EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

Bromacil Lithium Salt

FINAL

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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.

G. References

Aizenberg D, Sigler M, Zemishlany Z, Weizman A (1996). Lithium and male sexual function in affective patients. *Clin Neuropharmacol* **19**(6):515-9.

Altamirano-Lozano M, Roldan E, Bonilla E, Betancourt M (1998). Effect of metal compounds on boar sperm motility in vitro. *Adv Exp Med Biol* **444**:105-10; discussion 110-1.

Alvarez L (1988). Teratogenicity study of INN-976 in rats. Newark, DE: du Pont Inc., Haskell Laboratory for Toxicology and Industrial Medicine, 1988; Medical Res. No. 7977-001, Laboratory Project ID 605-87.

Amdisen A (1980). Serum concentration and clinical supervision in monitoring of lithium treatment. *Ther Drug Monit* **2**(1):73-83.

Amsterdam JD, Winokur A, Caroff S, Levin RM (1981). The effects of desmethylimipramine and lithium on human sperm function. *Psychoneuroendocrinology* **6**(4):359-63.

Amsterdam JD, Winokur A, Caroff S, Levin RM (1981). The effects of desmethylimipramine and lithium on human sperm function. *Psychoneuroendocrinology* **6**(4):359-63.

Aoki FY, Ruedy J (1971). Severe lithium intoxication: management without dialysis and report of a possible teratogenic effect of lithium. *Can Med Assoc J* **105**(8):847-8.

Baldessarini RJ, Tondo L, Hennen J, Viguera AC (2002). Is lithium still worth using? An update of selected recent research. *Harv Rev Psychiatry* **10**(2):59-75.

Banerji TK, Maitra SK, Basu A, Hawkins HK (1999). Lithium-induced alterations in the testis of the male roseringed parakeet (Psittacula krameri) : evidence for significant structural changes and disruption in the spermatogenetic activity. *Endocr Res* **25**(1):35-49.

Banerji TK, Maitra SK, Dey M, Hawkins HK (2001). Gametogenic responses of the testis in spotted munia (Lonchura punctulata; Aves) to oral administration of lithium chloride. *Endocr Res* **27**(3):345-56.

Banerji TK, Maitra SK, Dey M, Hawkins HK (2001). Gametogenic responses of the testis in spotted munia (Lonchura punctulata; Aves) to oral administration of lithium chloride. *Endocr Res* **27**(3):345-56.

Banerji TK, Parkening TA, Collins TJ (1982). Lithium: short-term and chronic effects on plasma testosterone and luteinizing hormone concentrations in mice. *Life Sci* **30**(12):1045-50.

Banerji TK, Parkening TA, Collins TJ, Rassoli A (1983). Lithium-induced changes in the plasma and pituitary levels of luteinizing hormone, follicle stimulating hormone and prolactin in rats. *Life Sci* **33**(16):1621-7.

Banerji TK, Parkening TA, Collins TJ, Rassoli AH, Legate LS (1986). Acute lithium treatment suppresses the proestrous LH surge in mice: chronic lithium leads to constant diestrus. *Brain Res* **380**(1):176-80.

Baptista T, Lacruz A, De Mendoza SR, Guillen MM, Burguera JL, De Burguera M *et al*. (2000). Endocrine effects of lithium carbonate in healthy premenopausal women: relationship with body weight regulation. *Prog Neuro-Psychopharmacol Biol Psychiat* **24**:1-16.

Bass AD, Yntema CL, Hammond WS, Frazer ML (1951). Studies on the mechanism by which sulfadiazine affects the survival of the mammalian embryo. *J Pharmacol Exp Ther* **101**:362-7.

Berridge MJ, Downes CP, Hanley MR (1989). Neural and developmental actions of lithium: a unifying hypothesis. *Cell* **59**(3):411-9.

Bishop JB, Morris RW, Seely JC, Hughes LA, Cain KT, Generoso WM (1997). Alterations in the reproductive patterns of female mice exposed to xenobiotics. *Fundam Appl Toxicol* **40**(2):191-204.

Blay SL, Ferraz MP, Calil HM (1982). Lithium-induced male sexual impairment: two case reports. *J Clin Psychiatry* **43**(12):497-8.

Bogdanffy MS (1989). Combined chronic toxicity/oncology study with bromacil (IN N976): two-year feeding study in rats. Newark, DE: du Pont Inc., Haskell Laboratory for Toxicology and Industrial Medicine, 1989; Haskell Project ID 186-89.

Bogdanffy MS (1991). Chronic toxicity study with bromacil (DPX-N976-136) One year feeding study in dogs (includes protocol). Newark, DE: du Pont Inc., Haskell Laboratory for Toxicology and Industrial Medicine, 1991; HLR-91.

Briggs GJ, Freeman RK, Yaffe SJ (2002). *Drugs in Pregnancy and Lactation.* Sixth ed. Philadelphia: Lippincott Williams and Wilkins.

Brock WJ (1988). Acute dermal toxicity study of IN N976-134 in rabbits. Newark, DE: du Pont Inc., Haskell Laboratory for Industrial Medicine, 1988 .

Brown NA, Mccarthy A, Wolpert L (1991). Further studies on the embryonic stage at which left/right asymmetry is specified in rat embryos. *Teratology* **43**(5):446.

Budavari S (1989). Bromacil. *The Merck Index*. 11th ed. Merck & Co., Rahway, New Jersey, p. 209.

Canolty NL, Ibrahim HS, Johnson MA (1989). Effects of dietary lithium carbonate on pregnant rats. *FASEB J* **3**(4):A1073.

CDPR (1997). Summary of Toxicology Data Bromacil. California Department of Pesticide Regulation, California Environmental Protection Agency, 11/14/97.

CDPR (2001). Summary of pesticide use report data 2000. Indexed by Chemical. Preliminary data. California Department of Pesticide Regulation. California Environmental Protection Agency. October 2001.

Chatterjee S, Roden K, Banerji TK (1990). Morphological changes in some endocrine organs in rats following chronic lithium treatment. *Anat Anz* **170**(1):31-7.

Chernoff N, Kavlock R J (1983). A teratology test system which utilizes postnatal growth and viability in the mouse. *Environ Sci Res* **27**:417-27.

Chernoff N, Kavlock RJ (1982). An in vivo teratology screen utilizing pregnant mice. *J Toxicol Environ Health* **10**(4-5):541-50.

Christensen S, Ottosen P D, Olsen S (1982). Severe functional and structural changes caused by lithium in the developing rat kidney. *Acta Pathol Microbiol Immunol Scand [A]* **90**(4):257-67.

Collins TJ, Chatterjee S, LeGate LS, Banerji TK (1988). Lithium: evidence for reduction in circulating testosterone levels in mice following chronic administration. *Life Sci* **43**(19):1501-5.

Cummings E, Heitcamp D (1981). Ventilatory sniffing in the albino rat. *Physiol Zool* **54**(2):230-6.

Czeizel A, Racz J (1990). Evaluation of drug intake during pregnancy in the Hungarian Case-Control Surveillance of Congenital Anomalies. *Teratology* **42**(5):505-12.

Dearani JA, Danielson GK (2000). Congenital Heart Surgery Nomenclature and Database Project: Ebstein's anomaly and tricuspid valve disease. *Ann Thorac Surg* **69**(Suppl. 4):S106-17.

Dostal LA, Anderson JA (1995). Developmental toxicity study in rats treated with the anticonvulsant, ralitoline. *Teratology* **51**:11-9.

du Pont (1987). Aqueous solubility of bromacil. Wilmington, DE: du Pont Inc., Agricultural Products Dept., Research Division, 1987; Report No. BR/PC-30-CA.

Epstein DD, Arnold E, Andrea J, Bass W, Bishop Y (1972). Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol Appl Pharmacol* **23**:288-325.

Fernandez M S, Izquierdo L (1983). Effect of LiCl on differentiation of mouse embryos beyond the blastocyst stage. *Arch Biol Med Exp (Santiago)* **16**(1):51-4.

Finlay C (1996a). Acute oral toxicity study with DPX-N976-188 (Hyvar(r) X-L) in male and female rats. Newark, DE: duPont & Co., Haskell Laboratory for Toxicology and Industrial Medicine, 1996a; Medical Research No. 10661-001, Haskell Laboratory Report No. 509-96.

Finlay C (1996b). Acute dermal toxicity study with DPX-N976-188 (Hyvar(r) X-L) in rats. Newark, DE: E. I. du Pont & Co., Haskell Laboratory for Toxicology and Industrial Medicine, 1996b; Medical Research No. 10661-001, Haskell Laboratory Report No. 470-96.

Flaherty B, Dean BS, Krenzelok EP (1995). Neonatal lithium toxicity as a result of maternal toxicity. *J Toxicol Clin Toxicol 1995;33(5):555* .

Flaherty B, Krenzelok EP (1997). Neonatal lithium toxicity as a result of maternal toxicity. *Vet Hum Toxicol* **39**(2): 92-3.

Frescura C, Angelini A, Daliento L, Thiene G (2000). Morphological aspects of Ebstein's anomaly in adults. *Thorac Cardiovasc Surg* **48**(4):203-8.

Fritz H (1988). Lithium and the developing rat kidney in transplacental target organ toxicity. *Arzneim-Forsch Drug Research* **38**(1):50-4.

Frolich JC, Leftwich R, Ragheb M, Oates JA, Reimann I, Buchanan D (1979). Indomethacin increases plasma lithium. *Br Med J* **1**(6171):1115-6.

Garcia-Palmer F, Weigensberg M, Freinkel N (1988). Lithium and the early postimplantation embryo: embryotoxicity and changes in myoinositol metabolism. *Clin Res* **36**(3):482a.

Ghadirian AM, Annable L, Belanger MC (1992). Lithium, benzodiazepines, and sexual function in bipolar patients. *Am J Psychiatry* **149**(6):801-5.

Ghosh D, Chaudhuri A, Biswas NM, Ghosh PK (1990a). Effects of lithium chloride on testicular steroidogenic and gametogenic functions in mature male albino rats. *Life Sci* **46**(2):127-37.

Ghosh D, Biswas NM, Chaudhuri A, Ghosh AK, Ghosh PK (1990b). Acid and alkaline phosphatase activities in lithium treated testis, prostate and seminal vesicles of adult albino rats: evidence of duration and dose dependent response. *Indian J Exp Biol* **28**(6):553-6.

Ghosh D, Biswas NM, Chaudhuri A, Ghosh PK (1990c). Direct effects of lithium chloride on testicular delta 5-3 beta and 17 beta-hydroxysteroid dehydrogenase activities in the rat--in vitro study. *Acta Physiol Hung* **76**(4):287-90.

Ghosh PK, Biswas NM, Ghosh D (1991a). Effect of lithium chloride on spermatogenesis and testicular steroidogenesis in mature albino rats: duration dependent response. *Life Sci* **48** (7):649-57.

Ghosh D, Biswas NM, Ghosh PK (1991b). Studies on the effect of prolactin treatment on testicular steroidogenesis and gametogenesis in lithium-treated rats. *Acta Endocrinol (Copenh)* **125**(3):313-8.

Ghosh PK, Biswas NM, Ghosh D (1991c). Effect of lithium chloride on testicular steroidogenesis and gametogenesis in immature male rats. *Acta Endocrinol (Copenh)* **124**(1):76-82.

Ghosh PK, Sarkar M, Ghosh P, Ghosh AK, Ghosh D (1989). Effect of lithium chloride on spermatogenesis, testicular delta 5- 3 beta- and 17 beta-hydroxysteroid dehydrogenase activities in toad (Bufo melanostictus). *Andrologia* **21**(3):199-203.

Gibbons BH, Gibbons IR (1984). Lithium reversibly inhibits microtubule-based motility in sperm flagella. *Nature* **309**(5968):560-2.

Giles JJ, Bannigan J (1993). Effects of lithium on neurulation stage mouse embryos. *Teratology* **48**(2):20A.

Giles JJ, Bannigan JG (1997). The effects of lithium on neurulation stage mouse embryos. *Arch Toxicol* **71**(8):519-28.

Glockner R, Jahne F, Schwarz S, Sourgens H, Bockers T (1989). Influence of treatment with lithium during pregnancy on reproductive performance of F1-female rats. *Proc. 6th International Trace Element Symposium.* Vol. 4. Leipzig/Jena: pp. 1270-6.

Goodnick PJ, Schorr-Cain CB (1991). Lithium pharmacokinetics. *Psychopharmacol* **27. 27**(4. 4):475-91, 475-91.

Gordon LR, Majka JA, Boorman GA (1996). Spontaneous nonneoplastic and neoplastic lesions and experimentally induced neoplasms of the testes and accessory sex glands. Mohr U, Dungworth DL, Capen CC, Carlton WW, Sundberg JP, Ward JM, Editor. *Pathobiology of the Aging Mouse*. Washington, DC: ILSI Press, pp. 421-5.

Gralla EJ, McIlhenny HM (1972). Studies in pregnant rats, rabbits and monkeys with lithium carbonate. *Toxicol Appl Pharmacol* **21**:428-33.

Gray LE Jr, Kavlock R J, Ostby J, Ferrell J, Rogers J, Gray K (1986). An evaluation of figure-eight maze activity and general behavioral development following prenatal exposure to forty chemicals: effects of cytosine arabinoside, dinocap, nitrofen, and vitamin A. *Neurotoxicology* **7**:449-62.

Gray LE Jr, Kavlock RJ (1984). An extended evaluation of an in vivo teratology screen utilizing postnatal growth and viability in the mouse. *Teratog Carcinog Mutagen* **4**:403.

Gray LE Jr, Kavlock RJ, Ostby J, Ferrell J (1983). Assessment of the utility of postnatal testing following prenatal exposure to forty chemicals. *Prog Clin Biol Res* **140**:39-62.

Greenspan BJ, Allen MD, Rebar AH (1986). Inhalation toxicity of lithium combustion aerosols in rats. *J Toxicol Environ Health* **18**(4):627-37.

Hallcher LM, Sherman WR (1980). The effects of lithium ion and other agents on the activity of myo-inositol-1-phosphatase from bovine brain. *J Biol Chem* **255**(22):10896-901.

Handelsman DJ, Yue DK, Turtle JR (1983). Hypogonadism and massive testicular infiltration due to amyloidosis. *J Urol* **129**(3):610-2.

Hansen DK, Walker RC, Grafton TF (1989). Comparison of lithium-induced embryotoxicity in mouse and rat embryos in vitro. *Teratology* **39**(5):457.

Hansen KD, Walker RC, Grafton TF (1990). Effect of lithium carbonate on mouse and rat embryos in vitro. *Teratology* **41**:155-60.

Hardman JG, Limibird LE, Gilman AG (editors) (2001). *Goodman and Gilman's The Pharmacological Basis of Therapeutics.* 10th ed. McGraw-Hill.

Haskell (1966). Long-term feeding tests with 5-bromo-3-secondary butyl-6-methyluracil (INN-976; "Hyvar" X; bromacil). Du Pont Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, 1966; Medical Res. No. MR-686, Report No. 21-66.

Hazleton (1966). "Hyvar" X Bromacil Weed Killer: reproduction study - rabbits. Falls Church, VA: Hazleton Laboraories, Inc., 1966; Project No. 201-163.

Heim W, Oelschlager H, Kreuter J, Muller-Oerlinghausen B (1994). Liberation of lithium from sustained release preparations. A comparison of seven registered brands. *Pharmacopsychiatry* **27**(1):27-31.

Hoffman RM (1988). Determination of the water solubility of bromacil, N976. Wilmington, DE: Du Pont Inc., Agricultural Products Dept., 1988; Report No. N976.A.

HSDB (2002a). Bromacil. *Hazardous Substances Data Bank*. via TOMES: National Library of Medicine, 2002a.

HSDB (2002b). Lithium Compounds. *Hazardous Substances Data Bank*. via TOMES: National Library of Medicine, 2002b.

Hsu JM, Rider AA (1978). Effect of maternal lithium ingestion on biochemical and behavioral characteristics of rat pups. Johnson FN, Johnson S, Ed. *Lithium in Medical Practice. Proceedings of the First British Lithium Congress*. Baltimore, MD: University Park Press, pp. 279-87.

Hullin RP, Srinivasan D P, Birch N J (1993). Monitoring lithium treatment. *BMJ* **306**(6872):269-70.

Ibrahim HS, Canolty NL (1990). Effects of dietary lithium on pregnant and lactating rats and their progeny. *Nutr Res* **10**:315.

Izquierdo L, Becker MI (1982). Effect of Li+ on preimplantaion mouse embryos. *J Embryol Exp Morph* **67**:51-8.

Jacobson SJ, Jones K, Johnson K, Ceolin L, Kaur P, Sahn D *et al*. (1992). Prospective multicentre study of pregnancy outcome after lithium exposure during first trimester. *Lancet* **339**(8792):530-3.

Jana D, Nandi D, Maiti RK, Ghosh D (2001). Effect of human chorionic gonadotrophin coadministration on the activities of ovarian d5-3B-hydroxysteroid dehydrogenase, and 17B-hydroxysteroid dehydrogenase, and ovarian and uterin histology in lithium chloride-treated albino rats. *Reprod Toxicol* **15**:215-9.

Johansen KT (1971). Lithium teratogenicity. *Lancet* **1**:1026-7.

Johansen KT, Ulrich K (1969). Preliminary studies of the possible teratogenic effect of lithium. *Act Psychiat Scan* **Suppl 207**:91-5.

Jurand A (1988). Teratogenic activity of lithium carbonate: an experimental update. *Teratology* **38**:101-11.

Kalin NH (1998). The contemporary use of lithium. *J Clin Psychiatry* **59**(Suppl. 6):35-6.

Kallen B, Tandberg A (1983). Lithium and pregnancy. A cohort study on manic-depressive women. *Acta Psychiatr Scand* **68**(2):134-9.

Kamboj VP, Kar AB (1964). Antitesticular effect of metallic and rare earth salts. *J Reprod Fertil* **7**:21-8.

Kehoe RF, Mander AJ (1992). Lithium treatment: prescribing and monitoring habits in hospital and general practice. *BMJ* **304**(6826):552-4.

Kern RH (1993). Bromacil lithium salt: description of beginning materials and synthesis, discussion of impurities. Wilmington, DE: Du Pont Inc., Agricultural Products Experimental Station, 1993; Laboratory Project ID AMR 2886-93.

Kersten L (1981). Toxicity and renal elimination of lithium in rats of different ages. *Arch Toxicol* **47**:135-44.

Kilts CD (1998). The ups and downs of oral lithium dosing. *J Clin Psychiatry* **59**(Suppl. 6):21-6.

Klein PS, Melton DA (1996). A molecular mechanism for the effect of lithium on development. *Proc Natl Acad Sci U S A* **93**(16):8455-9.

Klug S, Collins M, Nagao T, Merker HJ, Neubert D (1992). Effect of lithium on rat embryos in culture: growth, development, compartmental distribution and lack of a protective effect of inositol. *Arch Toxicol* **66**(10):719-28.

Koren G, Pastuzak A, Jacobson S, Nulman I (2001). The safety of commonly used antidepressants in pregnancy. Koren G, ed. *Maternal-fetal toxicology. A clinician's guide.* 3rd ed.New York: Marcel Dekker, pp. 85-104.

Kriegel H (1974). [On the question of the embryotoxic and teratogenic effects of actetylsalicylic acid and a combination preparation containing acetylsalicylic acid]. *Arzneim Forsch* **24**:1317-21.

Kristensen E, Jorgensen P (1987). Sexual function in lithium-treated manic-depressive patients. *Pharmacopsychiatry* **20**(4): 165-7.

Kristoff CA, Hayes PE, Barr WH, Small RE, Townsend RJ, Ettigi PG (1986). Effect of ibuprofen on lithium plasma and red blood cell concentrations. *Clin Pharm* **5**(1):51-5.

Krmpotic E, de la Torre R (1976). Effects of lithium on the fertility and devlopment of mice: evidence of synergistic effec with coxsackie virus. *Birth Defects Orig Artic Ser* . Vol. 13 (3D). p. 281.

Kuehl KS, Loffredo CA, Ferencz C (1999). Failure to diagnose congenital heart disease in infancy. *Pediatrics* **103**(4 Pt 1):743-7.

Laborde JB, Pauken CM (1995). Effect of lithium exposure throughout murine gestation. *Teratology* **51**(3):188.

Leonard A, Hantson P, Gerber GB (1995). Mutagenicity, carcinogenicity and teratogenicity of lithium compounds. *Mutat Res* **339**(3):131-7.

Levin RM, Amsterdam JD, Winokur A, Wein AJ (1981). Effects of psychotropic drugs on human sperm motility. *Fertil Steril* **36**(4):503-6.

Loevy HT (1973). Lithium ion in cleft palate teratogenesis in CD1 mice. *Proc Soc Exp Biol Med* **144**:644-6.

Lorimy F, Loo H, Deniker P (1977). [Clinical effects of long-term lithium treatment on sleep, appetite and sexuality]. *Encephale* **3**(3):227-39.

Luisier PA, Schulz P, Dick P (1987). The pharmacokinetics of lithium in normal humans: expected and unexpected observations in view of basic kinetic principles. *Pharmacopsychiatry* **20**(5):232-4.

MacLeod J, Swan RC, and Aitken A (1949). Lithium:its effect on human spermatozoa, rat testicular tissue and upon rats in vivo. [Abstract]. *Am J Physiol* **157**(2):177-183.

Malek (1989). Acute inhalation toxicity study with DPX-N976-137 (Hyvar milled) in rats. Newark, DE: du Pont Inc., Haskell Laboratory for Toxicology and Industrial Medicine, 1989; Medical Reserch No. 4581-755, Haskell Laboratory Report No. 670-89.

Manji HK, Potter WZ, Lenox RH (1995). Signal transduction pathways. Molecular targets for lithium's actions. *Arch Gen Psychiatry* **52**(7):531-43.

Marathe MR, Thomas GP (1986). Embryotoxicity and teratogenicity of lithium carbonate in Wistar rat. *Toxicol Lett* **34**:115-20.

Marcus WL (1994). Lithium: a review of its pharmacokinetics, health effects, and toxicology. *J Environ Pathol Toxicol Oncol* **13**(2):73-9.

Matsumoto N, Iijima S, Katsunuma H (1974). Placental transfer of lithium chloride and it effects on fetal growth and development in mice. *Teratology* **10**:89.

Matsumoto N, Iijima S, Katsunuma H (1975). Fetal body burdon of chemicals and its effect on fetal growth. *Teratology* **12**:203.

Messiha FS (1986a). Lithium and the neonate: developmental and metabolic aspects. *Alcohol* **3**(2):107-12.

Messiha FS (1986b). Effect of pre and postnatal lithium chloride ingestion on the developing mouse. *Vet Hum Toxicol* **28**(6):554-6.

Messiha FS (1989). Maternally-mediated neonatal lithium-cesium interaction in the mouse. *Physiol Behav* **46**(1):89-95.

Messiha FS (1992). Effect of maternal exposure to lithium salts on neonatal development. *Neurotoxicology 1992;13(2):498* **13**(2):498.

Messiha FS (1993). Maternally-mediated developmental lithium toxicity in the mouse. *Gen Pharmacol* **24**(1):9-15.

Moore JA, and an IEHR Expert Scientific Comittee (1995). An assessment of lithium using the IEHR Evaluative Process for Assessing Human Developmental and Reproductive Toxicity of Agents. IEHR Expert Scientific Committee. *Reprod Toxicol* **9**(2):175-210.

Mroczka DL, Hoff KM, Goodrich CA, Baker PC (1983). Effect of lithium on reproduction and postnatal growth of mice. *Biol Neonate* **43**:287-96.

Mullin LS (1991). Reproductive and fertility effects with bromacil multigeneration reproduction sudy in rats. Newark, DE: du Pont & Co., Haskell Laboratory for Toxicology and Industrial Medicine, 1991; Medical Research Project No. 8767-001, Haskell Laboratory Report No. 724-90.

Nandi DK, Ghosh D, Parua S, Debnath J (1994). Effect of lithium chloride on testicular delta 5-3 beta, 17 beta-hydroxy steroid dehydrogenase, acid phosphatase and gametogenesis in Bufo melanostictus. *Indian J Exp Biol* **32**(5):337-9.

Nelson SC, Herman MM, Bensch KG, Sher R, Barchas JD (1976). Localization and quantitation of lithium in rat tissue following intraperitoneal injections of lithium chloride. I. Thyroid, thymus, heart, kidney, adrenal, and testis. *Exp Mol Pathol* **25**(1):38-48.

Newell GW, Dilley JV (1978). Teratology and acute toxicology of selected chemical pesticides administered by inhalation. Menlo Park, CA: Stanford Research Institute, 1978; EPA-600/1-78-003.

Ng TB, Liu WK (1990). Toxic effect of heavy metals on cells isolated from the rat adrenal and testis. *In Vitro Cell Dev Biol* **26**(1):24-8.

Nilsson A, Axelsson R (1989). Psychopathology during long-term lithium treatment of patients with major affective disorders. A prospective study. *Acta Psychiatr Scand* **80**(4):375-88.

Nora JJ, Nora AH, Toews WH (1974). Lithium, Ebstein's anomaly, and other congenital heart defects. *Lancet* **Sept. 7, 1974**:594-5.

Ozdemir BH, Ozdemir OG, Ozdemir FN, Ozdemir AH (2002). Value of testis biopsy in the diagnosis of systemic amyloidosis. *Urology* **59**:201-5.

Park JM, Sridaromont S, Ledbetter EO, Terry WM (1980). Ebstein's anomaly of the tricuspid valve associated with prenatal exposure to lithium carbonate. *Am J Dis Child* **134**(7):703-4.

Parthasarathy R, Parthasarathy L, Ramesh TG, Devi CS, Vadnal RE (1992). The effects of lithium isotopes on the myo-inositol 1-phosphatase reaction in rat brain, liver, and testes. *Life Sci* **50**(19):1445-50.

Petrere JA, Rohn WR, Grantham LE II, Anderson JA (1993). Food restriction during organogenesis in rabbits: effects on reproduction and the offspring. *Fund Appl Toxicol* **21**:517-22.

Perez Romera E, Munoz E, Mohamed F, Dominguez S, Scardapane L, Villegas O *et al*. (2000). Lithium effect on testicular tissue and spermatozoa of viscacha (Lagostomus maximus maximus). A comparative study with rats. *J Trace Elem Med Biol* **14**(2):81-3.

Persson G (1977). Comparison of plasma lithium levels and their interindividual variations with coated lithium carbonate tablets and a medium-slow-release lithium sulphate preparation (Lithionit Duretter). *Acta Psychiatr Scand* **55**(2):147-52.

Petersen KP (1980). Effect of age and route of administration on LD50 of lithium chloride in the rat. *Acta Phamacol Et Toxicol* **47**:351-4.

Pinelli JM, Symington AJ, Cunningham KA, Paes BA (2002). Case report and review of the perinatal implications of maternal lithium use. *Am J Obstet Gynecol* **187**:245-9.

Prasad V, Sheard MH (1980). The acute and chronic effect of lithium on serum testosterone in rats. *Commun Psychopharmacol* **4**(2):147-52.

Ragheb M (1990). The clinical significance of lithium-nonsteroidal anti-inflammatory drug interactions. *J Clin Psychopharmacol* **10**(5):350-4.

Rane A, Tomson G, Bjarke B (1978). Effects of maternal lithium therapy in a newborn infant. *J Pediatr* **93**(2):296-7.

Raoof NT, Pearson RM, Turner P (1989). Lithium inhibits human sperm motility in vitro. *Br J Clin Pharmacol* **28**(6):715-7.

Rider AA, Hsu J M (1976). Effect of lithium ingestion and water restriction on rat dam and offspring. *Nutr Rep Int* **13**:567-77.

Rider AA, Simonson M, Weng YS, Hsu JM (1978). Effect on rat pup growth and behavior of maternal lithium ingestion and low protein diet. *Nutr Rep Int* **17**:595-606.

Rogers I, Varmuza S (1996). Epigenetic alterations brought about by lithium treatment disrupt mouse embryo development. *Mol Reprod Dev* **45**(2):163-70.

Roy U, Chattopadhyay S, Mukherjee BP (1999). The effects of lithium on reproductive physiology and maternal behaviour in rats. *Indian J Pharmacol 1999 Aug;31(4):306-10* **31**(4):306-10.

Sanchez RS, Murthy GG, Mehta J, Shreeve WW, Singh FR (1976). Pituitary-testicular axis in patients on lithium therapy. *Fertil Steril* **27**(6):667-9.

Sarver JW (1988). Acute oral toxicity study with IN N976-134 in male and female rats. Newark, DE: du Pont Inc., Haskell Laboratory for Toxicology and Industrial Medicine, 1988; Medical Research No. 4581-626, Haskell Laboratory Report No. 553-88.

Sarver JW (1997). Inhalation median lethal concentration (LC50) study with DPX-N976-188 (Hyvar(r) X-L) in rats. Newark, DE: duPont & Co., Haskell Laboratory for Toxicology and Industrial Medicine, 1997; Medical Research No. 10661, Haskell Laboratory Report No. HL-1997-00080.

Schluter G (1971a). [Effects of lithium carmine and lithium carbonate on the prenatal development of mice]. *Naunyn Schmiedebergs Arch Pharmacol* **270**:56-64.

Schmidt MM, Guan K, Wobus AM (2001). Lithium influences differentiation and tissue-specific gene expression of mouse embryonic stem (ES) cells in vitro. *Int J Dev Biol* **45**(2):421-9.

Schmuckler ME, Kern RH, O'Donnell T, Moore LA (1993). Physical and chemical characteristics of the lithium salt of bromacil. Wilmington, DE: Du Pont & Co., Agricultural Products Global Technology Division Experimental Station, 1993; Laboratory Project ID AMR 2853-93.

Schou M (1976). What happened later to the lithium babies? A follow-up study of children born without malformations. *Acta Psychiatr Scand* **54**(3): 193-7.

Schou M (1990). Lithium treatment during pregnancy, delivery, and lactation: an update. *J Clin Psychiatry* **51**(10):410-3.

Schou M (1998). Treating recurrent affective disorders during and after pregnancy. What can be taken safely? *Drug Saf* **18**(2):143-52.

Schou M, Amdisen A (1975). Letter: Lithium and the placenta. *Am J Obstet Gynecol* **122**(4):541.

Schou M, Goldfield MD, Weinstein MR, Villeneuve A (1973). Lithium and pregnancy. I. Report from the Register of Lithium Babies. *Br Med J* **2**(5859):135-6.

Schou M, Weinstein MR (1980). Problems of lithium maintenance treatment during pregnancy, delivery and lactation. *Agressologie* **21**(A):7-9.

Sechzer JA, Alexander GJ, Lieberman KW (1992). Maternal neglect and delayed development: effects of lithium intake in rats. *Anat Rec* **232**(4):79a-80a.

Sechzer JA, Lieberman KW, Alexander GJ, Weidman D, Stokes PE (1986). Aberrant parenting and delayed offspring development in rats exposed to lithium. *Biol Psychiatry* **21**(13):1258-66.

Seidenberg, JM, Becker RA (1987). A summary of the results of 55 chemicals screened for developmental toxicity in mice. *Teratog Carcinog Mutagen* **7**:17-28.

Seidenberg JM, Anderson DG, Becker RA (1986). Validation of an in vivo developmental toxicity screen in the mouse. *Teratog Carcinog Mutagen* **6**:361-74.

Sharma A, Rawat AK (1986). Teratogenic effects of lithium and ethanol in the developing fetus. *Alcohol* **3**(2):101-6.

Sheard MH, Marini JL, Giddings SS (1977). The effect of lithium on luteinizing hormone and testosterone in man. *Dis Nerv Syst* **38**(10):765-9.

Sheikha SH, Collins TJ, Rassoli AH, LeGate LS, Banerji TK (1987). Effects of lithium on the pituitary-gonadal axis in the rat: evidence for dose-dependent changes in plasma gonadotropin and testosterone levels. *Life Sci* **40**(18):1835-44.

Shen MR, Yang RC, Chen SS (1992). Effects of lithium and haloperidol on human sperm motility in-vitro. *J Pharm Pharmacol* **44**(6):534-6.

Sherman H, Kaplan AM (1975). Toxicity studies with 5-bromo-3-sec-butyl-6-methyl uracil. *Toxicol Appl Pharmacol* **34**:189-96.

Sherman WR, Munsell LY, Gish BG, Honchar MP (1985). Effects of systemically administered lithium on phosphoinositide metabolism in rat brain, kidney, and testis. *J Neurochem* **44**(3):798-807.

Sifton DW (ed) (2001). *Physicians' Desk Reference*. 55th ed. Montvale, NJ: Medical Economics Company, Inc.

Singer I, Rotenberg D (1973). Mechanisms of lithium action. *N Engl J Med* **289**(5):254-60.

Smithberg M, Dixit PK (1982). Teratogenic effects of lithium in mice. *Teratology* **26**:239-46.

Smithberg M, Dixit PK, Singer L (1984). Uptake and transfer of lithium in pregnancy and lactation in the mouse. *Proc Soc Exp Biol Med* **175**:164-8.

Smyth HF Jr., Carpenter CP, Weil CS, Pozzani UC, Striegel JA (1962). Range-finding toxicity data: List VI. *Am Ind Hyg Assoc J* **23**:95-107.

Smyth HF Jr., Carpenter CP, Weil CS, Pozzani UC, Striegel JA, Nycum JS (1969). Range-finding toxicty data: List VII. *Am Ind Hug Assoc J* **30**:470-6.

SRI (1977). Evaluation of selected pesticides as chemical mutagens 'in vitro' and 'in vivo' studies. Vol. PB-269 647. Stanford Research Institute, Menlo Park, CA, pp. .

Stern S, Frazer A, Mendels J, Frustaci C (1977). Distribution of the lithium ion in endocrine organs of the rat. *Life Sci* **20**(10):1669-74.

Szabo K T (1969). Teratogenicity of lithium in mice. *Lancet* **2**(7625):849.

Szabo KT (1970). Teratogenic effect of lithium carbonate in the foetal mouse. *Nature* **225**:73-5.

Szabo KT, Hawk AM, Henry M (1970). The teratogenic effect of lithium carbonate upon the palate of randombred mice. *Toxicol Appl Pharmacol* **17**:274.

Teixeira NA, Lopes R C, Secoli SR (1995). Developmental toxicity of lithium treatment at prophylactic levels. *Braz J Med Biol Res* **28**(2):230-9.

Tollefson G, Garvey MJ (1989). Spermatogenesis during extended lithium treatment. *Hillside J Clin Psychiatry* **11**(1):35-41.

Trautner EM, Pennycuik PR, Morris RH, Gershon S, Shankly KH (1958). Effects of prolonged sub-toxic lithium ingestion on pregnancy in rats. *Aust J Exp Biol Med Sci* **36**::305-22.

Troyer WA, Pereira GR, Lannon RA, Belik J, Yoder MC (1993). Association of maternal lithium exposure and premature delivery. *J Perinatol* **13**(2):123-7.

Tuchmann-Duplessis H, Mercier-Parot L (1973). [Influence of lithium on gestation and prenatal development of the rat and mouse]. *C R Seances Soc Biol Fil* **167**(2):183-6.

Tyrer SP, Peat MA, Minty PS, Luchini A, Glud V, Amdisen A (1982). Bioavailability of lithium carbonate and lithium citrate: a comparison of two controlled-release preparations. *Pharmatherapeutica* **3. 3**(4. 4):243-6, 243-6.

U.S. EPA (1988). Recomendations for and documentation of biological values for use in risk assessment. Cincinatti, OH: U.S. Environmental Protection Agancy, 1988; Document No. EPA/600/6-87/008.

U.S. EPA (1994). Proposed rule: addition of certainchemicals; toxic chemical release reporting; community right to know. *Fed Regist* **59 FR**:61432.

U.S. EPA (1996). Reregistration Eligibility Decision (RED): Bromacil. Washington, DC: U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, 1996; EPA 738-R-96-013.

Vacaflor L, Lehmann HE, Ban TA (1970). Side effects and teratogenicity of lithium carbonate treatment. *J Clin Pharmacol J New Drugs* **10**(6):387-9.

Wallis J, Miller R, McFadyen ML, Carlile JB (1989). A comparative study of standard and slow-release oral lithium carbonate products. *S Afr Med J* **76**(11):618-20.

Ward ME, Musa MN, Bailey L (1994). Clinical pharmacokinetics of lithium. *J Clin Pharmacol* **34**(4):280-5.

Warner JP (2000). Evidence-based psychopharmacology 3. Assessing evidence of harm: what are the teratogenic effects of lithium carbonate? *J Psychopharmacol* **14**(1): 77-80.

Weinstein MR, Goldfield M (1975). Cardiovascular malformations with lithium use during pregnancy. *Am J Psychiatry* **132**(5):529-31.

Wellings I (1993). The Submission of Product Chemistry Data for Lithium Salt of Bromacil. *Letter to Mario Fiol, U.S. Environmental Protection Agency*. Du Pont Agricultural Products, 1993.

White IG (1953). Studies on the alkali metal requirements of ram and bull spermatozoa. *Austral. J Biol Sci* **6**:716-24.

Wood CK (1980). Long-term feeding study in mice with 5-bromo-3-sec-butyl-6-methyluracil (INN-976; bromacil). Newark, DE: du Pont Inc., Haskell Laboratory for Toxicology and Industrial Medicine, 1980; ?

Wright TL, Hoffman LH, Davies J (1971). Teratogenic effects of lithium in rats. *Teratology* **4**:151-6.

Zalzstein E, Koren G, Einarson T, Freedom RM (1990). A case-control study on the association between first trimester exposure to lithium and Ebstein's anomaly. *Am J Cardiol* **65**(11):817-8.

Zellers JE (1987). Teratogenicity study of INN-976 in rabbits. Newark, DE: du Pont Inc., Haskell Laboratory for Toxicology and Industrial Medicine, 1987; Medical Res. No. 8187-001, Lab Project No. 527-87.