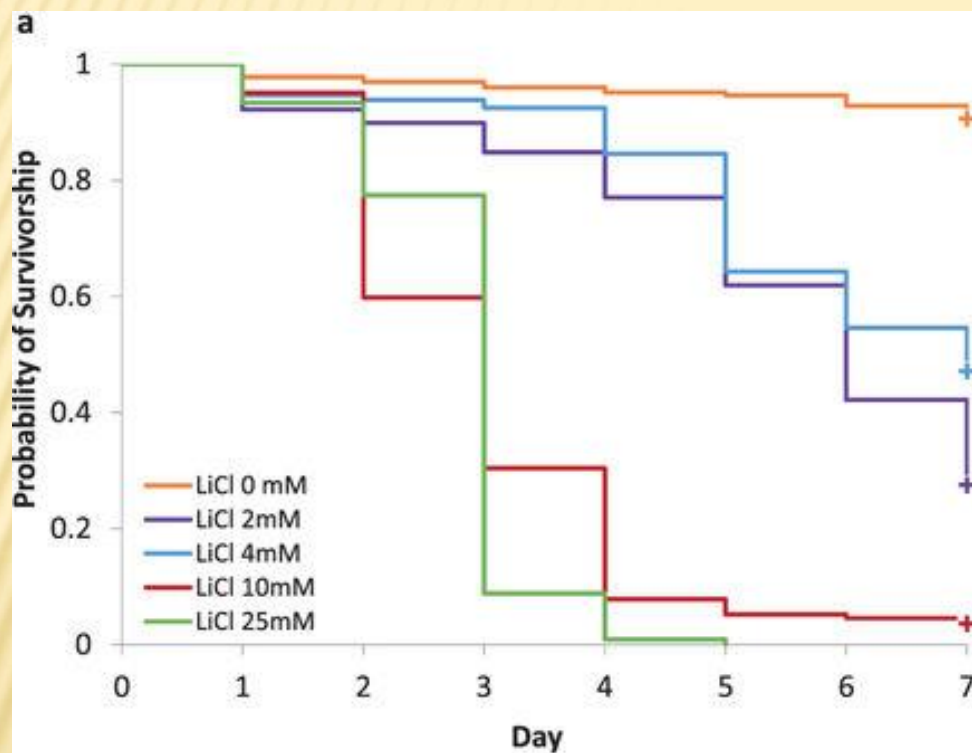
24 HOUR MITE MORTALITY TEST



- ✗ Mortality of phoretic Varroa mites kept on bees that were individually fed 10µl of a lithium chloride solution at concentrations ranging from 4mM to 100mM.
- ✗ The bees were fed LiCl only once at the beginning of the experiment, then they received sucrose syrup over five days.
- ✗ For each concentration, 12 cages with one bee and one Varroa mite each were analyzed.
- ✗ The survival of mites was significantly reduced compared to the control group when concentrations of 25mM and higher were fed to the bees ($P < 0.001$, log-rank test).

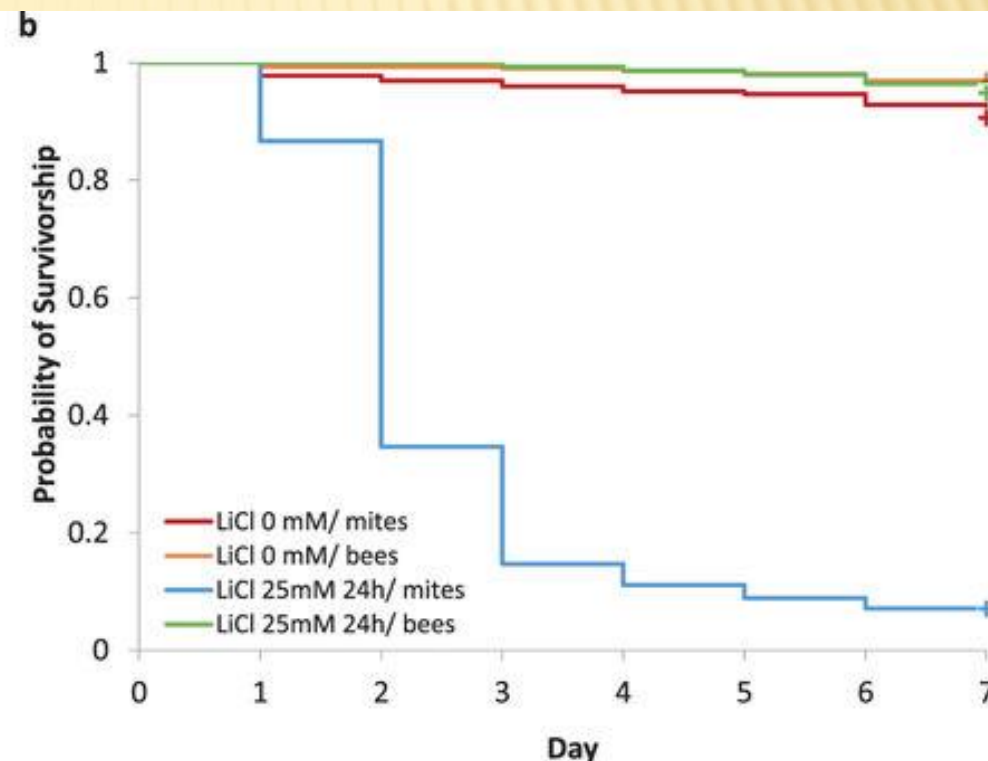
MORTALITY OF PHORETIC VARROA MITES AND HONEY BEES AFTER FEEDING LITHIUM CHLORIDE (LiCl) TO CAGED BEES

24 Hour Lithium Chloride Feeding



Kaplan-Meier survival curve of female Varroa mites kept on caged bees fed with LiCl in concentrations between 2mM–25mM ($n=33, 9, 9, 12$, and 9 cages for 0mM (control), 2mM, 4mM, 10mM and 25mM, respectively).

At all concentrations, the survival of mites in the treatment groups was significantly different from the control ($P<0.001$, log-rank test with Bonferroni correction).

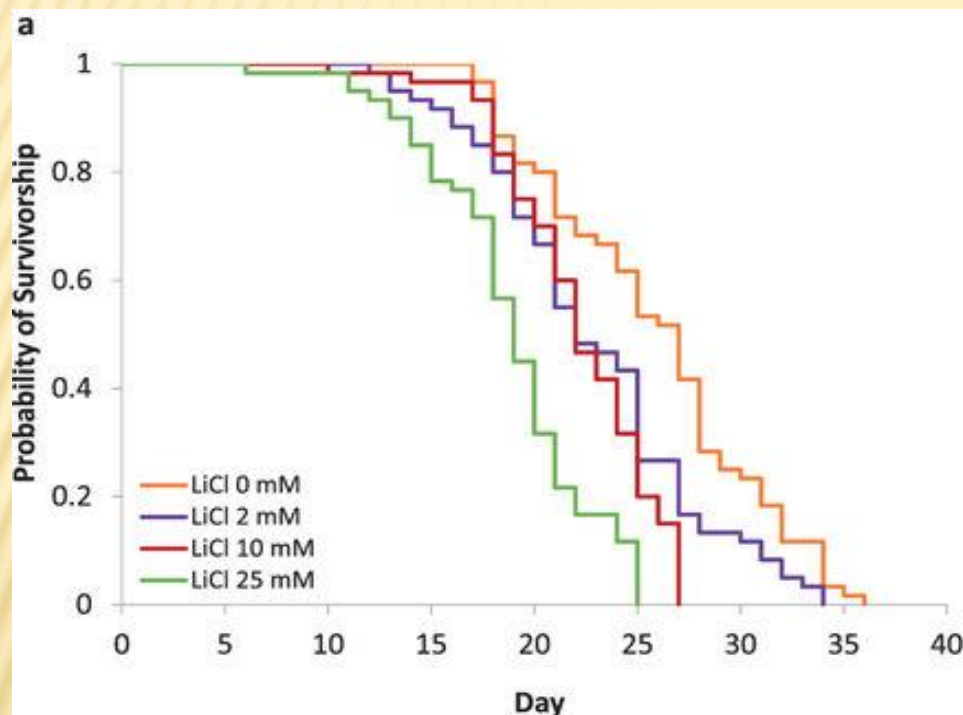


Kaplan-Meier survival curve of caged worker bees and female Varroa mites after 24h LiCl exposure ($n=9$ cages).

The survival of mites in the treatment group was significantly different from the control group ($P<0.001$, log-rank test) but there were no significant group differences in bee mortality.

MORTALITY OF HONEY BEES AFTER FEEDING LITHIUM CHLORIDE TO CAGED BEES

Continuous Lithium Feeding

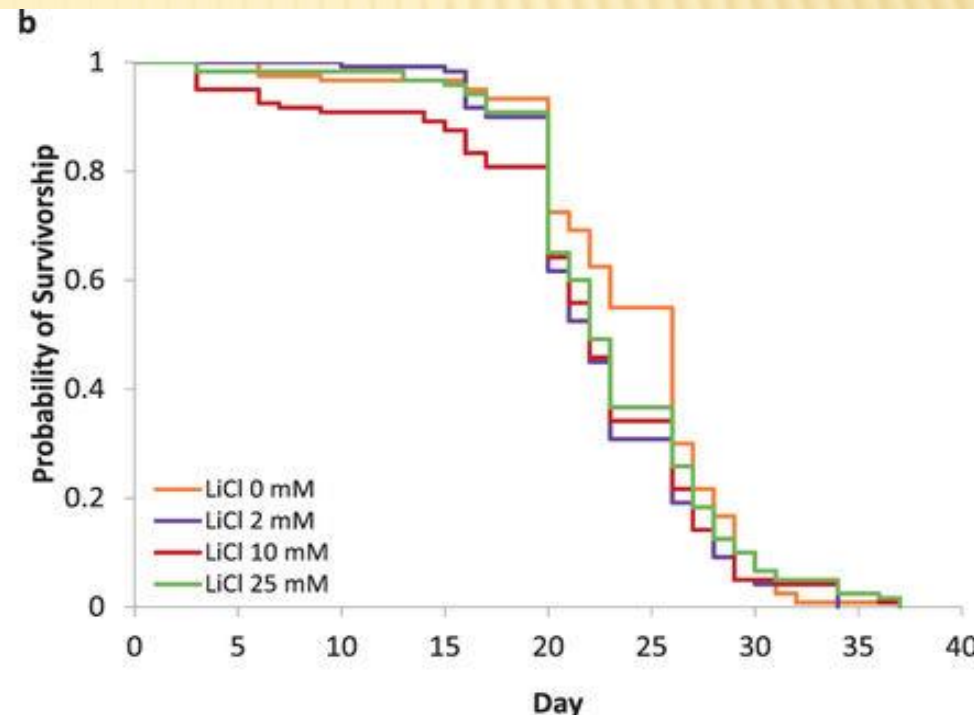


Kaplan-Meier survival curve of caged worker bees during chronic LiCl exposure.

LiCl diets at concentrations of 2mM, 10mM and 25mM were fed ad libitum till the death of the last bee (n=6 cages with 10 bees each).

The survival of all treated groups was significantly different from the sugar syrup control ($P < 0.01$, log-rank test with Bonferroni correction).

24 Hour Lithium Feeding



Kaplan-Meier survival curve of caged worker bees after a 24h LiCl exposure.

LiCl diets at concentrations of 2mM, 10mM and 25mM were fed ad libitum for the first 24h after hatching and then replaced by sucrose syrup (n=12 cages with 10 bees each).

The survival of all treated groups was not significantly different from the sugar syrup control ($P > 0.1$, log-rank test with Bonferroni correction).

EXPERIMENT 4 & 5 – TESTING OTHER SALTS

Testing acaricidal effect of various other salts

- ✖ Magnesium chloride, Lithium chloride, Sodium chloride at 25mM sucrose solutions
- ✖ 2 cages each with 50 bees and 25 mites were set up.
- ✖ Test lasted 7 days.

Agent	Per 25 mites	Per 50 bees
Lithium chloride LiCl	25 dead	3.2 dead
Sodium chloride NaCl	2.3 dead	1.7 dead
Magnesium chloride MgCl ₂	3.7 dead	50 dead 100% kill by day 5
Sucrose solution	1.7 dead	2 dead

In tests with magnesium chloride (MgCl₂) 100% of the caged bees died within five days, and due to the declining number of bees, the experiment was terminated before the effect on mites could be analyzed.

EXPERIMENT 6 – TESTING LITHIUM SALTS

Test of acaricidal effect of various lithium salts

- ✖ Li-citrate, Li-sulfate, Li-acetate, Li-lactate and Li-carbonate at 25mM sucrose solutions.
- ✖ Tested in cages of 50 bees with 25 mites.
- ✖ Test ran for 7 days.

Agent	Per 25 mites	Per 50 bees
Li-sulfate	25 dead	6.3 dead
Li-citrate	25 dead	3 dead
Li-acetate	25 dead	2.7 dead
Li-lactate	25 dead	5 dead
Li-carbonate	25 dead	1.7 dead

Even lower concentration of lithium salts killed mites.
4mM killed the same numbers of mites.

EFFICACY OF OTHER LITHIUM COMPOUNDS

	Control	Lithium Chloride		Lithium Lactate		Lithium Acetate		Lithium Carbonate		Lithium Sulfate		Lithium Citrate	
		LiCl		C ₃ H ₅ LiO ₃		C ₂ H ₃ LiO ₂		Li ₂ CO ₃		Li ₂ SO ₄		Li ₃ C ₆ H ₅ O ₇	
Molecular Weight		42.39g/Mol		96.01g/Mol		65.98g/Mol		73.89g/Mol		109.94g/Mol		209.92g/Mol	
No. of Li Ions	0	1		1		1		2		2		3	
		4mM	25mM	4mM	25mM	4mM	25mM	4mM	25mM	4mM	25mM	4mM	25mM
Cages	33	9	9	3	6	3	3	3	3	3	3	6	3
Mite mortality (%±SD)	9.4 (±6.2)	63	100	100	100	100	100	94.7 (±9.2)	100	100	100	100	100
Bee mortality (%±SD)	3.3 (±4.2)	4	5	4.7 (±2.3)	10 (±3.5)	9.3 (±4.2)	5.3 (±5.8)	2	3.3 (±4.2)	4 (±2)	12.7 (±7)	6	6

Mortality of phoretic Varroa mites and worker bees after feeding two concentrations of different lithium compounds over a maximum feeding period of seven days.

Concentration	Lithium chloride			Lithium citrate		
	Cages (n)	Bee mortality (%)	Mite mortality (%)	Cages (n)	Bee mortality (%)	Mite mortality (%)
1 mM	6	1	29	3	3	48
2 mM	9	3	72	6	4	96
4 mM	9	4	53	6	6	100
10 mM	12	7	96	6	4	100
25 mM	9	5	100	3	6	100

Comparison of the efficacy and side effects of lithium chloride and lithium citrate using concentrations for 1mM to 25mM over a maximum feeding period of seven days.

EXPERIMENT 7 – MINI HIVES

Acaricidal effect on Lithium chloride in mini-hives

- ✖ Under semi-field conditions mini-hives consisting of 2,000 - 3,000 bees each were treated with LiCl.
- ✖ Lithium chloride was dissolved in 70% sucrose with 2mM, 10mM and 25mM each.
- ✖ Bees were continuously feed for 23 days.
- ✖ Dead mites were documented daily.
- ✖ After 23 days, remaining bees were killed with Perizin (coumaphos) to count remaining mites.

	Remaining mites
Control sucrose solution	234
2mM LiCl	234
10mM LiCl	54
25mM LiCl	24

EXPERIMENT 8 - LARVAE

The effect of LiCl on larval development in vitro

- ✖ Larvae were obtained by caging the queen for 24 hours on broodless combs in the colony. 48 hour old larvae were transferred to petri dishes filled with larval food. That is about 5-6 days after egg was laid.
- ✖ Dishes were incubated at 34C and 95% humidity.
- ✖ Larvae were feed constantly with a mix of 53% royal jelly, 4% glucose, 8% fructose, 1% yeast. LiCl was added in 10mM, 25mM, 50mM or water for the control.

	Survival Rate
Control - Sucrose solution	51.2%
10mM LiCl	0%
25mM LiCl	0%
50mM LiCl	0%

All larvae were lost within 72 hours showing a strong lethal effect of LiCl on larvae. LiCl should ideally be applied when egg laying is decreased.

EXPERIMENT 9 – ARTIFICIAL SWARMS

✘ Artificial swarms

- ✘ LiCl was dissolved in sucrose at final concentrations of 25mM and 50mM.
- ✘ Each artificial swarm received 500ml of sucrose/LiCl mixture in a feeding balloon.
- ✘ The total volume was consumed by the bees.
- ✘ Mite fall was monitored for 7 days.
- ✘ Bees were killed with Perizin (coumaphos) to assess the number of remaining mites.

Lithium chloride concentration	Mite fall [Mean $\pm$ SD]			
	Swarms [n]	LiCl treatment	Perizin treatment	Efficacy [%]
25 mM	6	562.5 $\pm$ 149.4	65.5 $\pm$ 26.9	88.9
50 mM	3	178.3 $\pm$ 49.7	22.3 $\pm$ 3.1	89.6

LITHIUM AS A VARROACIDE

As a varroacide, lithium chloride (LiCl) displays some features that are unique in this combination:

- I. LiCl acts systemically via honey bee feeding (“easy-to-apply”),
- II. it is water soluble and will therefore not accumulate in beeswax which is a crucial problem for long-term treatment concepts using synthetic varroacides with lipophilic properties
- III. the oral toxicity of most lithium compounds to mammals is relatively low
- IV. it has no repellent effect (bees are not turned off) on the feeding solution within the relevant concentration range of 2–25 mM and
- V. it is available at moderate prices.

Highly promising is the fact that a single application of only 10 µl of LiCl in a 25 mM solution (corresponding to a dosage of 10.6 µg of LiCl) per individual bee is sufficient to kill phoretic mites.

A challenge for further research will be the development of a smart application technique for full-sized swarms and colonies to ensure that all bees receive the critical amount of the active compound.

SHOULD I TREAT MY HIVES WITH LITHIUM?

- ✘ The answer is no, at this time.
- ✘ First, it is not an approved treatment form yet.
- ✘ The study was preliminary and no field studies were done.
- ✘ Lithium is deadly to larvae, if fed directly, even in small concentrations.
- ✘ We don't know if nurse bees detox the lithium via their hypopharyngeal gland before they feed the larvae.
- ✘ We have no data telling us if the queen will be negatively effected by lithium.
- ✘ We have no long term data showing its effect on colony health.
- ✘ The study was based on lithium chloride, but we know that lithium citrate is as effective in smaller Mol quantities, as shown in one of their tests.
- ✘ Further studies are needed.

PATENT

- ✘ An international patent application, published under the Patent Cooperation Treaty (PCT), has been filed and was granted for all countries.
WO 2017/042240 A1
- ✘ SiTools Biotec is the patent holder and states that a product will be available within the next two years.
- ✘ It will be hard to enforce it since Lithium salts are widely available and a sugar solution holding the salt is easily made.
- ✘ We have yet again another product like oxalic acid. No one will be willing to produce studies that will satisfy the EPA, since the lithium salts can easily be purchased.

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