EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

Bromacil Lithium Salt

FINAL

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Reproductive and Cancer Hazard Assessment Section
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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that one of the mechanisms by which "a chemical is known to the state to cause cancer or reproductive toxicity [is] if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity" (Health and Safety Code Section 25249.8(b)). The "state's qualified experts" regarding findings of reproductive toxicity are identified as members of the Developmental and Reproductive Toxicant (DART) Identification Committee of the Office of Environmental Health Hazard Assessment's Science Advisory Board (Title 22, California Code of Regulations, Section 12301 (22 CCR 12301)). The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency.

Another of the mechanisms by which a chemical may be put on the Proposition 65 list is if the chemical has been formally identified as causing cancer or reproductive toxicity by an organization that has been designated by the State's qualified experts as "authoritative" for purposes of Proposition 65. One such "authoritative body" is the U.S. Environmental Protection Agency (22 CCR 12306).

Bromacil lithium salt was added to the Proposition 65 list of chemicals known to the state to cause reproductive toxicity on May 18, 1999, under the authoritative bodies provision of Proposition 65, based on formal identification as causing developmental toxicity by the U.S. Environmental Protection Agency (U.S. EPA) under its Toxic Release Inventory (TRI) process. In support of that formal identification, U.S. EPA stated that "bromacil lithium salt will dissociate into bromacil, which is soluble in aqueous solutions, and lithium ion", and cited only data on the developmental toxicity of lithium. Subsequent to the Proposition 65 listing, a Court of Appeal decision restricted the evidence that could be reviewed by OEHHA for potential authoritative bodies listings [Third District Court of Appeal, Western Crop Protection et al. vs. Gray Davis et al. (Case No. CO29727, May 9, 2000 as modified on denial of rehearing, June 8, 2000)]. In conducting the more restrictive review of the evidence, per that decision, OEHHA has found that there is no substantial evidence that the scientific criteria for listing bromacil lithium salt were met (22 CCR 12306(g)). As required by regulation (22 CCR 12306(j)), bromacil lithium salt was referred to the DART Identification Committee for a recommendation as to whether it should remain on the Proposition 65 list.

A public request for information relevant to the assessment of the evidence on the reproductive toxicity of this chemical was announced on March 22, 2002, in the *California Regulatory Notice Register*. This draft document provides the DART Identification Committee with information relevant to the reproductive toxicity of bromacil lithium salt, including studies not cited by U.S. EPA in the TRI process. The scope of the review here is broader than that previously reviewed by OEHHA during the

request for relevant information and notice of intent to list stages for bromacil lithium salt under that authoritative bodies mechanism. This is due to the fact that 22 CCR 12306(i) specifies a different inquiry for the DART Identification Committee than that undertaken by OEHHA during its review of the chemical to determine if it met the "sufficient evidence" standard specified in 22 CCR 12306(g). The determination by the Committee is distinct from OEHHA's determination whether U.S. EPA had sufficient data for its formal identification of bromacil lithium salt as causing reproductive (developmental) toxicity and calls for a review of all relevant information regarding bromacil lithium salt, not just that cited by U.S. EPA.

Because of the dissociation of this salt to bromacil and lithium ion, relevant data on the potential reproductive toxicity of bromacil and lithium are also provided. While this hazard identification document does not provide dose-response evaluation, exposure assessment, or determination of allowable or safe exposure levels, the document does provide information which may be useful in such appraisals. This document was released as the draft document *Evidence on the Developmental and Reproductive Toxicity of Bromacil Lithium Salt* in September 2002.

At their December 4, 2002, meeting, the Committee, by a vote of five in favor and none against (with two abstentions) found that bromacil lithium salt had been "clearly shown through scientifically valid testing according to generally accepted principles" to cause developmental and male reproductive toxicity.

The following is the final version of the document that was discussed by the Committee at their December 2002 meeting.

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A. Abstract

Bromacil lithium salt (CAS no. 53404-19-6) is an herbicide which inhibits photosynthesis. It is used for weed control on rights-of-way. In California, 4,478 lbs were applied in the year 2000. Bromacil lithium salt is produced for commercial use by dissolving bromacil in a solution of lithium hydroxide (LiOH). Bromacil is a weak organic acid, with limited solubility. Much greater solubility is achieved in the ionic form produced by reaction with lithium hydroxide.

Because of the dissociation of this salt to bromacil and lithium ion, relevant data on the potential reproductive toxicity of bromacil and lithium are also provided. Bromacil is a substituted uracil herbicide used on food crops and rights-of-way. Lithium is the least massive of the alkali metals. It is used for a number of industrial applications. It is also used extensively for treatment of bipolar disorder (manic-depression) and acute mania.

Bromacil is rapidly absorbed from the gastrointestinal (GI) tract. It is hydroxylated, and excreted mainly in urine. Lithium is also rapidly absorbed from the GI tract. It does not undergo metabolism in the body, and is excreted mainly in urine.

Bromacil and lithium both have relatively low acute and chronic toxicity. In acute studies in rats with bromacil and bromacil lithium salt, females were somewhat more sensitive than males. On a molar basis, in rats the acute oral LD_{50} of lithium was about three times the LD_{50} of bromacil. The LD_{50} of bromacil lithium salt was about one-half that of bromacil. This could be due to the toxicological effect of lithium, or it could be due to the fact that bromacil lithium salt was administered as a true solution, whereas bromacil was administered as a suspension (i.e. a particulate solid dispersed in water).

No data on the developmental or reproductive toxicity of bromacil lithium salt itself have been identified. There are several relevant studies of bromacil in experimental animals. There are also numerous relevant studies of lithium in humans and animals.

Developmental studies with bromacil in experimental animals have found indications of delayed development. These include reduced fetal weight, reduced skeletal ossification, and increased skeletal alterations.

Although early studies in women treated with lithium during pregnancy reported an increased risk of congenital cardiac malformations in infants, especially Ebstein's anomaly (displacement of the tricuspid valve), later studies did not find a significant association. Various factors may account for these inconsistent findings including different study designs, varying power to detect an association, difficulties in the accurate assessment of the outcome of concern, and lack of quantitative exposure assessment. The lack of data concerning lithium exposure may be especially important as the findings from animal studies of adverse developmental outcomes and a recent human study of premature birth suggest there is a threshold dose, below which lithium has no effect and above which it may.

Developmental studies with lithium in experimental animals have found adverse effects including death of the embryo, fetus, or pup, malformations, and retarded development (e.g. reduced fetal weight). While several studies have not found similar effects, multiple differences in study design make comparisons difficult. Developmental studies in rats by gavage found roughly similar molar levels of bromacil and lithium required to produce adverse developmental effects.

Reproductive and chronic studies with bromacil in experimental animals have not found indications of adverse female reproductive effects.

A small study with lithium in humans found no association with alterations of the menstrual cycle or reproductive hormones in healthy females treated at therapeutic doses of lithium. Two small studies found no association of female sexual dysfunction with lithium treatment. Some studies in mice and rats treated with lithium have found reduced female reproductive capacity (fraction pregnant or litter size). However, several other studies have not found such effects. Multiple differences in study design make comparisons difficult. Adverse changes in parenting behavior have been observed in rats treated with lithium orally or by injection, and pup death or developmental delay has been reported after lactational exposure to lithium.

Reproductive studies with bromacil in rats and mice, and chronic studies in rats and dogs have found no adverse male reproductive effects. A chronic study in mice found increased testicular atrophy among mice treated with high doses of bromacil in diet for 12-18 months. Testis weights in bromacil-treated mice that survived to terminal necropsy were not affected.

Several studies of lithium in humans have examined male reproductive endpoints. One study found reduced sperm viability after three weeks of lithium treatment, but no effect on other semen parameters. Another study found all semen parameters (including viability) to be in the normal range for men on long-term lithium therapy. Two studies found no alterations in male reproductive hormones associated with lithium treatment. Two cases of altered sexual function have been reported, but four studies found no association. In general, these studies were performed among small numbers of patients with affective disorders, and many of them did not include appropriate control groups or control for potential confounders.

One study in male and female mice treated orally with lithium observed reduced fertility. Two studies in rats treated orally observed no reduction in fertility. Decreased plasma testostersone was observed in mice treated in diet and rats treated in water, but not in rats treated in diet. Studies in animals treated by injection of lithium have observed adverse effects on sperm, testes histopathology, reduced testes weight, reductions in testosterone and alterations of other male reproductive hormones. Several in vitro tests have observed reduced sperm motility when treated with lithium. Inhibition of testicular activities of steroidogenic enzymes (3β- and 17β-hydroxysteroid dehydrogenase) and alteration in signal transduction pathways that involve inositol metabolism have been observed in rats following sc injection.

B. Introduction

The main focus of this document is bromacil lithium salt, which was listed as known to the state to cause reproductive (developmental) toxicity on May 18, 1999. However, because this salt dissociates to bromacil and lithium ion (U.S. EPA, 1994) and this dissociation creates the potential for exposure to bromacil and lithium, relevant data on the potential reproductive toxicity of bromacil and lithium are also provided.

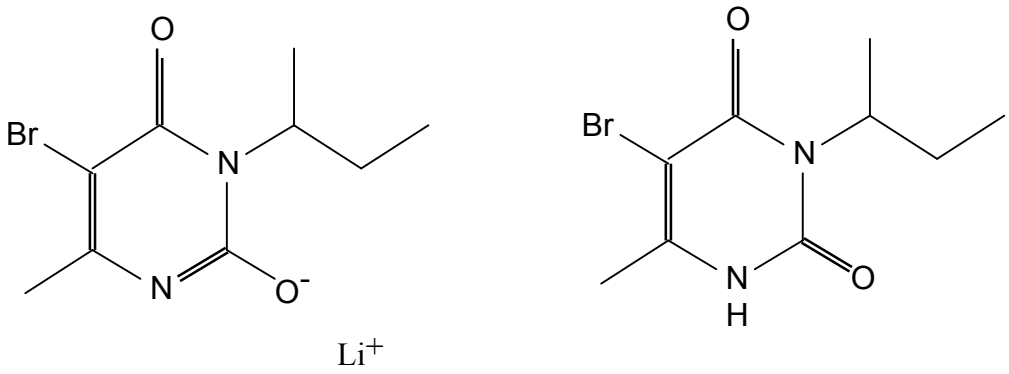
B.1. Chemical structure and main physical characteristics

Bromacil lithium salt (CAS No. 53404-19-6) is a light tan solid at room temperature. It has a mass of 267 D (Schmuckler et al., 1993). Bromacil lithium salt is not routinely produced as a solid. Rather, for commercial use it is prepared by dissolving bromacil in a solution of lithium hydroxide (LiOH). A laboratory preparation of bromacil lithium salt for regulatory study purposes used a ratio of 6 moles LiOH to one mole bromacil in methanol solvent (Wellings, 1993; Kern, 1993). OEHHA does not have information on the molar ratio or solvent used for commercial purposes. Commercial preparations supplied for regulatory testing purposes were reported to be an amber liquid which contained 21-22% bromacil lithium salt (Finlay, 1996a, 1996b; Sarver, 1997).

Bromacil (5-bromo-3-sec-butyl-6-methyluracil: CAS No. 314-40-9) is a white to light tan solid at room temperature. It has a mass of 261 D. It is prepared for use as a powder (HSDB 2002a). It is a weak organic acid, forming an ion with a valance of -1. Water solubility is pH dependent, and relatively low in deionized water (815 mg/L) (Budavari, 1989; du Pont, 1987; Hoffman, 1988). It has low volatility (vapor pressure 3.07×10^{-7} mmHg) (HSDB, 2002a).

Lithium (Li) is the least massive alkali metal (group IA). It has a mass of 6.94 D. In metallic form, it is a silvery-white solid at room temperature. It forms an ion with a valance of +1. It is naturally occurring in two isotopic forms, ^{7}Li (93%) and ^{6}Li (7%). In addition to the commonly used units of amount, such as milligrams (mg) or millimoles (mmol), amounts of lithium are frequently expressed as milliequivalents (meq). An equivalent refers to a mole of charge. Since the ionic form of lithium is +1, a meq is numerically identical to a mmol.

Figure 1. Structure of Bromacil lithium salt and Bromacil



Bromacil Lithium salt Bromacil

B.2. California use and exposure information

Bromacil lithium salt is an herbicide. The herbicidal portion is bromacil. Bromacil lithium salt is produced for commercial use by addition of bromacil to a solution of lithium hydroxide (LiOH) (Wellings, 1993; Kern, 1993). This produces a more concentrated solution than would addition of bromacil to, for example, water with approximately neutral pH, due to the relatively low solubility of bromacil at neutral pH. Data from the California Department of Pesticide Regulation (CDPR, 2001) indicate that application of bromacil lithium salt was 4,478 lbs in the year 2000 (most recent data). The greatest use was for weed control in rights-of-way.

Bromacil is described as "persistent and highly mobile" in the environment (U.S. EPA, 1996). The environmental fate of bromacil has been extensively studied (HSDB, 2002a). Degradation half lives have been estimated as 20 h for air, 75 d for soil and 36 d for sediment/water. Bromacil is highly mobile in soil and is found in groundwater in areas of agricultural use. Bioconcentrations factors (BCFs) in fish have been calculated at 2.8 and 26.5, suggesting minimal bioconcentration.

Bromacil residue tolerance has been established at 0.1 ppm in/on citrus and pineapple. Because bromacil lithium salt is not used on food crops, no residue tolerances have been established.

Virtually all lithium in nature occurs as the ion, Li^{+1}. Due to its ionic nature, lithium is not volatile; most lithium compounds in the atmosphere exist as particulates. It adsorbs poorly to soil. Most salt forms of lithium are somewhat to highly soluble. Lithium would be expected to migrate readily in water (HSDB, 2002b).

Lithium, in the forms lithium carbonate and lithium citrate, has been used therapeutically to treat manic-depressive or bipolar illness since the 1960s. The U.S. Food and Drug Administration approved the use of lithium in the treatment of acute mania in 1970, and approved its use for maintenance therapy in 1974. Extended or slow release formulations Lithobid and Eskalith were approved for use in the U.S. in 1982, although similar forms were available for use earlier in Europe (Kilts, 1998).

Normal plasma lithium concentration in humans is 15-20 μg/L (~2.5 μEq/L) (Kilts, 1998) (1 meq = 1 mM). Following oral adminstration of immediate release lithium formulation, plasma lithium concentrations will reach maximal values of 2-4 meq/L, 1000 greater than typical trace concentrations. Current label information in the Physician's Desk Reference (PDR) for treatment of acute mania recommends up to 340 mg of Li/day (serum levels of up to 1.5 meq Li/L) (equivalent to 600 mg of lithium carbonate [Li_2CO_3] three times per day), and for maintenance from 170 – 227 mg Li/day (serum levels of 0.6 – 1.2 meq Li/L) (equivalent to 300 mg of lithium carbonate three or four times per day (Sifton, 2001).

Others recommend lower ranges 0.8-1.2 meq Li/L for acute and 0.6-1.0 for maintenance therapy (NIMH/NIH cited in Moore et al., 1995). However, to achieve the recommended serum concentrations the prescribed dosage varies between individuals.

B.3. Pharmacokinetics

No information on the pharmacokinetics of bromacil lithium salt itself was located. The pharmacokinetics of bromacil and lithium are discussed below.

Pharmacokinetic studies have been conducted with labeled bromacil in rats (U.S. EPA 1996). Bromacil was rapidly absorbed from the GI tract, rapidly hydroxylated at side-chain sites, and excreted in urine as both the parent compound and metabolites. Tissue analysis indicates that it is widely distributed and does not accumulate with repeated dosing. Dermal absorption was estimated by U.S. EPA as 20% (U.S. EPA, 1996).

Lithium used to treat bipolar disorder is formulated as a lithium salt, lithium carbonate in the form of pill or capsule, or as lithium citrate in the form of a syrup. Lithium is available in rapid or immediate release, and slow or sustained release formulations.

Following oral ingestion of the standard immediate-release formulations, the lithium salt completely dissociates into ions. Lithium ions are absorbed rapidly and almost completely from the gastrointestinal tract by passive diffusion through pores in the small intestinal membrane. A small amount is actively transported in exchange for sodium (Kilts, 1998). Serum concentrations follow an open two-compartment model, with first order absorption into the central compartment (Ward et al., 1994).

Lithium ion does not undergo metabolism in the body. It is not bound to proteins in the plasma. In humans, a peak in plasma concentration of lithium occurs 2-4 hours after an oral dose with complete absorption occurring after 8 hours (Marcus, 1994). Since lithium has a low therapeutic index (i.e. where it is beneficial but not toxic) - as low as 2 or 3 - plasma or serum concentrations are monitored to assure safe use of the drug. Blood lithium concentration is usually measured at a trough of the oscillations that result from repetitive administration; however, the peaks can be two or three times higher at steady state. Even when concentrations of morning blood samples are in an acceptable range (~1 meq/L), intoxication may result when peaks are reached (Hardman et al., 2001).

Numerous pharmacokinetic studies of lithium have reported large interindividual variability in response to lithium administration (Persson 1977; Luisier et al., 1987, Wallis et al., 1989). In addition, there is some evidence that the pharmacokinetics differ in slow or sustained release lithium formulations compared with the standard release lithium formulations (Wallis et al., 1989; Persson, 1977; Ward et al., 1994; Tyrer et al., 1982). Some studies have shown sustained release formulations attain peak serum concentrations approximately 2 to 6 hours after administration with peak concentrations reaching only 50% of rapid release formulations (Ward et al., 1994). In a study comparing different brands, not all sustained release preparations conformed to the international standard for the formulation (Heim et al., 1994). In certain sustained release brands the release of lithium was similar to the rapid formulations. Side effects of lithium have been reportedly related to the maximal plasma concentration as well as its rate of increase following oral administration (Kalin, 1998; Hardman et al., 2001).

Lithium is initially distributed in the extracellular fluid and then is gradually accumulated in various tissues to different degrees. Lithium concentration in plasma is approximately twice that found in red blood cells, muscle, and cerebrospinal fluid, and similar to that found in umbilical cord blood, cardiac and lung tissue (Ward et al., 1994). It has been suggested that the activity of sodium-lithium countertransport mechanisms in red blood cells and perhaps muscle is responsible for the disproportionately low lithium concentrations in these tissues relative to plasma (Kilts, 1998). Passage through the blood-brain barrier is slower relative to serum levels with brain lithium concentrations exhibiting later peaks and slower rates of elimination (Kilts, 1998).

Lithium freely crosses the placental barrier. There is evidence that levels in the fetus are in equilibrium to those in the mother (Schou and Amdisen, 1975).

Approximately 95% of a single lithium dose is excreted through the kidneys. Less than 1% of ingested lithium leaves the human body in feces and 4-5% is excreted in sweat. Lithium is reabsorbed in the proximal tubules in tandem with sodium, with 80 percent of the filtered load being reabsorbed. The proximal reabsorption of these two ions is competitive, so a deficiency of sodium and sodium diuresis tends to increase the retention of lithium (Ward et al., 1994).

The elimination half-life of lithium seems to increase significantly over time. Goodnick et al. (1981) showed that as length of treatment in patients increased, from initiation, to

less than 1 year, to more than one year continuously, the elimination half-life increased in plasma (1.28 to 1.65 to 2.43 days), urine (1.12 to 1.85 to 2.40) and red blood cells (1.22 to 1.75 to 2.24). Similar results were reported by Nilsson and Axelsson (1989) who found a 25-30% increase in the elimination half-life.

The average half-life of lithium in the body varies between 20-24 hours in young adults to as long as 36 hours in elderly patients (Marcus, 1994). In pregnancy, plasma volume and glomerular filtration rate (GFR) increase. As lithium clearance also increases with GFR, increased doses of lithium are required to maintain therapeutic blood levels (Ward et al., 1994). At delivery GFR decreases to prepregnancy levels which requires a decrease in the lithium dose to avoid intoxication of the mother and the neonate (Schou, 1998).

Kilts (1998) states that lithium has the narrowest therapeutic index of any drug routinely prescribed in psychiatric medicine. It is poorly tolerated in one third or more of treated patients (Kilts, 1998) and necessitates repeated blood tests to assure ideal and relatively well-tolerated serum concentrations for long-term use (Baldessarini et al., 2002). Regular monitoring of serum levels is necessary (Amdisen, 1980, Hardman et al., 2001) since even stabilized regimens can be complicated by fever or other infection, changes in water or electrolyte balance, changes in dietary or activity habits, or for no apparent reason (Schou, 1989 cited in Hullin et al., 1993; Hardman et al., 2001). However, the monitoring of patients' serum levels may not be uniform across physicians. One study reported that, compared to hospital physicians, general practitioners were more likely to estimate serum concentrations less frequently, and their patients were more likely to experience raised lithium concentrations (Kehoe and Mander, 1992). One third of doctors were found not to respond to elevated lithium concentrations within the subsequent six weeks following detection. In addition, of the 458 patients in this study 56 had at least one reading of serum lithium concentrations above the therapeutic range, while in 18 patients dosages were reduced due to increasing lithium concentrations in the absence of any known predisposing factors or clinical signs.

Various drugs can also affect the concentration of blood lithium. Diuretics that lead to depletion of sodium can increase lithium retention. Nonsteroidal anti-inflammatory agents have been found to facilitate renal proximal tubular resorption of lithium and thus increase plasma concentrations to toxic levels. Frolich et al. (1979), found that 50 mg of indomethacin three times daily increased plasma lithium concentrations by 59 percent in patients and 30 percent in volunteers. Although indomethacin has been shown to be a particularly strong agent, interactions with ibuprofen and naproxen, and to a lesser extent aspirin, have also been reported (Kristoff et al., 1986; Ragheb, 1990; Hardman et al., 2001).

Although the mode of action of lithium in the treatment of bipolar disorder is not understood, much attention has focused on the effects of low concentrations of lithium on the metabolism of the biogenic monoamines, which have been implicated in the pathophysiology of mood disorders. In addition, effects of lithium on second-messenger

and other intracellular molecular mechanisms involved in signal transduction, and cell and gene regulation have also been investigated (Hardman et al., 2001).

B.3.1. Distribution of lithium in pregnant female, fetus and offspring

B.3.1.1. Overview

There are a considerable number of studies of the distribution of lithium in the pregnant female, fetus, and offspring of experimental animals. Some data are also available in humans. Lithium ion is readily absorbed by the oral route. It appears to distribute in rough proportion to body water. Lithium ion distributes to the fetus after prenatal exposure of the mother. Limited human data indicate that maternal and newborn lithium levels are nearly identical. In experimental animals, the concentrations in the fetus are typically about half the concentration in maternal serum or blood. Lithium ion is also taken up by the nursing offspring of treated mothers. Experiments using ip injection have found a peak of lithium in serum at the earliest time points tested, i.e. 15 to 30 minutes. By this route, plasma levels decline to about half the peak after four hours, and to very low levels in 24 hours. Lithium ion is excreted by the kidneys.

B.3.1.2. Individual studies

Some data are available relating human maternal and newborn lithium levels. Flaherty and Krenzelok (1997) (also Flaherty et al., 1995) report a 17 year old female treated with lithium for manic depression who gave birth at 37 weeks gestational age. The female's lithium level was 2.6 mmol Li/L. Immediately after birth, the infant's level was 2.1 mmol Li/L. Three days after birth the infant's level had declined to 1.4 mmol Li/L. The infant had normal renal function. Shou and Amdisen (1975) reported a series of measurements of maternal and newborn umbilical cord serum in women treated with lithium. There were nine new measurements and two measurements cited from published literature. The values for maternal and umbilical cord serum were essentially identical.

Smithberg et al. (1984) conducted a study of lithium uptake and transfer in non-pregnant, pregnant, and nursing 129/SvS1 mice. Mice were treated with lithium carbonate in water at 0, 1, or 2 mg/ml, corresponding to 0, 13.5, and 27.0 mmol Li/L, respectively. Low levels of lithium were found to be present in the mouse chow (0.064 ppm). Pregnant mice were treated from gd 7 or 8 to gd 18 or 19. Lactating mice were treated from birth to pnd 11 to 15. In control pregnant mice and fetuses, plasma lithium was below detection (limit not stated: by implication, less than 0.03 mmol Li/L). Plasma levels in pregnant females treated with lithium in water were approximately 3-4% the concentration in water. Plasma levels in pregnant females were approximately twice the levels in fetuses. Plasma levels in non-pregnant adult females were similar to pregnant females. Plasma lithium levels in control lactating females (i.e. 0 mmol Li/L in water) were barely detectable. Plasma lithium was about 60% higher in lactating females than pregnant females. Plasma lithium in lactating females treated with lithium was about 4 to 6 times greater than nursing offspring, although levels in control animals were similar, as summarized in Table 1.

Table 1. Plasma lithium concentrations in 129/SvS1 mice from study by Smithberg et al. (1984) [1]

Treatment group : mg Li_2CO_3/ml water (mmol Li/L water)	0 (0)	1 (13.5)	2 (27)
Pregnant adult	0	0.46 ± 0.06	1.09 ± 0.14
Fetus	0	0.22 ± 0.03	0.46 ± 0.10
Non-pregnant adult female	ND	ND	1.19 ± 0.44
Lactating adult	0.03 ± 0.01	0.75 ± 0.13	1.77 ± 0.09
Nursing offspring	0.03 ± 0.01	0.12 ± 0.03	0.41 ± 0.09

[1] Data are mmol Li/L plasma averages ± SE. ND: Not Determined. Numbers of animals were not reported. Data estimated by OEHHA staff from Figures 1 and 2 in Smithberg et al. (1984). Note some data were also reported in the text. OEHHA staff estimates and data in text agreed within rounding error.

Johansen and Ulrich (1969) treated pregnant Wistar rats with lithium in food with the target of achieving 0, 1, or 3 mmol Li/kg bw (0, 7 or 21 mg Li/kg bw) during gestation. Actual doses achieved were 0, 0.93 and 1.78 mmol Li/kg bw (0, 6.5 or 12.5 mg Li/kg bw), respectively. High dose values were lower than target due to reduced maternal food consumption. Maternal serum lithium levels were 0.14-0.21 and 0.41-0.48 mmol Li/L in the low and high treatment groups, respectively. Levels declined from gd 7 to 20. Fetal serum levels were approximately half of maternal levels on gd 20. Data from this study are summarized in Table 2.

Table 2. Serum lithium concentrations in Wistar rats from study by Johansen and Ulrich (1969) [1]

Treatment group : mmol Li/kg bw		0	0.93	1.78
Maternal	Gd 7	0.02	0.21	0.48
	Gd 14	0.02	0.18	0.43
	Gd 20	0.01	0.14	0.41
Fetus gd 20		ND	0.08	0.20

[1] Data are mmol Li/L serum averages (indices of variation were not reported). Data estimated by OEHHA staff from Figure 3 in Johansen and Ulrich (1969)

Several other studies have measured lithium concentrations in blood, serum, or plasma following oral treatment. Matsumoto et al. (1974) treated female ICR mice with lithium chloride at 400 mg/kg (9.4 mmol Li/kg) by gavage on gd 12. Four hours after administration, the maternal blood level was 1.6 mmol Li/L, and the level in fetuses was 0.83 mmol Li/kg bw. Mroczka et al. (1983) treated mating pairs of CFW mice with 10 or 50 mmol Li/L (70 or 350 ppm) in drinking water for two weeks. Plasma levels were 0.09 and 0.67 mmol Li/L, respectively. Two week old pups nursing from mothers maintained

on 50 mmol Li/L had plasma levels of 0.20 mmol Li/L. Szabo (1970a, 1970b; Szabo et al. 1970) treated female HaM/ICR mice with lithium carbonate for 10 days at 0, 100, 400 or 800 mg/kg/d (0, 2.7, 10.8 or 22 mmol Li/kg bw), and sampled blood three hours after the final dose. Whether the mice were pregnant or not, and the specific route of administration were not stated. Toxicity experiments reported in these articles used pregnant mice and gavage. Serum levels were 0.04, 0.45, 1.25, and 4.26 mmol Li/L, respectively, for the doses indicated. Two out of three mice treated at the high dose died.

Christensen et al. (1982) administered lithium in the diet to Wistar rats prenatally or prenatally and postnatally at 40 to 60 mmol Li/kg food (280 to 420 ppm). At 3 weeks post-delivery, in the group treated with lithium postnatally only, maternal plasma lithium concentration was 1.47 ± 0.24 mmol Li/L, and offspring plasma lithium was 0.51 ± 0.14 mmol Li/L. In the group treated with lithium prenatally and postnatally, maternal lithium was 1.15 ± 0.12, and offspring lithium was 0.54 ± 0.12 mmol Li/L. Gralla and McIlhenny (1972) treated rats with lithium carbonate by gavage. Rats treated for 3 days at 4.05 mmol Li/kg (28.4 mg Li/kg) had plasma lithium levels of 1.4 mmol Li/kg 3-5 hours after the last treatment. Tiexiera et al. (1995) treated mated female Wistar rats with lithium in water at 10 mmol Li/L (70 ppm) during pregnancy or during pregnancy and lactation. Maternal serum in rats treated during pregnancy only and during pregnancy and lactation were reported as 0.5 ± 0.1 mmol Li/L.

Gralla and McIlhenny (1972) also treated rabbits and monkeys with lithium carbonate by gavage. Rabbits treated at 1.08 mmol Li/kg (7.6 mg Li/kg) had peak plasma levels ranging from 1.5 to 2.4 mmol Li/kg one hour after treatment. Monkeys treated for 9 days at 0.675 mmol Li/kg (4.7 mg Li/kg) had plasma levels of at least 0.5 mmol Li/L four hours after the first treatment. At 24 hours after the third dose, plasma levels were 0.3-0.4 mmol Li/L, which were maintained through day 9.

There have also been several studies of lithium concentrations in serum after treatment by injection. Giles and Bannigan (1997) administered lithium carbonate at 300 mg/kg (8.1 mmol Li/kg) by ip injection to adult, non-pregnant CD-1 mice. Serum lithium levels peaked at 9.3-9.8 mmol Li/L from 15 minutes (the earliest time point tested) to 1 hour after administration. After 4 hours the level had fallen to approximately half of the peak value, and was below detection after 16 hours. Smithberg and Dixit (1982) treated non-pregnant 129 and A/J strain mice with lithium carbonate by ip injection at 0.625 to 5.0 mg/mouse (5 mg/mouse corresponds to 200 mg/kg, or 5.4 mmol Li/kg). Peak serum lithium levels of approximately 7 mmol Li/L were observed at 15 to 30 minutes after treatment (the earliest time points tested). Serum lithium levels declined to about half the peak after 4 hours, and less than one tenth of peak after 24 hours. Similar results were observed with pregnant mice, although the earliest time point tested was one hour. Johansen (1971) treated pregnant Wistar rats with 6.25 mmol Li/kg (43.8 mg Li/kg) by ip injection. Peak serum levels were observed 15 minutes after treatment at 9-10 mmol Li/L.

B.4. Nondevelopmental and reproductive (DART) toxicities.

Very little information is available on the toxicity of bromacil lithium salt per se. There are a considerable number of experimental animal studies of non-DART toxicity with bromacil, and both human and animal non-DART studies with lithium.

B.4.1. Non-DART toxicity of bromacil lithium salt

No human toxicity studies with bromacil lithium salt itself were located. In experimental animals, acute oral, inhalation, and dermal toxicity studies have been reported. These are described below. The only other toxicity studies with bromacil lithium salt that have been located are eye and dermal irritation studies.

Finlay (1996a)

Mature Sprague-Dawley rats were treated by gavage once with an undiluted commercial formulation of bromacil lithium salt (Hyvar ® X-L) at 2,000, 3,000, or 4,000 mg/kg for males and 500, 1,000, or 2,000 mg/kg for females. The active ingredient (bromacil lithium salt) was reported to be 21.9%. Thus, the doses of bromacil lithium salt were 440, 660, or 880 mg/kg for males and 110, 220, or 440 mg/kg for females. There were 5 rats/sex/dose. Rats were observed for 14 days following treatment. Mortality was observed at the highest doses in both sexes (see Table 3). The estimated LD_{50}s for the formulation were 3927 mg/kg for males and 1414 mg/kg for females. These correspond to LD_{50}s for bromacil lithium salt of 860 mg/kg for males and 310 mg/kg for females. Initial weight loss up to 24% was observed in some of the surviving rats, followed by resumed weight gain. Common clinical signs were ocular or oral discharge, immobility, labored breathing, hunched posture, and ruffled fur.

Table 3. Mortality data from rat acute oral toxicity study by Finlay (1996a)

Sex	Male			Female		
Formulation dose (mg/kg)	2,000	3,000	4,000	500	1,000	2,000
Bromacil lithium salt dose [mg/kg (mmol/kg)] [(1)]	440 (1.65)	660 (2.5)	880 (3.3)	110 (0.41)	220 (0.82)	440 (1.65)
Mortality (number dead/ number treated	0/5	0/5	3/5	0/5	0/5	5/5

[(1)] Bromacil lithium salt active ingredient reported as 21.9%.

Sarver (1997)

Mature Sprague-Dawley rats were treated once with a commercial formulation of bromacil lithium salt (Hyvar ® X-L) by inhalation for 4 hours at aerosol concentrations of 5.5 mg/L for males and 1.3, 2.2, 3.3, or 5.5 mg/L for females. The active ingredient (bromacil lithium salt) was reported to be 21.9%. Thus, the concentrations of bromacil lithium salt were 1.2 mg/L for males and 0.28, 0.48, 0.72, or 1.2 mg/L for females. There were 5 or 10 rats/sex/group. Rats were observed for up to 14 days after exposure. No male rats died. Mortality was observed in female rats at the two higher concentrations (see Table 4). Clinical signs commonly observed in male rats included ruffled fur and gasping. Clinical signs commonly observed in female rats included red ocular discharge, irregular respiration, gasping, lethargy, and immobility. In both sexes initial body weight loss was observed, followed by resumed weight gain. The LC_{50}s were estimated to be > 5.5 mg/L for males and 4.3 mg/L for females. These correspond to estimated LD_{50}s of > 204 mg/kg (> 0.76 mmol/kg) for males and 165 mg/kg (0.62 mmol/kg) for females.

Table 4. Mortality data from rat acute inhalation toxicity bromacil lithium salt study by Sarver (1997)

Sex	Male	Female			
Formulation concentration (mg/L)	5.5	1.3	2.2	3.3	5.5
Bromacil lithium salt concentration (mg/L) [(1)]	1.2	0.28	0.48	0.72	1.2
Bromacil lithium salt dose [mg/kg (mmol/kg)] [(2)]	204 (0.76)	49 (0.18)	84 (0.31)	126 (0.47)	209 (0.78)
Mortality (number dead/ number treated	0/5	0/10	0/10	6/10	2/5

[(1)] Bromacil lithium salt active ingredient reported as 21.9%.
[(2)] Inhalation dose calculated by OEHHA staff from chamber concentrations and body weights using the allometric equation for rats in U.S. EPA (1988).

Finlay (1996b)

Mature male and female Sprague-Dawley rats were treated with a commercial formulation of bromacil lithium salt by dermal exposure at 5,000 mg/kg for 24 hours. The active ingredient (bromacil lithium salt) was reported to be 21.9%. Thus, the dose of bromacil lithium salt was 1,200 mg/kg. The skin was shaved and intact, the application area was 37 cm^2, and the application site was occluded. There were 5 rats/sex. No mortality attributable to bromacil lithium salt treatment was observed. Initial body weight loss was observed, followed by resumed weight gain. Erythema was commonly observed, and there was edema in one rat and desquamation in one rat.

B.4.2. Non-DART toxicity of bromacil

No human studies of non-DART toxicity of bromacil were located. Acute, subchronic and chronic studies of bromacil have been conducted in connection with pesticide registration as reviewed by the CDPR (1997) and the U.S. EPA (1996).

B.4.2.1. Acute toxicity studies with bromacil

Sarver (1988)

Mature Sprague-Dawley rats were treated by gavage (suspension in water) with single doses of a commercial formulation of bromacil (Hyvar ® DF) at 1,200, 2,500, or 5,000 mg/kg for males and 1,200, 1,600, 2,500, or 5,000 mg/kg for females. The active ingredient (bromacil) was reported to be 80%. Thus, the doses of bromacil were 960, 2,000, or 4,000 mg/kg for males and 960, 1,280, 2,000, or 4,000 mg/kg for females. There were 10 rats/sex/dose. Rats were observed for 14 days. Mortality was observed at all doses in both sexes (see Table 5). The estimated LD_{50}s for the formulation were 2,000 mg/kg for males and 1,300 mg/kg for females. These correspond to LD_{50}s for bromacil of 1,600 mg/kg (4.9 mmol/kg) for males, and 1,040 mg/kg (4.0 mmol/kg) for females. All rats exhibited lethargy, and rats exposed at the highest dose exhibited labored breathing. Surviving rats exhibited initial weight loss, followed by weight gains.

Table 5. Mortality data from rat acute oral toxicity study with bromacil by Sarver (1988)

Sex	Male			Female			
Formulation dose (mg/kg)	1,200	2,500	5,000	1,200	1,600	2,500	5,000
Bromacil dose [mg/kg (mmol/kg)] [(1)]	960 (3.7)	2,000 (7.7)	4,000 (15.3)	960 (3.7)	1,280 (4.9)	2,000 (7.7)	4,000 (15.3)
Mortality (number dead/ number treated	2/10	6/10	10/10	2/10	8/10	10/10	9/10

[(1)] Bromacil active ingredient reported as 80%.

Malek (1989)

Mature Sprague-Dawley rats were treated with a commercial formulation of bromacil (Hyvar ® DF) by inhalation for 4 hours at particulate concentrations of 4.1, 5.1, or 5.2 mg/L for both males and females. The active ingredient (bromacil) was reported to be 80%. Thus, the concentrations of bromacil were 3.28, 4.08, or 4.16 mg/L. There were 5 rats/sex/group. Rats were observed for up to 14 days after exposure. One male rat died during exposure at the 5.1 mg/L concentration. No other rats died (see Table 6). Clinical signs observed in all rats included lethargy, and ocular and nasal irritation. In both sexes

initial body weight loss was observed, followed by resumed weight gain. The LC_{50}s were > 5.1 mg/L for males and females. This corresponds to estimated LD_{50}s of > 709 mg/kg (> 2.72 mmol/kg) for males and > 734 mg/kg (> 2.81 mmol/kg) for females.

Table 6. Mortality data from rat acute inhalation toxicity bromacil study by Malek (1989)

Sex	Male			Female		
Formulation concentration (mg/L)	4.1	5.1	5.2	4.1	5.1	5.2
Bromacil concentration (mg/L) [(1)]	3.28	4.08	4.16	3.28	4.08	4.16
Bromacil dose [mg/kg (mmol/kg)] [(2)]	563 (2.16)	694 (2.66)	709 (2.72)	584 (2.24)	717 (2.75)	734 (2.81)
Mortality (number dead/ number treated	0/5	1/5	0/5	0/5	0/5	0/5

[(1)] Bromacil active ingredient reported as 80%.
[(2)] Inhalation dose calculated by OEHHA staff from chamber concentrations and body weights using the allometric equation for rats in U.S. EPA (1988).

Brock 1988

Mature male and female New Zealand White rabbits were treated with a commercial formulation of bromacil (Hyvar ® 80 DF) by dermal exposure at 2,000 mg/kg for 24 hours. The purity of the active ingredient (bromacil) was reported as 80%, so the dose of bromacil was 1,600 mg/kg. The formulation, a powder, was moistened with water to form a paste. The skin was shaved and intact, the application area was 190 cm^2, and the application site was occluded. No mortality was observed. Slight initial body weight loss was observed in some rabbits, followed by resumed weight gain. Erythema was commonly observed, and edema was observed in one rabbit.

B.4.2.2. Subchronic, chronic, and carcinogenicity studies with bromacil

In chronic toxicity studies bromacil was administered in diet to mice, rats and dogs at diet concentrations up to 5000 ppm. In rats and dogs, the adverse effect identified at the LOEL was reduced food intake, body weight or weight gain (Table 7). Bromacil was not found to be carcinogenic and little target organ toxicity was identified. Effects on thyroid were noted in rats in an early study with a commercial formulation (Haskell, 1966) but were not seen in a later rat study using higher doses and a technical grade agent (Bogdanffy, 1989).

Table 7. Summary of chronic dog and rat studies

			Effects
Haskell 1966	dog, feed 3/sex/group	0, 50, 250, **1250** ppm, 0, 2, 9, **31** mg/kg/d[(2)] 2 years	no effects
Haskell 1966	rat, feed 36/sex/group	0, 50, **250**, 1250 ppm, 0, 2, **11**, 58 mg/kg/d[(2)] 2 years	reduced body weight
Bogdanffy 1991	dog, feed 5/sex/group	0, 50, **150**, 625 ppm, 0, 1, **5**, 17 mg/k/d[(1)] 1 year	reduced food intake
Bogdanffy 1989	rat, feed 62/sex/group	0, **50**, 250, 2500 ppm, 2, **12**, 103 mg/kg/d[(1)] 2 years	reduced body weight

[(1)] Mean of male and female doses; provided in study report.
[(2)] Mean of male and female doses; calculated by OEHHA staff from data in study report.

In contrast to the minimal toxicity detected in rat and dog studies, a chronic study in mice (Wood 1980) found a variety of target organ effects as well as increased tumor incidence. CD-1 mice were fed 0, 250, 1250 or 5000 ppm bromacil in diet for 18 months. The study featured a large group size (80 mice/sex/group). The doses calculated from food intake were 0, 40, 196 or 871 mg/kg/d for males and 0, 67, 330, or 1131 mg/kg/d for females. Treatment effects at the LOEL (250 ppm), as summarized by CDPR (1997), included "atrophy of immune system organs and pancreas; liver, testes and kidney necrosis and lung fibrosis." At higher doses effects include "testicular atrophy, atrial thrombus and aortic root necrosis, . . .liver adenoma and carcinomas (in males)." Amyloidosis was described in many tissues and was identified as the most common cause of death in the study. The LOEL for this study was 40 mg/kg/d based on liver necrosis as identified by U.S. EPA (1996). This study suggests a greater sensitivity of mice to bromacil than rats or dogs. Notably no reproductive or developmental toxicity studies have been conducted in mice.

Bromacil has been extensively tested for genotoxicity including assays for mutagenicity, clastogenicity and DNA damage (CDPR, 1997; U.S. EPA, 1996). Many of these tests, conducted from 1966 through 1988, did not contain cytotoxic doses, metabolic activation, and other currently required procedures (CDPR, 1997). As documented by DPR (1997), six of eight mutagenicity assays were negative; however, bromacil was found to be mutagenic in a drosophila dominant lethal test and mouse lymphoma assay for mutation frequencies. Chromosomal aberrations were not indicated in two mouse dominant lethal assays and two PCE micronucleus assays, but a human lymphocyte assay was positive. Unscheduled DNA synthesis and other DNA damage assays were negative. In reviewing these data, CDPR (1997) concluded that possible adverse effects were indicated for

mutagenicity and chromosome effects. However, U.S. EPA (1996), reviewing the same data, has concluded that bromacil is not mutagenic.

Cancer studies, combined with chronic toxicity, have been conducted in rats (Bogdanffy, 1989) and mice (Wood, 1980). The diet concentrations for rats were 0, 50, 250 or 2500 ppm and for mice were 0, 250, 1250 and 5000 ppm. No increase in tumor rates were seen in the rat study, although there was a trend toward increase in thyroid tumors. Increased incidence of liver adenomas and carcinomas combined was seen in the male mice at the 5000 ppm diet concentration. Hepatotoxicity was also prominent in the mouse but not the rat study. U.S. EPA has classified bromacil as a "possible human carcinogen." Bromacil is not listed under Proposition 65 as "known to the state to cause cancer."

B.4.3. Non-DART toxicity of lithium

B.4.3.1. Non-DART toxicity of lithium in humans

Adverse effects of lithium increase greatly with serum concentrations greater than 1.5 meq/L, while life threatening intoxication occurs at concentrations greater than 3.5 meq/L (Ward et al., 1994). Characteristics of acute lithium intoxication include vomiting, profuse diarrhea, coarse tremor, ataxia, nystagmus, coma and convulsions. Symptoms associated with milder toxicity, which can occur at the absorptive peak of lithium, include abdominal discomfort, diarrhea, sedation or confusion and fine tremor. Cases of severe overdose can result in potentially irreversible neurological damage, and coma or death (Baldessarini et al., 2002; Hardman et al., 2001).

Less severe adverse effects which can occur even at therapeutic blood levels of lithium include: nausea, daytime drowsiness, mild cognitive dulling, polyuria, polydipsia, weight gain, fluid retention, fine hand tremor, and dermatological reactions. In addition, patients on lithium occasionally develop benign diffuse, non-tender thyroid enlargement and serum thyroxin levels may decrease while thyroid-stimulating hormone may increase (Baldessarini et al., 2002).

Although lithium is used extensively in the treatment of bipolar disorder, there have been few investigations on the mutagenic potential of lithium compounds. Possible ways in which lithium could act on DNA include: lithium binds selectively to DNA; it competes with magnesium and could impair DNA synthesis and DNA repair (Leonard et al., 1995). In a recent review of the mutagenicity and carcinogenicity of lithium, Leonard et al. (1995) concluded that there is little evidence that lithium compounds have any significant clastogenic or mutagenic activity. No information was found on cancer caused by treatment with lithium.

B.4.3.2. Non-DART toxicity of lithium in experimental animals

Several studies have examined the non-DART toxicities of lithium in experimental animals. Table 8 shows acute lethal doses or concentrations. In mice, the LD_{50} for ip injection is about half that for gavage (Smithberg and Dixit, 1982). The rat inhalation LD_{50} shown in the table is for a lithium combustion aerosol, which was highly basic. The basicity likely contributed to mortality of the animals (Greenspan et al., 1986). The LD_{50} in the table is likely best interpreted as a lower limit for inhalation of lithium.

In addition to the above acute studies, a number of subchronic and chronic studies have been reported. Some of these are summarized below, along with ranges for LD_{50}s and LC_{50}s. In addition to lethality, a number of empirical observations of lithium toxicity have been made. Depending upon the treatment, these include reduced food consumption, reduced water consumption, weight loss, and drowsiness leading to stupor. In some cases (especially when lithium was administered in food), increased water consumption has been reported, coincident with increased urine output (e.g. Christensen et al., 1982). Although there is a very large literature on lithium, the mechanisms of toxicity in experimental animals do not appear to be well understood.

Table 8. Acute LD_{50}s from lithium treatment in experimental animals.

Reference	Duration	Species	Strain	Route	Sex	LD_{50} (mmol Li/kg)
Jurand (1988)	once	Mice	JBT/Jd	Injection (ip)	Female	11.9
Smithberg and Dixit (1982)	once	Mice	129 Sv/Sl	Injection (ip)	NR [1]	10.8
Smithberg and Dixit (1982)	once	Mice	129 Sv/Sl	Gavage	NR	16-22
Kersten (1981)	once	Rats	Wistar	Injection (ip)	Female	12.2 – 12.7 [2]
Petersen (1980)	once	Rats	Wistar	Injection (ip)	Male	12.1 – 15.7 [3]
Petersen (1980)	once	Rats	Wistar	Injection (sc)	Male	11.8-12.8 [3]
Petersen (1980)	once	Rats	Wistar	Gavage	Male	12.4 - 19.8 [3]
Smyth et al. (1962, 1969)	once	Rats	Wistar	Gavage	Male	19.2
Greenspan et al. (1986)	4 hours	Rats	F344	Inhalation	Female	11.0 [4, 5]
Greenspan et al. (1986)	4 hours	Rats	F344	Inhalation	Male	8.6 [4, 5]

[1] Not reported.
[2] Range of values for rats tested at 33, 55, 105, or 240 days of age.
[3] Range of values for rats tested at 6 weeks, 3 months, or 6 months of age.
[4] The exposure was to a lithium combustion aerosol, consisting of about 80% Li_2CO_3 and 20% LiOH and/or Li_2O. The authors point out that the apparent cause of death was respiratory tract damage, and that similar pathology and LC_{50} were obtained with a sodium combustion aerosol. The authors conclude that the cause of death was the caustic nature of the aerosol (i.e. highly basic), not lithium.
[5] Inhalation dose calculated by OEHHA staff from chamber concentrations and body weights using the allometric equation for rats in U.S. EPA (1988).

Mroczka et al. (1983)

Male and female CFW mice were treated with lithium chloride in water at 0, 10, 20, 30, 50, 100, or 200 mmol Li/L beginning at 6-8 weeks of age. The number of animals was not reported, but data suggest approximately 45 pairs of controls, and 85 pairs treated at 50 meq/L. Mice would not drink water containing 200 mmol Li/L, and all died within one week. Reduced water intake but no mortality was observed at 100 mmol Li/L.

Surviving mice were treated until reproduction ceased. No other systemic results were reported. The LC_{50} would be greater than 100 mmol Li/L.

Szabo (1969, 1970), Szabo et al. (1970)

Female HaM/ICR mice (pregnancy status not reported) were treated (apparently by gavage) with lithium carbonate at 0, 100, 400, or 800 mg/kg/d (0, 2.7, 10.8, or 22 mmol Li/kg/d) for 10 days. There were six controls, and three mice/lithium treated group. Two of three mice died after the 9th dose at 800 mg/kg/d. The LD_{50} would be 400-800 mg/kg/d (10.8-22 mmol Li/kg/d).

MacLeod et al. (1949)

Mature male (strain not reported) rats were injected (sc) with lithium chloride at 1, 3, 5, 10, or 15 mg LiCl/rat/day (0.14, 0.43, 0.72, 1.4, or 2.2 mmol Li/rat/day) for up to 34 days. Average body weight was stated to be 250 g, with range of 215-350. There were 2-3 rats/group. Clinical signs (increased reflex excitability and paralysis of hind limbs) were observed at 5, 10, and 15 mg Li/rat/day. Body weight loss was observed at 10 and 15 mg Li/day. Increased mortality (2/3) was observed at 15 mg lithium. The LD_{50} would be 10-15 mg/rat/d, corresponding to 40-60 mg/kg/d (5.8-8.6 mmol Li/kg/d).

Trautner et al. (1958)

Wistar rats (sex not specified) were treated with lithium chloride at 0, 10, 20, 30, or 50 mmol Li/L in drinking water for up to 2 years. At 50 mmol Li/L, reduced food and water intake, weight loss, fine muscular tremor, and drowsiness progressing to stupor were observed. All rats died in 2-3 weeks at this water concentration. Plasma concentrations exceeded 8 meq Li/L just before death. At 30 mmol Li/L, similar symptoms were observed. Some rats in this group died in 3-4 weeks, whereas others entered a "pseudo-stable" phase and did not die until up to 9 weeks at this water concentration. Plasma lithium concentrations were about 3 meq/L during the pseudo-stable phase, increasing before death. No effect on survival was observed at 20 or 10 mmol Li/L: most animals survived up to 2 years. A transient drop in water consumption was observed at 20 mmol Li/L. Plasma concentrations were 1.5-2.0 and 1 meq Li/L for the 20 and 10 mmol Li/L water concentrations, respectively. The LC_{50} would be 20-30 mmol Li/L.

B.4.4. Comparison of acute toxicities

Acute toxicity studies have been conducted with bromacil lithium salt, bromacil, and lithium. The studies with bromacil lithium salt and bromacil were conducted for pesticide registration purposes in Sprague-Dawley rats by the same laboratory and are

closely comparable. Comparison to acute studies with lithium are more complex due to experimental differences.

The acute bromacil lithium salt, bromacil, and lithium studies have been summarized in previous sections. The results are summarized in Table 9 below. In general, for bromacil and bromacil lithium salt, females were observed to be more sensitive than males. Bromacil lithium salt had lower lethal doses than bromacil. It is possible that this is directly due to toxic effects of lithium or of interactions between bromacil and lithium. However, it is also possible that it is due to the fact that the bromacil lithium salt was in a solution, whereas the bromacil was a suspension, i.e. a particulate solid dispersed in water. This could affect the absorption from the gastrointestinal tract or lungs, and increase the toxicity of bromacil lithium salt. With the exception of females exposed to bromacil lithium salt, the maximum doses used in inhalation studies did not result in lethality. For females treated with bromacil lithium salt, the inhalation route was more sensitive than the oral gavage route by approximately a factor of two. On a molar basis, for male rats the oral LD_{50} for lithium was approximately two to three times higher than the LD_{50} for bromacil. However, the LD_{50} for bromacil was in Sprague-Dawley rats, while the LD_{50} for lithium was in Wistar rats. Strain differences may play a role in the observed difference. Also, the form of lithium in this study was lithium chloride, which was in solution, in contrast to the suspension of bromacil. This could affect absorption.

Table 9. Acute oral and inhalation LD_{50}s for bromacil, bromacil lithium salt and lithium in rats. [1]

Compound	Bromacil		Bromacil lithium salt		Lithium
Strain	Sprague-Dawley	Sprague-Dawley	Sprague-Dawley	Sprague-Dawley	Wistar
Sex	Male	Female	Male	Female	Male
Oral gavage (mmol/kg)	6.1	4.0	3.2	1.2	12.4-19.8
Inhalation (4 hr.) (mmol/kg) [2]	> 2.7	> 2.8	> 0.76	0.62	NR

[1] References: bromacil oral (Sarver, 1988), bromacil inhalation (Malek, 1989), bromacil lithium salt oral (Finlay, 1996a), bromacil lithium salt inhalation (Sarver, 1997), lithium oral (Petersen, 1980).

[2] Inhalation dose calculated by OEHHA staff from chamber concentrations and body weights using the allometric equation for rats in U.S. EPA (1988).

C. Developmental Toxicity

C.1. Developmental toxicity of bromacil lithium salt

No studies of the possible developmental toxicity of bromacil lithium salt itself were located. However, several studies of bromacil in animals, and studies of lithium in humans and animals, were located.

C.2. Developmental toxicity of bromacil

No studies of the possible developmental toxicity of bromacil in humans were located. Several studies in experimental animals were found. Most of these studies were conducted for pesticide regulation purposes.

C.2.1. Developmental toxicity studies of bromacil in experimental animals

Newell and Dilley (1978)

An early developmental toxicity study that used aerosol inhalation reported effects on fetal weight and skeletal ossification. Bromacil was one of five chemicals tested in Sprague Dawley rats in a standard teratology format (dosing gestation day (gd) 7-14, fetal exam gd 20, gross, soft tissue and skeletal evaluations). Bromacil exposure was conducted 2 h/d at aerosol concentrations of 0, 38, 78 or 165 mg/m^3 in 8-10 animals/group (controls 19 animals/group). The bromacil was dissolved in DMSO and the aerosol was generated by a nebulizer as a spray. Both air and DMSO spray groups were used as controls. The aerosol particles were in the respirable range (0.3-3.0 μm).

The inhalation exposure was "nose-only." The doses were estimated in the report at 1.83, 3.75, and 7.92 mg/kg/day based on an assumed minute volume of 80 ml/min. Based on empirical data on rat minute volume (175 ml/min) (Cummings and Heitkamp, 1981) dose estimates would be approximately doubled (4, 8 and 17 mg/kg/d). There were no effects on dams' toxic signs, body weights or food intake. Body weight gain was not reported and analyzed. In fetuses, significant effects on body weight and number of caudal ossification centers were noted (Table 10). Caudal vertebral centra are the last component of the axial skeleton to ossify.

Table 10. Data from the rat inhalation developmental toxicity study (Newell and Dilley, 1978). [1]

Bromacil concentration (mg/m^3)		0 [2]	38	78	165
bromacil (mg/kg body weight/day) [3]		0	1.83	3.75	7.92
litters (number)		19	10	8	9
maternal weight gain (g)	treatment	38	36	26	32
	post-treatment	61	58	52	56
live litter size [4]		9.8	10.7	10.2	10.0
fetal weight (g)		4.3 ± 0.04	4.1 ± 0.09*	3.9 ± 0.07**	4.0 ± 0.09*
resorption (%/litter)		8.9 ± 3.1	4.7 ± 3.0	5.3 ± 2.2	7.6 ± 3.7
sternal ossification centers (no./litter)		6.0 ± 0.02	6.0 ± 0.0	5.9 ± 0.03	6.0 ± 0.03
caudal ossification centers (no./litter)		5.2 ± 0.2	4.5 ± 0.2**	4.8 ± 0.1*	4.7 ± 0.2*

[1] Data are numbers, averages, or averages ± Standard Error of the Mean (SEM).
[2] Combined DMSO and air control groups; these groups did not differ in implantation sites, fetal viability or weight; fetal skeletal findings were not reported separately for the 2 control groups.
[3] Converted by authors from air concentration and exposure duration assuming body weight of 200 g and minute volume of 80 ml/min
[4] Computed by OEHHA staff from mean implantations/litter and % mortality
* $p < 0.05$, test not stated
** $p < 0.01$, test not stated

Alvarez (1988)

A developmental toxicity study in Sprague-Dawley rats with oral administration reported effects on fetal viability and skeletal variations (Table 11). The dose levels were 0, 20, 75, 200 or 500 mg/kg administered by gavage on gd 7-16. The bromacil preparation was a suspension in methylcellulose. Maternal toxicity was similar to that found in the rabbit study (below), which consisted of a decrease in weight gain and food consumption early

in the dosing period followed by increase in these parameters after dosing was discontinued. Specifically, during the first two days of dosing maternal weight gain and food intake were significantly lower than control in the 75, 200, 500 mg/kg/d groups. Food intake, but not body weight gain, was also affected the second two days of dosing in the 200 and 500 mg/kg/d groups. No further effects on food intake and weight gain were seen later in dosing or after dosing. Maternal liver weight was greater in the 500 mg/kg/d group than controls (17.8 vs 16.4 g, p = 0.05). Alopecia was reported as a clinical observation.

Fetal toxicity including increased resorption and fetal variations was observed at the higher doses. The most common variations were extra vertebrae, rudimentary lumbar ribs and unilateral caudal shift of the ileum. The authors state that *in utero* survival was affected at the 200 and 500 mg/kg doses, although the group comparisons were not statistically significant because the control value was "near the upper limit of normal." The authors also stated that fetal weights were lower at 500 mg/kg; statistical analysis confirmed this only for the female fetuses.

Table 11. Data from the rat oral developmental toxicity study (Alvarez, 1988). [(1)]

bromacil (mg/kg body weight/day)		0	20	75	200	500
litters (no)		24	23	23	23	24
weight gain (g)	treatment	55 ± 3	53 ± 2	50 ± 1	44 ± 2*	45 ± 3*
	post treatment	77 ± 2	78 ± 3	87 ± 2*	84 ± 2	80 ± 2
resorptions (no./litter)		0.9	0.5	0.6	1.3	1.0
litter size		13.8 ± 0.4	13.2 ± 0.6	14.3 ± 0.4	13.8 ± 0.3	13.8 ± 0.5
fetal weight (g) [(2)]		5.14	5.20	5.32	5.16	4.99
skeletal variations (% affected/litter)		2.7	3.3	2.2	9.6†	50.1††
skeletal retardations (% affected/litter)		18.4	10.9	17.7	17.0	50.9††

[(1)] Data are numbers, averages, or averages ± SEM.
[(2)] Stunted fetuses excluded from means.
* $p < 0.05$, Dunnett's test
† $p < 0.05$, Mann Whitney U test
†† $p < 0.01$, Mann Whitney U test

Hazleton (1966), Sherman and Kaplan (1975)

Developmental toxicity studies using New Zealand white rabbits were performed in 1966 and 1987. No developmental toxicity was reported in the 1966 study, which was also

published in the open literature (Sherman and Kaplan, 1975). This study had a small group size (8-10) and groups were further subdivided at term for fetal exam or vaginal delivery. Bromacil was administered at 0, 50 or 150 ppm diet corresponding to 0, 1.2 or 6.2 mg/kg/d. No toxicity was reported in dams at any dose. No effects of treatment were reported on litter size, fetal/pup viability, resorptions, fetal/pup weight, fetal/pup length, gross malformation or skeletal ossification.

Zellers (1987)

A larger study used 20 New Zealand white rabbits per group given doses of 0, 30, 100, 300 or 500 mg/kg bromacil by gavage on gd 7-19 (Table 12). Maternal toxicity was detected at the two highest doses as statistically lower weight gain than controls early in the dosing period, followed by greater weight gain after discontinuation of dosing. Specifically, controls had an average total food intake of 562 g during dosing compared to 314 in the 300 mg/kg/d group and 206 g in the 500 mg/kg/d group. After dosing food intake were 1147, 1342 and 1467 g in the three groups. Alopecia was reported as a clinical observation. This is similar to the pattern of maternal toxicity reported in rats (Alvarez, 1988). Maternal body weights of the 500 mg/kg group were significantly lower than controls from gd 9 to 24 (term is gd 29).

Fetal toxicity was also similar to that in the rat study (Alvarez, 1988) which was conducted in the same laboratory at the same time. A statistically significant dose-related trend for increased resorptions per litter was reported; group comparisons were significant only for late resorptions in the 300 mg/kg/day group. The number of live females per litter was also significantly lower in the 300 and 500 mg/kg/day groups than in controls. There was no treatment effect on fetal weight. No significant group effects were noted for the incidence of malformation, variations or retardations in the gross or soft tissue. There was a significant dose trend for incidence of fetal skeletal variations with the 500 mg/kg group demonstrating a significantly higher percent affected (of fetuses). Skeletal malformations and retardations were not clearly affected by treatment.

Table 12. Data from rabbit oral developmental toxicity study (Zellers, 1987). [(1)]

bromacil (mg/kg body weight/day)		0	30	100	300	500
litters (no.)		16	17	17	14	16
weight gain (g)	treatment	51 ± 37	84 ± 27	132 ± 25	-140 ± 51*	-261 ± 41*
	post treatment	128 ± 43	140 ± 26	68 ± 52	200 ± 28	274 ± 29*
resorptions (no./litter)	total	0.5	0.5	0.3	1.1	1.0
	early	0.3	0.3	0.1	0.4	0.6
	late	0.2	0.2	0.2	0.8*	0.4
live litter size		8.1	7.4	8.4	6.6	6.9
fetal weight (g)		42.2	44.3	41.9	45.0	42.8
skeletal variations (% affected/litter)		59.9	66.4	62.8	65.7	87.3[†]
skeletal retardations (% affected/litter)		12.8	13.3	27.9	10.4	27.6

[(1)] Data are numbers, averages, or averages ± SEM.
* $p < 0.05$, Dunnett's test
[†] $p < 0.01$, Mann Whitney U test

C.2.2. Developmental endpoints from reproductive toxicity studies of bromacil

No effects attributable to prenatal exposure were reported in the bromacil multigeneration studies (Haskell, 1966; Mullin, 1991). In the Mullin (1991) study, at the high concentration, the average daily intake of bromacil during gd 0-14 for both generations was 169 mg/kg/d. These studies are described in section D.2.

C.3. Developmental toxicity of lithium

C.3.1. Developmental toxicity of lithium in humans

Early studies in a variety of mammalian and non-mammalian animal species provided evidence for teratogenic effects of lithium. In 1968 the Scandinavian Register of Lithium Babies was founded to investigate whether lithium was teratogenic in humans (Schou, 1990). This Register later merged with registries in the U.S.A. and Canada to form the International Register of Lithium Babies. Among other efforts to collect information, notices were placed in medical and psychiatric journals requesting reports to be submitted for babies born to woman who were taking lithium during at least the first trimester of pregnancy.

Findings from early Register studies suggested a higher rate of cardiovascular malformations, specifically Ebstein's anomaly, in lithium-exposed infants. Later studies had inconsistent results, and possible reasons for this are discussed below. Although Ebstein's anomaly is composed of a spectrum of several features, it is essentially characterized by the failure of the tricuspid valve to attach normally to the valve annulus. The resulting downward displacement of the tricuspid valve into the right ventricle produces an "atrialized" portion of the ventricle. The anatomical and functional abnormalities cause tricuspid regurgitation that results in right atrial and right ventricular dilatation and atrial and ventricular arrhythmias (Dearani and Danielson, 2000; Frescura et al., 2000). The incidence of this anomaly in the general population is 1 in 20,000. Data from the Register suggested a rate of Ebstein's anomaly 400 times greater in women taking lithium than in the general population (Nora et al., 1974).

C.3.1.1. Individual human studies

Schou et al. (1973)

In a 1973 study, Schou et al., using data collected by the International Register of Lithium Babies, reported on 118 births to mothers who had been taking lithium during the first trimester of pregnancy. Congenital malformations were considered to be macroscopic abnormalities of structure due to faulty development and present at birth. Of the 118 births five were stillborn and seven died within the first week of life; six of these 12 were malformed. A total of nine infants were malformed (7.6%). The cardiovascular system was involved in six of the nine cases; two of the six were Ebstein's anomaly. Two children had Down's syndrome. The authors acknowledged that with the retrospective study design there was an expected over-representation of adverse pregnancy outcomes in this population. Since the Register would have been more likely to receive reports of stillbirths, and babies with malformations of mothers taking lithium than of normal infants born to mothers taking lithium, a potential for bias exists within this study design. However, as this was the first study from the Register to report an excess of cardiac malformations associated with maternal lithium use during pregnancy, it is unlikely there was significant bias in the reporting of these specific defects. The authors did not note the unusually high occurrence of Ebstein's anomaly in this study. However, in a published letter, Nora et al. (1974) reported two cases of Ebstein's anomaly in women taking lithium out of 733 teratogenic histories obtained. Nora et al. also noted in this letter the 400 fold increase in the occurrence of Ebstein's anomaly over the expected frequency in the Register data from Schou et al. (1973).

Weinstein and Goldfield (1975)

In a 1975 study Weinstein and Goldfield reported on a total of 143 cases of lithium use during pregnancy from the Register of Lithium Babies. Thirteen (9.1%) babies were born with malformations, 11 (7.7%) of which were cardiovascular anomalies. Four of these 11 were cases of Ebstein's anomaly. The authors compared these rates with

published incidence data from several sources. Table 13 illustrates the comparison between the rates. These comparisons indicate that congenital heart malformations occurred six times more frequently in lithium treated pregnancies than expected when considering all malformations. In addition, Ebstein's anomaly occurred 30 times more frequently than expected when considering all forms of congenital heart malformations.

Table 13. Ratios between various malformations (Weinstein and Goldfield, 1975).

Malformations	Register of Lithium Babies	Expected
Ebstein's anomaly to all nontrivial anomalies	1:3.3	1:600
Ebstein's anomaly to all forms of congenital heart disease	1:2.5	1:80
Malformations of the tricuspid valve/ tricuspid atresia to all forms of congenital heart disease	1:2.0	1:44
All forms of congenital heart disease to all nontrivial anomalies	1:1.3	1:8

Kallen and Tandberg (1983)

In a cohort study conducted in Sweden, Kallen and Tandberg (1983) linked data from a discharge diagnosis registry with medical birth registry data to identify 350 women with manic-depressive disease who had given birth between 1973 and 1979. These data were also linked to congenital malformations registry data to search for any additional malformed infants and to obtain more detailed diagnoses. Information on drug use and other possible risk factors was obtained from records completed at the first prenatal care visit, usually in the 10-12th week of pregnancy. Complete information was obtained for 287 women. Fifty-nine infants were born to women who had been treated with lithium; in 41 of these infants lithium was the only drug the mother reported taking. A total of 228 infants were born to mothers with a current or subsequent diagnosis of manic-depressive disorder but who were not treated with lithium during pregnancy. No information was presented on specific dosage of drugs or serum levels of lithium. In this cohort the estimated relative risk for all malformations associated with exposure to lithium was 3.0 (95% confidence interval (CI), 1.2 – 7.7). The estimated relative risk of heart defects associated with lithium exposure was 7.7 (95% CI, 1.5 - 41.2). This estimate was based on four heart defects (6.8%) out of 59 exposed infants as compared with two (0.9%) out of 228 unexposed infants. None of these defects was Ebstein's anomaly. Due to the rarity of this defect and the small sample size of this study, the power to detect a significant increased risk was limited. In addition to the increase in congenital malformations in this cohort, there was also a higher neonatal risk of death in the lithium-exposed infants. Six of the 59 lithium-exposed infants and four of the 228 lithium-unexposed infants died during the neonatal period (relative risk = 5.4; 95% CI 1.6

– 18.4). However, a larger proportion of the deaths in the lithium-exposed infants were malformed infants. Data from this study are summarized in Tables 14 and 15.

Table 14. Drug use and birth outcomes (Kallen and Tandberg, 1983).

Disease state and drug exposure	Pregnancy outcome (number of infants)				
	Infants	Malformed infants	Heart defects	Neonatal deaths	Deaths and malformations
No disease before pregnancy	110	5	0	3	2
Disease, no drug	80	3	2 [(1)]	0	0
Psychotropic drug, no lithium	38	1	0	1	0
Lithium only	41	5	3	4	2
Lithium and other psychotropic drug	18	2	1	2	2
Total	287	16	6	10	6

[(1)] One of these infants had Down's syndrome.

Table 15. Lithium-exposed versus lithium-unexposed infants and birth outcomes (Kallen and Tandberg, 1983).

Drug exposure	Pregnancy outcome (number of infants)				
	Total infants	Malformed infants	Heart defects	Neonatal deaths	Deaths and malformations
Lithium only or with other psychotropic drugs	59	7	4	6	4
No lithium- but possibly other psychotropic drug	228	9	2	4	2
Total	287	16	6	10	6

Schou (1990)

In an updated report from the Register of Lithium Babies (Schou, 1990), data collected up until 1979 included 225 infants. Of these infants 25 (11%) had visible malformations, 18 (8%) of which were cardiovascular, with six being cases of Ebstein's anomaly. Ratios for these data comparable to those presented in Table 13 are as follows: Ebstein's anomaly to all nontrival anomalies -1:4.2; Ebstein's anomaly to all forms of congenital

heart disease – 1:3.0; and all forms of congenital heart disease to all nontrivial anomalies 1:1.4.

Zalzstein et al. (1990)

In a case-control study, Zalzstein et al. (1990) reviewed the records of 59 patients with Ebstein's anomaly born between 1971 and 1988 and diagnosed at The Hospital for Sick Children in Toronto, Canada, a tertiary referral center. Complete medical and drug exposure histories during pregnancy were obtained for all cases. Control patients included all patients diagnosed with neuroblastoma in the same hospital during the same period of time (n = 168). None of the mothers of the cases had manic depressive psychosis or were treated with lithium before or during pregnancy. Of the control patients, 2 women had depressive disorders; one of whom was treated for manic depressive disorder with lithium. No dose levels were reported. No significant association was observed between Ebstein's anomaly and exposure to lithium (odds ratio estimate = 0.9; 95% CI, 0.04-26.13). The authors calculated that this study would have been able to detect an increased risk of greater than 28-fold of an association between Ebstein's anomaly and exposure to lithium (power = 80%, alpha = 0.05).

The limitations of this study include the small number of cases and thus the lack of statistical power to detect risks less than a 28-fold increase. Furthermore, the study did not include terminated pregnancies or early postnatal mortality. Since lithium had been recognized as a probable teratogen some pregnancies may have been terminated early. Evidence of this was observed in a study by Jacobson et al. (1992), where there was a higher rate of therapeutic abortion in women taking lithium as compared to women not on lithium (10% versus 6%). In addition, early infant mortality may have been missed since the cases were identified from a tertiary referral center. Exposure assessment was not uniform for all cases in this study. Drug use information was taken from medical records; however, for 9 of the cases the information was obtained by interview. It has been noted by Warner (2000) that psychiatric case notes are often held separately from medical notes. If this were true in this study there would have been a possibility of under-reporting of lithium use.

Czeizel and Rasc (1990)

Czeizel and Rasc (1990) conducted a population-based case control study of the association between drug use during pregnancy and congenital abnormalities. In this study lithium was just one of many drugs evaluated for its teratogenic risk. Data from the Hungarian birth registry was linked to the country's malformation registry. This study examined the risk of major congenital malformations with exposure to lithium in malformed still- and liveborn cases diagnosed from birth till the age of one year. The investigators used a matched-pair case-control analysis, in which cases and three negative controls, newborns without any congenital abnormality, were matched for infant sex, birth week, and district of parents' residence. Information was collected on a total of

9,882 pairs. Lithium use was discordant in 7 pairs, 4 exposed cases with 3 exposed controls. (In matched case control studies a discordant pair is one in which the two subjects had different exposures to the risk factor of interest. These are the only pairs that are informative about the association between exposure and disease.) There were no pairs where both cases and controls were exposed. The odds ratio for all congenital malformations associated with exposure to lithium was 1.3 (95% CI 0.2 – 9.0). The findings of this study are limited by the limited exposure to lithium, the lack of specific information about cardiac anomalies and the lack of assessment of confounding factors.

Jacobson et al. (1992)

A prospective study by Jacobson et al. (1992) included 148 women taking lithium during the first trimester of pregnancy. The women were recruited between 1979 and 1991 from one of four teratogen information centers in the U.S.A. and Canada which they had contacted because of concern about exposure to lithium during pregnancy. Most of the women were from California. A control group consisted of women who had contacted the Canadian center because of concern about exposure to drugs without known teratogenic effects (n = 148). Women taking lithium were age-matched to controls (to within 2 years). Ten of the 148 patients were lost to follow-up postnatally; however, they were included in certain analyses as all had had prenatal echocardiography. Forty-six percent of patients had echocardiographs; none of the controls did as this was not deemed to be ethically justified. The mean daily lithium dose was 927 ± 340 (SD) mg, with a range of 50-2400 mg. Of the livebirths, four of the 105 exposed infants had malformations as compared with three of the 123 unexposed infants. (One therapeutic abortion in the exposed group is included in these numbers because of a diagnosis of Ebstein's anomaly in utero and the assumption that had the anomaly not been detected the pregnancy would have gone to term). The relative risk of any malformation associated with exposure to lithium during early pregnancy was 1.5 (95% CI, 0.4 –6.7). The relative risk for cardiac malformation was 1.1 (0.1 – 16.6) and for Ebstein's anomaly was 3.5 (0.1 – 84.9). Statistically significant differences in birthweight were observed between groups. The lithium exposed infants weighed a mean of 92 g more than the control infants (Mean = 3475 ± 660 g (SD) versus 3383 ± 566 g (SD) respectively, (p = 0.01 using Student's t-test for paired data). This is surprising since there were more cigarette smokers in the lithium group than in the controls (31.8% verse 15.5%; p = 0.002). The study reported no differences in gestational age between the groups.

One of the limitations of this study is that only 46% of the patients had echocardiographs. It is possible that various cardiovascular malformations including milder forms of Ebstein's anomaly may have been missed. In a study of factors associated with failure to diagnose congenital cardiovascular malformations in infancy, cases of Ebstein's anomaly were significantly overrepresented in a group of infants who died without diagnosis in life (Kuehl et al., 1999). Another limitation of the study is the small sample size and limited power as is evident by the wide confidence intervals.

Troyer et al. (1993)

A study by Troyer et al. (1993) reported an association between lithium therapy and an increased incidence of premature delivery. Of the 252 infants reported to the International Register of Lithium Babies between 1968 and 1983, the authors analyzed the data from those with complete information on the mother and infant (n = 84). Information included maternal age, daily lithium dose during each trimester, concurrent use of other medications, infant sex, gestational age, birth weight, and country of birth. Study infants were reported from 11 countries, but predominantly from Sweden (15%), Denmark (41%) and the United States (44%). Prematurity was defined as a gestational age less than 38 weeks from the last menstrual period. Infants with a birth weight greater than 2 standard deviations above the mean were considered large for gestational age (LGA). Infants from Scandinavian countries were compared with published Swedish growth standards. Standards from the United States were used for all other infants. Z scores were computed to evaluate the variability in birth weight at different gestational ages (Z = (Patient's birth weight – Mean birth weight for infants of same gestation age)/(1 SD of mean birth weight for infants of same gestational age)). Thirty-six percent of infants were born prematurely. Mothers of premature infants had taken significantly greater amounts of lithium during each trimester as compared with mothers of term infants (first trimester: premature vs term - 1270 mg lithium vs 984 mg lithium, respectively, $p < 0.05$; second trimester -1090 vs 680, $p < 0.01$; third trimester – 998 vs 726, $p < 0.01$). Thirty-seven percent of premature infants were LGA versus 15% of term infants (Z score 1.5, SD 1.8 versus 0.7, SD 1.3, respectively; $p < 0.01$). No direct correlation was seen between daily lithium intake and degree of prematurity or birth weight. There was no difference in maternal age between infants born prematurely or infants born at term. No other data on confounding factors for premature birth were noted.

An additional cohort of 350 Swedish women who were treated as inpatients for manic depression and gave birth within the same year was identified using record linkage of a diagnosis registry and a medical birth registry for the years 1973 to 1979. Available information on these women included age, parity, use of lithium, gestational age, and neonatal outcome. No information was available concerning the dosage or duration of medication administered. The cohort was divided into two groups, patients who were treated with lithium and patients who were not. In this cohort, 17% of infants were exposed to lithium: the mothers of 12% of whom were taking lithium alone, and the mothers of 5% of whom were taking lithium and another psychotropic drug. The percentage of premature infants in this cohort were compared with expected values derived from all birth in Sweden from 1973 to 1979 (n = 869,436). A significantly greater percentage of lithium-exposed infants were born earlier than 38 weeks (33%) compared with non-exposed infants born to mothers with manic-depressive disorder (13%) or to normal mothers (12%). The estimated relative risk of premature delivery in lithium-exposed infants compared to all non-exposed infants was 2.5 (95% CI, 1.6 – 4.0). In this cohort only 5% of lithium-exposed infants were LGA and there was no difference in Z scores for the lithium-exposed premature and term infant groups. There was no apparent control for potential confounders.

The design and results of these seven studies are collectively summarized for ease of comparison in Table 16.

Table 16. Summarized results of human studies of lithium exposure.

Reference	Study Design	Population	Results
Schou et al. 1973	Retrospective	Women identified through International Register of Lithium Babies. Lithium taken during the 1st trimester of pregnancy. No dose information. N = 118 births reported to register.	5 stillborn, 7 died within the 1st week of life; 6 of these 12 were malformed. Total of 9 malformed babies (7.6%) (6 involved cardiovascular system, 2 of which were cases of Ebstein's anomaly). Two children had Down's syndrome.
Weinstein and Goldfield, 1975	Retrospective	Updated report of Lithium Register. N = 143 births. (See Schou, 73).	13 malformed infants (9.1%). 11 involved cardiovascular system (7.7%), 4 of the 11 were Ebstein's anomaly.
Kallen and Tandberg, 1983	Cohort	Birth registry data linked with hospital in-patient and birth defects data for the period 1973-1979. N = 350 infants whose mothers were treated for manic-depressive illness. Compared to all births in Sweden for the same period.	All malformations – 7/59 in lithium-exposed infants, 9/228 in unexposed infants (RR = 3.0; 95% CI, 1.2 – 7.7). Heart defects 4/59 in exposed, 2/228 in unexposed (RR = 7.7; 95% CI, 1.5 - 41.2). None of these defects was Ebstein's anomaly. Neonatal deaths – 6/59 exposed infants, 4/228 unexposed infants (RR = 5.4; 95% CI 1.6 – 18.4).
Schou, 1990	Retrospective	Updated report from the Register of Lithium Babies. N = 225 infants.	25 infants (11%) had visible malformations, 18 (8%) of which were cardiovascular, and six were cases of Ebstein's anomaly.

Table 16. Summarized results of human studies of lithium exposure (continued).

Reference	Study Design	Population	Results
Zalzstein et al., 1990	Case control	59 cases born between '71 – '88 and diagnosed at tertiary care hospital with Ebstein's anomaly. 168 control children diagnosed with neuroblastoma.	None of the case mothers had manic depressive psychosis or were treated with lithium carbonate before or during pregnancy. One mother had been treated with lithium during pregnancy. No statistical difference in the rates of lithium exposure during the 1st trimester between the cases and controls.
Czeizel and Racz, 1990	Case control	Population based – linking the national data set on congenital anomalies with the national birth registry. 9,882 pairs were identified. Study evaluated a number of drugs for teratogenic risk.	4 exposed cases were compared with 3 exposed controls. Odds Ratio = 1.2 (95% confidence interval 0.2 – 9.0)
Jacobson et al., 1992	Prospective multicentered study	Women taking lithium during pregnancy were enrolled at 1 of 4 teratogen information centers (N=148). Mean daily lithium intake - 927 (SD ± 340) mg, with a range of 50-2400 mg. Controls, age-matched to patients, were enrolled from the Canadian Center when they called about drugs with no known teratogenic effects (n = 148)	All malformations – 4/105 lithium-exposed infants 3/123 unexposed infants (RR = 1.5; 95% CI 0.4 –6.7). Cardiac malformation - RR = 1.1 (95% CI 0.1 – 16.6). Ebstein's anomaly – RR = 3.5 (95% CI 0.1 – 84.9). Lithium-exposed infants weighed a mean of 92 g more than the control infants (Mean = 3475 ± 660g (SD) versus 3383 ± 566g (SD) respectively, (p = 0.01).
Troyer et al., 1993	Cohort	N = 84 maternal-infant records from the Lithium Registry. In addition, 350 Swedish women diagnosed with manic depression and gave birth the same year.	36% of infants in the register were premature. Women who delivered at <38 weeks gestation had higher prescribed levels of lithium than mothers who delivered at ≥38 weeks. More women (33%) taking lithium delivered at <38 wks than women not taking lithium (13%) compared with mothers not diagnosed with manic depression (12%).

In addition to the studies summarized above, there have been a number of case reports of adverse effects in infants born to mothers taking lithium. These are summarized in Table 17.

Table 17. Case reports of adverse effects in infants of mothers taking lithium.

Reference	Exposure	Results
Vacaflor et al., 1970	29 year old woman taking 900–1200 mg Li_2CO_3/d eight wks prior to conception and throughout pregnancy. Also taking 200 mg/d of chlorpromazine.	Infant born with bilateral club feet and meningomyelocele in the lumbar region.
Park et al., 1980	25 year old primigravida, dose - 1,200 mg Li_2CO_3/d throughout pregnancy. No other medication was taken.	Neonate was born with Ebstein's anomaly.
Aoki and Ruedy, 1971	29 year old woman, dose - 1800 mg of Li_2CO_3 /d beginning 5 weeks prior to onset of last menstrual period. Woman was also taking 300-1200 mg of chlorpromazine till 13th week of gestation.	Infant had bilateral talipes equinovarus with spastic paraparesis, aqueduct stenosis with mild hydrocephalus, spina bifida with sacral meningomyelocele.
Nora et al., 1974	2 women taking lithium during pregnancy.	Both infants born with Ebstein's anomaly.
Rane et al., 1978	34 year old woman, dose - 280 mg Li_2CO_3/d, decreased to 56 mg 3x day after 3-4 month of gestation. Also taking 5 mg levomepromazine and 5 mg nitrazepam at night.	Cardiovascular malformations – dextrocardia and situs solitus, patent ductus arteriosus and a juxtaductal aortic coarctation.
Pinelli et al., 2002	28 year old woman, dose - 1650 mg Li/d throughout gestation. Labetalol and betamethasone also taken at 30 weeks gestation. Delivery at 34 weeks after induction.	Infant had no congenital abnormality. At birth exhibited nephrogenic diabetes insipidus, cardiomegaly, hypoglycemia and hyperbilirubinemia. Echocardiogram identified a small patent ductus arterisosus. Exam at 1 month of age was normal.

Numerous case studies have reported other forms of lithium toxicity in the fetus and neonate. Reported effects include: "floppy baby syndrome" characterized by cyanosis and hypotonia; bradycardia; thyroid depression with goiter; atrial flutter; hepatomegaly; electrocardiogram abnormalities (T-wave inversion); cardiomegaly; gastrointestinal bleeding; diabetes insipidus; polyhydramnios; seizures; and shock. Some researchers report these effects to be transient and have no long-term sequelae (Briggs et al., 2002; Pinelli et al., 2002). Data from two studies which examined the long-term effects on neurodevelopment in the infant suggests normal behavioral development in childhood (Schou, 1976; Koren et al., 2001).

C.3.2. Developmental toxicity studies of lithium in experimental animals

There are a large number of studies of experimental animals relevant to the potential developmental toxicity of lithium. All of these studies were found in the open literature. The species studied included mouse, rat, rabbit, and monkey. Additionally, there are studies of farm animals, which are not reviewed here due to the large number of studies available in experimental animals. Most of these studies are by an oral route, although there are a number of injection studies in the mouse and two in rat. Many of these studies tested for the plasma or serum concentration of lithium. Generally the objective was to achieve plasma or serum concentrations in the same general range as used in human therapy, i.e., about 0.5 – 1.0 meq Li/L. Some studies used lithium carmine, a dye, or lithium hypochlorite, a bleaching agent. Due to the likely toxic effects of carmine and hypochlorite, these studies are not summarized in this document. There are several considerations with regard to hazard identification for the possible developmental toxicity of lithium. Most studies used a single dose or concentration. Some studies used very small numbers of animals. Maternal toxicity results were seldom reported, or were reported incompletely. Commonly, only a subset of developmental results were reported. Also commonly, qualitative results were reported in the text, but numerical data were not presented.

C.3.2.1. Developmental studies of lithium in mice by oral routes

Chernoff and Kavlock (1982, 1983)

Chernoff and Kavlock reported a screening study for a number of chemicals. The study was conducted in blocks consisting of a control group and one to three chemicals. One block of this study included lithium. In this block, mated female CD-1 mice were treated with lithium carbonate (Li_2CO_3) by gavage at 0 or 400 mg/kg/d (0 or 10.8 mmol Li/kg/d) for gestation day (gd) 8-12. Mice were allowed to give birth and nurse to postnatal day (pnd) 3. There were two lithium carbonate groups: one in which the lithium carbonate suspension was treated with a "Polytron" (presumably sonicated), the other not. There were 30 control females, and 25 in each lithium carbonate group.

There were no adverse effects on maternal mortality or body weight gain. For the Polytroned lithium carbonate only, reduced live litter size on pnd 1 and 3 (statistically significant) was observed. For the not Polytroned lithium carbonate, the live litter size was lower than controls, but the difference was not statistically significant. There were no effects on fraction pregnant, or pup weight on pnd 1 or 3. See Table 18 below.

Table 18. Results from mouse developmental study by Chernoff and Kavlock (1982, 1983). [(1)]

Dose [mg Li_2CO_3/kg/d (mmol Li/kg/d)]	0 (0)	400 (10.8)	400 (Polytron) (10.8)
Number of mated females	30	25	25
Number of females died	2	0	0
Number of females pregnant	12	17	16
Maternal weight gain (g)	4.1 ± 0.6	5.9 ± 0.4	5.6 ± 0.5
Number of pups alive on pnd 1	10.0 ± 0.4	9.1 ± 0.5	7.8 ± 0.8*
Number of pups alive on pnd 3	10.1 ± 0.4	9.1 ± 0.5	7.8 ± 0.8*
Pup weight pnd 1	1.69 ± 0.04	1.83 ± 0.03	1.81 ± 0.03
Pup weight on pnd 3	2.44 ± 0.07	2.64 ± 0.06	2.71 ± 0.07

[(1)] Data are numbers or averages ± SE.
* $p < 0.05$ statistically significant difference from control, ANOVA followed by t-test.

Gray et al, (1983, 1986), Gray and Kavloc, (1984)

This was a follow up study to the Chernoff and Kavlock study above. The objective was to examine additional postnatal endpoints. Mated female CD-1 mice were treated by gavage with lithium carbonate at 0 or 400 mg/kg/d (0 or 10.8 mmol Li/kg/d) for gd 8-12. It was not stated whether the dosing solution was treated with a "Polytron" or not. Females were allowed to give birth. Six randomly selected pups (3/sex) from the same treatment group were given to each dam on pnd 6. Pups were weaned on pnd 30. Offspring were housed 3 males and 3 females/cage for mating. "Obviously pregnant" females were removed to individual cages for gestation and delivery. There were 12 control litters, and 8 treated with lithium carbonate.

There was no effect on offspring viability on pnd 30 or figure 8 maze activity on pnd 22, 58, or 200. There was no effect on offspring vaginal patency on pnd 30, or the percentage which became pregnant, or age of parturition. Litter size was slightly lower, but the difference was not statistically significant. Data from this study are summarized in Table 19.

Table 19. Results from mouse developmental study by Gray et al. (1983, 1986), Gray and Kavlock (1984) [(1)]

Dose [mg Li_2CO_3/kg/d (mmol Li/kg/d)]		0 (0)	400 (10.8)
Number of litters		12	8
Pup viability pnd 30 (%)		86	88
Pup figure 8 maze activity	pnd 22	620	558
	pnd 58	816	768
	pnd 200	642	576
Vaginal patency pnd 30 (%)		90	89
Offspring pregnant (%)		100	100
Offspring age of parturition		67.7	66.6
Litter size		11.4	10.2

(1) Data are numbers, percentages, or averages. Indices of variation (e.g., SD) were not reported. There were no statistically significant differences from control.

Kriegel (1974)

Kriegel investigated "Togal," a drug containing aspirin, lithium, and quinine. One phase of the study used lithium citrate ($Li_3C_6H_5O_7$). Female NMRI mice were treated orally (apparently by gavage) with lithium citrate at 0 or 87 mg $Li_3C_6H_5O_7$/kg/d (0 or 1.24 mmol Li/kg/d) during gestation. There were 14 controls, and 50 treated with lithium. Females were sacrificed on gd 18. No maternal results were reported. No adverse effects on implantations, resorptions, fetal weight, or abnormalities of the brain were observed in the lithium treated group. Data from this study are summarized in Table 20

Table 20. Results from mouse developmental study by Kriegel (1974) [(1)]

Dose	0	87 mg $Li_3C_6H_5O_7$/kg/d (1.24 mmol Li/kg/d
Number of litters	14	50
Implantations/litter	11.8	13.7
Resorptions (%)	9.5	10.2
Fetal weight (g)	1.200 ± 0.028	1.230 ± 0.013*

(1) Data are numbers, averages, or average ± SE.

* $p < 0.001$ statistically significant difference from control (statistical method not reported).

Krmpotic and de la Torre (1976)

This study was reported in abstract only. ICR/SCI mice were treated with lithium chloride in water at 0 or 20 meq/L (0 or 20 mmol Li/L). The duration of treatment is not clear, nor is it clear whether treated animals were male or female. The numbers of animals treated were possibly 50 males and 100 females/group. Mice were mated one male to two females. Half of each group were sacrificed on gd 17, and half allowed to deliver. No systemic results were reported. Reduced fertility, and increased morbidity and mortality of offspring were reported.

Laborde and Pauken (1995)

This study was performed by staff of the National Center for Toxicological Research, U.S. Food and Drug Administration. It has been reported in abstract only. Mated female CD-1 mice were treated with lithium chloride in water at 0, 0.5, 1.0, 1.5, 2.0, or 2.5 mg lithium chloride/ml (0, 12, 24, 35, 47, or 59 mmol Li/L) for gd 0-17. Females were sacrificed on gd 17. Examination included fetal hearts by histological techniques. Reduced maternal food and water consumption, weight gain during gestation, and absolute weight gain were observed ("dose-related," statistical significance not reported). Reduced fetal weight and crown-rump length, and increased incidence of some skeletal and visceral defects with increasing lithium concentration were reported. There was no mention of specific heart defects. No effect on number of implants or live fetuses was observed.

Matsumoto et al. (1974, 1975)

Three studies were reported in abstract only. In experiment 1, pregnant female mice were treated orally with lithium chloride at 400 mg/kg (9.4 mmol Li/kg) on gd 12. Lithium concentration in maternal serum was measured at 1.8 meq/L after 4 hours. Lithium concentration in fetuses was 5.7 ug/g after 4 hours. Lithium concentration decreased quickly afterwards. In experiment 2, pregnant female mice were treated orally with lithium chloride at 400 mg/kg (9.4 mmol Li/kg) on gd 7, 8, or 9. Mice were sacrificed on gd 18. No maternal toxicity results were reported. Malformations were observed in the lithium chloride group (no results for controls were reported). In experiment 3, pregnant female mice were treated with lithium chloride in water at 20 or 40 meq/L (20 or 40 mmol Li/L) for gd 6-15. Mice were sacrificed on gd 18. No maternal toxicity results were reported. Reduced fetal weight and ossification centers in digits of hindlimbs and tail vertebrae were observed (statistically significant, but not reported at which concentrations).

Messiha (1986b)

Mated female Sprague-Dawley (as reported in the paper) mice were treated with lithium chloride in water at 0 or 1 meq Li/L (0 or 1 mmol Li/L) during gestation and lactation. There were 5 females/group. Females were allowed to deliver and nurse the offspring. Some offspring were sacrificed at weaning (23 days of age). Maternal consumption of lithium was 0.31 and 0.64 meq/kg/d (2.2 and 4.5 mg Li/kg/d) for gestation and lactation, respectively. No effect on maternal body weight, or absolute brain, kidney, liver, or spleen weights after lactation was observed. Results for 4-10 offspring/sex/group were reported at weaning. Disposition of other pups, if any, was not reported. In offspring, increased absolute kidney, liver, and spleen weights at weaning were observed (some statistically significant). There was no effect on offspring body weight or testes weight at weaning. Data from this study are summarized in Table 21.

Table 21. Selected results from mouse developmental study by Messiha (1986b) [(1)]

Concentration [meq Li/L (mg Li/L)]		0 (0)	1 (7)
Number of maternal mice		5	5
Body weight after lactation (g)		41.8 ± 4.0	42.3 ± 3.5
Number of offspring sacrificed at weaning	Male	4	8
	Female	10	4
Body weight at weaning (g)	Male	7.6 ± 1.4	7.5 ± 2.9
	Female	5.5 ± 1.1	7.3 ± 1.1
Liver weight at weaning (mg)	Male	366 ± 95	477 ± 80
	Female	284 ± 53	411 ± 35**
Kidney weight at weaning (mg)	Male	63 ± 14	77 ± 9
	Female	55 ± 7	65 ± 8*
Spleen weight at weaning (mg)	Male	42 ± 22	70 ± 18*
	Female	29 ± 6	55 ± 9***
Testes weight at weaning (mg)		42 ± 9	49 ± 7

[(1)] Data are numbers or averages ± SEM.
* $p < 0.05$ statistically significant difference from control. Student's t-test.
** $p < 0.005$ statistically significant difference from control. Student's t-test.
*** $p < 0.001$ statistically significant difference from control. Student's t-test.

Messiha (1986a, 1992, 1993)

Mated female Sprague-Dawley mice were treated with lithium chloride in water at 0 or 1 meq Li/L for mating (except for 6 hours/day when they were cohabited with untreated males), gestation and lactation. There were 5 females/group. Females were allowed to

deliver and nurse offspring. Offspring were weaned after 23 days and all were given water without lithium for two weeks. Maternal fluid intake was similar between groups during gestation, but lower in the lithium group during lactation (about 80% of control, statistically significant compared to control). Lithium intake was 0.31 and 0.64 meq Li/kg/d (2.2 and 4.5 mg Li/kg/d) for gestation and lactation, respectively. No effect on maternal body weight after delivery was observed. No effect on litter size at birth or newborn body weight was observed. Results were reported for 10-19 offspring/sex/group at weaning. Reduced offspring body weight at weaning was observed (statistically significant for males only). Results were reported for 5-13 offspring/sex/group at two weeks after weaning. No effect on offspring body weight two weeks after weaning was observed. Reduced brain, kidney, and testes weight two weeks after weaning was observed (some statistically significant). Data from this study are summarized in Table 22.

Table 22. Selected results from mouse developmental study by Messiha (1986a, 1992, 1993) [(1)]

Concentration [meq Li/L (mg Li/L)]		0 (0)	1 (7)
Number of maternal mice		5	5
Number of offspring examined at weaning	Male	13	13
	Female	19	10
Body weight at weaning (g)	Male	10.4 ± 1.9	7.0 ± 1.9*
	Female	8.3 ± 2.4	7.0 ± 2.0
Number of offspring sacrificed two weeks postweaning	Male	13	6
	Female	11	5
Body weight two weeks postweaning (g)	Male	20.4 ± 2.8	20.1 ± 1.3
	Female	17.5 ± 2.2	16.8 ± 1.4
Testes weight at two weeks postweaning (mg)		150 ± 16	99 ± 13***

[(1)] Data are numbers or averages ± SEM.
* $p < 0.05$ statistically significant difference from controls. Student's t-test.
*** $p < 0.001$ statistically significant difference from controls. Student's t-test.

Messiha (1989)

The objective of this experiment was to compare the effects of lithium treatment with the effects of cesium treatment, and the interaction between the two. Results for the control and lithium treated groups only are summarized here. Mated female Sprague-Dawley mice were treated with lithium chloride in water at 0 or 1 meq Li/L during gestation and lactation. There were three maternal mice/group. Females were allowed to deliver and

nurse offspring until weaning at 21-23 days of age. Offspring were then given water without lithium for two weeks. Equal numbers of offspring of each litter and both sexes were sacrificed at weaning and at the termination of the experiment. Maternal water consumption was similar in lithium treated groups and controls during gestation, but lower during lactation (lithium treated approximately 80% of controls, statistical significance not addressed). Maternal lithium intake was reported as 0.31 and 0.64 meq/kg/d (2.2 and 4.5 mg Li/kg/d) during gestation and lactation, respectively. Litter size was similar between control and lithium treated groups (12.0 ± 0.8 and 11.0 ± 1.0, respectively). Results for 8-12 offspring/sex/group were reported for organ weights at weaning and two weeks after weaning. At weaning, increased kidney, liver, and spleen weights were observed in lithium treated males and females (some statistically significant). Two weeks after weaning, lower kidney and liver weights, but no effect on spleen weight was observed in lithium treated males and females (statistically significant for kidney weight in females only). At weaning, increased testes weight was observed in lithium treated males (not statistically significant). Two weeks after weaning, reduced testes weight was observed in lithium treated males (statistically significant).

Seidenberg et al. (1986), Seidenberg and Becker (1987)

This was a screening test of a large number of chemicals, similar to the screening test by Chernoff and Kavlock, described above. In this test one of the blocks included lithium. Mated ICM/SIM female mice were treated by gavage with lithium chloride at 0 or 600 mg/kg/d (0 or 14 mmol Li/kg/d) for gd 8-12. Females were allowed to deliver. If delivery did not occur by gd 21 or 22, females were sacrificed. There were 28 mated females/group. Maternal mortality was higher in the lithium treated group than controls (2/28 vs. 0/28). No effect on maternal weight gain was observed. A lower number of pups/litter on pnd 1 and 3 was observed (not statistically significant, but authors indicate this was an effect). No effect on number of litters born, or pup weight on pnd 1 or 3 was observed. Data from this study are summarized in Table 23.

Table 23. Results from mouse developmental study by Seidenberg et al. (1986), Seidenberg and Becker (1987) [(1)]

Dose [mg lithium chloride/kg/d (mmol Li/kg/d)]	0 (0)	600 (14)
Number of mated females	28	28
Number of females died	0	2
Number of litters	13	15
Maternal weight change	4.3 ± 2.1	3.5 ± 3.2
Number of pups alive on pnd 1	11.3 ± 2.9	10.0 ± 4.2
Number of pups alive on pnd 3	10.9 ± 2.8	9.5 ± 4.2
Pup weight pnd 1	1.70 ± 0.16	1.81 ± 0.10
Pup weight on pnd 3	2.37 ± 0.34	2.56 ± 0.34

[(1)] Data are averages ± SD. No statistically significant differences from control.

Smithberg and Dixit (1982)

This paper mainly reported a series of injection studies (see Section C.3.2.2 below). In experiment 1b, mice of strain 129 Sv/Sl were treated with lithium carbonate at 10.0, 15.0, or 20.0 mg/mouse (400, 600 or 800 mg Li_2CO_3/kg; 75, 112 or 154 mg Li/kg) by gavage. Survival over 24 hours was reported. There were 5-16 animals/group. The LD_{50} was determined to be approximately 15-20 mg lithium carbonate/mouse (600-800 mg Li_2CO_3/kg, or 112-154 mg Li/kg). In experiment 4, mated female 129 Sv/Sl mice were treated with lithium carbonate at 2 mg/ml (50 mmol Li/L) in water for gd 1-18. There were 16 females in this group. There was no control group. A control group for the injection studies was treated by ip injection with water or sodium chloride on gd 9. There were 29 animals in this group. Females were sacrificed on gd 18 or 19. No maternal toxicity data were reported. Reduced frequency of females producing litters, litter size, and increased frequency of resorptions were observed in the lithium group compared to the injection control group. No effect was observed on frequency of abnormalities. Data from this study are summarized in Table 24.

Table 24. Selected results from mouse developmental study by Smithberg and Dixit (1982)

Treatment Group	Control (injection of water or NaCl solution on gd 9)	Li_2CO_3 in water, 2 mg/ml (50 mmol Li/L)
Number of maternal mice	29	16
Number of litters	12	2
Litter size	6.0	3.0
Resorptions (%)	18.4%	60%

(1) Data are numbers, averages, or percentages. No indices of variation (e.g. SD) were reported.

Szabo (1969, 1970), Szabo et al., (1970)

In experiment 1, female HaM/ICR mice (pregnancy status not reported) were treated (apparently by gavage) with lithium carbonate at 0, 100, 400, or 800 mg/kg/d (0, 2.7, 10.8, or 22 mmol Li/kg/d) for 10 days. There were six controls, and three mice in each lithium treated group. Plasma lithium concentrations were 0.04, 0.45, 1.25, and 4.26 meq/L for the control and three dose levels, respectively. Two of three mice died after the 9th dose at 800 mg/kg/d (22 mmol Li/kg/d). No other toxicity results were reported.

In experiment 2, a dose range finding study, mated female HaM/ICR mice were treated by gavage with lithium carbonate at 200, 300, or 465 mg/kg/d (5.4, 8.1, or 12.6 mmol Li/kg/d) for gd 6-15. Mice were sacrificed on gd 18. There were three to four litters per group. No maternal toxicity results were reported. Increased dead or resorbed implants, and lower litter size was observed at 465 mg/kg/d (12.6 mmol Li/kg/d). Increased cleft palate was observed at 300 and 465 mg/kg/d (8.1 and 12.6 mmol Li/kg/d).

In experiment 3, mated female HaM/ICR mice were treated by gavage with lithium carbonate at 0, 200, or 465 mg/kg/d (0, 5.4, or 12.6 mmol Li/kg/d) for gd 6-15. Mice were sacrificed on gd 18. Maternal mortality of 37% at 465 mg/kg/d (12.6 mmol Li/kg/d) was observed. No maternal mortality was observed at 200 mg/kg/d. No other maternal toxicity results were reported. There were 15-20 litters/group. Increased implants dead or resorbed, reduced litter size, and increased incidence of cleft palate were observed at 465 mg/kg/d (12.6 mmol Li/kg/d). Data from this study are summarized in Table 25.

Table 25. Results of mouse developmental study (experiments 2 and 3) by Szabo (1969, 1970), Szabo et al. (1970) [(1)]

Dose [mg Li_2CO_3/kg/d (mmol Li/kg/d)]		0 (0)	200 (5.4)	300 (8.1)	465 (12.6)
Experiment 2					
Number of litters		NA	3	4	4
Live fetuses/litter		NA	11.0	12.5	9.2
Dead or resorbed implants		NA	10.8%	5.7%	26.0%
Cleft palate	Number fetuses (number litters)	NA	0 (0)	3 (1)	11 (3)
	Percent fetuses (percent litters)	NA	0 (0)	6% (25%)	30% (75%)
Experiment 3					
Maternal mortality		Not reported	0	NA	37%
Number of litters		16	20	NA	15
Live fetuses/litter		11.4	12.2	NA	8.1
Dead or resorbed implants		12.4%	12.3%	NA	32.6%
Cleft palate	Number fetuses (number litters)	0 (0)	1 (1)	NA	19 (7)
	Percent fetuses (percent litters)	0 (0)	0.4% (5%)	NA	16% (47%)

[(1)] Data are numbers, averages, or percentages. No indices of variation (e.g., SD) were reported. No statistical analyses were reported.
NA: dose not used in that experiment.

C.3.2.2. Developmental studies of lithium in mice by injection

Bass et al. (1951)

In this early group of experiments, mated female albino mice were treated with lithium chloride and allowed to deliver litters. In experiment 1, mice were treated with 0 or 2 mg lithium chloride (0 or 0.047 mmol Li) by ip injection, twice daily on gd 6-7. There were 68-76 females/group. No maternal toxicity results were reported. Reduced number and percentage of full term deliveries were observed (statistically significant). No other results were reported. In experiment 2a, mice were treated with 0 or 1 mg lithium chloride (0.024 mmol Li) by ip injection, twice daily plus 0.5% (0.08% Li) of diet, or 2 mg lithium chloride (0.047 mmol Li) by ip injection, twice daily plus 1% (0.16% Li) of diet on gd 2-3. There were 50-54 females/group. No maternal toxicity results were reported. Reduced number and percentage of full term deliveries were observed (statistically significant). No other results were reported. In experiment 2b, mice were treated with 0 or 2 mg lithium chloride (0 or 0.047 mmol Li) by ip injection, twice daily plus 1% (0.16% Li) of diet on gd 12-13. There were 51-54 females/group. This experiment shared a control with experiment 2A. No maternal toxicity data were

reported. No effects on number or percentage of full term deliveries were observed. No other data were reported.

Giles and Bannigan (1993, 1997)

In experiment 1, non-pregnant CD-1 mice were injected ip with lithium carbonate at 300 mg/kg. After injection, the serum lithium level peaked at 10 mM at 1 hour, declined to 5 mM at 4-5 hours, and to 0 at 16 hr. In experiment 2, pregnant CD-1 mice were untreated, or injected ip with sodium chloride at 234 mg/kg, with sodium carbonate (Na_2CO_3) at 430 mg/kg, or lithium carbonate at 200, 300, 350, or 400 mg/kg (5.4, 8.1, 9.5, or 10.8 mmol Li/kg) on gd 8. Females were sacrificed on gd 18. There were 8-20 females/group. Profound hypothermia, muscular rigidity, and coma were observed at 350 and 400 mg Li_2CO_3/kg (9.5 and 10.8 mmol Li/kg). Mortality was observed at 350 and 400 mg Li_2CO_3/kg (9.5 and 10.8 mmol Li/kg) (2/15 and 5/15, respectively). No mortality was observed at 200 or 300 mg Li_2CO_3/kg. Increased resorptions were observed at 300, 350, and 400 mg/kg (8.1, 9.5, and 10.8 mmol Li/kg) (statistically significant). Increased malformed fetuses were observed at the same doses (not statistically significant). No effect was observed on fetal weight. In experiment 3, pregnant CD-1 mice were injected ip with sodium carbonate at 430 mg/kg or lithium carbonate at 300 mg/kg (8.1 mmol Li/kg) on gd 8. Some mice were sacrificed on each of gd 11, 12, 14, and 18. There were 4-20 females/group. No maternal toxicity results were reported. Variable increases in resorptions were observed on all sacrifice days. Increased malformations were observed on sacrifice days 11 and 12, but not 14, and possibly 18 (low incidence). Light and electron microscopic observation of the neural tube found cell death 3-17 hr after treatment, with cell debris cleared by 48 hr. No effect was observed on embryo or fetal weight

Jurand (1988)

In experiment 1, nonpregnant female JBT/Jd mice were injected ip with lithium carbonate at one of 5 doses from 300 to 440 mg/kg (8.1 to 11.9 mmol Li/kg). There were 12-17 females/group. The estimated LD_{50} for non-pregnant females was 440 mg/kg (11.9 mmol Li/kg). At the low dose of 300 mg/kg (8.1 mmol Li/kg) there were 1/15 deaths. At the high dose of 440 mg/kg (11.9 mmol Li/kg) there were 6/13 deaths. In experiment 2, mated female JBT/Jd mice were treated under light ether anesthesia with lithium carbonate by ip injection at 0 or one of 11 doses between 250 and 400 mg/kg (6.8 and 10.8 mmol Li/kg) on gd 9 plus 1 hour. Females were sacrificed on gd 13. There were 3-29 females/group. No maternal toxicity results were reported. Trends of increased proportions of dead embryos, retarded embryos, exencephaly, craniorachischisis, kinking of spinal cord, and dilation of 4th ventrical were observed. The doses where increases began varied depending upon endpoint. In experiment 3, mated female JBT/Jd mice were treated without ether anesthesia by ip injection with lithium carbonate at 0, 335, or 340 mg/kg (0, 9.1 or 9.2 mmol Li/kg) on gd 9 plus 1 hour. Females were sacrificed on gd 13. There were 8-10 females/group. No maternal toxicity

results were reported. Increased proportions of dead embryos, retarded embryos, exencephaly, craniorachischisis, kinking of spinal cord, and dilation of 4th ventrical were observed at both doses. In experiment 4, mated female JBT/Jd mice were treated by ip injection with lithium carbonate at 0 or 335 mg/kg (0 or 9.1 mmol Li/kg) on gd 9 plus 2 or 3 hours. Whether ether anesthesia was used was not stated. Females were sacrificed on gd 13. There were 10-12 females/group. No maternal toxicity data were reported. Increased fractions of dead embryos, retarded embryos, exencephaly, craniorachischisis, kinking of spinal cord, and dilation of 4th ventrical were observed at both treatment times.

Loevy (1973)

Mated female CD1 mice were untreated or treated by injection (specific route not reported) with 0 or 15.5 mg lithium chloride (0 or 0.366 mmol Li) on gd 11-12, 12-13, or 11-13. Mice were sacrificed on gd 17. There were 8-10 females/group. There were no maternal deaths. No other maternal toxicity results were reported. Increased proportions of resorptions and percentages of cleft palate were observed in all lithium chloride treated groups.

Schluter (1971a)

This article is in German with an English summary. Pregnant female NMRI/Han mice were injected (ip) with lithium carbonate at 0 or 40 mg/kg (0 or 1.1 mmol Li/kg) on gd 8. There were 10 females/group. No maternal toxicity results were reported. Increased resorptions (statistically significant) were observed. No increases in malformations or retarded fetuses were observed.

Smithberg and Dixit (1982)

In experiment 1a, mice of strain 129 Sv/Sl mice (sex not reported) were treated with lithium carbonate at 0, 5.0, 7.5, 10.0, 15.0, or 20.0 mg/mouse (0 or 0.14, 0.20, 0.27, 0.41, or 0.54 mmol Li/mouse) by ip injection. Survival over 24 hours was reported. There were 5-77 animals/group. The LD_{50} was determined to be approximately 10 mg/mouse (0.27 mmol Li/mouse). In experiment 2, mated female 129 Sv/Sl mice were treated by ip injection with lithium carbonate at 0.8, 1.6, 3.2, or 5.0 mg/mouse (0.022, 0.043, 0.087, or 0.135 mmol Li/mouse) on gd 8, at the same doses on gd 9, and at 0.8 or 5.0 mg/mouse on gd 10. Females were sacrificed on gd 18 or 19. There were 9-29 females/group. No maternal toxicity results were reported. An increased percentage of abnormal fetuses was observed at 5.0 mg/mouse. Abnormalities included fused ribs, vertebral defects, and exencephaly. No effect was observed on frequency of producing litters, litter size, or percent resorptions. In experiment 3, mated female A/J mice were treated by ip injection with lithium carbonate at 0.8 or 1.6 mg/mouse (0.022 or 0.043 mmol Li/mouse) on gd 8, with 0, 0.8 or 3.2 mg/mouse (0, 0.022, or 0.087 mmol Li/mouse) on gd 9, with 0.8 mg/mouse (0.022 mmol Li/mouse) on gd 10, with 0.8 mg/mouse (0.022 mmol Li/mouse)

on gd 12, 13 and 14, or with 3.2 mg/mouse (0.087 mmol Li/mouse) on gd 12, 13, or 14. Females were sacrificed on gd 18 or 19. There were 9-17 females/group. No maternal toxicity results were reported. Sporadic instances of rib and vertebral abnormalities were observed (especially on gd 8), but none were statistically significant. No effect was observed on frequency of producing litters, litter size, percent resorptions, or cleft lip and palate.

Tuchman-Duplessis and Mercier-Parot (1973)

Mated female Swiss mice were treated with lithium chloride by ip injection with 0, 100, or 150 mg/kg/d (0, 2.4, or 3.5 mmol Li/kg/d) for gd 7-12. There were 7-9 females/group. No effect was observed on maternal survival. No other maternal toxicity results were reported. No effects were observed on abortions, resorptions, litter size, or abnormalities.

C.3.2.3 Developmental studies of lithium in rats by oral routes

Canolty et al. (1989)

This study was available in abstract only. Pregnant Sprague-Dawley rats were treated with 0 or 750 ppm lithium carbonate (0 or 120 ppm Li; 0 or 17.3 mmol Li/kg food) in food during gestation. Another group was pair-fed to the lithium carbonate group. Mean serum lithium was 0.48 meq/L. Reduced net maternal body weight gain (maternal body weight minus uterus and contents) was observed in the lithium treated group. No significant effect was observed on maternal body weight. Increased fetal resorptions were observed in the lithium treated group. No significant effect was observed on litter size, fetal weight, litter weight, or gross malformations.

Fritz (1988)

In experiment 1, pregnant female Sprague-Dawley rats were treated with lithium carbonate at 100 mg/kg/d (2.7 mmol Li/kg/d) by gavage for gd 6-10, 11-15, or 16-20. There was no control group. There were 14-19 females/group. Females were sacrificed on gd 21. For gd 16-20 group, 7 out of 25 females died one day prior to expected delivery. In all groups, reduced body weight, food consumption, and polyurea were observed (as noted by author: no quantitative data reported). For the gd 16-20 group, compared to other two groups, a higher incidence of embryonic and fetal deaths, lower number of live fetuses/litter, and increased incidence of enlarged renal pelves were observed. The authors also remarked that fetal weight for all groups was below the normal range. No statistical tests were performed. Data from this experiment are summarized in Table 26.

Table 26. Selected data from rat developmental study, experiment 1, by Fritz (1988) [1]

Treatment group [all 100 mg Li_2CO_3/kg/d (2.7 mmol Li/kg/d)]	Gd 6-10	Gd 11-15	Gd 16-20
Number of litters	16	19	14
Embryonic and fetal deaths (% of implantation sites)	3.8%	7.0%	38.5%
Live fetuses/litter	12.7	11.8	8.8
Fetal weight (g)	4.91 ± 0.41	4.82 ± 0.81	4.48 ± 0.53
Enlarged renal pelves [fetuses affected/fetuses examined (litters affected/litters examined)]	0/67 (0/16)	3/75 (1/19)	7/41 (4/14)

[1] Data are numbers or averages (whether ± SD or ± SEM not reported). No statistical tests were reported.

In experiment 2, pregnant female Sprague-Dawley rats were treated with lithium carbonate at 0 or 100 mg/kg/d (0 or 2.7 mmol Li/kg/d) by gavage for gd 16-20. There were 20 mated rats/group. There were 17 control and 14 lithium carbonate treated pregnancies. One lithium treated female was found dead on gd 21, and one was found dead on pnd 1. About half were sacrificed on gd 21, and half allowed to give birth, nurse and wean pups. Pups were sacrificed on pnds 11-12 or 18-19. Reduced maternal body weight gain and food consumption, increased water consumption, and polyurea were observed in the lithium treated group (no statistics were reported). An increased incidence of enlarged renal pelves in fetuses (gd 21) was observed. A higher incidence of postnatal death from pnd 1-4 was observed. No renal changes were observed in pups sacrificed on pnd 11/12 or 18/19. Data from this experiment are summarized in Table 27.

In experiment 3, pregnant female Sprague-Dawley rats were treated with lithium carbonate at 0 or 60 mg/kg/d (0 or 1.6 mmol Li/kg/d) by gavage for gd 16-20. There were 20 females/group. Females were allowed to give birth. About half of the pups were cross-fostered immediately after birth. Pups were sacrificed on pnd 35-40. Reduced maternal body weight gain and food consumption, and increased water consumption during gd 16-20 were observed in the lithium treated group (statistically significant). No macroscopic changes were observed in maternal kidneys following weaning. Reduced litter size on pnd 1 was observed in the lithium treated group which was not cross-fostered (statistically significant). No effect was observed on pup body weights on pnd 4 or 21, on offspring on a negative geotaxis test or exploratory locomotion at 5 weeks or on offspring kidney relative weight or macroscopic pathology on pnd 35-40. Data from this experiment are summarized in Table 28.

Table 27. Selected data from rat developmental study, experiment 2, by Fritz (1988) [1]

Dose [mg Li_2CO_3/kg/d (mmol Li/kg/d)]	0 (0)	100 (2.7)
Number of females mated	20	20
Body weight gain (%) [2]	21.5%	11.5%
Number of females sacrificed gd 21	9	7
Enlarged renal pelves [fetuses affected/fetuses examined (litters affected/litters examined)]	0/133 (0/9)	20/93 (4/7)
Number of females allowed to litter and nurse	8	7
Number of females with live offspring pnd 4	7	4

[1] Data are numbers or percentages. No indices of variation (e.g. SD) were reported. No statistical tests were reported.
[2] Not clear if during gestation or during treatment.

Table 28. Selected data from rat developmental study, experiment 3, by Fritz (1988) [1]

Group		C		L	
Dose [mg Li_2CO_3/kg/d (mmol Li/kg/d)]		0 (0)		60 (1.6)	
Number of females total [2]		20		20	
Body weight gain gd 16-20 (%)		19%		12.9%**	
Food consumption (g/d)		23.1		18.4**	
Water consumption (ml/d)		46.6		60.0**	
Group (offspring cross-fostered)		CC	CL (0-->60)	LL	LC (60-->0)
Number of females [2]		9	10	8	10
Litter size on pnd 1		16.0 ± 2.1	(14.6) [3]	10.9 ± 5.8*	(14.9) [3]
Pup body weight (g)	pnd 4	8.0	8.7	8.6	8.1
	pnd 21	37.7	41.6	40.9	40.5

[1] Data are numbers or averages (± SD or SE not reported).
[2] The report appears to contain a typographical error in regard to number of females. The methods section states that the experiment began with 20 females per group. Table 3 of the report indicates there were 28 and 29 pregnant females in the control and lithium treated groups, respectively. Table 4 of the report implies that there were 9 + 10 (i.e. 19) controls and 8 +10 (i.e., 18) lithium treated pregnant females.
[3] Calculated from number of pups and females.
* $p < 0.05$ statistically significant difference from controls. Student's t-test, one tailed.
** $p < 0.01$ statistically significant difference from controls. Student's t-test, one tailed.

Gralla and McIlhenny (1972)

Five experiments were briefly reported in this paper: three in rats, and one each in rabbits and monkeys. The experiments in rabbits and monkeys are described below in section C.3.2.5. In rat experiment 1, Charles River albino male rats were treated by diet with lithium carbonate at 0, 0.27, 0.67 or 1.35 meq/kg/d (0, 1.9, 4.7 or 9.4 mg Li/kg/d) for 70 days and female rats were treated by gavage at 0, 0.675, 2.025, or 4.05 meq/kg/d (0, 4.7, 14.2, or 28.4 mg Li/kg/d) for 14 days. Males and females were mated according to zero, low, middle, or high dose levels. There were 20 animals/sex/dose. Half the females were sacrificed on gd 13, and the other half allowed to deliver and nurse pups for 21 days. In experiment 2, pregnant female Charles River albino rats were treated by gavage with lithium carbonate at 0, 0.675, 2.025, or 4.05 meq/kg/d (0, 4.7, 14.2, or 28.4 mg Li/kg/d) from gd 14 to pnd 21. Females were allowed to litter and nurse pups. There were 10 females/dose. In experiment 3, pregnant female Charles River albino rats were treated by gavage with lithium carbonate at 0, 0.675, 2.025, or 4.05 meq/kg/d (0, 4.7, 14.2, or 28.4 mg Li/kg/d) from gd 5-15. Females were sacrificed on gd 20. There were 20 females/dose.

Treatment of male and female rats by gavage at 4.05 meq/kg/d (4.05 mmol Li/kg/d) for 3 days resulted in an average plasma lithium concentration of 1.4 meq/L (similar in males and females). In reporting results, the authors frequently did not distinguish between experiments 1, 2, and 3. The authors stated that two females treated at 4.05 meq/kg/d (28.4 mg Li/kg/d) died. It is not clear if this refers to Experiment 1, 2, or 3, or all combined. The authors stated that there was no effect on parental body weight gain. No other parental results were reported. Reduced pup body weight was observed at the high dose on pnd 21 (statistically significant). However, it is not clear if this result refers to experiment 1 or 2. No effect was observed on pnd 1 or 4. The authors stated that no effects on fertility, implantation sites, litter size, offspring mortality, gross external or internal abnormalities were observed. Quantitative data were presented only for litter size and neonatal body weight, evidently from experiment 1 and/or 2. Data from these experiments are summarized in Table 29.

Table 29. Data from rat reproductive experiments 1 and/or 2, by Gralla and McIlhenny 1972 [(1)]

Dose [meq Li/kg/d (mg Li/kg/d)]		0 (0)	0.675 (4.7)	2.025 (14.2)	4.05 (28.4)
Litter size	Pnd 1	14	13	12	12
	Pnd 4	13	12	12	12
	Pnd 21	12	12	12	12
Pup weight (g)	Pnd 1	6	7	7	7
	Pnd 4	8	10	10	10
	Pnd 21	44	49	42	33*

[(1)] Data are averages (indices of variation, e.g. SD, were not reported).
* $p < 0.05$ statistically significant difference from controls. Statistical method not described.

Hsu and Rider (1978)

Mated female McCollum rats were treated with lithium (as lithium citrate) in water at 0 or 20 mM Li for pregnancy and lactation. There were 10 control and 13 lithium treated females during pregnancy. One pup from each litter was sacrificed at birth. About half the pups from each group were cross-fostered. Pups were weaned at 3 weeks of age. Males only were used for subsequent tests. No maternal toxicity results were reported. No effect was observed on pup birth weight, or relative spleen and kidney weight. Reduced relative liver weight at birth was observed in the lithium treated group (statistically significant). Reduced survival to weaning was observed in pups from mothers treated prenatally (statistically significant). Reduced body weight at weaning, and increased age of eye opening was observed in pups from mothers treated during lactation (statistically significant). Increased relative brain weight was observed at 3 weeks of age in prenatally and postnatally exposed pups (statistically significant). Increased maze running time at 4.5 months of age was observed in prenatally exposed males (statistically significant). Data from this study are summarized in Table 30.

Table 30. Selected results from rat developmental study by Hsu and Rider (1978) [(1)]

Group		Control (C)		Lithium-treated (L)	
Li concentration in water (mmol Li/L)		0		20	
Number of females		10		13	
Number of pups examined		8		11	
Birth weight (g)		6.31 ± 0.33		6.06 ± 0.32	
Relative pup organ weight at birth (mg organ/ g body)	Spleen	3.53 ± 0.34		3.51 ± 0.30	
	Kidney	7.58 ± 0.33		7.99 ± 0.34	
	Liver	46.8 ± 1.2		40.6 ± 2.2*	
Group (offspring cross fostered)		CC	CL (0-->20)	LL	LC (20-->0)
Number of pups examined		9	9	11	11
Survival to weaning (%)		100 ± 0	100 ± 0	82 ± 8*	84 ± 7*
Weaning body weight (g)		42.4 ± 1.7	31.3 ± 2.3**	30.4 ± 5.8**	38.0 ± 1.7
Age of eye opening (days)		14.3 ± 0.3	15.2 ± 0.2**	15.5 ± 0.2**	14.5 ± 0.3
Relative brain weight at weaning (mg brain/ g body)		3.062 ± 0.313	3.598 ± 0.721	4.236 ± 0.386*	3.441 ± 0.482

[(1)] Data a numbers or averages ± SEM.
* $p < 0.05$ statistically significant difference from controls by Student's t-test.
** $p < 0.01$ statistically significant difference from controls by Student's t-test.

Ibrahim and Canolty (1990)

Mated female Sprague-Dawley rats were treated with lithium carbonate in food at 0 or 1000 ppm (equivalent to 0 or 187.8 ppm Li; 0 or 27.1 mmol Li/kg food) during gestation and/or lactation. There were 11 controls and 13 lithium treated females during pregnancy. Females were allowed to give birth. After birth, the total litter size was reduced to 6, selecting males when possible. About half of each group was switched to the other group (i.e. females receiving no lithium during gestation were fed 1000 ppm during lactation, and vice versa). Reduced maternal body weight gain (62% of controls), food intake, and food efficiency during pregnancy was observed in the lithium treated group (statistically significant). Reduced litter size at birth (75% of controls), litter weight at birth, and mean birth weight were observed (statistically significant). No gross malformations at birth were observed. Reduced food intake (not statistically significant) and body weight (statistically significant) were observed at the end of lactation among both groups of dams treated with lithium during lactation. Reduced liver and kidney weights (absolute and relative) were observed in dams treated with lithium only during lactation (statistically significant). Slightly lower pup survival during lactation was

observed in prenatally treated litters (not statistically significant), and reduced pup survival was observed in litters treated prenatally and postnatally (statistically significant). Reduced litter weight at weaning was observed in both litters treated postnatally (statistically significant), but not in the litter treated only prenatally. Reduced pup weight at weaning was observed in the group treated postnatally only (statistically significant) but not the other groups. No effect was observed on pup liver or kidney weights. Data from this study are summarized in Table 31.

Johansen and Ulrich (1969)

Mated female Wistar rats were treated with lithium (salt not specified) by diet for gd 1-20 with concentrations targeted to achieve doses of 0, 1, or 3 meq Li/kg bw/d (0, 7, or 21 mg Li/kg/d). All animals were offered 25 g food/day. Females were sacrificed on gd 20. There were 11-13 females/group. The actual average doses of lithium were 0, 0.93 and 1.78 meq/kg bw/d (0, 6.5, and 12.5 mg Li/kg/d), due to reduced food intake in the high concentration group. The high dose group gained less weight than controls or lost body weight up to about gd 9. Body weight in this group was lower than controls for the remainder of gestation. Average maternal serum lithium concentrations were between 0.14-0.21 and 0.41-0.48 meq/L in the low and high dose groups, respectively (estimated by OEHHA staff from figure). Average fetal serum lithium concentrations were about half the maternal concentrations on gd 21. There was no effect on number of litters. The litter size was higher in the lithium treated groups than the control group, and the average fetal weight was lower. The authors stated that litter size and fetal weight were within "normal values." No gross malformations were observed. Data from this study are summarized in Table 32.

Table 31. Selected results from rat developmental study by Ibrahim and Canolty (1990) [(1)]

Group		Control (C)		Lithium-treated (L)	
Concentration in food [ppm Li_2CO_3 (ppm Li)]		0 (0)		1000 (187.8)	
Number of females		11		13	
Total food intake during gestation (g)		398 ± 10		320 ± 8*	
Body weight gain during gestation (g)		156 ± 5		97 ± 4*	
Litter size		12.5 ± 0.8		9.4 ± 0.6*	
Birth weight (g)		6.3 ± 0.1		5.7 ± 0.2*	
Litter weight (g)		76.0 ± 5		53.0 ± 3*	
Group (change of maternal feed)		CC	CL (0-->1000)	LL	LC (1000-->0)
Number of nursing females		5	6	6	7
Nursing female food intake during lactation (g)		929 ± 29	861 ± 110	844 ± 48	937 ± 61
Nursing female body weight at end of lactation (g)		269 ± 8	238 ± 10*	242 ± 2*	259 ± 4
Nursing female absolute organ weight (g)	Liver	18.97 ± 0.61	13.85 ± 1.77*	16.18 ± 0.63	17.85 ± 0.38
	Kidney	2.05 ± 0.04	1.79 ± 0.07*	1.73 ± 0.03*	1.90 ± 0.04*
Nursing female relative organ weight (g)	Liver	5.51 ± 0.08	3.69 ± 0.48*	5.32 ± 0.25	5.95 ± 0.13
	Kidney	0.59 ± 0.02	0.48 ± 0.02*	0.56 ± 0.01	0.63 ± 0.01
Litter size at weaning [(2)]		6.0 ± 0.1	6.0 ± 0.0	4.8 ± 0.5*	5.7 ± 0.2
Pup body weight at weaning (g)		58 ± 1	44 ± 3*	54 ± 4	57 ± 2
Litter weight at weaning (g)		351 ± 6	266 ± 19*	256 ± 27*	327 ± 14
Pup organ weight at weaning (g)	Liver	2.79 ± 0.21	2.22 ± 0.22	2.56 ± 0.18	2.62 ± 0.16
	Kidney	0.61 ± 0.03	0.52 ± 0.04	0.57 ± 0.04	0.60 ± 0.03

[(1)] Data a numbers or averages ± SEM.
[(2)] Litters reduced to 6 after birth.
* $p < 0.05$ statistically significant difference from controls by Student's t-test for results during gestation, or by ANOVA followed by Duncan's Multiple Range test for results during lacation.
** $p < 0.01$ statistically significant difference from controls by Student's t-test.

Table 32. Results from rat developmental study by Johansen and Ulrich (1969) [1]

Target dose [meq Li/kg bw/day (mg Li/kg/d)]	0 (0)	1 (7)	3 (21)
Actual dose [meq Li/kg bw/d (mg Li/kg/d)]	0 (0)	0.93 (6.5)	1.78 (12.5)
Number of females mated	11	13	13
Number of litters	10	13	12
Maternal weight gain during gestation (%) [2]	21.3%	20.4%	11.5%
Litter size	8.6	11.1	10.3
Number of fetuses resorbed (total for group)	1	4	3
Fetal weight (g)	3.56	3.12	3.22

[1] Data are numbers or averages. Indices of variation (e.g., SD) were not reported. No statistical tests were performed.
[2] Estimated by OEHHA staff from Figure 1, Johansen and Ulrich (1969).

Marathe and Thomas (1986)

Mated female Wistar rats were treated by gavage with lithium carbonate at 0, 50, or 100 mg/kg/d (0, 1.35, or 2.7 mmol Li/kg/d) for gd 6-15. Females were sacrificed on gd 20. There were 11-20 females/group. Maternal toxicity results were not reported. Reduced implantations, live litter size and fetal weight were observed at 100 mg/kg/d (statistically significant). Early and late resorptions were observed to be higher at 100 mg/kg/d (2.7 mmol Li/kg/d) (not statistically significant). Increased frequency of several skeletal malformations and alterations was observed at 100 mg/kg/d (2.7 mmol Li/kg/d). No visceral malformations were observed. Data from this study are summarized in Table 33.

Table 33. Selected results from rat developmental study by Marathe and Thomas (1986) [(1)]

Group [mg Li_2CO_3/kg/d (mmol Li/kg/d)]		0 (0)	50 (1.35)	100 (2.7)
Number of females		20	13	11
Implantations/litter		10.25 ± 0.68	8.69 ± 0.78	7.91 ± 0.71*
Live pups/litter		9.00 ± 0.68	8.23 ± 0.78	4.73 ± 1.29**
Resorptions/litter	Early	1.00 ± 0.24	0.31 ± 0.17	1.91 ± 0.97
	Late	0.25 ± 0.10	0.15 ± 0.10	1.18 ± 0.67
Fetal weight (g)	Male	3.65 ± 0.07	3.33 ± 0.10	2.42 ± 0.40**
	Female	3.32 ± 0.10	3.28 ± 0.08	2.24 ± 0.37**
Number of fetuses examined for skeletal alterations		95	107	54
Wavy ribs		0	0	51.85%
Shortening of bones	Radius and ulna	0	0	37.04%
	humerus	0	0	37.04%
	Tibia and fibula	0	0	33.33%
	femur	0	0	40.74%
Deformity in scapula		0	0	37.04%
Deformity in pelvic bones		0	0	33.33%

[(1)] Data are numbers, averages ± SE, or percentages.
* $p < 0.05$ statistically significant difference from controls, method not given.
** $p < 0.01$ statistically significant difference from controls, method not given.

Rider and Hsu (1976)

Mated female McCollum rats were treated with lithium citrate in water at 0 or 20 meq Li/L during pregnancy and lactation. Another group was restricted to the same amount of water as consumed by the 20 meq/L group. After birth, pups were culled and switched so that each maternal female from the control and lithium groups had 4 pups from her group and 4 pups from the other group (not including the water restricted group). The groups were designated CC, LL, CL, LC. There were 10 control, 13 lithium, and 9 water restricted females. Reduced water and food consumption (by 40% and 10%, respectively) were observed in the lithium treated group (statistically significant). Lower body weight gain during pregnancy was observed in the lithium treated group (not statistically significant). Reduced body weight gain during pregnancy was observed in the water restricted group (statistically significant). The average dose in lithium treated group was 2.3 meq Li/kg/d (16.1 mg Li/kg/d). Greater litter size, lower pup weight, and greater total litter weight were observed in the lithium treated group (not statistically significant). Reduced litter size, pup weight, and litter weight were observed in the water

restricted group (statistically significant). Reduced relative liver weight was observed in lithium treated pups at birth. No effect was observed on relative spleen or kidney weight. Reduced survival to weaning was observed in pups from dams treated during pregnancy (LL and LC vs. CC and CL) (statistically significant). Reduced weaning weight of pups nursed by lithium treated dams was observed. Data from this study are summarized in Table 34.

Table 34. Selected results from rat developmental study by Rider and Hsu (1976) [(1)]

Group		C		L		R
Description		Control		20 meq Li/L water (140 ppm)		Water restricted
Number of females mated		10		13		9
Number of females pregnant		9		11		6
Food intake (gd 15-17) (g)		21 ± 0.7		19 ± 0.5*		17 ± 0.4*
Fluid intake (gd 1-22) (ml/female)		45.6 ± 2.8		27.6 ± 0.7***		27.5 (restricted)
Maternal body weight at start of pregnancy (g)		224 ± 4.2		227 ± 2.0		219 ± 2.3
Maternal weight gain (g)	Gestation	102.9 ± 10.3		92.6 ± 4.6		57.8 ± 4.3***
	Lactation	34.3 ± 5.5		3.6 ± 10.4*		--
Litter size at birth		9.0 ± 0.9		11.2 ± 0.4		7.5 ± 1.3
Pup weight at birth (g)		6.4 ± 0.3		5.9 ± 0.2		5.7 ± 0.2
Litter weight at birth (g)		54.8 ± 6.0		64.9 ± 3.5		41.1 ± 7.0
Pup relative kidney weight at birth (mg/g)		7.58 ± 0.33		7.99 ± 0.34		--
Pup relative liver weight at birth (mg/g)		46.8 ± 1.2		40.6 ± 2.2*		--
Group (offspring cross-fostered)		CC	CL (0-->20)	LL	LC (20-->0)	R
Pup survival to weaning		100%	100%	82 ± 8%*	84 ± 7%*	92 ± 3%*
Pup weight at weaning (g)		42.4 ± 1.7	31.3 ± 2.3***	30.4 ± 5.8***	38.0 ± 1.7	35.6 ± 2.9

[(1)] Data are numbers or averages ± SE. -- Data not reported.
* $p < 0.05$ statistically significant difference from controls, method not given.
*** $p < 0.001$ statistically significant difference from controls, method not given.

Sharma and Rawat (1986)

Mated female albino (strain not reported) rats were treated by gavage with lithium (unspecified salt) at 0 or 7 mg/kg/d (0 or 1 mmol Li/kg/d) for gd 0-10. Females were sacrificed before birth of pups (day not specified). Numbers of animals were not reported. No maternal toxicity results were reported. Authors reported a reduction in fetal size, and numerous malformations, including cleft palate, hepatomegaly, brain liquifaction, lower and upper digit defects, clubbed foot, hydrocephaly, cardiomegaly, hydrohephrosis, and defects in ribs and sternum.

Teixeira et al. (1995)

Mated female Wistar rats were treated with lithium chloride in water at 0 or 10 mM (0 or 70 ppm) for gestation and lactation. An additional group was water restricted to the 10 mM lithium chloride group. Females were allowed to give birth and nurse the pups. Litters were culled to 8 pups on the day of birth. Pups from some of the lithium chloride and water restricted litters were cross fostered at birth. There were 13 females in the control group and 18-25 females in other groups. The following terminology was used by the authors: Control-NS (not stressed), Control-S (stressed: water restricted), LiPL (10 mM Li during pregnancy and lactation), LiP (10 mM Li during pregnancy only), LiL (10 mM Li during lactation only). Maternal serum monovalent ion levels were tested. It was observed that serum lithium was 0.5 mM during gestation and lactation. Serum potassium (K) was increased in the lithium treated group (statistically significant, 20-30%). No effect on serum sodium (Na) was observed. No other maternal results were reported. Lower litter sizes were observed in the lithium treated and water restricted groups (not statistically significant). The authors asserted that water restriction and lithium treatment resulted in reduced numbers of male pups (statistically significant), but data indicate that this was probably due to an unusually high percentage of males in Control-NS. Reduced percentage of pups with normal righting reflex at birth in lithium treated vs Control-NS and vs. Control-S was observed (both statistically significant). Reduced pup weight on pnd 21 in LiPL and LiL groups vs. Control-NS and Control-S was observed (statistically significant). Delayed eye opening in all lithium treated groups vs. Control-NS and vs. Control-S was observed (statistically significant). No effects on fertility (75%), percentage stillborn, malformations, or birth weight was observed. No effect on a pup retrieval test was observed. No effects on pinna detachment, cliff avoidance, or motor coordination of lithium groups vs. Control-S were observed, although there were some differences vs. Control-NS. Data from this study are summarized in Table 35.

Table 35. Selected results from rat developmental study by Teixeira et al. (1995). [1]

Group	Control-NS (0 mmol Li/L)	Control-S (0 mmol Li/L, water restrictred to Li group)		Li (10 mmol Li/L)	
Number of mated females	13	46		44	
Number of litters	13	46		44	
Stillborn pups [number (percentage)]	2/144 (1.3%)	4/439 (1.0%)		7/467 (1.5%)	
Litter size	11.1 ± 2.7	9.5 ± 2.6		9.6 ± 2.5	
Birth weight (g) [2]	5.8 ± 1.0	5.5 ± 1.0		5.6 ± 1.0	
Pups with righting reflex at birth	94.2%	78.5%*		70.5%*,#	
Pup retrieval test, latency (seconds)	13.1 ± 5.6	21.0 ± 10.4		15.0 ± 9.4	
Pup retrieval test, non-recovery	3%	5%		6%	
Group (offspring cross fostered)	Control-NS	Control-S	LiL (0-->10)	LiPL	LiP (10-->0)
Number of litters	13	21	25	18	22
Pup weight at weaning (pnd 21) (g) [2]	42 ± 4	37 ± 5@@	35 ± 7@@,$$	33 ± 5@@,$$	38 ± 5@@

[1] Data are numbers, percentages, or averages ± SD.
[2] Data estimated by OEHHA staff from Figure 1, Teixeira et al. (1995).
* $p < 0.05$ statistically significant difference from Control-NS group by Fisher's exact test.
# $p < 0.05$ statistically significant differences from Control-S group by Fisher's exact test.
@@ $p < 0.01$ statistically significant difference from Control-NS group by Duncan's test.
$$ $p < 0.01$ statistically significant difference from Control-S group by Duncan's test.

C.3.2.4 Developmental studies of lithium in rats by injection

Johansen 1971

This study was described in a short letter, similar in length to an abstract. Mated female Wistar rats were treated by injection with lithium chloride. Initial "loading dose" was by ip injection at 6.25 meq Li/kg (43.75 mg Li/kg) on gd 4, 7, or 9. A "maintenance" dose of 2 meq Li/kg (14 mg Li/kg) was administered by ip or sc injection until gd 19.

Controls were administered saline by ip or sc injection in equal volumes. Animals were sacrificed on gd 20. There were 8 controls and 42 total lithium treated (average 7/group lithium treated). Serum lithium was 9-10 meq/L at 15 minutes after injection. No maternal toxicity results were reported. In the fetuses, absent femur, tibia, and fibula bilaterally were observed in one fetus from the group treated from gd 4. The author commented that this may be spontaneous. No other gross malformations were observed. No other results were reported.

Tuchman-Duplessis and Mercier-Parot (1973)

Primiparous mated female Wistar rats were treated with lithium chloride by ip injection at a variety of concentrations and durations: control (it is not clear whether this group recived a sham injection), gd 1-12 at 100 or 150 mg/kg/d (2.4 or 3.5 mmol Li/kg/d), gd 4-12 at 100 or 150 mg/k/d, gd 7-9 at 200 mg/kg/d (4.7 mmol Li/kg/d), and gd 7-11 at 100, 200, or 250 mg/kg/d (2.4, 4.7, or 5.9 mmol Li/kg/d). There were 6-16 females/group. Increased percentage maternal mortality was observed for gd 1-12 at 150 mg/kg/d, and for gd 7-11 at 200 and 250 mg/kg/d. No other maternal results were reported. Increased abortions were observed for gd 1-12 at 100 and 150 mg/kg/d, for gd 7-9 at 200 mg/kg/d, and for gd 7-11 at 200 mg/kg/d (but not 250 mg/kg/d). Increased resorptions were observed for gd 1-12 at 100 and 150 mg/kg/d, for gd 4-12 at 150 mg/kg/d, and for gd 7-11 at 200 and 250 mg/kg/d. Reduced litter size was observed for gd 1-12 at 100 and 150 mg/kg/d, for gd 4-12 at 150 mg/kg/d, and for gd 7-11 at 250 mg/kg/d. Increased ocular abnormalities were observed for gd 7-11 at 250 mg/kg/d.

Wright et al. (1971)

Mated female Sprague-Dawley rats were treated with lithium chloride by ip injection at 0 or 50 mg/rat (8.4 mg Li/rat) (first injection) followed by 20 mg/rat (3.5 mg Li/rat) (subsequent injections) starting on gd 1, 4, 7, or 9, and continuing to gd 16. Rats were sacrificed on gd 17. Authors note that a "glucose slurry" was added to food if the rat's weight dropped below 200g (initial weights 220-250 g). There were 6 control females and 3 females/lithium treated group. It was implied that rats lost weight due to reduced food consumption, but no numerical data were reported. The doses used were described as "maximal sublethal" based on a trial series of injections. Serum lithium concentrations were 4.2 meq/L at 20 minutes after the initial injection. At 24 hours after the final injection, serum lithium ranged from 0.04 to 0.17 meq/L. Resorption frequency was observed to be higher in lithium treated rats. The frequency of malformations (eye, ear, cleft palate) was observed to increase in lithium treated groups. No effect on fetal weight or crown-rump length was observed (no numerical data was reported).

C.3.2.5. Developmental studies of lithium in other laboratory animals

Gralla and McIlhenny (1972)

As described above in Section C.3.2.3, three experiments in rats, and one experiment each in rabbits and monkeys, were very briefly reported in this paper. Mated New Zealand albino rabbits were treated with lithium carbonate by capsule at 0, 0.675 or 1.08 meq/kg/d (0, 4.7 or 7.6 mg Li/kg/d) from gd 5-18. There were 10 females/group. Females were sacrificed on gd 28. Treatment with 1.08 meq/kg/d resulted in plasma concentrations of 1.5 to 2.4 meq/L. Three rabbits treated at 1.08 meq/kg/d died late in pregnancy after prolonged anorexia and tremors. One mated but non-pregnant female treated at 0.675 meq/kg/d died. No other females died. The authors stated there was no effect on body weight gain. No other maternal results were reported. The authors stated that no effects on implantations, litter size, fetal death, fetal gross appearance or skeletal examination were observed. No quantitative data were presented.

Mated female rhesus monkeys were treated with lithium carbonate by capsule at 0 or 0.67 meq/kg/d (4.7 mg Li/kg/d) for gd 14-35. Females were allowed to deliver or were delivered by cesarian section about gd 120. There were 5 controls and 6 lithium treated females. Treatment at 0.67 meq/kg/d resulted in serum concentrations of 0.3-0.4 meq/L after the third dose. No maternal toxicity results were reported. By implication, no monkeys died. The authors stated that all progeny were normal. Offspring weights were reported and appeared generally lower in the lithium treated group, but the authors did not remark on this. OEHHA staff calculated the averages and standard deviations, and performed Student's t-test on the data. It was found that the average birth weight was lower in offspring from the lithium treated group, although the difference was not statistically significant. The pnd 7 body weight was reduced in offspring from the lithium treated group (statistically significant). No difference was found on pnd 30. Data from this study are summarized in Table 36.

Table 36. Data from monkey developmental study by Gralla and McIlhenny (1972) [(1)]

Group [meq Li/kg/d (mg Li/kg/d)]		0 (0)	0.67 [(2)] (4.7)
Offspring body weight [g/(n)]	Birth	476 ± 87 (5)	435 ± 20 (5)
	Pnd 7	486 ± 25 (4) [(3)]	426 ± 12* (5)
	Pnd 30	580 ± 45 (4)	578 ± 56 (5)

[(1)] Data are numbers or averages ± SD. Averages and SD calculated by OEHHA staff from individual animal data.
[(2)] Data exclude one pair of twins.
[(3)] One very small animal (332 g) in this group died between birth and pnd 7.
* $p < 0.05$ difference from control by Student's t-test. Calculated by OEHHA staff.

C.3.3. Developmental endpoints from reproductive toxicity studies of lithium

C.3.3.1. Reproductive toxicity studies of lithium in mice

Messiha (1986a)

In one of three experiments reported in this paper, female Sprague-Dawley mice were treated with lithium chloride in water at 0 or 1 meq Li/L (0 or 7 ppm) for 2 weeks before mating and throughout gestation. The number of treated females was not reported. No maternal toxicity results were reported. No effect on pup body weight (lithium group 1.7 ± 0.2 g, controls 1.5 ± 0.1 g) or absolute liver weight at 24-36 hours after birth was observed.

Mroczka et al. (1983)

In experiment 1, male and female CFW mice were treated with lithium chloride in water at 0, 10, 20, 30, 50, 100, or 200 meq Li/L (0, 70, 140, 210, 350, 700, or 1400 ppm) beginning at 6-8 weeks of age, and, after 2 weeks, mated for multiple rounds of reproduction, apparently until reproduction ceased. Lithium treatment of females was continued through gestation and lactation. The numbers of animals were not reported. At 200 meq Li/L mice would not drink and all died in 1 week. Reduced water intake at 100 meq Li/L was observed (no numerical data were reported). No other parental toxicity results were reported at 100 meq Li/L. No reproduction occurred at 100 meq Li/L. Plasma levels of lithium were reported as 0.09 and 0.67 meq/L for water concentrations of 10 and 50 meq Li/L. No parental toxicity results were reported for concentrations from 10 to 50 meq Li/L. Although the numbers of animals used were not reported, the data suggest there were approximately 45 pairs of controls and 85 pairs treated at 50 meq/L. Reduced number of litters/mating pair, increased time between litters, and increased pup death during lactation at 50 meq Li/L were observed. No effect on litter size at birth at 50 meq Li/L was observed. The authors stated that there was no effect on "pup size" at 50 meq Li/L, but no numerical data were reported. No developmental or reproductive results were reported for 10, 20, or 30 meq Li/L. Data from this study are summarized in Table 37.

Table 37. Selected results from mouse reproductive study by Mroczka et al. (1983) [1]

Concentration [meq Li/L (mmol Li/L)]	0 (0)	50 (50)
Number of litters (total)	252	283
Number of litters/mating pair [2]	5.6	3.3
Litter size at birth	8.29 ± 0.235	7.809 ± 0.193
Litter size at weaning	7.202 ± 0.251	5.444 ± 0.228***

[1] Data are numbers, averages, or averages ± SEM.
[2] Data estimated by OEHHA staff from Figure 1, Mroczka et al. (1983).
*** $p < 0.001$ statistically significant difference from controls by Student's t-test.

In experiment 2, male and female CFW mice were treated with lithium chloride beginning at 3 weeks of age, and mated for multiple rounds of reproduction beginning at 8 weeks of age. The water lithium levels were not explicitly stated, however the implication was that 0 and 50 meq Li/L (0 and 50 mmol Li/L) were used. No parental toxicity results were reported. The authors stated that no effect on pup weight at birth was observed, but no numerical data were presented. No litter size results were reported. Data presented only graphically indicated that weight gain during lactation was lower for lithium treated litters, and some differences persisted after weaning when offspring were switched to water without lithium. Some differences in absolute and/or relative brain, heart, liver, and kidney weights were observed in offspring from one to eight weeks of age.

C.3.3.2 Reproductive toxicity studies of lithium in rats

Christensen et al. (1982)

Female Wistar rats were treated with lithium chloride in food at 0 or 40 mmol Li/kg (280 ppm) food for 4 weeks, followed by 0 or 60 mmol Li/kg (420 ppm) food for 4 weeks, and during mating and gestation. There were initially 6 females/group. Females were allowed to give birth. Both groups were split so that half received control diet and half received lithium in food at 40 mmol Li/kg during lactation. Pups were assessed at 8-10 weeks postnatal. Plasma lithium in maternal animals after four weeks of treatment was 0.6 mM (0.6 meq/L). Increased water intake was observed in the lithium treated maternal group (5 fold during pre-mating treatment: statistically significant). Reduced body weight gain during gestation was observed in the lithium treated group (statistically significant). No effects were observed on litter size or pup weight at birth. In maternal animals, 3 weeks after delivery, plasma lithium was 1.15 and 1.47 mmol/L in the group treated pre- and postnatally and the group treated only postnatally, respectively. In pups, plasma lithium was 0.54 and 0.51 for the same groups, respectively. Offspring body weight at 8-10 weeks of age was similar to controls for the group treated prenatally, and lower than controls for both groups treated postnatally (not statistically significant). Kidney function was altered in offspring. In males, osmolality was reduced in both postnatally treated groups (statistically significant), but not the group treated only

prenatally. Females were not tested for urine osmolality. In females, inulin clearance was increased in the group treated pre-natally only, and reduced in both groups treated postnatally (all statistically significant). Males were not tested for inulin clearance. Data from this study are summarized in Table 38.

Table 38. Selected results from rat reproductive study by Christensen et al. (1982) [1]

Group treatment before and during gestation, mmol Li/kg food		0	40/60
Number of females mated		6	6
Number of females pregnant		6	5
Fluid consumption during the 7th week of pretreatment (ml/kg/d)		9 ± 3	50 ± 17**
Maternal weight gain during gestation (g)		98 ± 8	64 ± 8*
Litter size		11.1 ± 2.1	10.8 ± 1.3
Birth weight (g)		6.5 ± 0.7	6.2 ± 0.6
Offspring weighed at 8-10 weeks of age [2]	male	12	9
	female	8	8
Offspring weight at 8-10 weeks of age [2]	male	224 ± 26	227 ± 27
	female	169 ± 12	163 ± 18

[1] Data are numbers or averages ± SD.
[2] Data for offspring treated with lithium prenatally only.
* Statistically significant difference from controls, p not reported. Student's t-test.
** $p < 0.01$ statistically significant difference from controls. Student's t-test.

Glockner et al. (1989)

Female Wistar rats (P generation) were treated with lithium chloride at 0 or 20 mmol Li/L (140 ppm) in drinking water for 3 weeks before mating through gestation. Females were allowed to litter. Litters were culled to 6 pups, nursed and weaned. Female offspring (F1) were mated with untreated males, and allowed to litter. The numbers of females for the P generation were not reported. For the F1 generation females, there were 20 control and 30 from lithium treated mothers. In the P generation, reduced water consumption (80 ml/kg lithium treated vs. 140 ml/kg controls) was observed. Doses during pregnancy were 1.6, 1.98, and 2.08 mmol/kg/d for weeks 1, 2, and 3, respectively. Three weeks after the beginning of treatment, serum lithium was 0.96 mmol/L. No other P generation maternal results were reported. No developmental or reproductive results for the P/F1 litter were reported. In the F1 generation, no affect on maternal body weight or gain during gestation was observed. No other maternal F1 results were reported. For the F1/F2 litter, the authors state there was no effect on gestation length. However, Figure 1 shows lithium treated (in utero) mothers delivered less frequently on gd 21 than controls (18/30 vs. 18/20; p = 0.02, Fisher Exact Test). Also, no controls delivered on

gd 22, while 7/30 of the lithium treated group delivered on gd 22. For the F1/F2 litter, no effect on implantations, litter size, or pup body weight was observed. A reduced composite "skeletal ossification score" (statistically significant) in F2 pups from the lithium treated group compared to controls was observed. Data from this study are summarized in Table 39.

Table 39. Selected results from rat reproductive study by Glockner et al. (1989). [1]

Group (mmol Li/L water)	0	20
Implantations	12.5 ± 0.7	12.2 ± 0.5
Litter size	11.4 ± 0.5	11.2 ± 0.5
Pup body weight [2]	5.03 ± 0.06	4.98 ± 0.08
Pup skeletal ossification score (%) [3]	49%	42%*

[1] Data are averages ± SEM or percentages. All data refer to the F1/F2 litter.
[2] For pups born on gd 21.
[3] Estimated by OEHHA staff from Figure 3, Glockner et al. (1989)
* $p < 0.05$ statistically significant difference from controls, Mann-Whitney test.

Rider et al. (1978)

Female McCollum rats were treated with lithium citrate in water at 0 or 15 meq Li/L (0 or 105 ppm) for one month before mating, and during gestation and lactation. Females were then mated with untreated males. After mating, females were put on diets with 20% casein (adequate protein) or 10% casein (reduced protein). Pregnant females were allowed to give birth. At this stage of the study there were four groups: 20C, 20L, 10C, and 10L. The authors reported that there were 10-12 females/group at this stage. After delivery, pups were fostered to females fed a commercial stock diet. About half of the pups from lithium treated females were fostered to non-lithium treated females. All litters were reduced to 8 pups. Pups were weaned at 21 days of age, after which there was no further administration of lithium. This resulted in 6 groups: 20CC, 20LC, 20LL, 10CC, 10LC, and 10LL. The authors reported that there were 17 litters in the 20CC group, and 9-14 litters in the other groups. There appears to be some inconsistency, omission, or error in reporting, as the number of litters after fostering considerably exceeded the number of litters born. At three weeks of age, female pups representing at least 6 litters from each group were sacrificed for determination of organ weights. When pups were 4.5 months old, 1 male from each of 6 litters from each group was tested in a T-maze with a water reward. Ten days afterwards, the same animals were tested in an avoidance behavior test using water as an attractor and an electric shock.

Reduced body weight gain during pregnancy was observed in the lithium treated animals (statistically significant for 20L vs. 20C, but not for 10L vs. 10C). Reduced water consumption by lithium treated animals (75% of control) was observed. No effect on

food consumption on gd 15 or 16 was observed. Lithium intake was 1.17 and 1.19 meq Li/kg/d (8.2 and 8.3 mg Li/kg/d) for 20L and 10L groups, respectively. Serum lithium was 0.3-0.4 meq/L. Litter size and litter weight at birth were approximately 25% smaller in the lithium treated group with adequate protein (20%), but this was not statistically significant. No effect of lithium on pup weight at birth was observed.

No effects on pup survival to weaning or age of eye opening were observed. Lower pup weight at weaning was observed for pups nursed by lithium treated females (statistically significant for 10LL vs. 10CC, not others). Reduced relative spleen weight of female pups at weaning was observed for those nursed by lithium treated females (statistically significant for 10LL vs. 10CC only). No effect on female pup thymus, kidney, or liver weight at weaning was observed. No effect on male offspring weight at 13 weeks of age was observed. No effect of on male offspring maze times was observed. Male pups from females treated with lithium during pregnancy had a shorter avoidance time after electric shock (statistically significant for 20LL vs. 20CC and 10LL vs. 10CC, not others). Data from this study are summarized in Table 40.

Table 40. Selected results from rat reproductive/developmental study by Rider et al. (1978) [1]

Group		20C	20L	
mmol Li/L in water: premating and gestation (ppm)		0 (0)	15 (105)	
Number of mated females		11	12	
Number of deliveries (%)		9 (82%)	11 (92%)	
Original body weight (g)		226.0 ± 5.2	225.0 ± 3.6	
Weight gain (g)	Gestation	100 ± 6.9	80 ± 5.9*	
	Lactation	22.9 ± 6.1	17.6 ± 8.9	
Litter size		9.89 ± 0.93	7.45 ± 1.11	
Pup birth weight (g)		6.53 ± 0.15	6.56 ± 0.24	
Litter weight (g)		62.1 ± 6.9	46.5 ± 6.6	
Group (offspring fostered)		20CC	20LC (15-->0)	20LL
Number of litters [2]		17	10	14
Survival of pups to weaning		100%	91%	84%
Eye opening (days of age)		14.0 ± 0.13	13.8 ± 0.22	15.0 ± 0.26*
Pup weight at weaning (g) [3]		38.39 ± 1.19	38.78 ± 2.12	35.01 ± 1.04
Relative organ weight (g/100g body weight) [3]	Kidney	1.17 ± 0.04	1.16 ± 0.02	1.23 ± 0.03
	Liver	4.27 ± 0.11	4.10 ± 0.14	4.27 ± 0.09
	Spleen	0.4838 ± 0.037.2	0.4841 ± 0.0297	0.4224 ± 0.0170

[1] Data are numbers or averages ± SE. Data for rats on low protein diet (10% casein) were omitted from table.

[2] There appears to be an inconsistency between the number of deliveries reported for prenatal results (groups 20C and 20L) and the number of litters reported for postnatal results (groups 20CC, 20LC, and 20LL).

[3] These data refer to three week old females sacrificed for determination of organ weights. N = 6-9.

* $p < 0.05$ statistically significant difference from control, ANOVA and multiple range test.

Sechzer at al. (1986)

This study compared the effects of two stable lithium isotopes: ^{6}Li (atomic mass 6) and ^{7}Li (atomic mass 7). These are present in naturally occurring lithium (Li-N) at 7.4% and 92.6% abundance, respectively. Both of the pure isotopes and the natural mixture were administered separately to the rats in two experiments. In experiment 1, female Sprague-Dawley rats were treated with lithium chloride in water/orange juice solution with target doses of 0 or 2.0 meq Li/kg/day (0 or 14 mg Li/kg/d) for 10 days prior to mating, and for

gestation and lactation (to pnd 28). There were 20 mature females total (probably 5/group). Pups were culled to 8 per litter on pnd 3. Actual doses of lithium were reported to be 2.4 meq/kg/d (16.8 mg Li/kg/d) during gestation and 2.8-3.7 meq/kg/d (19.6-25.9 mg Li/kg/d) during lactation. Reduced grooming, alertness, and general activity were observed in the ^{7}Li and Li-N treated maternal females. Reduced food seeking activity was observed in ^{7}Li treated maternal females. Increased grooming and alertness was observed in ^{6}Li treated maternal females. The authors also noted that no cannibalization of pups occurred in lithium treated groups. No other maternal results were reported. The authors stated that lower birth weight was observed for all lithium treated litters (no numerical data were reported). Reduced grooming, nursing, and retrieval of pups by Li-N and ^{7}Li treated maternal rats was observed. Increased nest building, grooming, and nursing of pups by ^{6}Li treated maternal rats was observed. Delayed postnatal development in pups from lithium treated litters (eye opening, startle response, depth perception) was observed. Reduced spontaneous motor activity at 4 months was observed in all lithium treated pups (statistically significant only for ^{6}Li vs. controls). Data from this study are summarized in Table 41.

Table 41. Selected results from rat reproductive/developmental study, experiment 1, by Sechzer et al. (1986) [(1)]

Group (Li isotope)		Control	Li-N	^{6}Li	^{7}Li
Target dose [meq Li/kg/d (mg Li/kg/d)]		0 (0)	2 (14)	2 (14)	2 (14)
Number of pups examined for endpoints below		8 female 8 male	9 female 6 male	8 female 7 male	6 female 7 male
Pup weight (g)	Pnd 5	15.0	9.6	12.9	10.4
	Pnd 28	81.0	71.5	77.2	83.0
Age of eye opening (days)		12	18	18-20	19-20
Age of startle response (days)		12	15	14	15
Number of offspring examined for endpoint below		8 females 8 males	6 females 6 males	6 females 6 males	6 females 7 males
Spontaneous motor activity at 4 months		154	123	84*	131

[(1)] Data are numbers or averages. No indices of variation (e.g. SD) were reported.
* $p < 0.05$ statistically significant difference compared to controls, Student's t-test.

Experiment 2 was similar to experiment 1, except the target doses used were 0 or 4 meq Li/kg/d (0 or 28 mg Li/kg/d). No maternal results were reported. For 4 meq Li/kg/d, the authors stated that similar developmental delays to 2 meq Li/kg/d were observed, but of longer duration. No numerical data were reported.

Sechzer et al. (1992)

This study was reported in abstract only. Female rats (strain not reported) were treated with lithium (unspecified salt) in saccharin-sweetened water at 0 or 3 meq/kg/d (0 or 21 mg Li/kg/d) prior to breeding, and during gestation and lactation. Numbers of animals were not given. No maternal toxicity results were reported. Lithium treated females showed "maternal neglect" of pups: absence of nest building, short and infrequent periods of nursing, failure to retrieve pups, and poor grooming of pups. Pups showed developmental delays: reduced weight, delayed eye and ear opening, delayed appearance of depth perception, delayed initiation of startle response. At four months of age, spontaneous motor activity in the lithium treated offspring was 20% below that of controls.

Trautner et al. (1958)

Trautner et al. reported a number of experiments in rats. In experiment 1, Wistar rats (sex not specified) were treated with lithium chloride at 10, 20, 30, or 50 meq Li/L (70, 140, 210, or 350 ppm) in drinking water for up to 2 years. All rats died in 2-3 weeks at 50 meq/L. Plasma lithium concentrations exceeded 8 meq/L just before death. All rats died in 3-9 weeks at 30 meq/L. Plasma lithium concentrations were about 3 meq/L during a pseudo-stable phase, but increased shortly before death. There was no effect on survival at 20 or 10 meq/L: most animals survived up to 2 years. A transient drop in water consumption was observed at 20 meq/L. Plasma lithium concentrations were 1.5-2.0 and 1 meq/L for the 20 and 10 meq/L water concentrations, respectively. Possibly small increases in estrus cycle length and gestation period were observed in the group treated at 20 meq/L.

Experiments 2 and 3 from this study are discussed below in sections D.3.2.3 and E.3.2.2.

In experiment 4, female Wistar rats were treated with lithium chloride at 0 or 20 meq/L (0 or 140 ppm) in water for 3-7 weeks before mating and during gestation. Animals were allowed to give birth normally. Two series were reported: in series one there were 44 control and 16 lithium treated females, in series two there were 22 control and 13 lithium treated females. No maternal toxicity results were reported. Litter size in the lithium treated group was observed to be lower than controls in series 2, but not series 1. The authors comment that control litter size was low by historical standards in series one, and that the group was "much handled." The authors stated that no gross external malformations or difference in birth weight were observed in pups from lithium treated mothers. The authors also commented that early postnatal growth was slower in pups from mothers maintained on lithium. Except for litter size, no numerical data were reported. Data from this study are summarized in Table 42.

Table 42. Selected data from rat reproductive study, experiment 4, Trautner et al. (1958) [1]

Series	Series 1		Series 2	
Group : meq Li/L water (ppm)	0 (0)	20 (140)	0 (0)	20 (140)
Number of pregnancies	44	16	22	13
Total litter size	6.09 ± 2.195	6.19 ± 1.703	8.68 ± 1.426	5.69 ± 1.734
Live litter size	5.86 ± 2.501	6.00 ± 2.150	8.52 ± 1.662	5.38 ± 2.349

[1] Data are numbers or averages ± SD. No statistical tests were performed.

In experiment 5, female Wistar rats were treated with lithium chloride in water at 0 or 20 meq/L (0 or 140 ppm) for 3-7 weeks before mating and during gestation. Animals were laparotmized on gd 16-18 to examine numbers of corpora lutea, implants, and viable fetuses, then allowed to give birth. There were 31 females/group. No maternal toxicity results were reported. Reduced corpora lutea, implants, and viable fetuses were observed at 20 meq/L (statistically significant).

In experiment 6, female Wistar rats were treated with lithium chloride in water at 20 meq/L (140 ppm) for 3-7 weeks before mating, during gestation, and lactation. Animals were allowed to give birth. Pups were treated at the same concentration. At 6-7 months of age, female offspring were mated with untreated males. Females were laparotomized on gd 16-18. There were 14 lithium treated females. There were no concurrent controls in this experiment, but this group was compared to the control animals in experiment 5. The authors stated that no effect on maternal body weight at time of mating was observed. Reduced corpora lutea and viable fetuses (statistically significant), and lower implants (not statistically significant) compared to experiment 5 controls were observed. Data from this study are summarized in Table 43.

Table 43. Selected results from rat reproductive study, experiments 5 and 6, Trautner et al. (1958) [1]

Group	Control (0 meq Li /L)	1 generation (20 meq Li /L)	2 generation (20 meq Li /L)
Number of females	31	31	14
Corpora lutea/female	10.22 ± 1.316	9.02 ± 1.305**	8.93 ± 0.923**
Implantations/female	9.39 ± 1.726	7.90 ± 1.922**	8.42 ± 1.154
Viable fetuses/female	8.65 ± 1.872	7.23 ± 1.745**	7.29 ± 1.488*

[1] Data are numbers or averages ± SD.
* $p < 0.05$ difference from controls by Student's t-test.
** $p < 0.01$ difference from controls by Student's t-test.

C.3.4. In vitro developmental toxicity studies of lithium

A number of studies have examined the effects of lithium upon rodent embryos or embryonic stem cells in vitro. At sufficiently high concentrations, a variety of adverse effects were observed. The specific effects and concentrations required varied depending upon the study design and species.

C.3.4.1. Preimplantation embryos and lithium exposure

Fernandez and Izquierdo (1983)

Two cell stage mouse embryos were cultured in vitro in the presence of 0 or 15 mM lithium chloride for 66-70 hours. Reduced success in an in vitro implantation model was observed for lithium treated embryos.

Izquierdo and Becker (1982)

Two cell stage mouse embryos were cultured in vitro in the presence of 0 or one of several lithium chloride concentrations from 2 to 30 mM for 48 hours. Relative cell number was decreased in a concentration related manner at all concentrations of lithium chloride. Timing of blastulation was not affected.

Rogers and Varmuza (1996)

Two cell stage mouse embryos were cultured in vitro in the presence of 0, 0.9, 9.0, or 90 mM lithium chloride for 3-5 hours. Embryos were implanted in gd 0.5 pseudo-pregnant females and assessed on gd 9.5. Frequency of implantation was similar at all concentrations. Increased resorptions and abnormalities were observed at 90 mM lithium.

C.3.4.2. Postimplantation embryos and lithium exposure

Brown et al. (1991)

In this study, reported in abstract only, gd 9.0-9.5 Wistar rat embryos were cultured in 0 or 8 mM lithium chloride for 4 hours. Left/right asymmetry was assessed from heart looping and embryo turning. "Aberrations" of left/right asymmetry were observed in the lithium treated embryos.

Garcia-Palmer et al. (1988)

In this study, reported in abstract only, gd 9.5-10.5 rat (strain not given) embryos were cultured in vitro in the presence of 0, 1, 3, or 4 mM lithium (salt not given) for one day. Growth retardation and increased frequency of malformations were observed at all concentrations. The authors also state that myo-inositol distribution was altered, but the concentration at which this occurred was not reported.

Hansen et al. (1989, 1990)

This study compared the effects of lithium on mouse and rat embryos cultured in vitro. Mouse embryos were cultured from gd 8-10 and rat embryos from gd 9-11 or 10-12. The authors stated that these ages of embryos were chosen because mouse gd 8 and rat gd 10 embryos both have approximately 10 somites. Lithium concentrations (from lithium carbonate) were 0, 0.6, 1.2, 1.8, 2.4, or 5.0 mM. In rat embryos cultured from gd 10-12, retarded morphological development and increased frequency of non-viable embryos were observed at 1.8 mM and higher. No increase in open neural tubes was observed. In rat embryos cultured from gd 9-11, retarded morphological development was observed at all concentrations tested. Frequency of non-viable embryos was increased at 2.4 and 5.0 mM. Inconsistent increases in frequency of open neural tubes were observed. This observation was difficult to interpret due to the high frequency in controls. In mouse embryos, retarded morphological development was observed at 2.4 and 5.0 mM. No increase in frequency of non-viable embryos was observed. Increased frequency of open neural tubes was observed at 5.0 mM.

Klug et al. (1992)

Wistar rat embryos were cultured in vitro beginning on gd 9.5 for two days. In experiment 1, the embryos were cultured with lithium chloride at 0, 50, 100, 150, or 200 µg/ml (0, 1.4, 2.8, 3.5, 4.7 mmol Li/L) for 2 days. Crown-rump length was reduced (statistically significant, concentration-related) at 50 ug/ml and up. Dysmorphogenesis was increased at 150 µg/ml and up. In experiment 2, embryos at gd 9.5 were cultured with lithium chloride at 150 µg/ml plus myo-inositol at 0, 3.5, 7.0, 10.5, 28, or 56 mM. Myo-inositol had no beneficial effect on the lithium treated embryos. Crown-rump length was reduced at 3.5 mM myo-inositol and up. Increased gross structural deviations were observed at 10.5 mM myo-inositol and up.

C.3.4.3. Embryonic stem cells and lithium exposure

Schmidt et al. (2001)

This study examined the effects of lithium on differentiating mouse embryonic stem cells as part of a European Union screening validation. Embryonic stem cells were induced to form "embryoid bodies" and differentiate. They were exposed to 0, 0.1, 0.5, 1, or 5 mM lithium chloride. Cardiac muscle cell and skeletal muscle cell type differentiation was inhibited at 1 and 5 mM. No effect was observed on neuronal cell type differentiation.

C.4. Other relevant data.

The distribution of lithium in the pregnant female, fetus and offspring is discussed in section B.3.1.

C.5. Developmental toxicity: integrative evaluation of bromacil lithium salt

There are no developmental toxicity studies on bromacil lithium salt, and so evaluation of its toxicity therefore must rely on its dissociation products, bromacil and lithium.

C.5.1. Data on the developmental toxicity of bromacil

There are no human data on the developmental toxicity of bromacil. Four developmental toxicity studies of bromacil in experimental animals are available. All were performed with fixed designs and endpoint evaluations for pesticide registration purposes. Studies evaluating hypotheses concerning the characteristics and mechanism of bromacil on development were not available. Study results can therefore only be integrated in terms of consistency across studies. The interaction between maternal and fetal toxicity is also an issue integrating the results of the studies.

Bromacil effects were found on fetal weight and skeletal ossification and variations in three of the four studies; an oral study in rats, an inhalation study in rats and an oral study in rabbits (Newell and Dilley, 1978; Alvarez, 1988; Zellers, 1987). The fourth (oral rabbit) study, which did not report effects, used lower doses and a smaller sample size than the studies that reported effects (Hazleton, 1966).

Fetal weight and ossification effects occurred at a daily dose as low as 1.83 mg/kg/d in an inhalation study (Newell and Dilley, 1978), while oral studies (Zellers, 1987; Alvarez, 1988) using gavage administration had effective doses for these effects at 200 to 500 mg/kg/d. The lower effective dose in the inhalation study may have been due to the delivery of the bromacil as an aerosol dissolved in DMSO, a solvent with high epithelial penetrance. The inhalation route also avoids a first pass effect in the liver, where bromacil is metabolized.

Maternal toxicity must be considered in the interpretation of developmental toxicity findings. No maternal toxicity was noted in the inhalation toxicity study which monitored maternal weight, food intake and clinical observations. In the rat gavage study (Alvarez 1988), maternal weight gain and food intake were reduced during the first two days of the nine day dosing period in the three highest of the four dose groups. Food intake was also lower during the second two days in the two highest groups although weight gain recovered. Subsequently food intake and weight gain were not lower than controls and in fact were greater than controls after discontinuation of dosing. The rats in the highest dose groups also had heavier livers than controls at the end of the study.

To determine experimentally whether the brief period of reduced food intake and weight gain could be responsible for the fetal skeletal variations and retardation noted in the Alvarez (1988) study, a pair fed group would be necessary. Pair feeding groups are not part of the standardized study designs used for pesticide registration. However, developmental toxicity studies in open literature have used pair feeding during embryogenesis to investigate this issue. A study in rats (Dostal and Anderson, 1995) included a pair fed group with food intake restricted to 33% of control during organogenesis (gd 6-15). Pair fed dams lost 33 g during dosing as compared to a weight gain of 40g in controls. Over all of gestation the pair fed group gained 68 g as compared to 147 g in controls. Fetal weights were significantly lower (10%) in the pair fed group than in controls. No effects on skeletal ossification or variations were found in the fetuses from the pair fed group.

Developmental toxicity (increased skeletal variations) in the rabbit gavage study (Zellers 1987) was also accompanied by reduced maternal food intake and weight gain early in the dosing period. In a study from the open literature (Petrere et al. 1993) rabbits were restricted in their food intake during organogenesis to 68%, 34%, or 7% of ad lib fed controls. No effect of the food restriction on the incidence of skeletal variations was found in this study.

These data therefore suggest that fetal skeletal variations seen in the rat and rabbit bromacil studies were not due to reduced food intake.

C.5.2. Human data on developmental toxicity of lithium

Although earlier studies reported an increased risk of congenital malformations with exposure to lithium during pregnancy, specifically cardiac malformations, later studies did not find significant associations. Various factors may account for these inconsistent findings including different study designs, varying power to detect an association, difficulties in the accurate assessment of the outcome of concern (i.e. Ebstein's anomaly), and lack of quantitative exposure assessment. In regard to the latter, none of the studies examining the risks of teratogenicity reported serum lithium levels, and only one of the studies reviewed here reported the amount of lithium taken by the women, reported as mean daily dose (Jacobson et al., 1992). As mentioned previously, lithium has a narrow therapeutic window where it is beneficial but not toxic. Although it could be assumed

that lithium levels in all patients were within therapeutic range, many factors can affect serum lithium levels and even though monitoring of levels is recommended, research suggests that in some cases monitoring is not being conducted as regularly as needed (Kehoe and Mander, 1992).

Evidence for a threshold effect of lithium dosage on duration of gestation in humans has been shown by Troyer et al. (1993), as well as on adverse birth outcomes in animals. In the study by Jacobson et al. (1992), the authors did note that "the teratogenicity of lithium might be dose-related, as shown in animals" and hypothesized that had the doses used at the time the Scandinavian Register data were obtained been higher than present recommended doses, this could explain the discrepancy between the two study results. The authors then discounted this possibility by suggesting that "there is no evidence that lithium doses have changed during this time", citing Goodman and Gilman, (1985). However, the Jacobson study, which includes data collected from 1979 until 1991, reported lower mean daily doses of lithium (927 ± 340 mg/d (SD)) as compared with those reported in Troyer et al. (1993), which includes Scandinavian Register data from 1968 to 1983 (mean for included infants = 1089 ± 620 (SD); mean for excluded infants = 954 ± 435 (SD)). Thus varying levels of lithium exposure may explain the inconsistent findings of earlier versus later human studies.

Additional factors that may affect serum lithium levels which were not considered in these studies include the formulation of lithium, immediate versus slow release, as well as the use of anti-inflammatory drugs. Administration of slow release lithium formulations can result in lower peak lithium levels (Ward et al., 1994). Since lithium intoxication has been associated with peak lithium levels and the rate of rise following administration, it may be that these peak levels are more important with respect to potential teratogenicity than either the prescibed dosage of lithium or the lithium level traditionally monitored at 12-18 hours after the last dosage taken, approximately 9-15 hours after the peak level. With respect to anti-inflammatory drugs, it is now recognized that indomethacin as well as other nonsteroidal anti-inflammatory drugs can increase serum lithium levels. However, this was only reported in the late 1970's and none of the studies mentioned above reported collecting information from the patients on their use of such drugs during early pregnancy.

C.5.3. Data on the developmental toxicity of lithium in experimental animals

There are several considerations with regard to hazard identification for the possible developmental toxicity of lithium. Most studies used a single dose or concentration of lithium. Very small numbers of animals were used in some studies. Maternal toxicity results were seldom reported or reported incompletely. Commonly, only a subset of developmental results were reported, and qualitative results were typically reported in the text, but numerical data were not presented.

Many of the experimental animal studies which used one of the oral routes (i.e., gavage, drinking water, or food) have reported one or more adverse effects on developmental

endpoints following prenatal treatment. These include death, delayed development, and structural anomalies. Death of the embryo, fetus, or neonate has been observed in mice (Chernoff and Kavlock, 1982, 1983; Gray et al., 1983, 1986; Gray and Kavlock, 1984; Seidenberg et al., 1986; Seidenberg and Becker, 1987; Szabo, 1969, 1970, Szabo et al., 1970; Krmpotic and De la Torre, 1976; Mroczka et al., 1983; Smithberg and Dixit, 1982) and in rats (Fritz, 1988; Marathe and Thomas, 1986; Hsu and Rider, 1978; Rider and Hsu, 1976; Trautner et al., 1958; Canolty et al., 1989; Ibrahim and Canolty, 1990). Delayed development (e.g., reduced fetal or birth weight) has been observed in mice (Laborde and Pauken, 1995; Matsumoto et al., 1974, 1975; Messiha, 1989, 1992, 1993) and rats (Marathe and Thomas, 1986; Sechzer et al., 1986; Sharma and Rawat, 1986; Teixiera et al., 1995; Ibrahim and Canolty, 1990). Structural anomalies (e.g., cleft palate) have been observed in mice (Matsumoto et al., 1974, 1975; Szabo, 1969, 1970; Szabo et al., 1970b; Laborde and Pauken, 1995) and rats (Fritz, 1988; Marathe and Thomas, 1986; Sharma and Rawat, 1986).

In several studies adverse developmental effects were observed following exposure of the nursing mother to lithium. However, developmental effects which are due to postnatal exposure are outside of the purview of Proposition 65's consideration of developmental effects.

In many of the experimental studies which used oral routes, specific adverse developmental effects were reported not to occur. This raises the question of whether the adverse effects reported in other studies were reproducible. However, examination of the studies indicates that the study designs were generally not the same. Specifically, differences in species, strain, route, dose or concentration, duration, and endpoints for which results were reported usually preclude rigorous comparison of studies. In most cases, the differences in outcomes may be attributed to differing study designs.

The variety of adverse developmental effects which have been reported in experimental animal studies indicates that there may not be a unique lithium toxicity syndrome, such as the heart malformations reported in humans. In experimental animals, the specific adverse effects of lithium on development appear to vary according to study design. Only one study specifically mentioned that examination of the fetuses included special examination of the heart (Laborde and Pauken, 1995). This study was reported only in abstract, and no mention of effects on the heart was made.

The fact that many of the oral studies used a single dose or concentration may contribute to the finding of effects or lack of effects on specific endpoints. There is evidence that, at least in some cases, lithium has a very steep dose-response relationship. For example, in long term exposure of rats by drinking water reported by Trautner et al. (1958), all rats treated at 50 or 30 mmol Li/L died prematurely, whereas there was little or no effect on survival at 20 or 10 mmol Li/L. If the dose-response relationships for developmental effects are similarly steep, then small variations in dose or concentration could combine with other experimental variables to produce the variations in effects or lack of effects between studies.

Evaluation of the contribution, if any, of maternal or systemic toxicity to adverse developmental effects in these experimental animal studies is difficult. Many studies did not report maternal toxicity results at all, and those that did report results were not complete.

In vitro tests may shed some light on the mechanism(s) of lithium developmental toxicity. In vitro tests have found adverse effects on the conceptus at varying concentrations of lithium in the culture medium. Some of these effects occurred at concentrations similar to those found in the plasma or serum of animals treated by oral routes (i.e. up to 1-2 mmol Li/L), while others occurred only at higher concentrations.

There have been several studies that used injection as the route of exposure. In general, these studies have found adverse developmental effects. As with the oral studies, the specific effects observed vary. Also as with the oral studies, the specific study designs also vary, which may account for some of the differences in effects observed. Studies of the serum or plasma levels of lithium following injection or oral exposures suggest that injection produces a higher peak level of lithium than does oral exposure. As a result, the injection studies of lithium may be less relevant to hazard identification of bromacil lithium salt than oral lithium studies. However, the injection studies, in general, are supportive of the oral studies that observed adverse developmental effects of lithium treatment.

C.5.4. Comparison of bromacil and lithium developmental toxicity dose levels

As discussed earlier in this document, bromacil lithium salt applied as an herbicide is expected to dissociate readily into approximately equal molar quantities of bromacil and lithium. The exposures to bromacil and lithium that may result from application of bromacil lithium salt will not necessarily be equal, and may vary depending on the dispersion and persistence of each of the dissociation products. The levels of exposure to bromacil and lithium that are associated with developmental toxicity are discussed below, with the units of exposure expressed in mmol/kg/d for ease of comparison.

The study designs which were employed in the developmental studies for bromacil are mostly standard pesticide registration designs. In contrast, the developmental studies for lithium have a variety of designs, most of them unlike the standard pesticide registration designs. As a result, there are few opportunities to closely compare the results. One reasonably close comparison is the bromacil study by Alvarez (1988) to the lithium studies by Marathe and Thomas (1986) and Gralla and McIlhenny (1972) (experiment 3). These were all studies in rats by gavage for the middle 10-11 days of gestation. A more distant comparison can be to the lithium studies by Fritz (1988). This series of studies was in rats by gavage for 5 day periods during gestation. Effects and effective doses from these studies are summarized in Table 44.

Table 44. Comparison of effects from rat developmental studies by gavage using bromacil and lithium.

Reference	Chemical	Strain	Duration	DART effects	**NOEL**, LOEL and higher effective levels [mmol/kg/d (mg/kg/d)]
Alvarez (1988)	Bromacil	Sprague-Dawley	Gd 7-16	Increased resorptions? (not statistically significant)	**0.29**, 0.77, 1.9 (**75**, 200, 500)
				Increased skeletal variations	**0.29**, 0.77, 1.9 (**75**, 200, 500)
				Increased skeletal retardations	**0.77**, 1.9 (**200**, 500)
Gralla and McIlhenny (1972) Exp. 3	Lithium carbonate	albino	Gd 5-15	None	**4.05** (**28**)
Marathe and Thomas (1986)	Lithium carbonate	Wistar	Gd 6-15	Reduced implantations, litter size and fetal weight, increased resorptions, skeletal malformations and alterations	**1.35**, 2.7 (**9.4**, 18.7)
Fritz (1988) Exps. 1 and 2	Lithium carbonate	Sprague-Dawley	Gd 16-20	Increased embryonic and fetal death, reduced live litter size, enlarged renal pelves	2.7 (18.7)
Fritz (1988) Exp. 3	Lithium carbonate	Sprague-Dawley	Gd 16-20	Reduced litter size on pnd 1	1.6 (11.1)

In the bromacil study (Alvarez 1988), developmental effects were observed at 0.77 and 1.9 mmol/kg/d (200 and 500 mg/kg/d). Increased skeletal variations (statistically significant) were observed at both doses, and increased skeletal retardations (statistically significant) were observed at the high dose. The authors state that increased resorptions were observed at both doses. However, this effect was not statistically significant, and the response at the highest dose was lower than the response at the next highest dose. In the lithium carbonate study by Marathe and Thomas (1986), reduced implantations, litter size, and fetal weight, and increased resorptions, skeletal malformations, and alterations were observed at 2.7 mmol/kg/d (18.7 mg Li/kg/d). In the lithium carbonate study by

Gralla and McIlhenny (1972), the authors made a general statement that no developmental effects were seen at exposures up to 4.05 mmol/kg/d (28 mg Li/kg/d). However, no numerical data were presented, and the study is so briefly described that evaluation of its validity is very difficult. Using shorter exposure to lithium during a later period of gestation, Fritz (1988) found increased embryonic and fetal death, reduced live litter size, and enlarged renal pelves at 2.7 mmol/kg/d (18.7 mg Li/kg/d). Reduced litter size on pnd 1 was also observed at 1.6 mmol/kg/d (11.1 mg Li/kg/d).

Thus, overall, it appears that adverse developmental effects have been observed in rats at similar molar doses for bromacil (0.77 and 1.9 mmol/kg/d) and lithium (1.6 and 2.7 mmol/kg/d). Excluding the questionable effect on resorptions, the effects at these doses from bromacil were relatively less severe than were the effects from lithium. Maternal toxicity in the bromacil study by Alvarez (1988) was mild: approximately a 20% reduction in maternal weight gain during treatment, with some compensatory increase in weight gain after treatment ceased. Acute LD_{50}s with bromacil for rats of the same strain were reported to be 6.1 and 4.0 mmol/kg for males and females, respectively. Thus, the doses at which developmental effects were observed were about one-fifth and one-half of the LD_{50}. No maternal toxicity results were reported for the lithium study by Marathe and Thomas (1986), so this factor is very difficult to evaluate. Acute LD_{50}s with lithium for male rats of the same strain by gavage were reported to be 12.4-19.8 mmol/kg. Thus, the dose at which developmental effects were observed was perhaps (ignoring sex differences) about one-fifth of the LD_{50}.

D. Female reproductive toxicity

D.1. Female reproductive toxicity of bromacil lithium salt

No studies of the possible female reproductive toxicity of bromacil lithium salt were located. No human studies relevant to the possible female reproductive toxicity of bromacil were located. There are experimental animal studies relevant to the possible female reproductive toxicity of bromacil. These were conducted for pesticide registration purposes. There is one study of the effects of lithium administration on human female reproductive hormones, and two studies on female sexual function. There are also several experimental animal studies relevant to the possible female reproductive toxicity of lithium. These were found in the open literature.

D.2. Female reproductive toxicity of bromacil

Three studies directly involving reproduction were located. Also, several chronic studies examined reproductive tissues.

D.2.1. Reproduction studies with bromacil

Haskell (1966)

An early three-generation study was conducted in rats using 0 and 250 ppm bromacil (0, 11 mg/kg/d) in diet. To conduct this reproduction study, twelve CD rats/sex/group were removed from a larger chronic study after 12 weeks of exposure for a mating trial. Their offspring then provided the second and third generation. No effects on fertility or pregnancy outcome were reported. Pathology was conducted only for 10 rats/sex/group of the F3b weanlings and no treatment effects were reported.

Mullin (1991)

A two-generation study with one litter per generation was conducted in CD rats. The concentrations were 0, 50, 250 and 2,500 ppm bromacil in food. These corresponded to average doses for males and females for both generations during the premating period of 3, 18, and 181 mg/kg/d. Parental and developmental toxicity were elicited only in the F1 generation at the high dose and consisted of lower weights or reduced weight gain.

There were statistically significant reduced body weights in the high dose F1 males after weaning (about 8%); and the premating weight gain of the F1 males was significantly less than controls (8.4%). F1 females had significantly lower body weights prior to weaning on pnd 7 and 21 but not as adults. Premating weight gain of the F1 females was 7.6% lower than controls, though this difference was not statistically significant. Gestational and lactational weight gains were not affected; the high dose F1 females gained more weight than controls during lactation. No statistically significant effects on weight or weight gain were seen in the P generation. There was no organ pathology in the parents or offspring. Thus, general systemic toxicity was minimal at the doses used in this study, which were selected from rat chronic and subchronic studies.

No DART endpoints were affected by treatment (Table 45). These included mating and fertility indices, and offspring viability (litter size, live litter size, postnatal viability indices); viability was greater in the 2500 ppm group. Birthweight was not affected, although there were treatment effects on postnatal growth as described in the previous paragraph. There were no effects on reproductive organ weights, gross or histopathology of offspring.

Table 45. Fertility measures from the rat two-generation study (Mullin 1991). [1]

Bromacil concentration (mg/kg food)	0	50	250	2,500
bromacil dose (average for males and females for both generations during the premating period) (mg/kg/d)	0	3	18	181
P1 generation				
n (number of females)	30	30	30	30
mating index (%) [2]	100	100	100	96.7
fertility index (%) [3]	70	80	77	86
gestation length (days)	22	23	22	22
F1 generation				
n (number of females)	29	29	29	29
mating index (%)	90	97	83	100
fertility index (%)	65	68	83	72
gestation length (days)	23	23	23	23

[1] Data are numbers, averages, or percentages. Indices of variation (e.g. SD) were not reported. No statistically significant effects were observed.
[2] Number copulated/cohoused.
[3] Number delivered/copulated.

Bishop et al. (1997)

Bromacil was one of 29 agents evaluated in a screening test for "Total Reproductive Capacity" in mice. Bromacil was not one of the 17 chemicals found to affect Total Reproductive Capacity. In this paradigm, 30-36 female C57Bl/6 hybrid mice 10-12 weeks of age received one i.p. injection at a dose in the maximally tolerated range as determined from pilot studies. (This dose was not stated for bromacil). Total Reproductive Capacity was determined as the number of litters and number of offspring produced by each treated female when they were continually housed with a male for 347 days and each litter produced was removed at birth.

D.2.2. Reproductive organ effects with bromacil

Female reproductive organs were examined histopathologically in chronic dog, rat (Haskell 1966; Bogdanffy 1989; Bogdanffy 1991) (see Table 7) and mouse (Wood 1980) studies. Organ weights were not obtained. No treatment-related findings were reported in these studies.

D.3. Female reproductive toxicity of lithium

One study in humans which examined the effect of lithium treatment upon female reproductive hormones was located. Two studies of effects on female sexual function were located. There are several experimental animal studies with information relevant to the potential female reproductive toxicity of lithium. All of these studies were found in the open literature. The species studied were mouse and rat. These studies are by oral or injection routes. Some of these studies reported the plasma or serum concentration of lithium. Generally the objective was to achieve plasma or serum concentrations in the same general range as used in human therapy; i.e., about 0.5 – 1.0 meq Li/L. There are several considerations with regard to hazard identification for the possible female reproductive toxicity of lithium. Most studies used a single dose or concentration. Some studies used small numbers of animals. Maternal toxicity results were seldom reported, or were reported incompletely. Commonly, only a subset of female reproductive results were reported, and qualitative results were reported in the text, but numerical data were not presented.

D.3.1. Human female reproductive toxicity studies with lithium

Baptista et al. (2000)

In this study, healthy female premenopausal medical students were first evaluated for one menstrual cycle, and then randomly assigned to receive either lithium carbonate (n = 10) or placebo (n = 13) for the following menstrual cycle. Thus, for each woman the first menstrual cycle served as her baseline cycle. Hormone levels during treatment were monitored the same day as in the baseline cycle. The lithium carbonate was administered at 300 mg, three times per day. The women were evaluated for lutenizing hormone (LH), follicle stimulating hormone (FSH), 17-B estradiol, progesterone, prolactin, thyroxine, thyrotropin (TSH), cortisol, dehydroepiandrosterone sulfate, free testosterone, leptin, and an oral glucose test. At 15 hours after the last dose, serum lithium was measured at 0.31 meq Li/L. No effects on reproductive hormones were observed. All study subjects had normal menstrual cycles. The only significant effect observed was elevated TSH (approximately doubled in the lithium group, $p = 0.001$).

Ghadirian et al. (1992)

The authors for this retrospective study surveyed sexual function in 104 outpatients (45 men and 59 women) with bipolar disorder who were under treatment with lithium, either alone (35%) or in combination with benzodiazepines (49%), tricyclic antidepressants (17%), neuroleptics (17%), tryptophan (10%), or carbamazepine (1%). The patients were in a stable and euthyroid state at the time of the assessment. No control group was included in the study and the survey was self-completed. Female patients received lithium at an average dose of 943 mg/day. Average serum lithium in female patients was 0.58 mmol/L. The authors reported that among 59 female patients, 32% had a change in

menstruation, 40% had decreased sexual desire, 24% had increased sexual desire, 24% had decreased quality of orgasm, 22% had increased quality of orgasm, and 25% had difficulty in sexual functioning. The authors did not report separate results for men and women treated with lithium alone. When male and female patients were combined for statistical analysis, difficulties in sexual functioning were significantly more common in patients treated with a combination of lithium and benzodiazepines (49%) than in those treated with either lithium alone (14%) or lithium in combination with other drugs (17%). However, no relation was found between serum lithium level and sexual dysfunction scores. The authors concluded that lithium, when given alone, is unlikely to affect sexual function in bipolar patients; but when given in combination with benzodiazepines, it was associated with sexual dysfunction in about half of the patients.

Kristensen and Jorgensen (1987)

In this study, the authors surveyed via interview-questionnaire the sexual function in 24 patients (10 women; 26-59 years old) with major affective disorders who were given continuous lithium treatment for 6-24 months. Controls were 42 surgical outpatients (25 women) with no known psychiatric disease. Patients in the control group were matched to the lithium-treated group by age. The average 12-h serum lithium concentration in lithium-treated patients was 0.64 (0.5-1.0) mmol/L. Sexual dysfunctions were described by four (30%) female patients and eight (28%) controls; the difference between the two groups was not statistically significant. Changes in sexual function following lithium treatment were described as "none" for 6 women, "positive" for two women, and "negative" for two women. Limitations of this study include the retrospective design and the small number of subjects. In addition, since the presence of the disorder itself may affect sexual function it is important to include a control group of patients with bipolar disorder not treated with lithium, but perhaps with some other drug.

D.3.2. Experimental animal female reproductive toxicity studies with lithium

D.3.2.1 Female reproductive toxicity studies in mice with lithium by oral routes

Banerji et al. (1986)

In experiment 2, female C57BL/6 mice were treated with lithium chloride in food at 0 or 0.4% (0 or 94 mmol Li/kg food) for at least 15 days. There were 20 females/group. No systemic toxicity results were reported. Plasma lithium was measured at 0.84 and 0.82 meq/L on days 5 and 8 in smaller parallel treatment groups. All lithium treated mice ceased estrus cycling and entered constant diestrus by day 8.

Gray et al. (1983, 1986), Gray and Kavlock (1984)

This was a follow up study to the Chernoff and Kavlock (1982, 1983) study described in the Developmental Toxicity section above (section C.3.2.1). This study is also described in that section. The objective was to examine additional postnatal endpoints. Mated female CD-1 mice were treated by gavage with lithium carbonate at 0 or 400 mg Li_2CO_3/kg/d (0 or 10.8 mmol Li/kg/d) for gd 8-12. It was not stated whether the dosing solution was treated with a "Polytron" or not. Females were allowed to give birth. Six randomly selected pups (3/sex) from the same treatment group were given to each dam on pnd 6. Pups were weaned on pnd 30. Offspring were housed 3 males and 3 females/cage for mating. "Obviously pregnant" females were removed to individual cages for gestation and delivery. There were 12 control litters, and 8 treated with lithium carbonate.

There was no effect on offspring viability on pnd 30 or figure 8 maze activity on pnd 22, 58, or 200. Among female reproductive endpoints reported, there was no effect on offspring vaginal patency on pnd 30, or the percentage which became pregnant, or age of parturition. Litter size was slightly lower, but the difference was not statistically significant. Data from this study are summarized in Table 46 below.

Table 46. Results from mouse developmental/reproductive study by Gray et al. (1983, 1986), Gray and Kavlock (1984) [(1)]

Dose : mg Li_2CO_3/kg/d (mmol Li/kg/d)		0 (0)	400 (10.8)
Number of litters		12	8
Pup viability pnd 30 (%)		86	88
Pup figure 8 maze activity	pnd 22	620	558
	pnd 58	816	768
	pnd 200	642	576
Vaginal patency pnd 30 (%)		90	89
Offspring pregnant (%)		100	100
Offspring age of parturition		67.7	66.6
Litter size		11.4	10.2

[(1)] Data are numbers, percentages, or averages. Indices of variation (e.g. SD) were not reported. There were no statistically significant differences from control.

Krmpotic and de la Torre (1976)

This study is also described in the developmental toxicity section above (section C.3.2.1). This study was reported in abstract only. ICR/SCI mice were treated with lithium chloride in water at 0 or 20 meq/L (0 or 140 ppm) (the duration of treatment was not specified, nor whether males and/or females were treated, nor the number of animals

treated; there were possibly 50 males and 100 females/group). Mice were mated one male to two females. Half of each group were sacrificed on gd 17, and half allowed to deliver. No systemic results were reported. Reduced fertility, and increased morbidity and mortality of offspring were reported.

Messiha (1986a)

This study is also described in the developmental toxicity section above (C.3.3.1). In experiment 1, female Sprague-Dawley mice were treated with lithium chloride in water at 0 or 1 meq Li/L (0 or 7 ppm) for 2 weeks before mating and for gestation. There were 11 control and 10 lithium treated, but it is not clear if these refer to maternal or pup numbers. No maternal toxicity results were reported. No effect on pup body or absolute liver weight 24-36 hours after birth was observed.

Mroczka et al. (1983)

This study is also described in the developmental toxicity section above (section C.3.3.1.). In experiment 1, male and female CFW mice were treated with lithium chloride in water at 0, 10, 20, 30, 50, 100, or 200 meq Li/L (0, 70, 140, 210, 350, 700 or 1400 ppm) beginning at 6-8 weeks of age, and, after 2 weeks, mated for multiple rounds of reproduction, apparently until reproduction ceased. Lithium treatment of females was continued through gestation and lactation. The numbers of animals were not reported. At 200 meq Li/L mice would not drink and all died in 1 week. Reduced water intake at 100 meq Li/L was observed (no numerical data were reported). No other parental toxicity results were reported at 100 meq Li/L. No reproduction occurred at 100 meq Li/L. Plasma levels of lithium were reported as 0.09 and 0.67 meq/L for water concentrations of 10 and 50 meq Li/L. No parental toxicity results were reported for concentrations from 10 to 50 meq Li/L. Although the numbers of animals used were not reported, the data suggest there were approximately 45 pairs of controls and 85 pairs treated at 50 meq/L. Reduced number of litters/mating pair, increased time between litters, and increased pup death during lactation at 50 meq Li/L were observed. No effect on litter size at birth at 50 meq Li/L was observed. Authors state there was no effect on "pup size" at 50 meq Li/L, but no numerical data were reported. No developmental or reproductive results were reported for 10, 20,or 30 meq Li/L Data from this study are summarized in Table 47.

Table 47. Selected results from mouse reproductive study by Mroczka et al. (1983) [1]

Concentration in water: meq Li/L (ppm)	0 (0)	50 (350)
Number of litters (total)	252	283
Number of litters/mating pair [2]	5.6	3.3
Litter size at birth	8.29 ± 0.235	7.809 ± 0.193
Litter size at weaning	7.202 ± 0.251	5.444 ± 0.228***

[1] Data are numbers, averages, or averages ± SEM.
[2] Data estimated by OEHHA staff from Figure 1, Mroczka et al. (1983).
*** $p < 0.001$ statistically significant difference from controls by Student's t-test.

In experiment 2, male and female CFW mice were treated with lithium chloride beginning at 3 weeks of age, and mated for multiple rounds of reproduction beginning at 8 weeks of age. The water lithium levels were not explicitly stated; however, the implication was that 0 and 50 meq Li/L (0 and 350 ppm) were used. No parental toxicity results were reported. The authors stated that no effect on pup weight at birth was observed, but no numerical data were presented. No litter size results were reported. Data presented only graphically indicated that weight gain during lactation was lower for lithium treated litters, and some differences persisted after weaning when offspring were switched to water without lithium. Some differences in absolute and/or relative brain, heart, liver, and kidney weights were observed in offspring from one to eight weeks of age.

D.3.2.2. Female reproductive toxicity study in mice with lithium by injection

Banerji et al. (1986)

In experiment 1, female C57BL/6 mice were injected (ip) with lithium chloride at 0 or 5 meq/kg (0 or 35 mg Li/kg) at 10:00, 16:00, and 18:00 hours on the day of proestrus. Mice were sacrificed at 21:00 hours. There were 6-9 females/group. No systemic toxicity results were reported. Plasma lithium was measured at 1.66 meq/L at sacrifice. Reduced plasma luteinizing hormone (LH) (statistically significant) was observed. Increased plasma and pituitary follicle stimulating hormone (statistically significant) was observed. No effect on pituitary LH was observed.

D.3.2.3. Female reproductive toxicity studies in rats with lithium by oral routes

Christensen et al. (1982)

This study is also described in the developmental toxicity section above (section C.3.3.2.). Female Wistar rats were treated with lithium chloride in food at 0 or 40 mmol Li/kg food (0 or 280 ppm) for 4 weeks, followed by 0 or 60 mmol Li/kg food (0 or 420

ppm) for 4 weeks, and during mating and gestation. There were initially 6 females/group. Females were allowed to give birth. Both groups were split so that half received control diet and half received lithium in food at 40 mmol Li/kg during lactation. Pups were assessed at 8-10 weeks postnatal. Plasma lithium in maternal animals after four weeks of treatment was 0.6 mmol/L. Increased water intake was observed in the lithium treated maternal group (5 fold during pre-mating treatment: statistically significant). Reduced body weight gain during gestation was observed in the lithium treated group (statistically significant). No effects were observed on litter size or pup weight at birth. In maternal animals, 3 weeks after delivery, plasma lithium was 1.15 and 1.47 mmol/L in the group treated prenatally and postnatally and the group treated only postnatally, respectively. In pups, plasma lithium was 0.54 and 0.51 mmol/L for the same groups, respectively. Offspring body weight at 8-10 weeks of age was similar to controls for the group treated prenatally, and lower than controls for both groups treated postnatally (not statistically significant). Kidney function was altered in offspring. In males, osmolality was reduced in both postnatally treated groups (statistically significant), but not the group treated only prenatally. Females were not tested for urine osmolality. In females, inulin clearance was increased in the group treated pre-natally only, and reduced in both groups treated postnatally (all statistically significant). Males were not tested for inulin clearance. Data from this study are summarized in Table 48.

Table 48. Selected results from rat reproductive study by Christensen et al. (1982) [(1)]

Group treatment before and during gestation (mmol Li/kg food)		0	40/60
Number of females mated		6	6
Number of females pregnant		6	5
Fluid consumption at 7 weeks pretreatment (ml/kg/d)		9 ± 3	50 ± 17**
Maternal weight gain during gestation (g)		98 ± 8	64 ± 8*
Litter size		11.1 ± 2.1	10.8 ± 1.3
Birth weight (g)		6.5 ± 0.7	6.2 ± 0.6
Offspring weighed at 8-10 weeks of age [(2)]	male	12	9
	female	8	8
Offspring weight at 8-10 weeks of age (g) [(2)]	male	224 ± 26	227 ± 27
	female	169 ± 12	163 ± 18

[(1)] Data are numbers or averages ± SD.
[(2)] Data for offspring treated with lithium prenatally only.
* Statistically significant difference from controls, p not reported. Student's t-test.
** $p < 0.01$ statistically significant difference from controls. Student's t-test.

Glockner et al. (1989)

This study is also described in the developmental toxicity section above (section C.3.3.2.). Female Wistar rats (P generation) were treated with lithium chloride at 0 or 20 mmol/L (0 or 140 ppm) in drinking water for 3 weeks before mating through gestation. Females were allowed to litter. Litters were culled to 6 pups, nursed and weaned. Female offspring (F1) were mated with untreated males, and allowed to litter. The numbers of females for the P generation were not reported. For the F1 generation females, there were 20 control and 30 from lithium treated mothers. In the P generation, reduced water consumption (80 ml/kg lithium treated vs. 140 ml/kg controls) was observed. Doses during pregnancy were 1.6, 1.98 and 2.08 mmol/kg/d (11.2, 13.9 and 14.6 mg/kg/d) for weeks 1, 2, and 3, respectively. Three weeks after the beginning of treatment, serum lithium was 0.96 mmol/L. No other P generation maternal results were reported. No developmental or reproductive results for the P/F1 litter were reported. In the F1 generation, no affect on maternal body weight or gain during gestation was observed. No other maternal F1 results were reported. For the F1/F2 litter, the authors stated that there was no effect on gestation length. However, Figure 1 shows lithium treated (in utero) mothers delivered less frequently on gd 21 than controls (18/30 vs. 18/20;
p = 0.02, Fisher Exact Test). Also, no controls delivered on gd 22, while 7/30 of the lithium treated group delivered on gd 22. For the F1/F2 litter, no effect on implantations, litter size, or pup body weight was observed. A reduced composite "skeletal ossification score" (statistically significant) in F2 pups from the lithium treated group compared to controls was observed. Data from this study are summarized in Table 48.

Table 48. Selected results from rat reproductive study by Glockner et al. (1989). [1]

Group: mmol Li/L water (ppm)	0 (0)	20 (140)
Implantations	12.5 ± 0.7	12.2 ± 0.5
Litter size	11.4 ± 0.5	11.2 ± 0.5
Pup body weight [2]	5.03 ± 0.06	4.98 ± 0.08
Pup skeletal ossification score (%) [2, 3]	49%	42%*

[1] Data are averages ± SEM or percentages. All data refer to the F1/F2 litter.
[2] For pups born on gd 21.
[3] Estimated by OEHHA staff from Figure 3, Glockner et al. (1989)
* $p < 0.05$ statistically significant difference from controls, Mann-Whitney test.

Gralla and McIlhenny (1972)

This study is also described in the developmental toxicity section above (section C.3.2.3.). Five experiments were briefly reported in this paper: three in rats, and one each in rabbit and monkey. In rat experiment 1, Charles River albino male rats were treated by diet with lithium carbonate at 0, 0.27, 0.67 or 1.35 meq/kg/d (0, 1.89, 4.7 or 9.4 mg Li/kg/d) for 70 days and female rats were treated by gavage at 0, 0.675, 2.025, or 4.05 meq/kg/d (0, 4.7, 14.2 or 28.4 mg Li/kg/d) for 14 days. Males and females were mated according to zero, low, middle, or high dose levels. There were 20 animals/sex/dose. Half the females were sacrificed on gd 13, and the other half allowed to deliver and nurse pups for 21 days. In experiment 2, pregnant female Charles River albino rats were treated by gavage with lithium carbonate at 0, 0.675, 2.025, or 4.05 meq/kg/d (0, 4.7, 14.2 or 28.4 mg Li/kg/d) from gd 14 to pnd 21. Females were allowed to litter and nurse pups. There were 10 females/dose. In experiment 3, pregnant female Charles River albino rats were treated by gavage with lithium carbonate at 0, 0.675, 2.025, or 4.05 meq/kg/d (0, 4.7, 14.2 or 28.4 mg Li/kg/d) from gd 5-15. Females were sacrificed on gd 20. There were 20 females/dose.

Treatment of male and female rats by gavage at 4.05 meq/kg/d for 3 days resulted in an average plasma lithium concentration of 1.4 meq/L (similar in males and females). In reporting results, the authors frequently did not distinguish between experiments 1, 2, and 3. The authors stated that two females treated at 4.05 meq/kg/d died. It is not clear if this refers to Experiment 1, 2, or 3, or all combined. The authors stated that there was no effect on parental body weight gain. No other parental results were reported. Reduced pup body weight was observed at the high dose on pnd 21 (statistically significant). However, it is not clear if this result refers to experiment 1 or 2. No effect was observed on pnd 1 or 4. The authors stated that no effects on fertility, implantation sites, litter size, offspring mortality, gross external or internal abnormalities were observed. Quantitative data were presented only for litter size and neonatal body weight, evidently from experiment 1 and/or 2. Data from this study are summarized in Table 49.

Table 49. Data from rat reproductive experiments 1 and/or 2, by Gralla and McIlhenny 1972. [(1)]

Dose: meq Li/kg/d (mg Li/kg/d)]		0 (0)	0.675 (4.7)	2.025 (14.2)	4.05 (28.4)
Litter size	Pnd 1	14	13	12	12
	Pnd 4	13	12	12	12
	Pnd 21	12	12	12	12
Pup weight (g)	Pnd 1	6	7	7	7
	Pnd 4	8	10	10	10
	Pnd 21	44	49	42	33*

[(1)] Data are averages. Indices of variation (e.g. SD) were not reported.
* $p < 0.05$ statistically significant difference from controls. Statistical method not described.

Rider et al. (1978)

This study is also described in the developmental toxicity section above (section C.3.3.2.). Female McCollum rats were treated with lithium citrate in water at 0 or 15 meq Li/L (0 or 105 ppm Li) for one month before mating, and during gestation and lactation. Females were mated with untreated males. After mating, females were put on diets with 20% casein (adequate protein) or 10% casein (reduced protein). Pregnant females were allowed to give birth. At this stage of the study there were four groups: 20C, 20L, 10C, and 10L. The authors reported that there were 10-12 females/group at this stage. After delivery, pups were fostered to females fed a commercial stock diet. About half of the pups from lithium treated females were fostered to non-lithium treated females. All litters were reduced to 8 pups. Pups were weaned at 21 days of age, after which there was no further administration of lithium. This resulted in 6 groups: 20CC, 20LC, 20LL, 10CC, 10LC, and 10LL. The authors reported that there were 17 litters in the 20CC group, and 9-14 litters in the other groups. There appears to be some inconsistency, omission, or error in reporting, as the number of litters after fostering considerably exceeded the number of litters born. At three weeks of age, female pups representing at least 6 litters from each group were sacrificed for determination of organ weights. When pups were 4.5 months old, one male from each of 6 litters from each group was tested in a T-maze with a water reward. Ten days afterwards, the same animals were tested in an avoidance behavior test using water as an attractor and an electric shock as the aversive stimulus.

Reduced body weight gain during pregnancy was observed in the lithium treated animals (statistically significant for 20L vs. 20C, but not for 10L vs. 10C). Reduced water consumption by lithium treated animals (75% of control) was observed. No effect on food consumption on gd 15 or 16 was observed. Lithium intake was 1.17 and 1.19 meq Li/kg/d (8.2 and 8.3 mg Li/kg/d) for 20L and 10L groups, respectively. Serum lithium was 0.3-0.4 meq/L. Litter size and litter weight at birth were approximately 25% smaller in the lithium treated group with adequate protein (20%), but this was not statistically significant. No effect of lithium on pup weight at birth was observed.

No effects on pup survival to weaning or age of eye opening were observed. Lower pup weight at weaning was observed for pups nursed by lithium treated females (statistically significant for 10LL vs. 10CC, not others). Reduced relative spleen weight of female pups at weaning was observed for those nursed by lithium treated females (statistically significant for 10LL vs. 10CC only). No effect on female pup thymus, kidney, or liver weight at weaning was observed. No effect on male offspring weight at 13 weeks of age was observed. No effect of on male offspring maze times was observed. Male pups from females treated with lithium during pregnancy had a shorter avoidance time after electric shock (statistically significant for 20LL vs. 20CC and 10LL vs. 10CC, not others). Data from this study are summarized in Table 50.

Table 50. Selected results from rat reproductive study by Rider et al. (1978). [1]

Group		20C	20L	
mmol Li/L in water: premating and gestation (ppm)		0 (0)	15 (105)	
Number of mated females		11	12	
Number of deliveries (%)		9 (82%)	11 (92%)	
Original body weight (g)		226.0 ± 5.2	225.0 ± 3.6	
Weight gain (g)	Gestation	100 ± 6.9	80 ± 5.9*	
	Lactation	22.9 ± 6.1	17.6 ± 8.9	
Litter size		9.89 ± 0.93	7.45 ± 1.11	
Pup birth weight (g)		6.53 ± 0.15	6.56 ± 0.24	
Litter weight (g)		62.1 ± 6.9	46.5 ± 6.6	
Group (offspring fostered)		20CC	20LC (15-->0)	20LL
Number of litters [2]		17	10	14
Survival of pups to weaning		100%	91%	84%
Eye opening (days of age)		14.0 ± 0.13	13.8 ± 0.22	15.0 ± 0.26*
Pup weight at weaning (g) [3]		38.39 ± 1.19	38.78 ± 2.12	35.01 ± 1.04
Relative organ weight (g/100g body weight) [3]	Kidney	1.17 ± 0.04	1.16 ± 0.02	1.23 ± 0.03
	Liver	4.27 ± 0.11	4.10 ± 0.14	4.27 ± 0.09
	Spleen	0.4838 ± 0.037.2	0.4841 ± 0.0297	0.4224 ± 0.0170

[1] Data are numbers or averages ± SE. Data for rats on low protein diet (10% casein) were omitted from table.

[2] There appears to be an inconsistency between the number of deliveries reported for prenatal results (groups 20C and 20L) and the number of litters reported for postnatalt results (groups 20CC, 20LC, and 20LL).

[3] These data refer to three week old females sacrificed for determination of organ weights. N = 6-9.

* $p < 0.05$ statistically significant difference from control, ANOVA and multiple range test.

Sechzer et al. (1986)

This study is also described in the developmental toxicity section above (section C.3.3.2.). This study compared the effects of two stable lithium isotopes: ^{6}Li (atomic mass 6) and ^{7}Li (atomic mass 7). These are present in naturally occurring lithium (Li-N) at 7.4% and 92.6% abundance, respectively. Both of the pure isotopes and the natural mixture were administered separately to the rats in two experiments. In experiment 1, female Sprague-Dawley rats were treated with lithium chloride in water/orange juice solution with target doses of 0 or 2.0 meq Li/kg/d (0 or 14 mg Li/kg/d) for 10 days prior to mating, and for gestation and lactation (to pnd 28). There were 20 mature females

total (probably 5/group). Pups were culled to 8 per litter on pnd 3. Actual doses of lithium were reported to be 2.4 meq/kg/d (16.8 mg Li/kg/d) during gestation and 2.8-3.7 meq/kg/d (19.6-25.9 mg Li/kg/d) during lactation. Reduced grooming, alertness, and general activity were observed in the ^{7}Li and Li-N treated maternal females. Reduced food seeking activity was observed in ^{7}Li treated maternal females. Increased grooming and alertness was observed in ^{6}Li treated maternal females. The authors also noted that no cannibalization of pups occurred in lithium treated groups. No other maternal results were reported. The authors stated that lower birth weight was observed for all lithium treated litters (no numerical data were reported). Reduced grooming, nursing, and retrieval of pups by Li-N and ^{7}Li treated maternal rats was observed. Increased nest building, grooming, and nursing of pups by ^{6}Li treated maternal rats was observed. Delayed postnatal development in pups from lithium treated litters (eye opening, startle response, depth perception) was observed. Reduced spontaneous motor activity at 4 months was observed in all lithium treated pups (statistically significant only for ^{6}Li vs. controls). Data from this study are summarized in Table 51.

Table 51. Selected results from rat reproductive/developmental study, experiment 1, by Sechzer et al. (1986). [(1)]

Group (Li isotope)		Control	Li-N	^{6}Li	^{7}Li
Target dose [meq Li/kg/d (mg Li/kg/d)]		0 (0)	2 (14)	2 (14)	2 (14)
Number of pups examined for endpoints below		8 female 8 male	9 female 6 male	8 female 7 male	6 female 7 male
Pup weight (g)	Pnd 5	15.0	9.6	12.9	10.4
	Pnd 28	81.0	71.5	77.2	83.0
Age of eye opening (days)		12	18	18-20	19-20
Age of startle response (days)		12	15	14	15
Number of offspring examined for endpoint below		8 females 8 males	6 females 6 males	6 females 6 males	6 females 7 males
Spontaneous motor activity at 4 months		154	123	84*	131

[(1)] Data are numbers or averages. No indices of variation (e.g. SD) were reported.
* $p < 0.05$ statistically significant difference compared to controls, Student's t-test.

Experiment 2 was similar to experiment 1, except the target doses used were 0 or 4 meq Li/kg/d (0 or 28 mg Li/kg/d). No maternal results were reported. For 4 meq Li/kg/d, the authors stated that similar developmental delays to 2 meq Li/kg/d were observed, but of longer duration. No numerical data were reported.

Sechzer et al. (1992)

This study is also described in the developmental toxicity section above (section C.3.3.2.). This study was reported in abstract only. Female rats (strain not reported) were treated with lithium (unspecified salt) in saccharin sweetened water at 0 or 3 meq/kg/d (0 or 21 mg Li/kg/d) prior to breeding, and during gestation and lactation. Numbers of animals were not given. No maternal toxicity results were reported. Lithium treated females showed "maternal neglect" of pups: absence of nest building, short and infrequent periods of nursing, failure to retrieve pups, and poor grooming of pups. Pups showed developmental delays: reduced weight, delayed eye and ear opening, delayed appearance of depth perception, and delayed initiation of startle response. At four months of age, spontaneous motor activity in the lithium treated offspring was 20% below that of controls. If these developmental delays were a result of aberrant parenting, they would be regarded as a female reproductive effect.

Teixeira et al. (1995)

This study is also described in the developmental toxicity section above (section C.3.2.3.). Mated female Wistar rats were treated with lithium chloride in water at 0 or 10 mM (0 or 70 ppm) for gestation and lactation. An additional group was water restricted to the 10 mM lithium chloride group. Females were allowed to give birth and nurse the pups. Litters were culled to 8 pups on the day of birth. Pups from some of the lithium chloride and water restricted litters were cross fostered at birth. There were 13 females in the control group and 18-25 females in other groups. The following terminology was used by the authors: Control-NS (not stressed), Control-S (stressed: water restricted), LiPL (10 mM Li during pregnancy and lactation), LiP (10 mM Li during pregnancy only), LiL (10 mM Li during lactation only). Maternal serum monovalent ion levels were tested. It was observed that serum lithium was 0.5 mM during gestation and lactation. Serum potassium (K) was increased in the lithium treated group (statistically significant, 20-30%). No effect on serum sodium (Na) was observed. No other maternal results were reported. Lower litter sizes were observed in the lithium treated and water restricted groups (not statistically significant). The authors asserted that water restriction and lithium treatment resulted in reduced numbers of male pups (statistically significant), but data indicate that this was probably due to an unusually high percentage of males in Control-NS. Reduced percentage of pups with normal righting reflex at birth in lithium treated vs Control-NS and vs. Control-S was observed (both statistically significant). Reduced pup weight on pnd 21 in LiPL and LiL groups vs. Control-NS and Control-S was observed (statistically significant). Delayed eye opening in all lithium treated groups vs. Control-NS and vs. Control-S was observed (statistically significant). No effects on fertility (75%), percentage stillborn, malformations, or birth weight was observed. No effect on a pup retrieval test was observed. No effects on pinna detachment, cliff avoidance, or motor coordination of lithium groups vs. Control-S were observed, although there were some differences vs. Control-NS. Data from this study are summarized in Table 51.

Table 51. Selected results from rat developmental study by Teixeira et al. (1995). [1]

Group	Control-NS (0 mmol Li/L)	Control-S (0 mmol Li/L, water restrictred to Li group)		Li (10 mmol Li/L)	
Number of mated females	13	46		44	
Number of litters	13	46		44	
Stillborn pups [number (percentage)]	2/144 (1.3%)	4/439 (1.0%)		7/467 (1.5%)	
Litter size	11.1 ± 2.7	9.5 ± 2.6		9.6 ± 2.5	
Birth weight (g) [2]	5.8 ± 1.0	5.5 ± 1.0		5.6 ± 1.0	
Pups with righting reflex at birth (%)	94.2%	78.5%*		70.5%*,#	
Pup retrieval test, latency (seconds)	13.1 ± 5.6	21.0 ± 10.4		15.0 ± 9.4	
Pup retrieval test, non-recovery (%)	3%	5%		6%	
Group (offspring cross fostered)	Control-NS	Control-S	LiL (0-->10)	LiPL	LiP (10-->0)
Number of litters	13	21	25	18	22
Pup weight at weaning (pnd 21) (g) [2]	42 ± 4	37 ± 5@@	35 ± 7@@,$$	33 ± 5@@,$$	38 ± 5@@

[1] Data are numbers, percentages, or averages ± SD.
[2] Data estimated by OEHHA staff from Figure 1, Teixeira et al. (1995).
* $p < 0.05$ statistically significant difference from Control-NS group by Fisher's exact test.
# $p < 0.05$ statistically significant differences from Control-S group by Fisher's exact test.
@@ $p < 0.01$ statistically significant difference from Control-NS group by Duncan's test.
$$ $p < 0.01$ statistically significant difference from Control-S group by Duncan's test.

Trautner et al. (1958)

Parts of this study are also described in the developmental toxicity section above (section C.3.3.2.). Trautner et al. reported a number of experiments in rats. In experiment 1, Wistar rats (sex not specified) were treated with lithium chloride at 10, 20, 30, or 50 meq Li/L (70, 140, 210, or 350 ppm) in drinking water for up to 2 years. All rats died in 2-3 weeks at 50 meq/L. Plasma lithium concentrations exceeded 8 meq/L just before death. All rats died in 3-9 weeks at 30 meq/L. Plasma lithium concentrations were about 3

meq/L during a pseudo-stable phase, but increased shortly before death. There was no effect on survival at 20 or 10 meq/L: most animals survived up to 2 years. A transient drop in water consumption was observed at 20 meq/L. Plasma lithium concentrations were 1.5-2.0 and 1 meq/L for the 20 and 10 meq/L water concentrations, respectively. Possibly small increases in estrus cycle length and gestation period were observed in the group treated at 20 meq/L.

In experiment 2, male and female Wistar rats were treated with lithium chloride at 0 or 25 meq/L (0 or 175 ppm) in water for 17 days before mating. Matings included control x control, lithium x control, and lithium x lithium. Lithium treated females were kept on lithium through gestation. There were 6 rats/sex/group. The authors remarked that the females remained "healthy" through pregnancy. No other maternal or systemic toxicity results were reported. A lower frequency of pregnancies in lithium treated females was observed. Data from this study are summarized in Table 52.

Table 52. Results from rat reproductive study, experiment 2, by Trautner et al. (1958)[(1)]

Group (F x M)	C x C	C x Li	Li x C	Li x Li
Female treatment: mmol Li/L (ppm)	0 (0)	0 (0)	25 (175)	25 (175)
Male treatment: mmol Li/L (ppm)	0 (0)	25 (175)	0 (0)	25 (175)
Number of rats mated/sex	6	6	6	6
Number of female rats pregnant	5	4	2	2
Live litter size	7.8	8.0	6.5	5.5

[(1)] Data are numbers or averages. No indices of variation (e.g., SD) were reported.

In experiment 3, female and male Wistar rats were treated with lithium chloride in water at 0 or 20 meq/L (0 or 140 ppm). The duration of treatment was not specified: possibly it was the same as experiment 2, above. Matings included lithium x control, and lithium x lithium. There were 7-17 rats/sex/group. No maternal or systemic toxicity results were reported. No effect on frequency of pregnancy in lithium x control matings was observed, although there may have been a lower frequency in lithium x lithium matings. Data from this experiment are summarized in Table 53.

Table 53. Results from rat reproductive study, experiment 3, by Trautner et al. (1958)[1]

Group (F x M)	C x Li	Li x C	Li x Li
Female treatment: mmol Li/L (ppm)	0 (0)	20 (140)	20 (140)
Male treatment: mmol Li/L (ppm)	20 (140)	0 (0)	20 (140)
Number of rats mated/sex	15	17	7
Number of female rats pregnant	14	16	5

[1] Data are numbers.

In experiment 4, female Wistar rats were treated with lithium chloride at 0 or 20 meq/L (0 or 140 ppm) in water for 3-7 weeks before mating and during gestation. Animals were allowed to give birth normally. Two series were reported: in series one there were 44 control and 16 lithium treated pregnant females, in series two there were 22 control and 13 lithium treated pregnant females. No maternal toxicity results were reported. Litter size in the lithium treated group was observed to be lower than controls in series 2, but not series 1. The authors comment that control litter size was low by historical standards in series 1, and that the group was "much handled." The authors stated that no gross external malformations or difference in birth weight were observed in pups from lithium treated mothers. The authors also commented that early postnatal growth was slower in pups from mothers maintained on lithium. Except for litter size, no numerical data were reported. Data from this experiment are summarized in Table 54.

Table 54. Selected data from rat reproductive study, experiment 4, Trautner et al. (1958). [1]

Series	Series 1		Series 2	
Group: meq Li/L water (ppm)	0 (0)	20 (140)	0 (0)	20 (140)
Number of pregnancies	44	16	22	13
Total litter size	6.09 ± 2.195	6.19 ± 1.703	8.68 ± 1.426	5.69 ± 1.734
Live litter size	5.86 ± 2.501	6.00 ± 2.150	8.52 ± 1.662	5.38 ± 2.349

[1] Data are numbers or averages ± SD. No statistical tests were performed.

In experiment 5, female Wistar rats were treated with lithium chloride in water at 0 or 20 meq/L (0 or 140 ppm) for 3-7 weeks before mating and during gestation. Animals were laparotmized on gd 16-18 to examine numbers of corpora lutea, implants, and viable fetuses, then allowed to give birth. There were 31 females/group. No maternal toxicity results were reported. Reduced corpora lutea, implants, and viable fetuses were observed at 20 meq/L (statistically significant). Data from this experiment are summarized in Table 55.

In experiment 6, female Wistar rats were treated with lithium chloride in water at 20 meq/L (140 ppm) for 3-7 weeks before mating, during gestation, and lactation. Animals were allowed to give birth. Pups were treated at the same concentration. At 6-7 months of age, female offspring were mated with untreated males. Females were laparotomized on gd 16-18. There were 14 lithium treated females. Note that there were no independent controls, but this group was compared to the animals in experiment 5. The authors stated that no effect on maternal body weight at time of mating was observed. Reduced corpora lutea and viable fetuses (statistically significant), and lower implants (not statistically significant) compared to experiment 5 controls were observed. Data from these experiments are summarized in Table 55.

Table 55. Selected results from rat reproductive study, experiments 5 and 6, Trautner et al. (1958). [(1)]

Group	Control: 0 meq Li/L (0 ppm)	1 generation: 20 meq Li/L (140 ppm)	2 generation: 20 meq Li/L (140 ppm)
Number of females	31	31	14
Corpora lutea/female	10.22 ± 1.316	9.02 ± 1.305**	8.93 ± 0.923**
Implantations/female	9.39 ± 1.726	7.90 ± 1.922**	8.42 ± 1.154
Viable fetuses/female	8.65 ± 1.872	7.23 ± 1.745**	7.29 ± 1.488*

[(1)] Data are numbers or averages ± SD.
* $p < 0.05$ difference from controls by Student's t-test.
** $p < 0.01$ difference from controls by Student's t-test.

D.3.2.4. Female reproductive toxicity studies in rats by injection

Jana et al. (2001)

Female Wistar rats were injected (sc) with lithium chloride at 0 or 1.6 mg/kg/d (0.04 mmol Li/kg/d) or with lithium chloride at 1.6 mg/kg/d (0.04 mmol Li/kg/d) plus human chorionic gonadotropin (hCG) at 25 ug/kg/d for 28 days. There were 6 females/group. No effect on female body weight was observed. Plasma lithium was measured at 0.2, 1.6, and 1.5 meq Li/L for the 3 groups, respectively. Following treatment with lithium alone, reduced relative ovary and uterus weight and number of estrus cycles over the 28 day period (statistically significant) was observed. Increased length of estrus cycles (statistically significant) was observed. Reduced ovarian hydroxysteroid dehydrogenase activities (statistically significant) was observed. Reduced numbers of healthy ovarian follicles and increased numbers of regressing follicles (statistically significant) were observed. Following treatment with lithium plus hCG, there was little difference from controls. Treatment with hCG thus appeared to reverse or ameliorate all effects of lithium in this study.

Roy et al. (1999)

In experiment 1, female Wistar rats were injected (ip) with lithium chloride at 1.5, 3, or 5 meq/kg (no 0 control) (10.5, 21, or 35 mg Li/kg), twice per week. The duration was not clear, possibly it may have been 6 weeks. There were 12 females/group. No systemic toxicity results were reported. A dose-related lengthening of estrus cycle length was observed. Estrus cycle lengths were 7-8, 8-11, and 10-11 days at 1.5, 3, and 5 meq/kg, respectively.

In experiment 2, female Wistar rats were injected (ip) with lithium chloride at 0 or 3 meq/kg (0 or 21 mg Li/kg) on the day of estrus. Females were mated and allowed to give birth. There were 12 females/group. No systemic or maternal toxicity results were reported. No effect on frequency of pregnancy or litter size was observed.

In experiment 3, pregnant female Wistar rats were injected (ip) with lithium at 3 meq/kg (21 mg Li/kg) on gd 6, 12, and 18 and pnd 3, 9, and 15. Females were allowed to deliver and nurse pups. The number of animals was not reported. One half of the lithium treated females died during "difficult delivery." The average number of fetuses in lithium treated females was reduced from 8 to 4. Lithium treated mothers ignored the pups and did not nurse, leading to death of pups.

In experiment 4, nursing Wistar rats were injected (ip) with lithium at 3 meq/kg (21 mg Li/kg) on pnd 3, 9, and 15. The number of females was not reported. No maternal toxicity results were reported. Mothers ignored the pups and did not nurse, leading to death of pups.

In experiment 5, non-pregnant, pregnant, and nursing female Wistar rats were injected (ip) with lithium at 0 or 3 meq/kg/d (0 or 21 mg Li/kg/d) at 6 day intervals, on gd 6, 12, and 18, or on pnd 3, 9, 15, respectively. Systemic and maternal toxicity results were not reported. Severely reduced serum LH was observed in all lithium treated groups. In the brain, lithium was measured at 0.268-0.294 µg/g, and in serum lithium was 2.25-2.57 µg/ml, for all groups.

D.4. Other relevant data.

No information on the distribution of bromacil or lithium to female reproductive organs was located. Distribution of lithium in pregnant female, fetus, and offspring was reviewed in section B.3.1.

D.5. Female Reproductive Toxicity: Integrative Evaluation of Bromacil Lithium Salt

D.5.1. Female reproductive toxicity of bromacil lithium salt

There are no female reproductive toxicity studies on bromacil lithium salt, and so evaluation of its toxicity therefore must rely on its dissociation products, bromacil and lithium. As discussed below, studies to date have provided no evidence of female reproductive toxicity for bromacil. Studies of lithium in animals have provided evidence of effects on female fertility, litter size and maternal behavior.

D.5.2. Data on the female reproductive toxicity of bromacil

The database of animal studies for considering female reproductive toxicity of bromacil is limited. No human studies are available.

No effects of bromacil on female fertility indices were found in two rat multigeneration studies conducted for pesticide registration (Haskell 1966, Mullin 1991). The earlier study had small group size and one dose of bromacil that produced no systemic toxicity. The second, larger study used a dose range that produced minimal general toxicity, which consisted of reduced (<10%) body weight and body weight gain in the F1 generation during periods of rapid growth before and after weaning. The absence of a maximally tolerated dose in these studies limits their ability to identify reproductive toxicity.

No treatment-related effects on female reproductive organ pathology were reported in chronic studies with bromacil in dogs, rats and mice or in the rat multigeneration studies. Reproductive organ weights were measured only in the dog studies and no effects were reported.

D.5.3. Human data on the female reproductive toxicity of lithium

One study of the effects of lithium treatment on female human reproductive hormones was located. This study used a relatively brief exposure (one month) to therapeutic doses of lithium. No indication of alterations to reproductive hormone levels or perturbation of the menstrual cycle was observed. Whether any of the reproductive hormones or the menstrual cycle would be perturbed by other dosing regimens (e.g., longer exposure) cannot be answered by this study.

One study on the effects of various treatments, including lithium, for bipolar disorder on human female sexual function was located. The relatively small number of subjects taking lithium alone, and lack of data for females taking lithium alone, make this study difficult to evaluate. By multiple regression analysis, the authors concluded that lithium by itself was not associated with changes in human sexual function. Another study found no association of sexual dysfunction with lithium use for 6-24 months by 10 women with

major affective disorders. The lithium treated women were compared to a control group of 25 female surgical outpatients.

D.5.4. Data on the female reproductive toxicity of lithium in experimental animals

There are several considerations with regard to hazard identification for the possible female reproductive toxicity of lithium. Most studies used a single dose or concentration of lithium. Very small numbers of animals were used in some studies. Maternal or systemic toxicity results were seldom reported or reported incompletely. Commonly, only a subset of reproductive results were reported and qualitative results were reported in the text, but numerical data were not presented.

Fertility was observed to be adversely affected by lithium treatment in some studies, but not others. In mice, a continuous breeding protocol found complete failure of reproduction at 100 mmol Li/L in water. The same study found reduced total number of litters and increased time between litters at 50 mmol Li/L. Both males and females were treated in this study (Mroczka et al., 1983). Another study in mice reported reduced fertility at 20 mmol Li/L in water. However, this study was reported only in abstract, and is difficult to evaluate (Krmpotic and de la Torre, 1976). A screening study in mice found no effect on fertility in the F1 generation when the parental females were treated at 10.8 mmol Li/kg/d for gd 8-12 (Gray et al., 1983, 1986; Gray and Kavlock, 1984). A study in rats found no effect on litter size or birth weights, but a reduced composite "skeletal ossification score" in the offspring of F1 generation females when the parental (P) females were treated at 20 mmol Li/L during premating and gestation (Glockner et al., 1989). A series of studies by one group found reduced fertility in rats. In Wistar rats, reduced frequency of pregnancy was observed in females, but not males, treated at 25 mmol Li/L in water for 17 days before mating. A similar experiment found no effect at 20 mmol Li/L. A subsequent experiment found reduced litter size when females were treated for 3-7 weeks before mating and during gestation at 20 mmol Li/L. A final experiment found reduced corpora lutea, implantations, and viable fetuses in both the first and second generations treated at 20 mmol Li/L (Trautner et al., 1958). Other studies in rats have not found indications of reduced fertility. No reduction in fraction of females becoming pregnant or litter size was observed in Wistar rats treated at 40/60 mmol Li/L in water for 8 weeks before mating and during gestation (Christensen et al., 1982). The authors stated that there was no effect on fertility or litter size when Charles River albino female rats were treated at 0, 0.675, 2.025, or 4.05 mmol Li/kg/d by gavage for 14 days and males were treated at 0, 0.27, 0.67 or 1.35 mmol Li/kg/d in food for 70 days before mating (Gralla and McIllheny, 1972). No reduction in fraction pregnant was observed in McCollum rats treated at 15 mmol Li/L in water for one month before mating and during gestation. Litter size was lower, but the difference was not statistically significant (Rider et al., 1978).

One study in mice found cessation of estrus cycling when mice were treated at 94 mmol Li/kg food (Banerji et al., 1986). Rats injected (sc) with 0.04 mmol Li/kg/d for 28 days were found to have a number of changes to female reproductive parameters, including

reduced ovary and uterus weight, reduced number of estrus cycles over the 28 day period, increased length of estrus cycles, reduced number of healthy ovarian follicles, and reduced ovarian hydroxysteroid dehydrogenase activities. Coadministration of hCH reversed or ameliorated all the observed lithium effects (Jana et al., 2001). Rats injected with lithium at 1.5 to 5 mmol Li/kg/d had lengthened estrus cycles (Roy et al., 1999).

In mice, injection (ip) of 5 mmol Li/kg three times during the day of proestrus suppressed the expected surge of plasma LH, although pituitary LH was not affected (Banerji et al., 1986). In rats, injection (ip) of 3 mmol Li/kg/d resulted in severe reductions in serum LH, with similar results for non-pregnant, pregnant, and lactating rats (Roy et al., 1999).

Several studies have provided evidence of female reproductive toxicity manifested as adverse effects on the developing offspring exposed to lithium via lactation. Although under Proposition 65 developmental toxicity cannot be identified on the basis of such effects, they may be considered with regard to identification of female reproductive toxicity.

Mroczka et al. (1986a) reported increased mouse pup mortality after gestational and lactational exposure to 50 meq Li/l in water, and decrease weight gain during lactation in offspring of mice treated with lithium in water from age three weeks onwards. Reduced body weight in all rat pups and reduced relative spleen weight in female pups at weaning after was reported after lactational exposure to lithium (Rider et al., 1978). Studies by one group have observed aberrant parenting behavior by female rats during oral lithium treatment. This group compared the effects of the isotopes ^{6}Li and ^{7}Li with naturally occurring lithium. Naturally occurring lithium is composed of 7.4% ^{6}Li and 92.6% ^{7}Li. Female Sprague-Dawley rats were treated in drinking water for 10 days prior to mating and for gestation and lactation with a target dose of 2.0 mmol Li/kg/d. Maternal animals treated with naturally occurring lithium and ^{7}Li displayed less self-grooming and alertness. Those treated with ^{6}Li displayed increased self-grooming and alertness. Reduced grooming, nursing, and retrieval of pups were observed in maternal rats treated with ^{7}Li and naturally occurring lithium, but opposite results were observed for the isotope ^{6}Li. It was observed that all lithium treated groups of pups had indications of retarded development, including greater age at eye opening and startle response development. Also, all had lower spontaneous motor activity at four months of age. However, no significant effect on pup weight was observed (Sechzer et al., 1986). A subsequent experiment by the same group, using 3 mmol Li/kg/d, found “maternal neglect” of pups and developmental delays in the pups. This experiment was reported only in an abstract, and is hard to evaluate (Sechzer et al., 1992). In another experiment, female Wistar rats treated at 10 mmol Li/L in water for gestation and lactation were not different from controls in a pup retrieval test (Teixeira et al., 1995). Rats injected (ip) with 3 mmol Li/kg three times during gestation and three times during lactation also ignored their pups and did not nurse, leading to death of pups. The same result was observed when rats were injected only during lactation (Roy et al., 1999).

In the above studies, female reproductive capacity (fraction pregnant or litter size) was observed to be adversely affected by lithium treatment in some oral studies, but not in

others. To some extent this can be attributed to higher doses or concentrations in the studies in which effects were observed than those studies in which no effects were observed. However, there is also some overlap. Differences in species, strain, and duration of treatment likely also play a role. The observation of cessation of estrus cycling in a high concentration mouse oral study is supportive of the reduced fertility in another high concentration mouse oral study. Injection studies suggest that perturbation of the hormones of the hypothalamic-pituitary-gonadal axis may be involved. Parental neglect of pups has been observed in two oral studies in rats (by the same group) and one injection study in rats. This could be attributed to alterations in hormones, or to a direct effect on behavior.

E. Male Reproductive Toxicity

E.1. Male reproductive toxicity of bromacil lithium salt

No information regarding the possible male reproductive toxicity of bromacil lithium salt in laboratory animals with bromacil lithium salt as the testing agent or in humans exposed to bromacil lithium salt was identified by OEHHA.

E.2. Male reproductive toxicity of bromacil

No human studies relevant to the possible male reproductive toxicity of bromacil were located. One two-generation reproductive study in rats and two dominant lethal studies in mice conducted in connection with pesticide registration for bromacil were identified. In addition, acute, subchronic and chronic toxicity studies on bromacil, reviewed by OEHHA and summarized under Section B.4.2. (non-DART toxicity), contained information relevant to possible effects of bromacil on the weights and morphology of reproductive organs in male animals.

E.2.1. Multigeneration reproductive study with bromacil

Mullin (1991)

This is a two-generation reproductive study with one litter per generation conducted in CD rats. Detailed information about this study was described above under Section D.2.1, "Female reproductive studies with bromacil." No effects on male fertility indices or male reproductive organs were reported. Notably the highest dose in this study produced minimal general toxicity, as reflected in reduced body weight gain in the F1 generation.

E.2.2. Dominant lethal studies with bromacil

SRI (1977)

This is a dominant lethal study conducted in mice. Bromacil was one of 20 pesticides evaluated with a mouse dominant lethal assay in conjunction with other genotoxicity

assays. Male proven breeder ICR/SIM mice were used. The maximum dose was selected from acute (two-week) toxicity studies to produce less than 20% weight loss, no mortality and no reduction in mating. Bromacil was administered in feed at concentrations of 0, 1250, 2500 or 5000 mg/kg diet for seven weeks, followed by weekly mating for eight weeks. Data tables showed no significant effect on fertility for any week. CDPR reviewed this study and concluded that no adverse dominant lethal effects occurred (CDPR, 1997).

Epstein et al. (1972)

This is a dominant lethal study in which bromacil was one of 174 agents screened for dominant lethal effect. ICR mice were treated with bromacil by i.p. injection at a dose of 150 mg/kg/d one time or orally at 750 or 1000 mg/kg/d for five days. The number of pregnancies, implantations and early resorption were determined during eight subsequent weeks of mating with untreated females. Agents were classified in four categories: (1) causing statistically significant effects relative to controls (9% of agents); (2) causing statistically significant effects but with internal inconsistencies in the data (4%); (3) causing effects within control limits (74%); and (4) causing effects outside control limits but not statistically significant (13%). Bromacil fell in the last category in that early deaths were higher than control norms (95% CI) during at least one week of the mating period in the group that received 1000 mg/kg/d orally for five days. In addition, the number of pregnancies produced was lower than group norms for both the 750 and 1000 mg/kg/d groups. The weekly pregnancy rate exceeded 30% in 99% of 450 control groups but was 19% in the 750 mg/kg/d bromacil group and 29% in the 1000 mg/kg/d group. No detailed data or specific discussion of results for bromacil were presented in the report.

E.2.3. Male reproductive effects of bromacil in subchronic or chronic studies

Haskell (1966); Bogdanffy (1989); Bogdanffy (1991)

These studies are summarized above in section B.4.2.2. No treatment related effects on testes weight, gross appearance or histopathology were reported in chronic studies in rats or dogs.

Wood (1980)

In the chronic study in mice reported by Wood (1980), testis weights in bromacil-treated animals that survived to terminal necropsy were not affected, but regarding the "bromacil-related non-neoplastic histomorphological findings", the author stated that,

> "In the testes of mice from the test groups…, a dose-dependent increase in the incidence of testicular tubule atrophy was observed in all male test groups. Increased incidence of spermatocyte necrosis, sperm calculi and mild interstitial

cell hypertrophy/hyperplasia were observed in male mice from the 1250 and 5000 ppm group."

Organ weights and pathology data on the male reproductive organs are summarized in Table 56 below. Compared to the control group, it appears that there is a notable increase in the incidences of several pathological changes in the testes or epididymides in the middle and/or high dose groups. Since no statistical analysis for the pathology data was provided in the report, OEHHA staff performed a statistical analysis and found that the incidences for all reported morphological changes (except the incidence of sperm calculi/plugs) in the testes of animals exposed to 5,000 ppm in diet (871 mg/kg/d) bromacil were significantly increased, compared to that in the control group ($p<0.05$ or 0.01). The incidence of increased number of sloughed or degenerating spermatocytes in the epididymis (bilateral) was also significantly increased at the high dose level. At the middle dose level (1250 ppm in diet or 196 mg/kg/d), the incidences of bilateral atrophy, sloughing germ cells/germ cell necrosis, and arteritis in the testes were also significantly increased ($p<0.05$ or 0.01). At the low dose level (250 ppm or 40 mg/kg/d), only the incidence of unilateral testicular atrophy was significantly increased, but this incidence at the middle dose level was not significantly higher than that of the controls.

Considering the high mortality in all groups, high incidences of amyloidosis and testicular atrophy in bromacil-treated animals, OEHHA reviewed pathology data for each individual animal on incidences of amyloidosis and atrophy (either bilateral or unilateral) in the testis and further analyzed the data as presented in Table 57. Among the animals that died prior to terminal necropsy, the incidence of testicular atrophy was significantly increased at middle and high doses. Among the animals that survived to terminal necropsy, the incidence of testicular atrophy was increased in all treated groups, but was only statistically significant at the low and high dose groups. In addition, OEHHA staff found that amyloidosis was present in 24% of the cases of testicular atrophy occurring in the two highest dose groups (1250 and 5000 ppm) and 21% of the males in all dose groups. It should be noted, as summarized in section B.4.2.2, that other severe damage (e.g., liver cancers) likely related to bromacil treatment were also observed in animals at the middle and high dose groups.

Table 56. Data on male reproductive organs from a chronic (18-month) study of bromacil administered in diet to mice (Wood 1980). [1]

Bromacil mg/kg/d		0	40	196	871
Data at terminal necropsy [2]					
mortality		51/80	49/80	47/80	45/80
body weight (g)		44.3	43.2	43.9	41.8
Testes weight (g)		0.47	0.43	0.46	0.43
Testes wt./body wt. (%)		1.07	1.00	1.05	1.05
Testes Pathology (numbers of animals) [3] [4]		79	80	79	76
Testes	interstitial cell hyperplasia/hypertrophy	14	17	25	30**
	amyloid, tubular/intertubular	2	5	5	14**
	Atrophy, bilateral	8	8	24**	21**
	Atrophy,unilateral	3	13*	6	13**
	necrosis/sloughing germ cells	4	8	14*	23**
	sperm calculi/plugs	-	4	-	4
	Arteritis	2	6	9	10*
Epididymis Pathology (numbers of animals)[3] [4]		75	80	78	78
Epididymis	increased sloughed/degenerating spermatocytes, bilateral	2	4	5	10*
	increased sloughed/degenerating spermatocytes, unilateral	2	4	4	4
	Aspermia, bilateral	3	2	4	9
	Aspermia, unilateral	2	8	7	3

[1] Data are numbers or averages. No indices of variability (e.g. SD) were reported.
[2] No statistically significant effects for body or organ weight data.
[3] Pathology data were the number of cases (animals) for mice that died prior to terminal necropsy (78 weeks) and mice examined at terminal necropsy. Statistical analysis was not provided for pathology data in the study report. "-" indicates that the study authors did not report any data for the group.
[4] Statistical analysis by x^2 test, Yates correction conducted by OEHHA staff. Compared to the control group, *: $p<0.05$; **: $p<0.01$.

Table 57. Incidences of testicular amyloid and atrophy (bilateral and unilateral combined) from the Wood study (1980). [(1)]

Bromacil mg/kg/d		0	40	196	871
Early death	Total No. examined	50	49	47	42
	Amyloid (%)	4 (8.0%)	13 (26.5%)*	2 (4.3%)	11 (26.2%)*
	Atrophy (%)	10 (20.0%)	12 (24.5%)	23 (48.9%)**	18 (42.9%)*
Terminal necropsy	Total No. examined	29	31	32	34
	Amyloid (%)	0 (0)	3 (9.7%)	0 (0)	5 (20.6%)*
	Atrophy (%)	1 (3.4%)	8 (25.8%)*	7 (21.9%)	16 (47.1%)**

[(1)] Data are numbers or percentages. Data from the original report by Wood (1980). Pathology data was missing in a few animals in the control, middle, and high dose groups.
*p<0.05, **p<0.01, compared to the control with Fisher's exact probability test.

E.3. Male reproductive toxicity of lithium

E.3.1. Human studies

E.3.1.1. Overview

Lithium, in the form of lithium carbonate (Li_2CO_3), is one of the most effective and frequently used drugs for treatment of bipolar disorder (Manji et al., 1995). Possible adverse effects of lithium on the male reproductive functions of men have been investigated in a number of human studies. Major findings from the human studies are summarized below.

E.3.1.2. Effects of lithium on semen quality in men

Amsterdam et al. (1981)

The authors investigated the effect of lithium or desmethylimipramine (DMI) on sperm count, viability, and motility in ejaculates among nine patients with affective disorder (40.3 ± 5.4 years old) and nine physically healthy men as controls (28.6 ± 2.6 years old). Four of the nine patients received lithium carbonate for a minimum of three weeks at doses sufficient to maintain plasma levels of lithium from 0.6 to 1.4 mmol Li/L for at least three weeks. The remaining five patients received DMI. Sperm count, viability and morphology were determined by routine microscopic analysis. Sperm viability was evaluated as the ratio of living sperm to total sperm count and expressed as the ratio of sperm viability after three weeks of treatment to the sperm viability at the beginning of the treatment. Sperm motility, expressed as motility index, was measured spectrophotometrically as an increase in absorbance (at 545 nm) as the sperm swim

upward into the light path. The authors found that change in sperm viability over the three-week period was significantly reduced in the patients (76.5 ± 4.7%), compared to the control (105.0 ± 4.6%, $p < 0.001$, one-tailed). Sperm motility (motility index) was increased by 353% in two patients, remained unchanged in one, and not calculated for one, in four patients that received lithium treatment. Similarly, sperm viability was significantly decreased in the five DMI patients; sperm motility was decreased by 77% in two DMI patients, increased by 420% in one, remained unchanged in one, and was not calculated for one ($p < 0.01$, one-tailed). Sperm count and morphology were unaltered by drug treatment. The conclusions that can be drawn from this study are limited by the very small number of subjects and the potential confounding by the differences in age of the control subjects (28.6 ± 2.6) versus the patients (40.3 ± 5.4, $p < 0.05$).

Levin et al. (1981)

The authors evaluated semen quality among nine patients (mean age 40 ± 9 years) diagnosed as having clinical depression and nine healthy volunteer semen donors (mean age 29 ± 3 years) with normal sperm characteristics. Four of the nine patients received treatment with lithium carbonate for three weeks. The authors for this study appear to be the same research group as those in the report published by Amsterdam et al. (1981), in which Levin was the second author. The human subjects reported in this paper appeared to be the same as those reported by Amsterdam et al (1981). The authors reported that there were no significant differences in sperm count, viability, or motility between the two groups at the beginning of lithium treatment in the patients. Three weeks of continuous therapy with lithium carbonate resulted in a significant decrease in sperm viability (55% in the lithium group vs. 71% in the control; $p < 0.01$), but no significant change in sperm count or motility. Relevant data reported by the authors, in the formats that are different from those that were reported by Amsterdam et al. (1981), are presented in the Table 58.

Table 58. Data on semen parameters from a three week study of lithium chloride in humans (Levin et al., 1981). [(1)]

	Control (n = 9)		Patients on Lithium (n = 4)	
	Initial	3 wks	Pretreatment	3 wks
Count (10^6/ml)	90 ± 13	82 ± 13	45 ± 7	46 ± 7
Viability (%)	68 ± 4	71 ± 4	70 ± 4	55 ± 4*
Motility Index	8.8 ± 0.8	8.4 ± 1.0	12 ± 2.0	14 ± 2.8
Semen pH	8.0 ± 0.3	8.0 ± 0.2	8.0 ± 0.2	8.0 ± 0.2

[(1)] Data are averages ± SE.
* $p < 0.05$ compared to pretreatment value, by Student's t-test.

Tollefson and Garvey (1989)

The authors analyzed ten male bipolar outpatients (mean age 34 yrs) who had been on lithium therapy (0.5-0.9 meq/l) for an average of 15 months and each subject had been at stable mood for at least six months. Semen samples were collected by masturbation and immediately analyzed for semen quality (including sperm count, viability, motility, and other routine parameters) at Day 0 , Day 35 and Day 70. The authors stated that "no significant deviations were observed either within (3 visits) or between subjects relative to these parameters", but detailed information about statistical analysis was not reported. All the values were within the normal range in the normal human population. Although the authors reported no differences in the measured parameters between patients and an age-matched control group, no data were presented for the control group. It should be noted that the patients were already on lithium treatment for an average of 15 months before the 70-day study period.

E.3.1.3. Effects of lithium on plasma sex hormones levels in men

Sanchez et al. (1976)

The authors analyzed plasma follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels in ten patients undergoing lithium therapy for 2-48 months. Plasma lithium levels ranged from 0.45 to 1.20 mmol/L. The ages of patients ranged from 42 to 60 years old. No control group was included. Testosterone levels ranged from 0.28 to 4.4 ng/ml with a mean of 2.83 ng/ml and were below the normal range (4-10 ng/ml) in seven of the ten patients. FSH levels were high in two patients (one with a normal testosterone level and one with a testosterone level of 3.25 ng/ml) and LH levels were high in one (with normal FSH and testosterone levels). The authors stated that "the low testosterone levels showed no correlation with age, duration of therapy, or lithium

levels." However, no detailed information about statistical analysis was reported.

Sheard et al. (1977)

As part of a study to investigate the effects of lithium on impulsive aggressive behavior, the authors measured serum LH and testosterone levels weekly in 34 male prisoners (16-24 years old) who received lithium carbonate for up to three months and compared the levels to those in 32 age-matched prisoners who received placebo during the experimental period. Hormone levels were also compared with levels obtained from a one month pre- and a one month post-treatment control period. The study was conducted over a three-year period. All subjects were free from psychosis or somatic illness. The authors observed that a significant reduction of serious aggressive behavioral incidents occurred in the third month on lithium treatment and a significant rise in serum LH in the 8^{th}, 11^{th}, and 12^{th} week of treatment, but not in any other weeks compared with LH levels in the control group and with the last week of the pre and post control months in the treatment group. There was no change in serum testosterone levels through the experimental period.

E.3.1.4. Effects of lithium on sexual functions in men

Lorimy et al. (1977)

This report was published in French with an abstract in English. According to the information provided in the abstract, it appears that the authors tried to determine the incidence of long-term lithium treatments on sleep, appetite and sexual behavior. The study was conducted in fifty outpatients of both sexes who were on lithium treatment for at least six months and whose mood disturbances were stabilized. The authors found that half of the subjects considered that lithium modified their sexuality towards a decrease of desire without modifying their capacities of realization. No information on the total number of male patients among those surveyed or the number of male patients who reported decreased desire was available.

Blay et al. (1982)

This is a clinical case report. The authors reported two cases of male sexual dysfunction associated with lithium therapy for bipolar disorder. One patient (42 years of age) experienced loss of libido and impaired erection after one-month treatment with lithium carbonate. Serum lithium level fell to zero and sexual dysfunction in this patient disappeared on the second day after the patient switched to a blind placebo substitution. When lithium treatment resumed, his sexual impairment shortly reappeared. The second patient (58 years of age) had sexual dysfunction similar to that in the first one and the symptoms remitted spontaneously after 2 months of continued lithium therapy.

Kristensen and Jorgensen (1987)

In this study, the authors surveyed via interview-questionnaire the sexual function in 24 patients (18-59 years of age; 14 men) with major affective disorders who were given continuous lithium treatment for 6-24 months, and among 42 surgical outpatients (17 men) with no known psychiatric disease as the control group. Patients in the control group were chosen based on the same age and selection criteria as the lithium-treated group. The average 12-h serum lithium concentration in lithium-treated patients was 0.64 (0.5-1.0) mmol Li/L. Sexual dysfunctions, including erectile dysfunction, premature ejaculation, retarded ejaculation, and reduced libido, were reported by five (36%) male patients and five (29%) controls; the difference between the two groups was not statistically significant. Limitations of this study include the retrospective design and the small number of subjects. In addition, since the presence of the disorder itself may affect sexual function it is important to include a control group of patients with bipolar disorder not treated with lithium, but perhaps with some other drug.

Ghadirian et al. (1992)

The authors for this retrospective study surveyed sexual function in 104 outpatients (45 men and 59 women) with bipolar disorder who were under treatment with lithium, either alone (35%) or in combination with benzodiazepines (49%), tricyclic antidepressants (17%), neuroleptics (17%), tryptophan (10%), or carbamazepine (1%). The patients were in a stable state at the time of assessment. No control group was included in the study and the survey was self-completed. Male patients received lithium at an average dose of 1080 ± 269 mg/day. Serum lithium in male patients was 0.61 ± 0.22 mmol/L. The authors reported that among 45 male patients, 43% had decreased sexual desire, 40% difficulty in getting or maintaining an erection, 36% decreased quality of orgasm, and 32% decreased quantity of ejaculate. The majority of these changes were mild. Overall, 58% had no change in sexual function, 23% mild changes, 9% moderate changes, and 9% great changes. When both male and female patients were included for statistical analysis, difficulties in sexual functioning were significantly more common in patients treated with a combination of lithium and benzodiazepines (49%) than in those treated with either lithium alone (14%) or lithium in combination with other drugs (17%). However, no relationship was found between serum lithium level and sexual dysfunction scores. The authors concluded that lithium, when given alone, is unlikely to affect sexual function in bipolar patients; but when given in combination with benzodiazepines, it was associated with sexual dysfunction in about half of the patients.

Aizenberg et al. (1996)

This study evaluated sexual function and behavior in 35 bipolar and schizoaffective male patients (43.3 ± 9.6 years old) under lithium treatment. All the patients were in euthymic state and were receiving lithium as the sole medical treatment. Eleven patients (31.4%) reported sexual dysfunction on at least two items of the sexual function questionnaire. The authors found that 23 and 20% of patients reported reduction in frequency of sexual thoughts and loss of erection during sex, respectively. Difficulties in achieving and maintaining erections (ease of arousal) were reported in 14% of patients. Almost all patients reported pleasure during sexual activity and were satisfied with their sexual performance. There was no difference in serum lithium levels in patients with and without sexual dysfunction. No statistical correlation was found between sexual function scores and serum lithium levels.

E.3.1.5. In vitro studies in human sperm

MacLeod et al. (1949)

This early report by MacLeod et al. in 1949 investigated effects of lithium chloride on the anaerobic and aerobic lactic acid production and the motility of sperm obtained from normal human semen. No detailed information on the design of experiments or statistics was reported. The authors found that exposure to lithium chloride for four hours at concentrations from 6 mM to 25 mM caused decreases in motility and lactic acid production in a dose-dependent manner.

Levin et al. (1981)

In this study, no EC_{50} for an effect on sperm motility was found in human sperm treated in vitro with up to 10 mM lithium carbonate (0.0039 – 10 mM). Sperm motility in this study was measured by the "turbidimetric method", in which a semen sample (100 µl) is layered on the bottom of a specially constructed cuvette containing 0.6 ml of modified Lopata's medium and the sperm motility is measured spectrophotometrically as an increase in absorbance as the sperm swim upward into the light path. It is unknown if there is any difference in the sensitivity and efficiency between the turbidimetric and transmembrane methods.

Raoof et al. (1989)

In this study, semen samples collected by masturbation from seven healthy volunteers were incubated in phosphate buffer saline (PBS) containing 1.0-100 mM lithium chloride for two hours. The sperm motility was measured by the method of transmembrane migration. The authors found that lithium chloride significantly reduced sperm motility with an EC_{50} of 6.4 mM lithium. The authors stated that concentrations of as high as 3.2 mM lithium were observed in the semen samples from individual healthy volunteers treated with therapeutic doses of lithium, but detailed data regarding lithium concentrations in semen samples were not provided.

Shen et al. (1992)

The authors incubated semen samples from healthy donors with lithium carbonate for two hours at concentrations of lithium ranged from 1.0 to more than 13 mM (according to the figure presented by the authors). Sperm motility, measured by the method of transmembrane migration, was apparently decreased in a dose-dependent manner, but no statistical analysis was reported. The authors reported an EC_{50} at 10 mM following a 2-hr treatment.

E.3.2. Male reproductive toxicity of lithium in experimental animals

Information regarding the male reproductive toxicity of lithium following oral administration or subcutaneous or intraperitoneal injections is provided in a number of studies in experimental animals including mice, rats, or rodents captured from the wild. The direct effect of lithium on male reproductive organs was also investigated in several studies using in vitro models. Major findings from these studies are summarized below.

E.3.2.1. Studies in mice by oral routes.

Mroczka et al. (1983)

This study was also discussed in this document under Section D.3.2.1 for the "Female Reproductive Toxicity of Lithium". In this study, both male and female CFW mice were treated with lithium chloride in drinking water at 0, 10, 20, 30, 50, 100, or 200 meq Li/L (0, 70, 140, 210, 350, 700 or 1400 ppm) beginning at 6-8 weeks of age. After two weeks, the animals were mated for a period not reported by the authors, but for multiple rounds of reproduction, apparently until reproduction ceased. Female animals were exposed to lithium during gestation and lactation periods. Plasma levels of lithium were 0.09 and 0.67 mmol/L in the 10 and 50 mmol Li/L-water groups, respectively. Mice treated with lithium chloride at 200 meq Li/L did not drink water and died within one week. Animals at 100 meq Li/L survived but did not reproduce. Reduced numbers of litters/mating pair, increased interval time between litters, and increased postnatal mortality were observed in mice treated with 50 meq Li/L. The authors did not report any data on the general or reproductive effects of lithium in mice treated with lower levels of lithium chloride (10, 20, or 30 meq Li/L in drinking water).

Collins et al. (1988)

In this study, plasma levels of LH and testosterone were measured in male C57BL/6 mice (50-60 days old) fed with diet containing 0 or 0.4 % lithium chloride (94 mmol Li/kg food) for 15 or 30 days. Ten animals per group were used. Mean concentration of plasma lithium was 1.839 ± 0.39 mmol/L after 30 days of treatment. Significantly decreased body weights, increased water consumption, and increased urine production were observed in lithium-treated animals. Plasma testosterone levels were significantly reduced after both 15 days and 30 days treatment (approximately 400 pg/ml in lithium-treated groups vs. 1000-1,200 pg/ml in the control groups, according to the figures reported by the authors). The authors observed no significant change in plasma or pituitary levels of LH.

E.3.2.2. Studies in rats by oral routes

Trautner et al. (1958)

The studies reported by Trautner et al. (1958) were also reported under Section D.3.2.1 for the "Female Reproductive Toxicity of Lithium." The authors conducted several series of studies to investigate the possible effect of lithium chloride administered via drinking water on the pregnancies of treated adult Wistar rats. Male rats in two out of the six series of experiments reported by the authors were exposed to lithium chloride.

In Experiment 2, male and/or female rats were treated at 0 or 25 meq Li/L (0 or 175 ppm) in water for 17 days before mating. When lithium treated males were mated with either

control or lithium treated females, no apparent effect of lithium on male fertility (number of female rats becoming pregnant or litter size) was observed, compared to that in the control group (control male mated with control female; see Table 52). The number of female pregnant rats (two out of six females) and the average litter size per pregnant rat (5.5 or 6.5 per pregnancy) in lithium-treated females mated to control or lithium-treated males appeared to be lower than that in lithium-treated males mated to control females (four or five out of six females and 7.8-8.0 per pregnancy), but no statistical analysis was performed.

In the series of Experiment 3, the animals were treated with 0 or 20 meq Li/L in drinking water for a period not reported by the authors. Consistent with the findings from Experiment 2, the fertility (number of female rats becoming pregnant) in control females mated to lithium-treated males (16/17; 94%) was comparable to that in control females mated to control males (14/15; 93%), but it appeared to be lower in lithium-treated females mated with lithium-treated males (5/7; 71%). No statistical analysis was reported. The authors did not report any data on litter size in this experiment.

In all other series of experiments, the authors observed a reduction in the numbers of female rats becoming pregnant, the numbers of corpora lutea, implants, and viable fetuses per pregnant female in lithium-treated females mated to control males.

Gralla and McIlhenny (1972)

This study was also reported under Section D.3.2.3 for the "Female Reproductive Toxicity of Lithium." The authors reported several series of experiments on the metabolism, developmental and reproductive effects of lithium in rats, rabbits, or monkeys following administration of lithium carbonate either via diet or by gavage. In experiments that are relevant to the possible male reproductive effect of lithium, male albino rats (from Charles River) were treated in diet with lithium carbonate at 0, 0.27, 0.67 or 1.35 meq/kg/d (0, 1.9, 4.7 or 9.5 mg Li/kg/d) for 70 days before mating and then mated to females that received lithium carbonate treatment for 14 days prior to mating. The authors did not report detailed findings from each specific experiment, but generally stated that "maternal parameters such as fertility, average number of implantation sites, average litter size, body weight gain and offspring body weight at 20 days gestation, offspring mortality and gross appearance after transverse sectioning or skeletal staining revealed no differences between treated and control groups." More information about this study is provided in section D.3.2.3.

Prasad and Sheard (1980)

The authors investigated the effect of lithium on serum testosterone levels in rats following either one or several weeks of treatment. In the one-week treatment study, seven mature male Sprague-Dawley rats were treated with lithium chloride in drinking water at 15 meq Li/L (105 ppm) for one week after monitoring of their serum

testosterone levels for five days. The authors found no effect of lithium on body weights; no other general toxicity was reported. Compared to the serum testosterone levels before treatment (3.09 ± 0.44 ng/ml), lithium treatment caused gradual and significant decrease in serum testosterone levels during the 7-day treatment period (from 2.09 ± 0.51 to 1.32 ± 0.34, 1.03 ± 0.22, and 0.66 ± 0.14 ng/ml on day 1, 3, 5, and 7 of treatment, respectively). Serum lithium levels ranged from 0.21 to 0.36 meq/L during the 7-day treatment period.

In another experiment reported in the same paper, a group of seven mature male Sprague-Dawley rats were fed with diet containing lithium chloride at 20 mmol Li/kg diet (140 ppm) for one week, with diet containing 40 mmol Li/kg diet (280 ppm) for one week, and then with diet containing 60 mmol Li/kg diet (420 ppm) for three and one half weeks (total of five and one half weeks). Serum testosterone levels in lithium-treated animals were measured at the end of treatment and compared to those in another group of seven animals that received no lithium treatment. Serum levels of lithium were 0.62 ± 0.04 meq Li/L in lithium-treated animals. Serum levels of testosterone in both control and lithium-treated animals showed wide variation, ranging from 3.7 to 7.7 ng/ml in the control and 2.1-9.1 ng/ml in lithium-treated group. No significant difference in the means of serum testosterone levels between the treated and the control groups was observed (5.28 ± 0.67 in the treated vs. 5.44 ± 0.44 in the control, $p > 0.05$)

Chatterjee et al. (1990)

Mature male Fischer 344 rats were treated with lithium chloride at 0 or 0.4% in diet for 15 or 30 days. The number of animals was not reported. Blood levels of lithium were 0.94 ± 0.04 and 1.46 ± 0.22 meq Li/L for the 15- and 30-day treatments, respectively. The authors observed decreased body weights and increased adrenal weights. Lithium treatment for 15 or 30 days led to significant reduction in the epithelial height of the follicular cells and in an increase in colloidal content in the thyroid, suggesting a hypothyroid condition. The size of the seminal vesicles was markedly reduced after 15 days of treatment and the reduction became dramatic after the 30-day treatment. Morphologically, the epithelial height in the seminal vesicle was reduced markedly; the mucosal pseudostratified columnar epithelium regressed to an almost squamous epithelium. No discernible morphological changes were evident in the testis.

E.3.2.3. Studies with lithium by subcutaneous injection

A series of studies (summarized in Table 59) conducted by Ghosh et al. investigated the male reproductive effects of lithium chloride following subcutaneous injection in Wistar rats. In one of these studies, groups (8 rats/group) of adult male Wistar rats (150-170 g, about nine months old) were treated by subcutaneous (sc) injection with lithium chloride at doses of 0, 1, 2, 4 mg/kg/d (equivalent to 23.59, 47.18, or 94.36 μmol Li/kg/d) for 7, 14, or 21 days (Ghosh et al., 1990a). Plasma levels of lithium 24 hr after the last injection on day 21 were 0.32 ± 0.02, 0.58 ± 0.04, or 0.92 ± 0.10 mmol Li/L for the three treated groups, respectively. No data on general toxicity were reported. The authors

found that treatment with lithium chloride at 2 mg/kg/d for 21 days caused a significant reduction of plasma follicle stimulating hormone (FSH), LH, prolactin (PRL) and testosterone along with inhibition of testicular activities of delta 5-3 beta hydroxysteroid dehydrogenase (3β-HSD) and 17 beta hydroxysteroid dehydrogenase (17β-HSD), two key steroidogenic enzymes. Spermatogenic (gametogenic) activity was also significantly reduced as indicated by reduction in the number of Type A spermatogonia and Step 7 spermatids in seminiferous tubules at Stage VII of the seminiferous cycle when compared to controls. The degree of detrimental effects of lithium on the testis was more prominent at the dose of 4 mg/kg/d. The authors concluded that lithium administration caused significant adverse effects on testicular activities when plasma lithium concentration was below or within the therapeutic range in humans. In addition to the findings summarized above, Ghosh et al. (1990b; 1991a; 1991b) also reported that daily sc injection of 2 or 4 mg/kg lithium chloride for 14 days and 21 days, but not for seven days, showed a significant inhibition in the activity of acid phosphatase in the testis, prostate and seminal vesicle of adult male Wistar rats; increased activity of alkaline phosphatase and decreased organ weights were found in the testis, prostate and seminal vesicle of the animals treated with lithium chloride at doses of 2 and 4 mg/kg/d for 21 days. The authors also found that administration of bovine PRL at a dose of 0.25 mg/kg/d was protective against lithium-caused testicular damages. Adverse effects of lithium chloride on the male reproductive system in immature Wistar rats (35 days of age) were similar to those observed in the adult rats of the same strain (Ghosh et al., 1991c). Based on the findings by Ghosh et al. as summarized in Table 59, the lowest observed effect level (LOEL) of lithium chloride for causing adverse male reproductive effects following subcutaneous injection is 2 mg/kg/d (47.18 μmol Li/kg/d); the no observed effect level (NOEL) is 1 mg/kg/d (23.59 μmol Li/kg/d).

E.3.2.4. Studies with lithium by intraperitoneal or intratesticular injection

The effect of lithium compounds on the pituitary-gonadal axis or reproductive organs of male animals following intraperitoneal injection was investigated in a number of studies summarized in Table 60.

Three studies summarized in Table 60 were conducted in adult male Sprague-Dawley rats by the research group of Banerji et al. (Banerji et al., 1982, 1983; Sheikha et al., 1987). The studies mainly focused on the endocrine effects of lithium chloride following intraperitoneal injection for a short period of time. A comparison of the study designs and the major findings among the studies conducted by the research group of Banerji et al. is presented in Table 61. It appears that repeated treatments with lithium chloride at high doses may cause decreases in plasma level of testosterone in male rats, but the effect of lithium chloride on plasma levels of FSH, LH or testosterone in mice may be different from those observed in rats. The effects of lithium on blood levels of pituitary hormones in rats or mice following ip injection may depend on the dose and duration of treatment.

In addition to the studies by Banerji et al., the study by Perez Romera et al. (2000) compared the male reproductive effects of lithium chloride following ip injection in adult Wistar rats to those in viscacha, a nocturnal rodent captured from the wild. The authors

treated groups (four per group) of male viscachas (weighing 5.5-6.5 kg) or male Wistar rats (weighing 180-230 g) by daily ip injection with 1.0 mmol/kg/d (7 mg/kg/d) for 35 days. Blood concentrations of lithium after 35-day treatment were 0.616 ± 0.007 mmol/l and 0.146 ± 0.003 mmol/l in viscachas and rats, respectively. The authors observed disorganized seminiferous tubules with reduced diameter and germ cell degeneration in the testes of viscacha treated with lithium chloride. The sperm number in the caudae epididymis of lithium-treated viscacha was also significantly decreased (351 ± 77 X 10^6/ml vs. 693 ± 39 X 10^6/ml in the controls; $p < 0.05$). Increased proportions of dead sperm or sperm with poor motility were also significantly increased in lithium-treated viscachas. However, none of the effects observed in viscachas was found in rats treated with the same dose of lithium chloride. It should be noted that the blood lithium concentration in rats was considerably lower than that in lithium-treated viscachas.

Table 59. Male reproductive effects of lithium in rats in vivo (sc injection).

Animals	Treatment	General Toxicity	Reproductive Effects	References
Male Wistar rats, 150-170g, 10 rats/group	Sc injection, 0, 1, 2, 4 mg/kg/d for 7, 14 or 21 days.	Not reported.	Decreased activity of acid phosphatase, decreased activity of alkaline phosphatase, and decreased organ weights in the testis, seminal vesicles, and prostate at 2- and 4-mg/kg/d doses after 14-day treatment. NOEL= 1 mg/kg/d	Ghosh et al., 1990a
Male Wistar rats, 150-170g, 8 rats/group	Sc injection, 0, 1, 2, 4 mg/kg/d lithium chloride for 21 days.	Plasma lithium levels were 0.32, 0.58, and 0.92 meq/L for the three dose groups, respectively. No general toxicity data were reported.	Decreased plasma levels of FSH, LH, PRL and T and in the activities of 3beta-HSD and 17beta-HSD in testicular tissues at 2- and 4-mg/kg/d doses. Decreased numbers of Type A spermatogonia and Step 7 spermatids in Stage VII seminiferous tubules. NOEL= 1 mg/kg/d	Ghosh et al., 1990b
Male Wistar rats, 150-170g, 10 animals/group	Sc injection, 0 or 2 mg/kg/d lithium chloride for 7, 14 or 21 days.	Plasma lithium levels were 0.65, 0.68, and 0.69 meq/L after 7-, 14- and 21-day treatment, respectively. No general toxicity were data reported.	Decreases in plasma levels of FSH, LH, PRL and T and in the activities of 3beta-HSD and 17beta-HSD in testicular tissues were observed after 14- and 21-day treatment. The nuclear area of Leydig cells and the numbers of Type A spermatogonia, preleptotene spermatocytes, and Step 7 spermatids in Stage VII seminiferous tubules were decreased after 21-day treatment.	Ghosh et al., 1991a.
Male Wistar rats, 90 days old, 10 animals per group	Sc injection, 0, 2.0 mg/kg/d lithium chloride or 2.0 mg/kg/d plus 0.25 mg/kg/d prolactin (PRL) for 21d	No effect on body weights. No other general toxicity data were reported.	Decreases in testicular weights, the numbers of spermatogonia and step 7 spermatids, serum levels of FSH, LH, PRL, and T, and activities of testicular 3β- and 17β-HSD. Supplemental treatment with PRL attenuated the lithium effects.	Ghosh et al., 1991b
Male Wistar rats, 35 days of age, 35-38 g, rats/group	Sc injection, 0, 2.0 mg/kg/d lithium chloride for 15, 20 or 25 days.	Plasma lithium levels ranged from 0.52 to 0.61 after treatment for 15-25 days. No effect on the body weights. No other general toxicity data were reported.	Relative organ weights of the testis, prostate, and seminal vesicles were reduced after 20-day treatment. Decreased plasma levels of FSH, LH, PRL and T, in activities of 3beta-HSD and 17beta-HSD in testicular tissues, and decreased numbers of germ cells in Stage VII seminiferous tubules.	Ghosh et al., 1991c

Table 60. Male reproductive effects of lithium following ip or intratesticular injection.

Animals	Treatment	Non-repro. Effects	Endocrine or Reproductive Effects	References
Wistar rats, 180-230 g, 4/group	Ip injection, 1.0 mmol/kg/d lithium chloride for 35 days	Not reported.	No effect on the morphology of testis or the epididymal sperm number and motility. Sex hormone levels not measured.	Perez Romera et al. (2000)
Male SD rats, 50 days of age. 9-19 rats per group	Ip injection, 0 or 2.5 meq/kg, twice daily for 2 days or once daily for 7 days.	Plasma lithium levels were 0.88 (2d) and 3.55 meq/L (7d). No report on general toxicity.	Increased plasma level of LH with 2-day treatment. Decreased plasma levels of LH and PRL with 7-day treatment. No effect on plasma FSH level. Or on the pituitary tissue level of FSH, LH and PRL.	Banerji et al. (1983)
Male SD rats, 250-300g. 5-11 animals/group	Ip injection, 0 or 2.5, or 5.0 meq/kg lithium chloride, twice daily for 1 d; 0, 2.5, or 3.5 meq/kg, twice daily for 5 days	Not reported.	Increased plasma FSH and decreased T levels in 2.5 meq/kg X 5d group; reduced plasma levels of LH, FSH, and T at 3.5 or 5.0 meq/kg doses.	Sheikha et al. (1987)
Male C57BL/6 mice, 9-10 months old, 10-14 animals per group	Ip injection, 0 or 2.5 meq/kg, twice daily for 7 days; 0 or 1.25 meq/kg, twice daily for 21 days	Not reported.	Increased plasma testosterone in the 2.5 meq/kg X 7d group; no effect on plasma levels of T or LH in other treated groups.	Banerji et al. (1982)
Viscacha (nocturnal rodent), 5.5-6.5 kg, 4/group	I.p. injection, 1.0 mmol/kg/d lithium chloride for 35 days	Not reported.	Hypospermatogenesis; decreased sperm count, motility, and viability.	Perez Romera et al. (2000)
Adult albino rats, strain and number per group not reported.	Single intratesticular injection of 0.08 mmol/kg lithium nitrate to the left testis; right testis served as the control.	Not reported.	"mild disorganization of the seminiferus epithelium and the interstitium" was observed at 2 days after injection, but not at 7 days. No effect on the testicular weight or the morphology of spermatozoa in the ductus deferens.	Kamboj and Kar (1964)

Table 61. Endocrine effect of lithium chloride as observed by Banerji et al. [1]

Reference	Banerji et al. (1982)	Banerji et al. (1982)	Banerji et al. (1983)	Banerji et al. (1983)	Sheikha et al. (1987)	Sheikha et al. (1987)
Animals	C57 mice	C57 mice	SD rats	SD rats	SD rats	SD rats
Treatment	Ip inj., 2.5 meq/kg, twice/d for 7d	Ip inj,1.25 meq/kg, twice/d for 21d	Ip inj., 2.5 meq/kg, twice/d for 2d	Ip inj., 2.5 meq/kg, once/d for 7d	Ip inj, 2.5, 5.0meq/kg, twice/d for 1d	Ip inj, 2.5, 3.5meq/kg, twice/d for 5d
Daily dose (meq/kg/d)	5.0	2.5	5.0	2.5	5.0 or 10.0	5.0 or 7.0
Lithium blood levels (meq/l)	0.9 ± 0.1	1.84 ± 0.14	0.88 ± 0.05	3.55	1.01 ± 0.04 4.98 ± 0.29	0.95 ± 0.02 4.63 ± 0.54
Plasma FSH			No effect	No effect	2.5: No effect 5.0: decrease	2.5: increase 5.0: decrease
Plasma LH	No effect	No effect	Increase	Decreased	No effect	2.5: No effect 5.0: decrease
Plasma PRL			No effect	Decreased		
Plasma Testosterone	Increased	No effect			2.5: No effect 5.0: decrease	2.5: decrease 5.0: decrease
Pituitary FSH, LH or PRL			No effect	Increased PRL in ctrl and treated rats.[2]	No effect	No effect

[1] Blank fields indicate the endpoint was not measured in the respective study.
[2] PRL levels in plasma and pituitary tissues increased notably in controls injected with saline.

E.3.2.5. Male reproductive toxicity of lithium in vitro

The male reproductive effects of lithium were investigated in a number of in vitro studies, as summarized below in Table 62. It appears that exposure to lithium as lithium chloride can cause decreased motility of sperm obtained from ram, boar, or bull (White, 1953; Altamirano-Lozano et al. 1998); it also causes diminished activities of 3β-HSD and 17β-HSD in testes isolated from rats (Ghosh et al. 1990c). Although no effect of lithium chloride on production of testosterone in cultured rat testicular cells was reported by Ng and Liu (1990), it should be noted that production of testosterone is the function of Leydig cells but the authors did not report any data on the identity or purity of the cultured cells. Based on the brief description of the method used for cell isolation, it is highly likely that the cells the authors used were a mixture of testicular cells including germ cells, Sertoli cells, and interstitial cells.

Table 62. Male reproductive effects of lithium observed in studies in vitro.

Species	Experimental System	Treatment and Endpoints	Effects	References
Bull	Sperm incubation	Incubated with 4 mmol Li/L for 4 hr. Sperm motility.	Decreased sperm motility.	White, 1953
Ram	Sperm incubation	Incubated with 4 mmol Li/L for 4 hr. Sperm motility.	Decreased sperm motility.	White, 1953
Wistar rats	Whole testis organ incubation	Incubation with 2.5 mmol Li/L for x hr. Activities of 3β-HSD and 17β-HSD.	Decreased activities of 3β-HSD and 17β-HSD.	Ghosh et al., 1990c
Rats, strain not reported (body weight 120-160 g)	Mixed testicular cell culture	Incubation with 0, 1, 10, or 100 μM lithium chloride for 2 hrs. Cell viability and production of Testosterone (T).	No effect on viability or LH-stimulated production of T.	Ng and Liu, 1990
Boar	Sperm incubation	Incubation with 0, 0.05-2.30 mmol Li/L for 1-4 hr. Sperm motility	Decreased sperm motility at all concentrations after 4-hr exposure.	Altamirano-Lozano et al., 1998.

E.3.2.6. Male reproductive effects of lithium in non-mammalian species.

The male reproductive effects of lithium have been investigated in three studies conducted in non-mammalian species. The major findings from these studies are summarized in Table 63. The data provided in these reports suggest that exposure to lithium chloride at doses used by the study authors causes severe adverse effects in the testes of non-mammalian animals captured from the wild during the reproductively active seasons.

Table 63. Male reproductive effects of lithium in non-mammalian species.

Species	Treatment	Non-reproductive Effects	Reproductive Effects	References
Male Roseringed Parakeet (Psittacula krameri); No. of bird/group not reported.	Intramuscular injection, 0 or 0.5 meq/kg (21.2 mg/kg) of lithium chloride, twice daily for 5 or 10 days	Serum lithium levels were 0.14-0.25 meq/l. Decreased body weights.; no effect on feeding behavior.	Decreased testis weights and extensive degeneration. No effect on Leydig cell morphology.	Banerji et al., 1999
Male Spotted Munia (Lonchura punctulata); No. of bird/group not reported.	Gavage, 0, 2.5 or 5.0 mEg/kg/d (106 or 212 mg/kg) for 5 or 10 days.	No effect on behavior.	Decreased testis weights and extensive testicular degeneration.	Banerji et al., 2001
Indian toad (Bufo melanostictus), 8 animals/group	Injection, 200 µg/toad/alternate day for 7, 14, and 21 days.	Not reported.	Decreases in testis weights, 3β- and 17β-HSD activity, and spermatogenic activity.	Nandi et al., 1994

E.4. Mechanistic considerations

There are no data regarding accumulation of lithium in male reproductive organs in men. In the report by Raoof et al. (1989), the authors stated that as high as 3.2 mM of lithium was observed in semen samples from individual healthy volunteers treated with therapeutic doses of lithium, but detailed data regarding lithium concentrations in semen samples are not available. Accumulation of lithium in the pituitary and testis has been observed in rats following intraperitoneal injection with lithium chloride, but the amount of lithium in the testis of treated rats as compared to that in the blood was not reported (Nelson et al., 1976; Stern et al., 1977). Following a single sc injection of 10 meq Li/kg lithium chloride, lithium levels in the testes of male Harlan Sprague-Dawley rats (180 g of weight) reached the highest level of approximately 2.0-2.2 meq Li/kg tissue at about 1.5 hr after injection and remained high until about 12 hrs after injection (Sherman et al., 1985).

Lithium is the most widely used treatment for bipolar affective disorder. It not only treats the acute episode of mania, but also reduces the frequency and severity of recurrent episodes of mania and depression in patients with bipolar or unipolar disorders (Manji et al., 1995). Although the molecular mechanism underlying the therapeutic actions of lithium have not been fully elucidated, several hypotheses have been proposed. Many of the proposed mechanisms have suggested an inhibitory effect on components of various neurotransmitter signaling pathways, such as cyclic AMP (cAMP), cyclic GMP formation, G proteins, or inositol phosphate metabolism. Lithium has also been shown to substitute for monovalent cations, interface with divalent cations and thus influence multiple neurotransmitter systems. The most widely accepted model is the inositol depletion hypothesis. It is based on the observation that lithium inhibits inositol monophosphatase (IMPase) and could thereby deplete the cell of an endogenous source

of inositol. Cells then would become unable to generate inositol 1,4,5-trisphosphate (IP3) in response to extracellular signals and thus IP3-dependent responses would be blocked (Singer and Rotenburg, 1973; Hallcher and Sherman, 1980; Berridge et al., 1989; Manji et al., 1995). Enzyme glycogen synthase kinase-3β (GSK-3β) has been recently proposed to be an alternative target of lithium. GSK-3β was the protein kinase responsible for the inhibitory phosphorylation of glycogen synthase (Klein and Melton, 1996). All of the possible molecular targets discussed above, such as cAMP, cGMP, G proteins, IP3, GSK-3beta, play important roles in establishing and maintaining the normal function of the male reproductive system in mammals. Lithium has been shown to inhibit the activity of myo-inositol 1-phosphatase prepared from rat testes (Parthasarathy et al., 1992). Concentrations of myo-inositol-1-P in the testes of male Harlan Sprague-Dawley rats were increased from 0.11±0.01 mmol/kg dry tissue weight in the control group to 0.31±0.02 mmol/kg dry tissue weight at six hour after a single sc injection of 10 meq/kg lithium chloride (Sherman et al., 1985). Ghosh et al. have proposed that some of the manifestations of lithium-caused male reproductive damages observed by the authors may be due to an effect of lithium on cyclic AMP system by inhibition of adenylate cyclase, but the authors did not provide any experimental data to support their hypothesis (Ghosh et al., 1991c). Gibbons and Gibbons (1984) found that low concentrations of lithium reversibly inhibited the microtubule-based movement of reactivated sea urchin sperm flagella. The authors noted that the action of lithium is directed primarily towards one or more regulatory sites through which Ca^{2+} modulates the asymmetry of flagellar waveform, rather than towards dynein ATPase itself. The authors concluded that lithium inhibited the sperm adenylate cyclase, but this action was not likely relevant to its inhibition of normal motility.

In addition to morphological changes in the testis of rats following sc injection with lithium chloride, Ghosh et al. (1990a, 1991a) and others (e.g., Sheikha et al., 1987) also observed decreased testosterone levels in plasma in rats after repeated treatment with lithium (see above), with or without any change in plasma levels of pituitary hormones (FSH, LH, or PRL). The observed effect of lithium on plasma testosterone levels could result from the actions of lithium on the pituitary-gonadal axis or on the testis directly. Ghosh et al. had observed that lithium inhibited activities of 3β-HSD and 17β-HSD following either in vivo treatment via sc injection or in vitro in cultures of whole testicular organs (Ghosh et al., 1990b, 1991a, 1991b, 1991c).

E.5. Male reproductive toxicity: integrative evaluation of bromacil lithium salt

E.5.1. Male reproductive toxicity of bromacil lithium salt

No information regarding the male reproductive toxicity of bromacil lithium salt in humans or animal species is currently available.

E.5.2. Data on male reproductive toxicity of bromacil

There is no information available regarding the possible male reproductive effects of bromacil in humans.

In animals, no effect on male fertility or reproductive organs was found in one two-generation reproductive study conducted in rats and one dominant lethal study in mice (Mulling, 1991; SRI, 1977). In another dominant lethal study in mice, the authors stated that bromacil was one of those chemicals "causing effects outside control limits but not statistically significant" (Epstein et al., 1972). However, no detailed data about the experiments with bromacil or specific discussion on the findings related to bromacil were presented in the report.

No treatment-related effects of bromacil on the male reproductive organs were reported in chronic studies conducted in rats or dogs. However, relatively detailed information on the possible effects of bromacil on organ weights and morphology of mice following chronic treatment was reported by Wood (1980). Based on the data reported by the study author, statistical analysis by OEHHA staff has found that incidences of several pathological changes in the testis or epididymis were significantly increased among animals treated with bromacil in diet. Notably, the incidences of testicular atrophy among bromacil-treated mice that died prior to terminal necropsy was not significantly higher than in the control group, but this incidence among mice that were treated with bromacil and survived to terminal necropsy was significantly increased in a dose-dependent manner, compared to that of the controls that survived to the terminal necropsy. As mentioned above under "non-DART toxicity", amyloidosis was a common finding in the mouse chronic study and was the major cause of death. The author for the report stated that:

> "Pathological evaluation of the mice that died during the study revealed a variable increase in the incidence of amyloidosis in numerous organ systems which could be attributed as the cause of death in the majority of these mice. The incidence of amyloidosis among the study groups was not, however, clearly related to the dietary concentration of bromacil."

Amyloidosis is a common disease in some strains of mice and can be a cause of Leydig cell atrophy (Gordon et al., 1996). Case studies from the human literature confirm that oligospermia, testicular atrophy, and infertility can occur as secondary events to amyloidosis and amyloid deposition in testes (Ozdemir et al., 2002; Handelsman et al., 1983). Gordon et al. (1996) cited an occurrence of 7.3% testicular amyloidosis in CD-1 mice, the strain used in the bromacil chronic study. The incidence of testicular

amyloidosis in the control group of the bromacil chronic study in mice was 8.7% among animals that died prior to terminal necropsy and zero among those examined at terminal necropsy. However, the incidences of degenerative changes in the testis (testicular atrophy, sloughing germ cells, or germ cell degeneration) were obviously higher than that of testicular amyloidosis, especially among the animals that were subject to terminal necropsy. The authors also reported no case of Leydig cell atrophy; in contrast, significantly increased incidence of interstitial cell hyperplasia was observed in animals treated with 5000-ppm bromacil. In addition, examination of the individual data by OEHHA staff found that amyloidosis were present in 24% of the cases of testicular atrophy occurring in the two highest dose groups (1250 and 5000 ppm) and 21% of the males in all dose groups. Collectively, the data presented in this study report do not indicate a strong correlation between testicular amyloid and testicular atrophy.

E.5.3. Male reproductive toxicity of lithium

E.5.3.1 Major findings on the male reproductive toxicity of lithium

Fertility

There is no information available regarding the effect of lithium on fertility in men. In animals, information on the effect of lithium on fertility (pregnancy rate, the number of litters per mating pair, or interval time between litters) was provided in one study in mice (Mroczka et al., 1983) and two studies in rats (Trautner et al., 1958; Gralla and McIlhenny, 1972). Lithium chloride was used and administered via drinking water in the mouse study by Mroczka et al. (1983) and in the rat study by Trautner et al. (1958).

The rat study by Gralla and McIlhenny probably found no effect of lithium on fertility, implantations, or litter size following treatment of male rats with lithium carbonate in diet for 70 days prior to mating with female rats treated with lithium carbonate by gavage for 14 days prior to mating, based on the general statement by the study authors. Similarly, Trautner et al. (1958) reported no apparent effect of lithium on the number of pregnant female rats mated to male rats treated with lithium chloride at doses of 20 or 25 mmol Li/L in drinking water, but detailed information about the study design and the findings from the experiments was not reported, small numbers of animals per group were used, and no statistical analysis was performed. Thus, based on the limited information provided in these two studies in rats, it is difficult to determine if exposure to lithium causes adverse effect on male fertility in rats.

In the mouse study by Mroczka et al. (1983), the authors observed reduced numbers of litters per mating pair, increased interval time between litters, and increased postnatal mortality in the offspring among animals exposed to 50 mmol Li/L in drinking water. Plasma lithium level was 0.67 mmol/L in mice exposed to 50 mmol Li/L in drinking water. Since both male and female animals were exposed to lithium chloride via drinking water, it is not clear if the observed effects resulted from the exposure of the female or of the male animals or both. This study was also reviewed by Moore et al. in “an assessment of lithium using the IEHR evaluative process for assessing human developmental and

reproductive toxicity of agents" (Moore et al., 1995). Moore et al. (1995) stated that the observed effects on fertility in mice may be considered as information relevant to the male reproductive toxicity of lithium.

Effects on semen quality in men or sperm parameters in animals

Three reports provided information relevant to the possible effect of lithium on semen quality in male patients with affective disorder (Amsterdam et al., 1981; Levin et al., 1981; Tollefson and Garvey, 1989) and one study investigated the effect of lithium on epididymal sperm count and motility in Wistar rats and viscacha (a wild nocturnal rodent) (Perez Romera et al., 2000). The possible effect of lithium on sperm motility has also been studied in four in vitro studies using human sperm (MacLeod et al., 1949; Levin et al., 1981; Raoof et al., 1989; Shen et al., 1992) and in two in vitro studies using sperms from bull, ram, or boar (White, 1953; Altamirano-Lozano et al., 1998).

No obvious lithium treatment-related effect on semen quality was reported among ten bipolar patients studied for a period of 70 days (Tollefson and Garvey 1989). Decreased sperm viability, but no effect on sperm count or motility, among four patients who received lithium carbonate treatment was reported by Amsterdam et al. (1981) and Levin et al (1981), respectively. Both studies, by Amsterdam et al. (1981) and Levin et al. (1981), respectively, were conducted in a very small number of patients (four patients with nine controls), using an uncommon method for sperm motility measurement. It appears that the same study was reported in these two separate papers.

Limited information about the possible effect of lithium on sperm number or quality in animals is available. As observed by Perez Romera et al. (2000), treatment with lithium chloride by i.p. injection at 1.0 mmol Li/kg/d for 35 days caused significant decrease in the number, motility and viability of epididymal sperm in viscacha (wild nocturnal rodents captured during the period of maximal reproductive activity), but not in Wistar rats treated concurrently with viscacha. The authors only used four animals per group. Quantitative data on sperm parameters in rats were not reported. Blood lithium concentrations were 0.616 ± 0.007 mmol/L and 0.146 ± 0.003 mmol/L in viscacha and rats, respectively. The authors stated that the difference in blood concentrations of lithium might explain the discrepancy in their findings between rats and viscacha.

Decrease in sperm motility after direct exposure of human sperm or animal sperm to lithium chloride has been observed in three in vitro studies using human sperm (MacLeod et al., 1949; Raoof et al., 1989; Shen et al., 1992) and in studies using sperm collected from bull, ram or boar (White, 1953; Altamirano-Lozano et al., 1998). However, the study by Levin et al. (1981) using human sperm found that incubation with lithium carbonate at concentrations up to 10 mM did not cause significant reduction in sperm motility. It should be noted that the method for sperm motility measurement used in the study by Levin et al. (1981) was different from that used by Raoof et al. (1989) or Shen et al. (1992) in their studies of human sperm.

Effects on male reproductive organs

There is no information regarding the possible effect of lithium on male reproductive organs in men. There are a number of studies that provided information relevant to the effect of lithium on organ weights and/or morphology of male reproductive organs in experimental animals.

Decreased testis weights have been observed in rats following subcutaneous injection with lithium chloride at a dose of 2.0 mg/kg/d (equivalent to 47.2 μmol/kg/d) for 2-3 weeks (Ghosh et al., 1990a; 1991a; 1991c). No other studies that provided information relevant to the male reproductive effects of lithium have evaluated or reported the effect of lithium on testicular weights. Lithium-caused histopathological changes in the testis have been observed in Wistar rats following sc injection (Ghosh et al., 1990a; 1991a; 1991b; 1991c), in viscacha following ip injection (Perez Romera et al., 2000), but not in Wistar rats following ip injection (Perez Romera et al., 2000) or in Fischer 344 rats following treatment via diet with lithium chloride (Chatterjee et al., 1990). Ghosh et al. (1990a, 1991a, 1991b, 1991c) had investigated the testicular effects of lithium in Wistar rats following sc injection with lithium chloride at different doses for a different period of time and had consistently reported that the numbers of Type A spermatogonia and Step 7 spermatids in Stage-VII seminiferous tubules of rats were significantly decreased after sc injection of lithium chloride for 2-3 weeks at doses ≥ 2.0 mg/kg/d (equivalent to 47.2 μmol/kg/d). Plasma levels of lithium in rats with pathological changes in the testes ranged between 0.58-0.92 meq/L (mmol/L). Similar to the findings in rats as described above, Perez Romera et al. (2000) reported that ip injection of 1.0 mmol/kg/d (7 mg/kg/d) lithium chloride for 35 days caused reduced diameter, epithelial disorganization, and decreased number of germ cells (mostly round and elongated spermatids) in the seminiferous tubules of viscacha. Blood lithium concentration in lithium-treated viscacha was 0.616 mmol Li/L, which is comparable to that in rats in the studies by Ghosh et al (1990a; 1990b). However, no histopathological changes were observed in two other rat studies in which the animals either received ip injection of lithium chloride at a dose of 1.0 mmol/kg/d for 35 days (Perez Romera et al., 2000) or were exposed to lithium chloride in diet for 15 or 30 days (Chatterjee et al., 1990). Notably, the blood lithium concentration in rats in the study by Perez Romera et al. (2000) was 0.146 mmol Li/L, which is obviously lower than that in animals that had marked pathological changes in the testis. The lithium levels in plasma of rats treated with lithium chloride in diet by Chatterjee et al. (1990) were 0.94 and 1.46 mmol Li/L after 15 and 30 days of treatment, respectively. While Ghosh et al. (1990a, 1990b, 1991a, 1991b, 1991c) generally used 8-15 Wistar rats per group in their studies, Perez Romera et al. (2000) used four Wistar rats per group; the number of Fischer rats per group was not reported by Chatterjee et al. (1990). All studies used paraffin section for histopathological evaluation, but the testicular tissues were fixed in Bouin's fluid in studies by Ghosh et al. and Perez Romera et al., respectively, or in 10% neutral formalin in the study by Chatterjee et al.

In addition to changes in organ weights and morphology of the testis, decreased weight/size and/or histopathological changes in seminal vesicles were observed in rats following treatment with lithium chloride via diet (Chatterjee et al., 1990) or by sc injection (Ghosh et al., 1990a, 1991c). Ghosh et al. (1990a, 1991c) also reported decreased prostate weights in rats following sc injection with lithium chloride. Both the

seminal vesicles and the prostate are accessory reproductive glands whose size and function are regulated by levels of testosterone. No other studies reported information relevant to the effect of lithium on accessory reproductive organs.

It should be noted that treatment with lithium also leads to decreased testicular weights with degenerative changes in the testis of non-mammalian species (Ghosh et al., 1989; 1990c; Banerji et al., 1999; 2001).

Effects on sex hormone levels in men or in experimental animals

Two reports provided information about sex hormone levels (FSH, LH, or testosterone) in male patients treated with lithium (Sanchez et al., 1976; Sheard et al., 1977). The study by Sanchez et al. was performed in ten patients aged 42-60 years (average 53 years) with no control group and no information on statistical analysis. No clear evidence on a correlation between testosterone level and lithium treatment was provided by this study. The study by Sheard et al. did not find any consistent changes in testosterone or LH that may be related to lithium treatment.

In animals, decreased plasma levels of testosterone were observed in mice following oral treatment with lithium chloride in diet (Collins et al., 1988), in rats treated with lithium chloride in drinking water (Prasad and Sheard, 1980) or by sc injection (Ghosh et al., 1990a, 1991a, 1991b, 1991c), or by ip injection (Sheikha et al., 1987), but not in rats treated with lithium chloride in diet (Prasad and Sheard, 1980). Plasma levels of testosterone in C57BL/6 mice following ip injection with 1.25 meq/l lithium chloride (8.75 ppm) twice a day for 21 days remained at the levels similar to those in the control animals, but were significantly increased when the animals were ip injected with 2.5 meq/L (17.5 ppm) lithium chloride twice a day for seven days. Decreased plasma levels of FSH, LH, and PRL were also observed by Ghosh et al. in rats following sc injection (Ghosh et al., 1990a, 1991a, 1991b, 1991c), but Collins et al. (1988) reported no change in plasma or pituitary level of LH in mice treated with lithium chloride in diet. Following ip injection in mice or rats, plasma levels of FSH or LH might remain unchanged, or decreased or increased, as observed by the research group of Banerji et al., depending on the dose regime (see Table 61 for detailed information). Overall, it appears that treatment with lithium chloride at relatively high doses and/or for relatively long-time period causes decrease in plasma levels of testosterone, with or without notable changes in plasma levels of LH and/or LH.

Effects on sexual function in men or on sexual behavior in experimental animals

There is no information available regarding the possible effect of lithium on sexual behavior in experimental animals. Information about the effect of lithium on sexual function in men was provided in five reports (Lorimy et al., 1977; Blay et al., 1982; Kristensen and Jorgensen, 1987; Ghadirian et al., 1992; Aizenberg et al., 1996). Although possible loss of libido and impaired erection was observed in two patients reported by Blay et al. (1982), none of the other four reports provided evidence that may support a correlation between impaired sexual function and lithium treatment. However,

limitations in the study design or data analysis should be noted. For example, all the studies or clinical reports used self-reported questionnaires to evaluate the sexual function among patients with affective disorders. The possible effect of affective disorder per se on sexual function among the patients was not addressed in the reported surveys. In addition, only the survey by Kristensen and Jorgensen (1987) included a control group which consisted of surgical outpatients (not healthy volunteers).

F. Summary

F.1. Developmental toxicity

No studies on the possible developmental toxicity of bromacil lithium salt itself have been identified. There are several studies of bromacil in experimental animals and of lithium in humans and experimental animals that have data relevant to developmental toxicity. The designs of these studies vary widely.

The standard units of dose, i.e. mg/kg/d, make it difficult to compare toxicities of bromacil and lithium. This is because the mass of bromacil is much greater than the mass of lithium (approximately 38 fold). If it is assumed that a formulation of bromacil lithium salt contains approximately equal moles of bromacil and lithium, a different unit of dose is required. This report follows the approach of Moore et al. (1995) and expresses bromacil and lithium in mmol/kg/d (or meq/kg/d, which is numerically equivalent).

Studies of bromacil in experimental animals have observed relatively mild forms of developmental toxicity. A developmental study in rats by inhalation found small, but statistically significant reductions in fetal weight and caudal ossification at relatively low doses (0.007 to 0.030 mmol/kg/d) (Newell and Diley, 1978). A later developmental study in rats by gavage found no effect on fetal weight at doses up to 1.9 mmol/kg/d. This study did find increases in skeletal variations at 0.77 and 1.9 mmol/kg/d with a NOEL of 0.29 mmol/kg/d. In addition, increased incidence of skeletal retardation was observed at 1.9 mmol/kg/d, with a NOEL of 0.77 mmol/kg/d (Alvarez, 1988). A two generation reproductive study in rats found no effect on birth weight at doses up to 0.66 mmol/kg/d (Mullin, 1991). A developmental study in rabbits by gavage also found no effect on fetal weight at doses up to 1.9 mmol/kg/d. This study did find increased skeletal variations at 1.9 mmol/kg/d, with a NOEL of 1.15 mmol/kg/d (Zellers, 1987). For comparison purposes, a series of acute lethality studies in rats found apparent differences in sensitivity between oral an inhalation routes of from two to six fold, depending upon assumptions about the cause of differences (see section B.4.4.). The experimental animals studies with bromacil were performed mainly for pesticide registration purposes. Although most of these studies were reported in great detail, only a mild or minimal degree of maternal or systemic toxicity was achieved. There is little information on what might occur at higher doses.

Lithium is used in the treatment of bipolar (manic depressive) disorder, and in treatment of acute mania. In human studies and case reports, the most commonly observed association is between maternal lithium treatment during gestation and cardiac abnormalities in infants, most specifically Ebstein's anomaly. Although earlier studies reported an increased risk of congenital abnormalities with exposure to lithium, later studies did not find a significant association. Various factors may account for these inconsistent findings including the different study designs; the small sample size of some studies, and thus the limited power to detect a significant effect; and the difficulties in the accurate assessment of the outcome of concern. However, the lack of quantitative exposure measures to lithium may be especially important as the findings from animal studies of adverse developmental outcomes and a recent human study of premature birth suggest a threshold effect of lithium.

There are a large number of studies of the possible developmental effects of lithium in experimental animals. Death of the embryo, fetus, or young pup, malformations, and retarded development (e.g. reduced fetal weight) have been observed in many studies in mice and rats. Although some studies did not find effects on one or more of these endpoints, this can generally be attributed to differences in study design. In developmental studies in mice treated by gavage, adverse effects (reduced litter size and malformations) have been observed at doses between 10.8 and 14 mmol/kg/d (Chernoff and Kavlock, 1982, 1983; Seidenberg et al., 1986; Seidenberg and Becker, 1987; Szabo 1969, 1970; Szabo et al., 1970). In developmental studies in rats treated by gavage, adverse effects (reduced litter size, increased resorptions, reduced fetal weight) have been observed at 1.6 or 2.7 mmol/kg/d (Fritz, 1988; Marathe and Thomas, 1986). There are several considerations with regard to hazard identification concerning the experimental animal studies with lithium. Most had very minimal reporting of maternal or systemic toxicity, and only a subset of developmental endpoints were reported. Frequently, qualitative results were reported, but numerical data were not. Also, many studies used only a single dose or concentration of lithium. This may be particularly important since at least some forms of lithium toxicity have extremely steep dose-response curves.

There have not been reports of heart anomalies in experimental animals. However, many of the studies did not appear to employ techniques which would detect such an effect. One study, reported in abstract only, indicated that special techniques were used to examine possible heart anomalies. However, the abstract did not report on the results of the in vitro heart examinations (Laborde and Pauken, 1995).

Maternal toxicity was present in both of the oral bromacil developmental studies. However, the toxicity was minimal up to the highest dose used in the studies, 1.9 mmol/kg/d. Food restriction studies which resulted in greater maternal effects did not result in comparable developmental effects. Evaluation of maternal toxicity in lithium developmental studies is hampered by generally poor reporting.

Comparison of the doses which elicited adverse effects in the oral gavage bromacil and lithium rat studies suggests that they are of similar magnitude on a molar basis. Relatively mild adverse developmental effects were observed in the bromacil studies at

0.77 and 1.9 mmol/kg/d. More severe adverse developmental effects were observed in the lithium studies at 1.6 and 2.7 mmol/kg/d, with no observed adverse effects occurring at 1.35 mmol/kg/d or, in one study, at 4.05 mmol/kg/d. It is plausible that both bromacil and lithium would make a contribution to adverse developmental effects in bromacil lithium salt.

F.2. Female reproductive toxicity

No studies on the possible female reproductive toxicity of bromacil lithium salt itself have been identified. There are several studies of bromacil in experimental animals, three studies of lithium in humans, and several studies of lithium in experimental animals which have data relevant to female reproductive toxicity. The considerations or limitations of the studies relevant to female reproductive toxicity are similar to those described for developmental toxicity.

There are two reproduction studies with bromacil. Both males and females were treated in these studies. The average female premating dose for the high concentration in the later study was 0.75 mmol/kg/d. No adverse effects on fertility or other female reproductive endpoints were observed. Chronic dog, rat, and mouse studies found no effects on reproductive organ histopathology.

One study examined short-term effects of lithium on healthy human females. Treatment for one month with a therapeutic dose of lithium had no effect on menstruation or reproductive hormones. Two studies examined the effects of lithium on women with affective disorders. No association of lithium use by itself and sexual dysfunction was found.

In one study in female mice treated with lithium in food at a relatively high concentration (94 mmol Li/kg food), the mice ceased estrus cycling (Banerji et al., 1986). In another study in mice, both sexes were treated with lithium in drinking water. All mice died at 200 mmol/L. There was no reproduction at 100 mmol/L. Reduced number of litters per mating pair, and increased time between litters was observed at 50 mmol/L (Mrozca et al., 1983). In one early rat drinking water study, reduced female fertility (fraction pregnant) was observed at 25 mmol/L, but not at 20 mmol/L. Rats treated at 20 mmol/L had reduced litter size compared to controls. This was largely due to reduced numbers of corpora lutea (Trautner et al., 1958). Several other studies have not found effects on female reproductive capacity (fraction pregnant, litter size). However, differences in study design may account for some of the differences. Injection studies suggest that alterations of the hormones of the hypothalamic-pituitary-gonadal axis may be involved.

Two studies reported increased pup mortality and/or decrease weight gain after lactational exposure to lithium (Mroczka et al., 1986a; Rider et al., 1978). One group has reported that female rats treated with lithium in drinking water at 2.8-3.7 mmol Li/kg/d displayed less self-grooming and alertness than controls. These rats also neglected their pups. Delayed postnatal pup development was observed (Sechzer et al., 1986, 1992).

Another group did not find altered parenting in rats treated at 10 mmol Li/L (Teixeira et al., 1995). However, rats treated by injection at 3 mmol Li/kg three times during lactation ignored their pups, resulting in pup death (Roy et al., 1999).

F.3. Male reproductive toxicity

No studies on the possible male reproductive toxicity of bromacil lithium salt itself have been located. Several relevant studies with bromacil in experimental animals were retrieved. There have also been several studies with lithium in humans and in animals. The considerations or limitations of the studies relevant to male reproductive toxicity are similar to those described for developmental toxicity.

Treatment with bromacil had no effect on male fertility or reproductive organs in a two-generation reproductive study in rats. In one dominant lethal study in mice, no reproductive effects were observed. In another study, bromacil was described as "causing effects outside control limits but not statistically significant." No treatment-related effects on male reproductive organs were observed in chronic studies in rats and dogs. A chronic study in mice found increased testicular atrophy among mice treated with high doses of bromacil in diet for 12-18 months. Amyloidosis was also commonly observed in the mice, but did not correlate strongly with testicular effects. Testis weights in bromacil-treated mice that survived to terminal necropsy were not affected in this study.

There are several studies of human males treated with lithium. One study with 10 men on long-term lithium therapy for bipolar disorder found major semen parameters (volume, sperm density, sperm motility, abnormal forms) to be within the normal range. Another study with four treated and nine control men found a decrease in viable sperm in patients treated for three weeks with lithium carbonate compared to pretreatment values. No effect on sperm count (density) or motility was observed. Two studies found no association of lithium treatment with alterations of male reproductive hormones. One report of two clinical cases observed altered sexual function in two patients treated with lithium. However, four other reports found no association between lithium treatment and altered sexual function. In general, these studies were conducted among relatively small numbers of patients with affective disorders; many of them did not include control groups or control for potential confounders.

There have been several studies of lithium treatment by oral routes in experimental animals. A study in mice treated with lithium in water found reduced fertility. However, both males and females were treated in this study. Two studies in rats (one by diet, one by water) found no effect on male fertility. However, limited reporting of data makes it difficult to evaluate these studies. In rats treated by diet, no testicular histopathology was observed, although alterations of the seminal vesicles were observed. Decreased plasma testosterone was observed in mice treated in diet and rats treated in water, but not in rats treated by diet.

There have also been a number of studies of lithium treatment by injection routes in experimental animals. One study by ip injection found reduced number, motility, and viability of epidiymal sperm and testes histopathology in viscacha (a wild nocturnal rodent captured during the reproductively active season). No effect was seen in rats treated at the same dose, but the serum lithium level was much higher in the viscacha than the rat. Reduced testis weight was observed in rats following sc injection. Several studies in rats by one group have observed testicular histopathology and reduced weights in seminal vesicles and prostate following sc injection. Testicular activities of 3β- and 17β-HSD were significantly decreased in rats following sc injection. Reduced plasma testosterone was observed in rats treated by sc or ip injection. However, unchanged or increased plasma testosterone was observed in mice treated by ip injection, depending on the dose and duration of treatment. Alterations in other male reproductive hormones have been observed, but the exact effect (decrease, increase or no change) may be dependent on animal species and dosing regime.

Reduced sperm motility has been observed in several in vitro studies using sperm from humans or farm animals, but not in one study which measured sperm motility with a method different from that used by others. The lithium concentrations where substantial effects were observed were considerably higher than normal therapeutic serum levels. However, there is no reliable information on the relationship between serum and semen lithium levels.

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