# Additional Toxicology Studies

## In this guide



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46. This section summarises several other studies that have contributed to the understanding of the toxic effects of boron across species and exposure durations, which show effects at similar dose levels to the Heindel et al (1992), Price et al. (1996) and Weir and Fisher (1972) studies, and drawn from the ATSDR (2010) review.

47. Dixon et al. (1976) studied the effects of sodium tetraborate on reproduction in male rats following acute and subchronic exposure. In the acute study, adult male Sprague–Dawley rats (10 animals per group) were given single oral doses of sodium tetraborate at 0, 45, 150 or 450 mg boron/kg bw. Fertility was assessed by serial mating trials in which each male was mated with a series of untreated virgin females in sequential 7 day periods (for up to 70 days). The females were sacrificed 9 days after the end of their breeding periods (when they could be 9–16 days pregnant), and uteri and fetuses were examined, though no evaluation is reported. Male rats were sacrificed on days 1 and 7, and at subsequent 7-day intervals, for histopathological examination of the testes. No effect on male fertility was found at any dose in this study. Testicular lesions were not reported. For this study, a US EPA evaluation of boron reported a NOAEL of 450 mg boron/kg bw for reproductive effects in male rats following single-dose oral exposure. In the sub-chronic study, rats were exposed to drinking water boron concentrations of 0.3, 1.0 and 6.0 (maximum dose equivalent to 0.84 mg B/kg bw/day). Groups were selected randomly at 30, 60 and 90 days and noted for body weight and weight of the testis, prostate, and seminal vesicles as well as changes in serum chemistry (sodium, potassium, chloride, carbon dioxide, total proteins, albumin, calcium, alkaline phosphatase, total bilirubin, blood urea nitrogen (BUN), glucose and serum glutamic-oxalic transaminase (SGOT), serum glutamic pyruvate transaminase, fructose, zinc and phosphate). Boron treatment did not affect LH and FSH in plasma. The sub-chronic tests failed to indicate any reproductive effects, changes in serum chemistry and body weight and weight of the testis, prostate, and seminal vesicles.

48. A study by Lee et al. (1978) investigated the effects of dietary boron exposure on male Sprague-Dawley rats by administering 0, 500, 1000, and 2000 ppm of boron (as borax) in food for 30 to 60 days (equivalent to 0, 2.8, 5.7, and 11.3 mg B/kg bw/day). Eighteen male rats per group were examined for changes in fertility, testicular and epididymal histology, enzyme activities, hormone levels, and boron accumulation. Rats receiving 500 ppm (2.8 mg B/kg bw/day) showed no significant adverse effects. However, exposure to 1000 and 2000 ppm resulted in testicular atrophy, germ cell depletion, reduced seminiferous tubular diameter, and increased testicular boron levels. These morphological changes were linked

to decreased activities of post-meiotic germ cell markers, while other enzyme activities increased, likely due to the relative enrichment of Sertoli cells and spermatogonia. Hormonal analysis revealed elevated plasma FSH, variable LH changes, and normal testosterone levels. Serial mating studies demonstrated dose-dependent reductions in fertility, with prolonged infertility at the highest dose. Rats exposed to 2000 ppm (11.3 mg B/kg bw/day) exhibited persistent germinal aplasia and infertility, lasting at least 8 months post-exposure. The NOAEL was determined to be 2.8 mg B/kg bw/day, based on testicular atrophy. These findings suggest that boron accumulation in the testes leads to progressive germ cell depletion and long-term reproductive dysfunction.

49. Dixon et al. (1979) investigated the effects of dietary boron exposure to boron on male Sprague-Dawley rats by administering 0, 500, 1000, and 2000 ppm of boron (as borax) in food for 30 to 60 days (equivalent to 0, 25, 50, and 100 mg B/kg bw/day). Eighteen male rats per group were examined for correlations between enzyme activity (hyaluronidase (H), lactate dehydrogenase isoenzyme-X (LDH-X), dehydrogenases of sorbitol (SDH), a-glycerophosphate (GPDH), glucose--phosphate (G6PDH), malate (MDH), glyceraldehyde-3-phosphate (G3PDH), and isocitrate (ICDH)) and testicular histology and androgen activities of the male accessory organs. There was a significant decrease in tubular diameter across all the doses in the 60-day treatment groups. Male fertility was unaffected at 500 ppm. There was significant loss of germinal elements, testicular atrophy, reduced spermatocytes and spermatogenic cells at 1000 ppm. At 2000 ppm, several germinal aplasia, testicular atrophy, infertility and irreversible damage was noted in some cases. There was no dose-related decrease in litter size or fetal death in utero. Plasma FSH levels were elevated at higher doses, however, LH and testosterone remained unchanged. No dominant lethal effects were observed, and a testicular boron concentration of 6-8 ppm was associated with infertility. Overall, the authors established a NOAEL of 25 mg B/kg bw/day based on dose-related tubular germinal aplasia, which was noted to be reversible at low doses.

50. Seal and Weeth (1980) conducted a 70-day study on Long-Evans hooded rats to further evaluate boron toxicity in drinking water and investigate its physiological effects at high concentrations. Male rats (15 per group) were given 0, 150, or 300 mg B/L, corresponding to recalculated doses of 0, 23.7, and 44.7 mg B/kg bw/day. Rats exposed to 150 mg B/L showed a 7.8% reduction in body weight, while those at 300 mg B/L had a 19.8% decrease. High-dose rats exhibited atrophic scrotal sacs, coarse fur, and elongated toenails. Testes and seminal vesicle weights significantly reduced, and spermatogenesis was severely

impaired at 300 mg B/L, with only 3 out of 15 rats producing spermatozoa. Plasma protein and triglycerides were reduced at high doses, and bone calcium levels decreased at 300 mg B/L, indicating possible bone metabolism disruption. The study identified 23.7 mg/kg bw/day as the lowest observed adverse effect level (LOAEL) based on impaired spermatogenesis.

51. Settimi et al. (1982) studied the effects of sodium tetraborate exposure in 2 month old Wistar rats. Male rats (20 per dose group) received either 0 or 3 g/L sodium tetraborate (0 – 20.8 mg B/kg bw/day as reported by ATSDR, 2010) in drinking water for 3 to 14 weeks. The study found increased cerebral succinate dehydrogenase activity after 10 and 14 weeks, along with elevated RNA concentration and acid proteinase activity in the brain at 14 weeks. In the liver, NADPH-cytochrome c reductase activity and cytochrome b5 content in the microsomal fraction decreased after 10 and 14 weeks, while cytochrome P-450 concentration was reduced at 14 weeks. There were no significant effects on body weight or liver, kidney, and testis weights compared to controls. The results support the hypothesis that the borate anion exerts its toxic effects by interfering with flavin metabolism in flavoprotein-dependent pathways.

52. Fail et al. (1991) evaluated the potential reproductive toxicity of boric acid in Swiss CD-1 mice using the Reproductive Assessment by Continuous Breeding protocol. Male and female mice were exposed to boric acid through feed at concentrations of 0, 1000, 4500, or 9000 ppm (0, 27, 111 and 220 B/kg bw/day) for 27 weeks. Fertility effects were observed during a 14-week cohabitation period, where 4500 ppm partially reduced fertility, and 9000 ppm resulted in complete infertility, with no litters, dead or alive, produced at the highest dose. Among litters born at 4500 ppm, live litter size and body weight were significantly reduced. A crossover mating trial confirmed that males were the most affected, as 4500 ppm-exposed males mated with control females had significantly lower fertility rates and mating indices. Necropsy findings after 27 weeks of exposure showed dose-related reductions in male reproductive organ weights, increased abnormal sperm morphology, decreased sperm concentration and motility, and seminiferous tubule degeneration. In females, 4500 ppm exposure led to significantly reduced kidney/adrenal and liver weights, while kidney/adrenal weight reductions were also seen in males. Further assessment of the F1 generation, where the last litters of control and 1000 ppm females were reared to 74 days and mated within their treatment groups, showed normal fertility but decreased adjusted mean body weight in F2 pups. Overall, males were identified as the most sensitive sex for boron toxicity.

53. Harris et al. (1992) conducted a study on Swiss CD-1 mice to assess reproductive and developmental toxicity of boric acid. Male and female mice were orally exposed via gavage, with the female group receiving daily doses for 19 days and co-habited with treated male mice after 7 days to evaluate reproductive toxicity, and a second group exposed during gestation (GD 8-14) and allowed to litter for observations through to postnatal day 4 (PND 4) to assess developmental toxicity. Dose levels used were 0, 120, 400 and 1200 mg/kg/day boric acid (0, 29.8, 69.92 and 209.76 B/kg bw/day). Significant testicular toxicity, including germ cell loss and reduced testis weight, was observed at the highest dose (1200 mg/kg/day). No effects were seen on epididymal weight or sperm density. Pregnant females at high doses exhibited increased post-implantation loss, and there was a reduction in live births at the highest dose, though no neonatal mortality occurred between postnatal days 1 and 4.

54. In a study conducted by Ku et al. (1993), the reversibility of testicular lesions was evaluated in F344 rats administered boric acid in feed at concentrations of 0, 3,000, 4,500, 6,000, or 9,000 mg/kg (0, 26, 38, 52 or 68 mg B/kg bw/day) for 9 weeks. Recovery was assessed for up to 32 weeks post-treatment. Mild spermiation inhibition was observed at 3000 ppm from week 5, while 4500 ppm caused severe spermiation inhibition by week 2, leading to a 72–97% reduction in epididymal sperm count. Higher doses (6000 and 9000 ppm) resulted in progressive testicular atrophy, appearing by week 9 at 6000 ppm and as early as week 6 at 9000 ppm. Even after 32 weeks post-treatment, no recovery from testicular atrophy was observed in higher dose groups. No boron accumulation in the testes beyond levels found in blood was detected during the 9-week exposure period. Following treatment, serum and testis boron levels in all dose groups declined to background levels. Increased serum FSH and LH levels indicated a gonadotropin response to testicular damage.

55. Chapin et al. (1997) investigated whether elevated dietary boric acid levels affected bone-related parameters, including serum electrolytes, bone structure, and strength. In the first study, male rats consumed diets containing 3000, 4500, 6000, or 9000 ppm boric acid (52.5, 78.8, 105 or 157.5 mg B/kg bw/day) for nine weeks, with serum calcium, phosphorous, potassium, chloride, and boron levels monitored during and after exposure. The second study included both male and female rats consuming diets with 200, 1000, 3000, or 9000 ppm boric acid (3.5, 17.5, 52.5 or 157.5 mg B/kg bw/day) for 12 weeks, assessing serum calcium, phosphorous, and magnesium levels, bone boron concentration, and bone structure and strength. The control diet contained 20–40 ppm boric acid. The authors have not provided a reason for this. Serum and bone boron

concentrations were measured at weekly intervals and at 8, 16, 24, and 32 weeks post-exposure. Boron concentrations in bone were elevated in all treated groups, reaching up to four times the levels found in serum. Following cessation of exposure, serum and urinary boron levels returned to control values, while boron levels in bone remained three times higher than control levels up to 32 weeks post-exposure.

56. Yoshizaki et al. (1999) conducted a three-week study on Wistar rats to evaluate the effects of boric acid on male reproductive parameters. Male rats (20 per group) received oral doses of 50, 150, and 500 mg/kg/day of boric acid (8.8, 26, 88 mg B/kg bw/day) via drinking water. Results showed that all parameters of epididymal sperm analysis were affected at the highest dose (500 mg/kg/day), with sperm number, motility, velocity, and amplitude of lateral head displacement also impacted at 150 mg/kg/day. Morphological examinations revealed seminiferous tubule atrophy and multinucleated giant cells in the testes at 500 mg/kg/day. The NOAEL in this study was 50 mg boric acid/kg bw/day (equivalent to 8.8 mg boron/kg bw/day).

57. Sabuncuoglu et al. (2006) exposed male albino Sprague–Dawley rats (24 per group) to boric acid at doses of 0, 100, 275, or 400 mg/kg bw/day (0, 17.5, 48.1 and 70 mg B/kg bw/day). Kidneys were collected on days 10, 30, and 45 following sacrifice, and kidney weights, boron concentrations, and histopathological changes were assessed. Significant boron accumulation was observed in kidney tissue across all experimental groups, with a marked decrease in boron concentration on day 45 compared to day 30. Histopathological degenerative changes, particularly in proximal tubular cells, were dose- and time-dependent. The study concluded that subacute boric acid exposure led to dose-dependent kidney tissue damage in all exposed groups.