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A toxicological evaluation of lithium orotate

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MCMD: Pharmaceutical lithium carbonate is 18.78% lithium; the typical typical dose is 300-600 mg 2-3 times/day, providing 113-339 mg of lithium/day, which can be 5 - 300x times the nutritional dose!

suggested nutritional dose is 1-20 mg (click for link)

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ABSTRACT

Lithium orotate, the salt of lithium and orotic acid, has been marketed for decades as a supplemental source of lithium with few recorded adverse events. Nonetheless, there have been some concerns in the scientific literature regarding orotic acid, and pharmaceutical lithium salts are known to have a narrow therapeutic window, albeit, at lithium equivalent therapeutic doses 5.5–67 times greater than typically recommended for supplemental lithium orotate. To our knowledge, the potential toxicity of lithium orotate has not been investigated in pre-clinical studies; thus, we conducted a battery of genetic toxicity tests and an oral repeated-dose toxicity test in order to further explore its safety. Lithium orotate was not mutagenic or clastogenic in bacterial reverse mutation and in vitro mammalian chromosomal aberration tests, respectively, and did not exhibit in vivo genotoxicity in a micronucleus test in mice. In a 28-day, repeated-dose oral toxicity study, rats were administered 0, 100, 200, or 400 mg/kg body weight/day of lithium orotate by gavage. No toxicity or target organs were identified; therefore, a no observed adverse effect level was determined as 400 mg/kg body weight/day. These results are supportive of the lack of a postmarket safety signal from several decades of human consumption.

1. Introduction

Lithium orotate (LO) is commercially available as a monohydrated salt (InChIKey OZCFLLGHXKDGIL-UHFFFAOYSA-M) with a molecular weight of 180.1 g/mol and a molecular formula of $C_5H_5LiN_2O_5$ (or $LiC_5H_3N_2O_4{\bullet}H_2O$) and is comprised of approximately 3.86% lithium, 86.14% orotate, and 10.01% water. The structural formula of LO, monohydrate is given in Fig. 1. Like other lithium salts, LO dissociates in solution to release free lithium ions and, in the specific case of the orotate salt, orotic acid (OA).

According to Devadason, LO has been used as a lithium supplement worldwide for more than three decades, and in the United states and Europe, LO has been used for more than four decades (Devadason, 2018). Devadason also noted reported beneficial effects of LO supplementation, such as feeling calmer, improved responses to stressors, and behaving less impulsively, but acknowledged the lack of evidence of the safety of LO. These effects were noted as similar, but attenuated in comparison, to the effects of lithium carbonate (LC) and were also compared to early work with lithium and epidemiological data in postulating that LO could be a viable supplemental source of lithium, which was suggested to be a necessary trace element for optimal human health. While data definitively demonstrating a nutritional requirement for lithium or its reported benefits is limited, nonetheless, a search of the National Institutes of Health Dietary Supplement Label Database returned a variety of LO-containing products, confirming its availability in supplemental form, with suggested uses ranging from 1 to 20 mg elemental lithium per day (although one product was labeled as containing 120 mg lithium). Due to this availability of LO, and given the

Abbreviations: 2AA, 2-aminoanthracene; ALT, alanine aminotransferase; ANOVA, analysis of variance; FOB, functional observation battery; GLP, good laboratory practice; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; LC, lithium carbonate; LO, lithium orotate; MPCE, micronucleated polychromatic erythrocyte; NDI, nephrogenic diabetes insipidus; NOAEL, no observed adverse effect level; OA, orotic acid; OECD, Organisation for Economic Co-operation and Development; SCE, sister chromatid exchange; SPF, specific pathogen-free; TBIL, total bilirubin; TG, test guideline.

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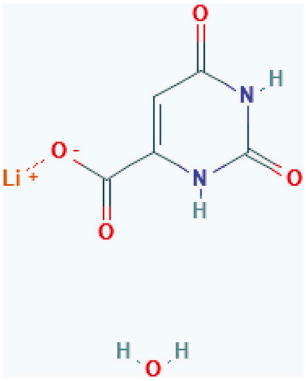

Fig. 1. Lithium orotate monohydrate structural formula PubChem identifier *CID 46942246*, URL: https://pubchem.ncbi.nlm.nih.gov/compound/46942246#section=2D-Structure.

known potential toxicity of approved pharmaceutical preparations of lithium salts (e.g., LC) at therapeutic dose levels and concerns regarding the safety of orotic acid, its safety should be evaluated. Yet to our knowledge, formal preclinical and clinical safety assessments have not been conducted.

Lithium, present at a concentration of approximately 20 ppm in the Earth's crust, is an element found in almost all rocks (hence its name, for the Greek *lithos*, meaning stone), and because of this, also, in trace amounts, in soils and ground waters; therefore, it is naturally found in plant foods and drinking waters (Ramoju et al., 2020; Schrauzer, 2002; Szklarska and Rzymski, 2019). Dietary intake of lithium has been reported as primarily from vegetables, grains, and, in some locations, drinking water and, to a lesser extent, from dairy products, meat, nuts, and other foods and beverages and has been estimated to range from approximately 0.24 to 1.5 μg/kg body weight (bw) daily (United States Environmental Protection Agency (EPA) et al., 2008), while even lower intakes have been reported in Poland and Belgium (Szklarska and Rzymski, 2019), although higher intakes have been reported by Schrauzer (9.3–44.3 μg/kg bw daily based on an average adult weight of 70 kg) (Schrauzer, 2002) and Moore (34–75 μg/kg bw daily from food and municipal drinking water combined) (Moore, 1995), suggesting wide geographical variation. A role of lithium as an essential nutrient has been suggested and a provisional Recommended Dietary Allowance of 1000 μg has been proposed (Schrauzer, 2002), This suggestion and proposal were based on a) its presence in the food supply, b) its detection in human organs and fetal tissues, c) epidemiological data demonstrating an inverse association between drinking water concentrations and behavioral deficits (and possibly mortality rates (Szklarska and Rzymski, 2019)), and d) animal data demonstrating behavioral and/or development abnormalities in rats and goats fed low-lithium diets. Nonetheless, other authors have been less willing to commit to this idea (Perez-Granados and Vaquero, 2002; Szklarska and Rzymski, 2019), and to our knowledge, it has not be taken up by any authoritative scientific bodies.

for 150# person: 0.24 ug = aprox 15 mcg ; 44 ug = 3 mg; 75 ug = 5.25 mg

Regardless of whether or not any potential for beneficial effects of relatively low dietary exposures are relevant in humans, adverse events associated with pharmaceutical use (e.g., LC, lithium citrate) at daily dosages providing approximately 113–338 mg elemental lithium (titrated to achieve serum lithium concentrations of 0.6–1.4 mmol/L) include renal, neurological, psychiatric, thyroid, parathyroid, cardiovascular, dermatologic, hematologic, and gastrointestinal effects with the most common adverse effect being nephrogenic diabetes insipidus (NDI) and the kidney being the primary target organ in both males and females (United States Environmental Protection Agency (EPA) et al., 2008). Such effects are known to occur over the range of therapeutic serum lithium concentrations with dose-dependent increases in severity and are usually reversible if caught early and treatment is discontinued. Nonetheless, permanent organ damage, and even death, can occur, even without prior warning, in stable patients, following months to years of treatment.

OA is an intermediate in synthesis of pyrimidines, and as such, is an endogenous metabolic component in humans (Loffler et al., 2016). In addition to its role in pyrimidine biosynthesis, OA may have other important functions in human health, such as a role in embryonic development, but more work remains to fully elucidate such potential roles. OA is also present in the diet at low levels, with bovine milk being the primary dietary source and containing 20–100 ppm (20–100 mg/L) (Durschlag and Robinson, 1980b; EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2009; Loffler et al., 2016). Like the case with lithium, there may be a balance between background dietary levels of OA intake, which do not appear harmful, and that of excessive intake with the potential to cause toxicity. In rats, at least, exogenous exposure to OA at 1% in the diet results in hepatic lipid accumulation, which can be adverse, and the development of which may require the presence of other dietary components (Durschlag and Robinson, 1980a, 1980b; Hebbachi et al., 1997; Sidransky and Verney, 1965; Sidransky et al., 1963). In vitro experiments demonstrated a common molecular mechanism by which OA can induce hepatic lipogenesis in human hepatoma cell lines and primary rat hepatocytes (Jung et al., 2011). The mechanism, which is likely the result the effects of OA on gene transcription (Griffin et al., 2004; Jung et al., 2011; Wang et al., 2011), was confirmed in vivo in rats (Jung et al., 2011), suggesting a potential concern that OA could induce fatty liver in humans. Tumor promoting effects have also been observed in studies in rats, mice, and hamsters (Chu and Malmgren, 1964; Columbano et al., 1982; Kokkinakis and Albores-Saavedra, 1994; Kokkinakis et al., 1991; Laconi et al., 1993; Laurier et al., 1984; Lin and Tung, 1965; Rao et al., 1983, 1984, 1985; Sarma et al., 1986; Vasudevan et al., 1994) and may also be related to OA effects on gene transcription (Manjeshwar et al., 1999). Also, of note, is the inconsistent nature of a smaller body of evidence showing tumor inhibiting properties of OA (Rogers, 1957a, 1957b; Sidransky and Verney, 1970). Finally, OA elevations are seen in some disorders of pyrimidine metabolism in humans, and in rats, elevated OA has been implicated in insulin resistance and endothelial dysfunction leading to hypertension as well as renal effects similar to those observed in diabetes (Choi et al., 2015; Dumler et al., 1979; Loffler et al., 2016; West et al., 2017).

The above notwithstanding, only a small number of adverse events and case reports of LO toxicity have been reported. For example, searches of adverse event report databases maintained by the United States Food and Drug Administration, European Medicines Agency, and World Health Organization returned only 21 entries that could be confirmed as related to non-pharmaceutical preparations of lithium or orotate and/or LO at recommended supplemental doses and of these, only eight could be confirmed as related specifically to LO. Only a single case study (of an intentional overdose of LO) was found (Pauze and Brooks, 2007). The patient was an 18-year-old female who presented to the emergency department complaining of nausea and vomiting after intentionally ingesting 18 capsules of LO, containing 120 mg each; she had a serum lithium concentration of 0.31 mmol/L on admission, approximately 90 min following the ingestion. Assuming 120 mg of LO monohydrate per capsule, the patient ingested approximately 83.4 mg of elemental lithium (approximately 74% of the starting dose of prescription lithium preparations). In addition to her reported symptoms, the patient presented with a mild, diffuse tremor without rigidity, and her repeat serum lithium was 0.40 mmol/L 1 h later. Nausea and tremor are known adverse effects of lithium, although her serum levels were below the therapeutic range. She was treated with intravenous fluids and an anti-emetic medication and monitored; 3 h following admission her tremor and nausea had resolved.

Some authors have argued that LO has a lesser potential for adversity relative to LC and other pharmaceutical salts due to its reaching therapeutic tissue concentrations at lower ingested doses (Marshall, 2015;

Nieper, 1973; Sartori, 1986). The proposed mechanism is "directed intracellular transport", facilitated by the orotate carrier molecule, and resulting in release of the lithium ion at the intracellular site of action while it remains sequestered by the orotate carrier in the bloodstream (Nieper, 1973). This ability of directed transport is reported to be furthered by the affinity of orotates to tissues in which the pentose phosphate pathway is active (e.g., the blood brain barrier and glial cells). However, if this were accurate, toxicity of LO would be expected to be greater than that of LC, as toxic effects are generally directly due to concentrations reaching the target tissue rather than serum concentrations, which only indirectly measure target tissue exposure, and the above case report, appears consistent with that principle. Additionally, this was not born out in pharmacokinetic assessments (Kling et al., 1978; Smith, 1976; Smith and Schou, 1979), including after oral administration (Smith, 1976). When administered to rats by intraperitoneal injection, LO (200 mEq Li/L) resulted in statistically significantly higher serum, brain, kidney, and heart lithium levels and reductions in renal clearance of lithium, creatinine clearance, and urine flow rate relative to a molar equivalent dose of LC; kidney weights were also increased, and the kidneys appeared large and pale (Kling et al., 1978; Smith and Schou, 1979). When lower doses were administered to rats by gavage (0.5–1.0 mEq Li/kg bw) as a single bolus or continuously in the diet (increased by 15 mEq Li/kg diet at 4-day intervals until reaching 60 mEq) for 20 days, no differences in absorption, distribution, or excretion (not assessed in the repeated dose study) were observed between LO, LC, or lithium chloride (Smith, 1976). Thus, it is more likely that the lack of a postmarket safety signal is due to the low recommended servings sizes of LO relative to LC, which range from 1 to 20 (versus 113–338) mg elemental lithium daily. Nonetheless, there are many unknowns with respect to supplemental use of LO. For example, there is no direct monitoring of recommended use compliance, as illustrated by the case report of Pauze and Brooks, duration of use, serum lithium concentrations, or signs of toxicity, all of which are monitored under physician supervision with use of prescription lithium preparations. Thus, adverse effects may progress unmonitored in the case of excessive use or until cumulative effects of chronic use manifest as overt pathology. For these reasons, we have now investigated LO in a battery of preclinical toxicological investigations.

2. Material and methods

2.1. Test item

Lithium orotate monohydrate (InChIKey OZCFLLGHXKDGIL-UHFFFAOYSA-M), a white solid powder containing 3.6–5.0% elemental lithium on a wet basis, is synthesized from starting materials lithium hydroxide and OA. In addition to lithium content, food grade specifications include qualitative identification and limits for residual solvents, heavy metals, and microbes. LO monohydrate (lot number 699024), was provided by Pure Encapsulations (Sudbury, MA, USA) and contained 3.82% elemental lithium.

2.2. Care and use of animals

The animal studies were conducted under the permission of the Institutional Animal Care and Use Committee of Toxi-Coop Zrt. During the acclimation and treatment periods, ad libitum access was provided to food (ssniff® SM R/M-Z + H, ssniff Spezialdiäten GmbH, Soest, Germany) and potable water. The 28-day study was additionally conducted according to the National Research Council Guide for Care and Use of Laboratory Animals (National Research Council, 2011) and in compliance with the principles of the Hungarian Act 2011 CLVIII (modification of Hungarian Act 1998 XXVIII) and Government Decree 40/2013 regulating animal protection.

2.3. Bacterial reverse mutation test

Bacterial tester strains (Moltox, Inc., Boone, NC, USA) *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 *uvrA* were exposed to test item concentrations of 5000, 1600, 500, 160, 50, and 16 μg/plate suspended in an ultrapure water (ASTM Type 1) vehicle with and without a metabolic activation system (S9-mix). The test solutions were prepared freshly just prior to exposure of the bacterial cultures using serial dilutions and heating and/or placing in an ultrasonic bath for about 15 min until dissolution of the test item was achieved. The vehicle (ultrapure water) and test item concentrations (the high concentration was the maximum concentration recommended for soluble non-cytotoxic substances) were chosen based on preliminary solubility and cytotoxicity tests, and the Good Laboratory Practice (GLP) (OECD, 1998) compliant main mutagenicity tests were conducted in triplicate as an initial test utilizing a plate incorporation procedure and a confirmatory test utilizing a pre-incubation procedure. Colonies were counted manually, and means, standard deviations, and mutations rates were calculated. A concentration was considered cytotoxic if a concentration-related reduction in the number of revertant colonies compared to the concurrent vehicle-control, a reduction in the number of revertant colonies compared to the historical vehicle-control range, the appearance of pinpoint colonies, and/or reduced background lawn development were observed. A concentration-related increase in the number of revertant colonies and/or a reproducible increase compared to the concurrent vehicle-control, of at least 2-fold in tester strains *S. typhimurium* TA98, TA100, or *E. coli* WP2 *uvrA* or 3-fold in tester strains *S. typhimurium* TA1535 or TA1537, occurring in at least one strain at one or more concentrations with or without S9-mix, were considered a positive response for mutagenicity of the test item while the absence of both criteria was considered a negative response.

The experiments were conducted in accordance with OECD test guideline (TG) 471 (OECD, 1997) with reference to procedures described by Ames et al. (1975), Maron and Ames (1983), Kier et al. (1986), Venitt and Parry (1984), and Mortelmans and Zeiger (2000). Positive controls (strain specific) utilized in the experiments without S9-mix were 4-Nitro-1,2-phenylenediamine (TA98), sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and methyl methanesulfonate (WP2 *uvrA*) while 2-aminoanthracene (2AA) was utilized with all strains in the experiments with S9-mix. The three former positive controls were obtained from Merck KGaA (Darmstadt, Germany) while the latter two were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The S9-mix was prepared in the laboratory utilizing a rat liver S9 fraction (Moltox, Inc., Boone, NC, USA), which was checked by the manufacturer for promutagen activation of ethidium bromide, cyclophosphamide, benzo(a)pyrene and 2AA and confirmed in the laboratory with 2AA.

2.4. In vitro mammalian chromosomal aberration test

The test item was suspended in distilled water (MAGILAB Kft., Budapest, Hungary) and short-term and long-term experiments were carried out in duplicate utilizing V79 male Chinese hamster lung cells (European Collection of Authenticated Cell Cultures, Salisbury, England). Treatment formulations were prepared directly prior to use as follows, in brief: the stock suspension was stirred until homogeneity was achieved and diluted with additional distilled water to obtain the lower concentration suspensions, and these suspensions were further diluted with the V79 cell culture medium (Dulbecco's Modified Eagle's medium (Sigma Aldrich Chemie GmbH, Schnelldorf, Germany) with or without S9 mix to obtain the final treatment concentrations. In the short-term experiment without S9-mix, cells were exposed to concentrations of 1250, 1875, and 2500 μg/mL for 3 h and sampling was conducted after 20 h (1.5 cell cycles) and in the short-term experiments with S9-mix, cells were exposed to 312.5, 625, 1250, and 2500 μg/mL for 3 h and sampling was made at 20 and 28 (2 cell cycles in order to cover a

potential mitotic delay) hours. In the long-term experiments, cells were exposed to 312.5, 625, 1250, and 1875 μg/mL for 20 h without S9-mix and sampling was made at 20 and 28 h. The vehicle (distilled water) and experimental concentrations were chosen based on preliminary solubility and cytotoxicity tests and the high concentrations were selected on the basis of limiting cytotoxicity (55 ± 5%, assessed based on a reduction in the relative increase in cell count). The experiments were conducted in compliance with GLP and in accordance with OECD TG 473 (OECD, 2016a) with reference to procedures described by Preston et al. (1981) and Brusick (1989)). Positive controls (Sigma Aldrich Chemie GmbH, Schnelldorf, Germany) were cyclophosphamide and ethyl methanesulfonate (EMS), with and without S9-mix, respectively. EMS is a known clastogen described in the cited literature and verified by the historical database of the laboratory.

2.5. In vivo mammalian micronucleus test

Twenty-five 8-week-old male specific pathogen free (SPF) Hsd Win: NMRI mice (Toxi-Coop, Budapest, Hungary), weighing 31.4–36.8 g at the start of treatment, were randomized to five groups of five animals and administered the test item, suspended in 1% methylcellulose at doses of 0 (vehicle-control), 500, 1000, and 2000 mg/kg bw or the positive control, cyclophosphamide, dissolved in sterile water (MAGILAB Kft., Budapest, Hungary), at a dose of 60 mg/kg bw. The test solutions were prepared fresh, stirred until homogeneity was reached, and administered by gavage within 2 h of preparation on two occasions separated by a 24 h interval. The positive control was administered once by intraperitoneal injection. The vehicle-control was prepared in the laboratory using methylcellulose (Sigma Aldrich Chemie GmbH, Schnelldorf, Germany) and distilled water (MAGILAB Kft, Budapest, Hungary). Prior to both treatment and sacrifice, measurements of body weight were obtained, and animals were monitored immediately after treatment and at regular intervals until sacrifice for mortality, morbidity, and clinical signs of toxicity. All animals were sacrificed by cervical dislocation 24 h after the final treatment and bone marrow slides were prepared for scoring under light microscopy using samples obtained from both femurs of each animal. The vehicle, doses, and sex of the animals were determined based on a preliminary solubility test and a preliminary toxicity test utilizing 2 mice/sex/group, and the main study was conducted in compliance with GLP and according to OECD TG 474 (OECD, 2016b) with reference to the procedures described by Salamone and Heddle (1983).

2.6. 28-Day repeated-dose oral toxicity study in rats

Forty-two to 45 day-old male and female SPF Han:WIST rats (Toxi-Coop, Budapest, Hungary) weighing 176–201 g and 116–150 g, respectively, at the start of treatment were randomly assigned to four groups of 10 rats/sex and administered the test item, suspended in 1% methylcellulose, by gavage at doses of 0 (vehicle-control), 100, 200, and 400 mg/kg bw/day. The vehicle-control was chosen on the basis of stability and homogeneity over the range of relevant test concentrations during the preliminary work to validate the analytical method for determination of the test item in the dosing formulations and was prepared in the laboratory using methylcellulose (Molar Chemicals Kft., Halásztelek, Hungary) and distilled water (Parma Produkt Kft., Budapest, Hungary). Recovery of LO from the vehicle was 98.8% of the nominal concentration at about 5 mg/mL and 95.5% at about 40 mg/mL. LO was stable at 5 mg/mL and 40 mg/mL concentrations for at least 4 h at room temperature and for two days in a refrigerator at 5 ± 3 °C. Concentrations, stability, and homogeneity of the test solutions were verified analytically prior to the start of the study, and concentrations were checked twice (Weeks 1 and 4) during the study. Cage-side and detailed clinical observations and measurements of body weight and food consumption were made throughout the course of the study. A functional observation battery (FOB) was conducted during the last week using a modification of the procedure described by Irwin (1968). Blood samples for clinical pathology evaluations were collected from the retro orbital venous plexus 24 h following the final treatment and after overnight deprivation of food, except that, due to a technical error in the laboratory, the male animals were not fasted. Animals were placed under deep narcosis using Isofluran CP® anesthesia (Medicus Partner Kft., Biatorbágy, Hungary) for blood collection and then immediately sacrificed by exsanguination from the abdominal aorta. At necropsy, gross pathological examinations were conducted, organ weight measurements were made, and tissues were collected, preserved, and processed for histopathological examinations. The study was conducted in compliance with GLP and according to OECD TG 407 (OECD, 2018). Optional examinations added to the study included ophthalmological examination of all animals prior to initiation of dosing and control and high-dose animals during the final study week and ovary and uterine weights.

2.7. Statistics

Statistical analyses were performed with SPSS PC + software, version 4 (SPSS, Inc., Chicago, IL, USA), and Microsoft Excel version 2016 (Microsoft, Hungary) was used to check for linear trends. A *P*-value of <0.05 was considered statistically significant in all tests.

2.7.1. In vitro mammalian chromosomal aberration test

Following blind scoring of the duplicate slides, the results were pooled, and a chi-square test was used to compare the number of aberrations (with and without gaps) and the number of cells with aberrations (with and without gaps) in test and concurrent positive control groups to the concurrent negative controls. Adequate regression analysis was used to check the data for linear trends in the number of cells with aberrations (without gaps).

2.7.2. In vivo mammalian micronucleus test

Kruskal-Wallis non-parametric one-way analysis of variance (ANOVA) was used to compare the frequencies of micronucleated polychromatic erythrocytes (MPCEs) observed in the test and positive control groups to those of the negative control group. Adequate regression analysis was used to check the data for linear trends in the frequency of MPCEs.

2.7.3. 28-Day repeated-dose oral toxicity study in rats

All quantitative data (body weights, body weight gains, food consumption, feed efficiency, clinical pathology parameters, and absolute and relative organ weights) were subjected to statistical analyses; male and female data were evaluated separately. Between group variance was checked using Bartlett's homogeneity of variance test, and normality of heterogeneous data was assessed with the Kolmogorov-Sivmirnov test. Homogenous and normally distributed heterogenous data were subjected to a one-way ANOVA to assess for statistical significance followed by Duncan's Multiple Range test to check positive results for inter-group statistical significance while non-normal data were evaluated using the Kruskal-Wallis non-parametric one-way ANOVA followed by the Mann-Whitney *U* test for inter-group comparisons. Relevance of qualitative data (clinical and functional observations, ophthalmoscopy, and gross and histopathological findings) was assessed by frequency and severity of observations without statistical analyses.

3. Results

3.1. Bacterial reverse mutation test

No limiting precipitation or cytotoxicity of the test item were observed in the initial and confirmatory mutation tests, and the test item concentrations did not cause concentration-related increases or biologically relevant fold-increases in revertant colonies compared to the

vehicle-control in any tester strain with or without metabolic activation (see supplementary data, Tables S2 and S3). The positive controls demonstrated the expected increases compared to their vehicle-controls and all positive and negative controls fell within the corresponding historical control ranges of the laboratory.

3.2. In vitro mammalian chromosomal aberration test

No precipitation or relevant alterations in pH or osmolarity were observed in any of the experiments, and cytotoxicity ranged from 52 to 58% at the highest concentrations. No dose-related or statistically significant increases in structural aberrations were observed in test item-treated cells compared to the concurrent or historical negative controls, and all results were within the 95% control limits of the historical negative control data (see supplementary data, Table S4). No polyploid or endoreduplicated metaphases were observed in any of the experiments. The negative and positive controls were also within the distribution of the respective historical control data and the positive controls induced the expected statistically significant increases in structural aberrations compared to the negative controls.

3.3. In vivo mammalian micronucleus test

No mortality or adverse reactions to treatment were observed in mice of the test item dose groups or negative (vehicle) and positive control groups. No statistically significant increases in MPCE frequencies were observed in the test item dose groups compared to the negative control group, no dose-related increases were observed, and all results were within the 95% control limits of the historical negative control data (see supplementary data, Table S5). The ratios of immature to total erythrocytes in the dose groups were similar, but slightly decreased, compared to the negative control group. The positive control induced the expected statistically significant increases in MPCE frequency compared to the concurrent and historical negative controls and the negative and positive controls were compatible with the laboratory's positive and negative historical control data, respectively.

3.4. 28-Day repeated-dose oral toxicity study in rats

3.4.1. Mortality, clinical observations, and ophthalmology

There were no unscheduled deaths during the study. One male animal of the 400 mg/kg bw/day group exhibited piloerection, decreased body tone, and an enlarged abdomen (which felt hard on the days of detailed examinations) beginning on Days 5, 11, and 19, respectively, while piloerection was observed transiently (Days 8–14) in a single female animal of the 400 mg/kg bw/day group. In the male animal, the observed signs correlated with a net weight loss and low food consumption over the course of the study, with obvious underdevelopment and undernourishment at the gross pathological examination and moderate decreases of prostatic and seminal vesicular secretions and lymphocyte depletion in the thymus at the histopathological examination. No abnormal behavior or clinical signs were observed in the other animals. No functional abnormalities were observed during the FOB and no eye alterations were observed at the ophthalmologic examinations.

3.4.2. Body weights and food consumption

All groups gained weight during the study with no overall effect on body weight development of male or female group animals (Fig. 2). While mean group body weights of the 400 mg/kg bw/day group males were lower compared to the control throughout the study, the differences were only statistically significant on Days 7, 10, and 14. Mean group food consumption was largely comparable throughout (with only a few sporadic statistical decreases and increases), and the body weight effect could be largely attributed to the single male animal discussed above (see supplementary data, Tables S10 and S11, for full details).

3.4.3. Clinical pathology

A few statistically significant alterations in hematological parameters (Table 1) in the male and female test item groups compared to controls were slight (within, or marginal to, the historical control ranges) and were not correlated to histological findings. Likewise, a few statistically significant alterations in clinical chemistry parameters in the male and female test item groups compared to the control were, with the exception of total bilirubin (TBIL) in the female groups, well within the historical control ranges, and were without correlating histopathology. While there was a dose-related increase in alanine aminotransferase (ALT) in both male and female animals, the increases were well under one-fold at the 400 mg/kg bw/day dose level, and while TBIL was statistically significantly decreased in the 400 mg/kg bw/day group compared to the control in female animals, all female groups were above the historical control range (with the control group being highest). The clinical chemistry results are presented in Table 2.

3.4.4. Necropsy and histopathology

A few statistically significant changes in absolute and relative organ weights in the male and female test item groups compared to their respective controls (see supplementary data, Table S14) were within their corresponding historical control ranges and without correlating histopathology. Nonetheless, statistically significant, dose-related increases in liver weights were observed in both males (relative to body weight) and females (absolute and relative to both body and brain weights) and correlated with the statistically significant dose related

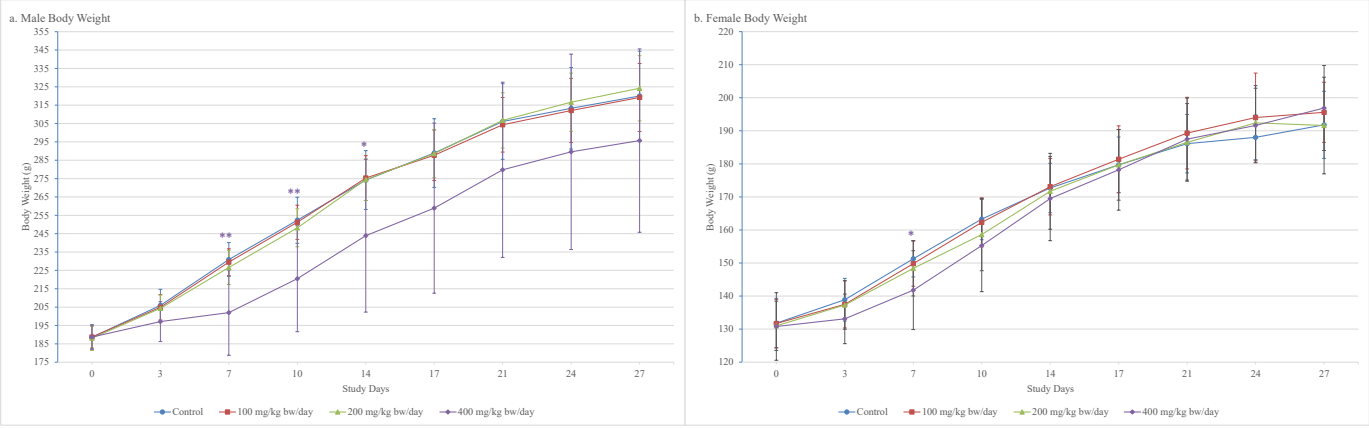

Fig. 2. Male and Female Body Weight Development. (a) Male body weights (*p < 0.05, **p < 0.01; Mann-Whitney *U* test). (b) Female body weight (*p < 0.05; Duncan's multiple range test).

Table 1
Results of the hemotological evaluation.

Group		WBC	NEU	LYM	MONO	EOS	BASO	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	RET	PT	APTT
mg/kg bw/day		[x109/L]	[%]	[%]	[%]	[%]	[%]	[x1012/L]	[g/L]	[L/L]	[fL]	[pg]	[g/L]	[x109/L]	[%]	[sec]	[sec]
Male																	
0 (Control)	Mean	5.40	10.97	85.69	1.40	1.63	0.07	8.18	158.20	0.46	56.20	19.34	344.40	778.30	2.27	10.36	13.51
(n = 10)	SD	1.22	3.55	4.64	0.51	0.98	0.07	0.25	3.49	0.01	1.23	0.38	9.56	109.91	0.31	0.21	1.29
100	Mean	5.59	12.22	84.12	1.38	1.93	0.08	8.31	157.60	0.46	55.02	18.97	345.00	743.00	2.47	10.20	12.76
(n = 10)	SD	1.16	4.04	5.16	0.58	1.13	0.06	0.33	5.27	0.01	1.50	0.42	8.33	103.37	0.34	0.15	0.69
	±%	4	11	−2	−1	18	14	2	0	−1	−2	−2	0	−5	9	−2	−6
200	Mean	5.30	15.32	80.73	1.99	1.57	0.06	8.34	158.50	0.46	54.94	18.99	345.60	780.80	2.58	10.26	12.59
(n = 10)	SD	1.47	9.68	11.33	0.89	1.04	0.05	0.25	3.98	0.01	0.99	0.39	5.04	114.92	0.29	0.26	1.40
	±%	−2	40	−6	42	−4	−14	2	0	0	−2	−2	0	0	14	−1	−7
	SS														[a]		
400	Mean	5.62	16.88	79.14	2.38	1.30	0.06	8.11	153.20	0.45	55.91	18.92	338.40	850.20	3.64	10.22	12.71
(n = 10)	SD	0.92	6.49	7.50	1.16	0.46	0.05	0.54	6.65	0.02	1.85	0.64	7.99	158.04	0.89	0.26	1.54
	±%	4	54	−8	70	−20	−14	−1	−3	−1	−1	−2	−2	9	60	−1	−6
	SS		[a]		[a]				[a]						**		
Test for Significance	NS	U	NS	DN	NS	NS	NS	DN	NS	NS	NS	NS	NS	U	NS	NS	
Historical Control Range		4.9–11.2	7.6–16.2	78.8–89.0	1.3–3.3	0.4–7.9	0.0–0.2	7.80–8.76	151–172	0.45–0.50	54.4–59.8	18.1–20.8	334–356	638–1100	2.37–4.04	9.8–10.7	9.9–15.8	
Female																	
0 (Control)	Mean	4.39	15.56	79.39	1.81	2.92	0.07	8.34	161.78	0.45	54.50	19.42	356.11	786.67	2.38	9.88[c]	13.21[c]
(n = 9[b])	SD	1.15	5.04	6.22	0.46	2.35	0.05	0.35	4.89	0.02	0.90	0.32	9.53	126.08	0.26	0.17	1.23
100	Mean	3.99	16.72	79.44	2.09	1.39	0.06	8.30	156.80	0.45	53.73	18.92	352.20	821.30	2.52	9.75	12.75
(n = 10)	SD	0.86	7.44	7.86	0.80	0.56	0.07	0.19	4.80	0.01	1.75	0.56	6.16	107.40	0.52	0.21	0.91
	±%	−9	7	0	15	−52	−10	−1	−3	−2	−1	−3	−1	4	6	−1	−3
	SS											[a]					
200	Mean	4.61	15.80	80.66	2.03	1.15	0.02	8.34	157.80	0.44	53.25	18.90	355.10	906.80	2.76	9.92	13.63
(n = 10)	SD	1.23	3.48	4.14	0.64	0.63	0.04	0.37	5.20	0.02	1.02	0.37	4.91	173.76	0.51	0.21	1.16
	±%	5	2	2	12	−61	−70	0	−2	−3	−2	−3	0	15	16	0	3
	SS											[a]					
400	Mean	4.29	20.61	74.78	2.55	1.60	0.07	8.10	153.80	0.44	54.01	18.99	352.10	911.50	2.84	9.86[d]	12.99[d]
(n = 10)	SD	1.31	10.85	12.06	0.83	0.95	0.07	0.33	5.87	0.02	2.10	0.60	6.06	139.99	0.51	0.11	0.65
	±%	−2	32	−6	41	−45	5	−3	−5	−4	−1	−2	−1	16	19	0	−2
	SS								**								
Test for Significance	NS	NS	NS	NS	NS	NS	NS	DN	NS	NS	DN	NS	NS	NS	NS	NS	
Historical Control Range		3.5–8.3	6.5–26.9	67.7–90.8	0.9–3.2	0.6–4.4	0.0–0.2	7.47–8.85	145–172	0.41–0.49	52.6–60.0	17.8–20.8	327–361	768–1230	1.86–3.44	9.5–10.0	9.6–19.1	

Abbreviations: ±%, percent change compared to control; APTT, activated partial thromboplastin time; BASO, basophil granulocytes; DN, Duncan's multiple range test; EOS, eosinophil granulocytes; HCT, hematocrit; HGB, hemoglobin; LYM, lymphocyte; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MONO, monocyte; NEU, neutrophil granulocytes; NS, not significant; PLT, platelet count; PT prothrombin time; RBC, red blood cell (erythrocyte); RET, reticulocyte; SD, standard deviation; SS, statistically significant compared to control; T, T-test versus control; U, Mann-Whitney *U* test versus control; WBC, white blood cell.

[a] $p < 0.05$; **$p < 0.01$.

[b] Measurement of hematological parameters was not possible in one female animal in the control group due to coagulation of the blood sample (evaluation of coagulation parameters was not affected because those are collected in a separate tube).

[c] n = 10.

[d] n = 9; measurement of coagulation parameters was not possible in one female animal in the 400 mg/kg bw/day group due to coagulation of the blood sample.

Table 2
Results of the clinical chemistry evaluation.

Group		ALT	AST	ALP	TBIL	CREA	UREA	GLUC	CHOL	Pi	Ca++	Na+	K+	Cl-	ALB	TPROT	A/G
mg/kg bw/day		[U/L]	[U/L]	[U/L]	[μmol/L]	[μmol/L]	[mmol/L]	[mmol/L]	[mmol/L]	[mmol/L]	[mmol/L]	[mmol/L]	[mmol/L]	[mmol/L]	[g/L]	[g/L]	
Male																	
0 (Control)	Mean	33.70	85.40	174.40	1.72	21.40	5.53	7.17	1.85	2.66	2.63	143.63	4.45	100.37	43.54	61.65	2.49
(n = 10)	SD	5.83	11.68	24.82	0.23	2.22	0.43	0.38	0.28	0.11	0.05	1.73	0.19	1.34	2.36	2.35	0.53
100	Mean	37.00	100.70	165.50	1.77	21.80	6.17	7.06	1.91	2.53	2.63	143.16	4.67	100.15	44.28	61.40	2.65
(n = 10)	SD	5.33	26.77	34.05	0.28	2.53	0.95	0.67	0.30	0.18	0.05	1.49	0.45	1.88	0.94	2.27	0.41
	±%	10	18	−5	3	2	12	−1	3	−5	0	0	5	0	2	0	6
200	Mean	38.60	93.80	175.70	1.84	21.40	6.44	7.08	1.95	2.61	2.68	143.24	4.36	99.38	44.44	61.51	2.64
(n = 10)	SD	3.69	18.62	41.53	0.33	2.22	0.77	0.45	0.21	0.15	0.05	1.70	0.32	1.78	1.09	2.53	0.27
	±%	15	10	1	7	0	16	−1	5	−2	2	0	−2	−1	2	0	6
	SS	[a]					[a]										
400	Mean	46.80	96.70	168.50	2.03	21.30	6.62	6.71	1.81	2.71	2.73	143.58	4.32	100.02	43.57	58.84	2.94
(n = 10)	SD	9.80	16.13	36.21	0.45	3.68	0.89	0.93	0.19	0.23	0.10	1.81	0.46	2.33	1.43	2.97	0.58
	±%	39	13	−3	18	0	20	−6	−2	2	4	0	−3	0	0	−5	18
	SS	**					**				**					[a]	
Test for Significance	U	NS	NS	NS	NS	DN	NS	NS	NS	DN	NS	NS	NS	NS	DN	NS	
Historical Control Range		35–61	72–136	123–262	0.8–2.3	17–29	5.6–10.9	4.0–5.9	1.7–3.0	2.5–3.3	2.6–3.0	141.4–147.6	4.3–5.6	94.5–101.8	39.9–45.8	55.5–63.4	2.0–3.1	
Female																	
0 (Control)	Mean	32.40	92.20	83.50	2.87	27.70	5.74	5.78	1.48	2.22	2.59	143.20	4.37	101.36	50.53	65.48	3.41
(n = 10)	SD	9.19	23.77	30.39	0.43	2.79	0.83	0.87	0.24	0.30	0.07	1.40	0.77	1.27	1.90	2.16	0.36
100	Mean	31.60	79.30	86.60	2.43	26.80	5.89	5.99	1.64	2.12	2.55	142.26	4.03	100.33	47.70	63.52	3.03
(n = 10)	SD	4.53	10.50	27.21	0.28	2.35	0.83	1.24	0.34	0.20	0.04	1.91	0.23	1.41	2.32	2.66	0.27
	±%	−2	−14	4	−15	−3	3	4	10	−4	−2	−1	−8	−1	−6	−3	−11
	SS														[a]		[a]
200	Mean	35.60	95.10	79.80	2.69	25.50	6.47	5.25	1.66	2.11	2.61	141.94	4.22	100.28	49.64	64.71	3.34
(n = 10)	SD	11.80	26.88	20.53	0.66	2.80	0.80	0.63	0.26	0.13	0.10	1.69	0.60	1.68	2.77	3.13	0.39
	±%	10	3	−4	−6	−8	13	−9	12	−5	1	−1	−3	−1	−2	−1	−2
400	Mean	46.80	91.60	97.90	2.20	27.00	6.55	5.82	1.95	2.20	2.65	140.91	4.15	100.39	47.53	62.96	3.11
(n = 10)	SD	8.89	17.56	19.94	0.40	1.89	0.94	0.59	0.44	0.28	0.06	1.33	0.32	1.45	3.64	3.56	0.43
	±%	44	−1	17	−23	−3	14	1	31	−1	2	−2	−5	−1	−6	−4	−9
	SS	**			**				**			**			[a]		
Test for Significance	DN	NS	NS	DN	NS	NS	NS	DN	NS	NS	DN	NS	NS	DN	NS	DN	
Historical Control Range		26–60	67–145	66–188	0.9–2.0	21.0–32.0	4.8–12.2	3.3–6.2	1.4–2.5	1.8–2.8	2.5–2.7	139.8–147.3	3.7–4.7	97.0–103.7	42.0–50.3	58.7–68.4	2.2–3.8	

Abbreviations: ±%, percent change compared to control; A/G, albumin/globulin ratio; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ca++, calcium; CHOL, total cholesterol; Cl–, chloride; CREA, creatinine; DN, Duncan's multiple range test; GLUC, glucose; K+, potassium; Na+, sodium; NS, not significant; Pi, inorganic phosphorous; SD, standard deviation; SS, statistically significant compared to control; TBIL, total bilirubin; TPROT, total protein; U, Mann-Whitney *U* test versus control.

[a] $p < 0.05$; **$p < 0.01$.

increases in ALT observed in both sexes (Subsection 3.4.3 and Table 2). All gross and histopathological (Table 3) lesions observed were common background pathology seen in control rats, occurred with similar frequencies in the concurrent control and treated animals, and/or occurred in individual animals and were not accompanied by degenerative or inflammatory changes (see supplementary data, Tables S15 and S16, for full details).

4. Discussion and conclusions

4.1. Discussion

Genetic toxicity tests of other lithium salts, though limited, are in general agreement with our results showing a lack of genotoxic potential of LO. Lithium hypochlorite (LHC), a pool sanitizer, did not exhibit mutagenicity in an Ames test or an in vitro mammalian mutation test using the hypoxanthine-guanine phosphoribosyl transferase gene (albeit the latter results were equivocal with S9-mix due to excessive cytotoxicity at the high concentration) (Weiner et al., 1990). And while LHC did cause damage to chromosomes in an in vitro chromosomal aberrations test, it was not clastogenic in the in vivo (gavage administration) bone marrow chromosomal aberrations test in rats. Statistically significant increases in sister chromatid exchange (SCE) frequency were not observed in the blood of 23 patients receiving continuous lithium therapy for one to eight years compared to age-matched controls (Garson et al., 1981), and in his report on developmental and reproductive toxicity of lithium, Moore reported that an SCE assay by Latimer et al. (1980) did not show increased frequency in human lymphocytes of subjects on long-term lithium treatment (Moore, 1995). In contrast, OA, at 1% in the diet of rats (approximately 500–1000 mg/kg bw/day), has been shown to induce DNA strand breaks in alkaline elution assays (Rao et al., 1984, 1985); however, we note that this assay ignores the critical importance of intercellular differences in DNA damage (Kang et al., 2013).

In our work with LO, the test item did not induce reverse point mutations by base substitutions or frameshifts in histidine-requiring *S. typhimurium* or tryptophan requiring *E. coli* when exposed to the test item up to the maximum recommended concentration for soluble noncytotoxic substances (5000 μg/plate). Likewise, the test item did not cause increases in chromosomal aberrations in mammalian cells up to the maximum feasible concentrations based on limiting cytotoxicity (2500 μg/mL in the short-term experiments and 1875 μg/mL in the long-term experiments) nor did the test item induce micronuclei in the bone marrow of mice administered up to a limit dose (2000 mg/kg bw administered twice at a 24 h interval).

To the best of our knowledge, lithium salts have not been evaluated in chronic, subchronic, or, until now, subacute oral repeated-dose toxicity studies. The 28-day repeated dose study of LO in rats, conducted as part of the current work, was largely unremarkable in outcomes. Most interesting, were the findings in a single male animal of the 400 mg/kg bw/day group. At the end of the study, in correlation with body weight and food consumption measurements, this animal appeared grossly underdeveloped and undernourished. Individually, this animal exhibited a neutrophilic granulocytosis (see supplemental data, Table S12) approximately double the historical control range, which is consistent with observations of benign granulocytosis observed with lithium toxicity in humans (United States Environmental Protection Agency (EPA) et al., 2008), but other hematological and clinical chemistry markers were unremarkable in terms of known effects of lithium. This animal also had weights of the testes (absolute and relative to brain weight), epididymides (absolute), and seminal vesicles and prostate together (absolute and relative to both body and brain weights) that were below those of the respective historical control ranges. At the histological examination, the animal exhibited lesions of the male reproductive system. Decreased secretions of moderate degree of the prostate and seminal vehicles were observed. No other histological lesions of the male reproductive system were observed although analyses of sperm motility were not performed.

Thakur et al. observed complete blockage of prostatic and seminal vesicle secretions in rats administered LC at doses of 800 and 1100 mg/kg bw/day for 90-days, but no effects were observed at the 500 mg/kg bw/day dose level (Thakur et al., 2003). In addition, absolute (but not relative) weights of the testes, epididymides, seminal vesicle, and prostate; daily sperm production and cauda epididymis sperm numbers; serum testosterone; and interstitial fluid volume were all statistically significantly reduced in the 800 and 1100 mg/kg bw/day groups, and the percentage of sperm with abnormal morphology was statistically significantly reduced in all dose groups, including the low dose of 500 mg/kg bw/day. Histologically, moderate to severe degeneration of spermatogenic cells, prominent vacuolization of Sertoli cell cytoplasm, and other degenerative changes in reproductive tissues were observed in the high-dose group (1100 mg/kg bw/day) males. Finally, a statistically significant reduction in male fertility index, which was not recovered following 30 days of no treatment, was observed in the 800 and 1100 mg/kg bw/day groups when the male rats were mated with untreated females. In our study, spermatogenic and Sertoli cells were normal in all 400 mg/kg bw/day group males, and we did not observe adverse effects on spermatogenesis. It is notable that in terms of lithium exposure, Thakur's dose groups were approximately 6-, 10-, and 13-fold greater compared to our high dose group; however, it is difficult to draw definitive conclusions related to exposure due to a lack of comparative toxicokinetic data. Smith found similar absorption and distribution profiles between lithium equivalent doses of LC and LO administered to rats by gavage or in the diet (Smith, 1976). While we calculated Smith's gavage doses to be similar to those of our low and mid doses and their dietary dose to be approximately 3-fold greater than our high dose (EFSA Scientific Committee, 2012), it was also approximately one half and one quarter of Thakur's low and high doses, respectively. It is also notable that Thaker's study did not report any of the clinical observations, body weight and food measurement decreases, or gross appearance abnormalities observed in the single 400 mg/kg bw/day group male in our study. Thus, while it is not possible to definitively rule out an effect of the test item on this single male rat, neither is it possible to definitively rule in an effect of the test item. As such, these observations

Table 3
Summary of histopathology findings.

Organs	Observations	Control	100 mg/kg bw/day	200 mg/kg bw/day	400 mg/kg bw/day
Male	**Number of Animals Examined**	**10**	**2**	**2**	**10**
Kidneys	Dilation, pelvis	1	2	2	2
Prostate	Decreased amount of secrete, moderate	0	[a]	[a]	1
Seminal vesicle[b]	Decreased amount of secrete, moderate	0	[a]	[a]	1
Thymus	Lymphocyte depletion, mild	0	[a]	[a]	1
Female	**Number of Animals Examined**	**10**	**2**	**1**	**10**
Kidneys	Dilation, pelvis	1	2	1	0
Stomach	Acute hemorrhage, mild	0	[a]	[a]	1
Thymus	Acute hemorrhage, mild	0	[a]	[a]	1
Uterus	Dilation	2	[a]	[a]	3

Data represent number of animals with the observation.
Organs without lesions in 10 of 10 control and high-dose animals or without gross lesions at necropsy not shown.
[a] , not examined (only gross lesions were examined in the low- and mid-dose animals).
[b] Seminal vesicle with coagulating gland.

were considered individual findings of indeterminate cause and were not considered due to administration of the test item.

Statistically significant, dose-related increases in ALT and liver weights were observed in both sexes in our study. These statistically significant increases remained within the corresponding historical control ranges and were not associated with any correlating histopathology; therefore, while not ruled out as test item-related, they were considered adaptive changes. Increased liver weights have also been observed in rats fed with orotic acid; however, these were associated with accumulations of neutral lipid, and sometimes with necrosis and inflammatory infiltrates (Durschlag and Robinson, 1980a, 1980b; Hebbachi et al., 1997), none of which were observed in our study.

There are several limitations of our 28-day study that are worthy of brief discussion. Thyroid and parathyroid hormone assays were not conducted. While these assays are not required by the OECD 407 TG, they may have been useful given the ability of lithium to induce hypothyroidism and hyperparathyroidism. Nevertheless, the histological architecture of the thyroid and parathyroid glands of all control and 400 mg/kg bw/day male and female animals appeared normal during the histopathological examinations and no hypercalcemia indicative of hyperparathyroidism was observed. Additionally, it has been suggested that hypothyroid effects of lithium may be secondary to altered renal clearance of iodine (Moore, 1995), and no test item-related renal affects were observed in our study; however, fluid intake was not monitored, and urinalysis was not performed during the study. The kidney is the primary target organ of lithium toxicity, and NDI the most common presentation of renal pathology associated with, lithium therapy. NDI results in reduced concentrating ability of the kidney (which is the most common finding in human studies of lithium adverse events), which can present with both polydipsia and polyurea (United States Environmental Protection Agency (EPA) et al., 2008). As such, measurements of fluid intake and the urinalysis parameters of volume and osmolarity may have been particularly insightful. However, no histopathological lesions commonly observed with NDI were observed, and dilation of the renal pelvis, the only renal pathology that was observed (Table 3), was present in all groups, including the control, at low frequency and is a very common, benign background lesion observed in rats (Frazier et al., 2012; Hard et al., 1999; Johnson et al., 2013; NTP and DHHS, 2014). Finally, no serum lithium concentrations were obtained during our study. These may have been particularly interesting to see individually in the 400 mg/kg bw/day male rat discussed above.

4.2. Conclusions

LO was unequivocally negative for exhibiting genotoxic potential in a standard battery of tests recommend by ICH. In a 28-day repeated dose oral toxicity study, the test item did not cause signs of toxicity and no target organs were identified. The NOAEL was determined as 400 mg/kg bw/day, the highest dose tested. These results are supportive of the history of use of LO without significant postmarket safety signal generation and both open the door, and illustrate the need, for additional studies on this widely available form of lithium.

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CRediT authorship contribution statement

Timothy S. Murbach: Writing – original draft, preparation, Visualization. **Róbert Glávits:** Formal analysis, Data curation, Writing – review & editing. **John R. Endres:** Conceptualization, Resources, Writing – review & editing, Funding acquisition. **Gábor Hirka:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. **Adél Vértesi:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization, Supervision, Project administration. **Erzsébet Béres:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization, Supervision, Project administration. **Ilona Pasics Szakonyiné:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Authors Timothy Murbach and John Endres are salaried employees of AIBMR Life Sciences, Inc. (Seattle, WA, USA). AIBMR was contracted by the study sponsor, as an independent third party, to determine appropriate study protocols and dose selections, place the studies, approve the study plans, and monitor the toxicological studies herein described and to analyze and interpret the resulting data and prepare the manuscript. Author Gábor Hirka is owner and Managing Director at Toxi-Coop Zrt. (with test facilities in Budapest (28-day study) and Balatonfüred (genotoxicity studies), Hungary); authors Adél Vértesi, Erzsébet Béres, and Ilona Pasics Szakonyiné are salaried employees of Toxi-Coop; and author Róbert Glávits is an independent contractor to Toxi-Coop. Toxi-Coop was contracted by AIBMR to develop the study plans and conduct, analyze and interpret, and report the results of the toxicological studies herein described. The authors declare no additional conflicts of interest in regard to the research, authorship, and/or publication of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yrtph.2021.104973.

Data availability

The data sets generated during these studies (Tables S1–S16) are available in the supplementary data files.

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