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Toxicologic Studies on Borax and Boric Acid

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Toxicologic Studies on Borax and Boric Acid. WEIR, ROBERT J., JR. and FISHER, RUSSELL S. (1972). *Toxicol. Appl. Pharmacol.* 23, 351–364. In Sprague-Dawley rats the acute po LD50 values for borax were 4.5 g/kg and 4.98 g/kg in males and females, respectively; boric acid, 3.45 g/kg in males and 4.08 g/kg in females. In Long-Evans male rats the LD50 values for borax and boric acid were 6.08 g/kg and 3.16 g/kg, respectively. In 90 day feeding studies, rats tolerated both borax and boric acid at 525 ppm boron equivalent. Dogs displayed no adverse effect at 175 ppm of borax (as boron equivalent) and 525 ppm of boric acid (as boron equivalent). In rats, high boron levels at 1750 and 5250 ppm of both compounds caused growth suppression, decreased food utilization efficiency, degeneration of gonads and skin desquamation on the paws and tails. At the 1750 ppm level, both boron compounds produced testicular atrophy in all male dogs. Two year dietary feeding studies indicated that both borax and boric acid could be tolerated by rats and dogs at 350 ppm boron equivalent. Rats fed dietary boron compounds at 1170 ppm levels showed toxic signs which were found to be similar to those observed in subchronic studies. Testicular degeneration was also observed in rats and dogs fed 1170 ppm levels. Reproduction studies revealed that rats fed both borax and boric acid at 1170 ppm boron equivalent were sterile. Both compounds at 350 ppm boron equivalent had no adverse effect on fertility, lactation, litter size, weight and appearance.

Borax has been known as a cleaning agent since the days of the ancient Greek and Roman empires, and also has a long record of use as a food preservative in Europe and America. This has been restricted in recent decades largely because it tended to disguise, and therefore render marketable, food that had deteriorated (Valdes-Dapena and Arey, 1962).

The ubiquitous occurrence of boron in nature and the fact that it has been shown to be an essential element for plant growth (Johnson and Fisher, 1930; Shkolnik, 1935) has stimulated speculation that boron may also be essential to animal life. An excess of soil boron is toxic to plants (Barton-Wright, 1933; Miller, 1938), and effective herbicides have been developed to exploit this finding.

Human poisoning by boron compounds has been reported where large amounts were taken po, or absorbed when boric acid solutions were used for irrigation of body cavities or the gastrointestinal or urinary tracts (Goldbloom and Goldbloom, 1953; Valdes-Dapena and Arey, 1962). Poisoning has also been reported following

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the absorption of boric acid when it was misused as body powder or ointment over large areas of denuded or burned skin (Goldbloom and Goldbloom, 1953; Valdes-Dapena and Arey, 1962). Locksley and Farr (1955) have concluded that the tolerance of adult men to borax appears to be greater than one might anticipate from the literature. They administered borax iv in doses up to 20 g (2.12 g boron) without dangerous toxic effects in 10 patients who were receiving treatment for brain tumors by neutron capture therapy, utilizing B10 as the capture element.

Much research has been performed to evaluate the toxic effects in animals exposed to boron compounds, especially borax and boric acid (Frost and Richards, 1945; Pfeiffer and Jenney, 1950; Ploquin, 1967). The widespread occurrence and use of boron compounds, together with the limited information on complete chronic toxicity studies on borax and boric acid, were important considerations in prompting the studies reported here.

METHODS

Both borax ($Na_2B_4O_7 \cdot 10H_2O$) and boric acid (H_3BO_3) of ACS grade were supplied by the U.S. Borax Research Corporation, Anaheim, California. The boron equivalent is generally referred to throughout the text. Values for deriving these equivalents are as follows.

Weight boric acid ($H_3BO_3 \times 0.1750$ = equivalent weight boron
Weight borax $\times$ 0.1134 = equivalent weight boron

Acute oral toxicity of boron compounds. Albino rats of Sprague-Dawley[1] and Long-Evans[2] strains, 6 groups of 5 rats each, were fasted and dosed by stomach intubation with a single dose of borax or boric acid (50% w/v in 0.5% aqueous methyl cellulose or distilled water suspension). In the dog study, borax and boric acid were administered po by capsule to fasted mongrel dogs. Food and water were freely available during observation periods after the administration of the test material, i.e., 7 days for dogs and 14 days for rats.

Subchronic oral toxicity of boron compounds. Ten young male and 10 female albino rats (Sprague-Dawley strain,[1] weighing 81–119 g) per group were placed for a period of 90 days on dietary concentrations of borax or boric acid at 52.5, 175, 525, 1750 and 5250 ppm as boron equivalent added with thorough mixing to the basal diet[3] on a w/w basis. In addition, five young male and five female beagle dogs per group were placed for a period of 90 days on dietary concentrations of borax or boric acid at 17.5, 175 and 1750 ppm as boron equivalent added to the laboratory diet.[4]

In both studies all animals were individually caged. Body weights and food consumption were measured at weekly intervals. Hematologic studies included packed cell volume, hemoglobin, erythrocyte count, total and differential leukocyte counts on all dogs initially, at 2 and 4 wk and at termination. Biochemical studies including

[1] Charles River Pathogen Free.
[2] This work was conducted by Dr. R. W. Fogleman of Hazleton Nuclear Science Corporation, a subsidiary of Hazleton Laboratories. The supplier of the Long-Evans strain rat was Diablo Laboratories.
[3] Purina Laboratory Chow.
[4] Wayne Dog Feed.

blood urea nitrogen, blood sugar, serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase were performed at the same time. Urine samples were analyzed for specific gravity, pH, protein, sugar, bilirubin, acetone and sediment at similar intervals. Survivors were sacrificed after 90 days on the diet. At necropsy the weights of brain, thyroid, liver, spleen, kidney, adrenals and testes were recorded. The tissues preserved in buffered formalin and studied histopathologically were brain, pituitary, thyroids, lung, heart, liver, spleen, kidneys, adrenals, pancreas, small and large intestines, urinary bladder, testes, ovary (for rat only), bone and bone marrow.

Chronic oral toxicity of boron compounds. The control group of 70 male and 70 female weanling rats (Sprague-Dawley strain[1]) received the basal diet.[3] Test groups of 35 male and 35 female weanling rats each received a diet containing borax or boric acid at 117, 350 and 1170 ppm as boron equivalent, for a period of 2 yr. In the dog study, the control group of 4 male and 4 female young beagles received the basal diet.[4] Test groups of 4 male and 4 female dogs received a laboratory diet containing borax or boric acid at 58, 117 and 350 ppm as boron equivalent for 2 yr. An additional test group of dogs received borax and boric acid at 1170 ppm as boron equivalent in the diet for 38 wk.

All animals were individually housed and provided with free access to the diet and drinking water. Data on body weight, food consumption and toxic signs were recorded regularly. Samples of blood for hematologic studies (as described previously) were taken from representative rats in each group, and from all dogs at seven intervals during the 2 yr feeding period. Biochemical studies and urine analyses (as described previously) were carried out on all dogs at similar intervals. Pooled urine samples from 5 rats each were analyzed at 6, 18 and 24 mo. Five rats of both sexes from each group at 6 and 12 mo, and all survivors at 2 yr were sacrificed and necropsied. One male and one female dog from each group were sacrificed and necropsied after 1 yr of study; the remaining dogs were sacrificed at the end of 2 yr, and gross necropsies were performed. Organ weights (organs involved were described previously) were recorded and organ weight/body weight rations were calculated. Sections of tissues involved (as described previously) were examined for histopathologic alteration. The studies on additional groups of dogs which received 1170 ppm boron equivalent as borax or boric acid were identical to the 2 yr study described above except for the 38 wk early termination.

Reproduction study. Cesarean-derived rats[1] (weighing 110–150 g) were randomized in groups of 8 males and 16 females. The animals received borax or boric acid at 117, 350 and 1170 ppm as boron equivalent in the diet.

Prior to initiation of the first breeding phase, the animals were maintained in individual cages and fed their respective diets for 14 wk. Body weight and food consumption were recorded weekly. After the 14 wk feeding period, 1 male and 2 females were placed in each breeding cage. At 24 hr after birth, the litters were reduced to a maximum of 8 progeny to be raised. The first filial generation (F_{1A}) was carried through weaning and discarded. The parental generation (P_1) was rebred to produce their second litter (F_{1B}). At the time of weaning, 16 females and 8 males each from the control and test groups were selected at random and designated the second parental generation (P_2) for continuation of the reproduction study. These animals were bred to produce the F_{2A} and F_{2B} litters as before. The F_{2B} litter became the P_3 generation and were bred to produce the F_{3A} and F_{3B} litters. With the exception of the P_1, P_2 and P_3 control and test groups, necropsies were performed on all rats.

Statistical method. Numerical deviation from the control observations were evaluated by conventional statistical tests using $P < 0.05$ as the fiducial limit (Snedecor, 1956).

RESULTS

Acute Oral Toxicity of Boron Compounds

Rats. The LD50 values with confidence limits are presented in Table 1. In male Sprague-Dawley rats, the LD50 was 4.50 g/kg for borax and 3.45 g/kg for boric acid. The LD50 for female Sprague-Dawley rats was 4.98 g/kg for borax and 4.08 g/kg for boric acid. In male Long-Evans rats LD50 values were found to be 6.08 g/kg and 3.16 g/kg for borax and boric acid, respectively. The signs of toxicity included depression, ataxia, convulsion and death and were similar for borax and boric acid.

Dogs. When either borax or boric acid was administered po to dogs at various dose levels from 1.54 to 6.51 g/kg borax or from 1.0 to 3.98 g/kg boric acid, all animals, excluding a few at the low dose levels, vomited within the first hour. The severity of the emetic response varied with dosage. There were no deaths during the 14 day post-dosage observation period.

Subchronic Oral Toxicity of Boron Compounds

Rats. The physical appearance of the rats receiving either borax or boric acid at levels at and below 525 ppm boron were generally comparable to those of the controls throughout the study. Rats fed 1750 and 5250 ppm of boron as borax or boric acid had a rapid respiration, inflamed eyes, swollen paws, and desquamated skin on the paws and tails. These animals appeared excited when handled. All males had a shrunken scrotum during the last weeks of the study. One rat each at 52.5 and 1750 ppm of boron (in borax) died during the study. At 5250 ppm of boron, both borax and boric acid killed all rats within 3 to 6 wk.

Growth and food utilization efficiency were significantly reduced for males fed borax at 1750 ppm boron content and for both males and females at 5250 ppm boron. Boric acid at 525 ppm (or less) as boron equivalent did not affect the growth, food consumption and food efficiency. At 1750 ppm boron levels, boric acid reduced growth and food consumption in both males and females.

Borax at 52.5 ppm as boron equivalent caused an increase in the weight of brain, spleen, kidneys and ovaries in female rats, while boric acid at the same level caused an increase in liver weight; no changes of organ weights occurred in male rats. Increase in kidney weight was observed in males fed borax at 175 ppm boron content. Rats which received either borax or boric acid at 525 ppm of boron showed organ weights comparable to those of the controls. Male rats fed boron compounds at 1750 ppm boron content had a significant decrease in body weight and the weights of liver, spleen, kidneys and testes (Table 2); borax at this level also caused a reduction in brain weight, while boric acid lowered adrenal weight. Female rats which received the same dose levels of either borax or boric acid had decreases in body weight and weights of liver, spleen and ovaries; in addition, boric acid caused a fall in adrenal weight (Table 3).

An increase in brain/body weight ratio occurred in female rats fed borax at 52.5 ppm as boron equivalent, while boric acid at the same dose level was accompanied by a decrease in brain/body weight ratio in male rats. Borax caused an increase in kidney/

TABLE 1

LD50 VALUES FOR BORON COMPOUNDS IN RATS

			LD50 g/kg			
			Borax		Boric acid	
Strain	Sex	Body weight (g)	Confidence limits	Boron equivalent	Confidence limits	Boron equivalent
Sprague-Dawley[a]	M	267–310	4.50 (4.14–5.01)	0.51	3.45 (2.95–4.04)	0.60
Sprague-Dawley[a]	F	206–248	4.98 (4.31–5.76)	0.56	4.08 (3.64–4.56)	0.71
Long-Evans[b]	M	85–118	6.08 (3.54–10.4)	0.69	3.16 (estimate)	0.55

[a] Sprague-Dawley rats received boron compounds in 0.5% aqueous methyl cellulose suspensions.
[b] Long-Evans rats received boron compounds in distilled water suspensions.

TABLE 2

CHANGES OF BODY WEIGHT (g) AND ORGAN WEIGHTS (g) FOR MALE RATS RECEIVING DIETARY BORAX OR BORIC ACID (1750 PPM AS BORON EQUIVALENT) FOR 90 DAYS[a]

	Control	Borax	Boric acid
Body	477 ± 31	215 ± 90[b]	268 ± 44[b]
Brain	2.13 ± 0.17	1.86 ± 0.11[b]	1.97 ± 0.15
Thyroids	0.019 ± 0.004	0.015 ± 0.002	0.016 ± 0.004
Liver	16.85 ± 1.35	7.04 ± 1.42[b]	7.41 ± 1.61[b]
Spleen	0.78 ± 0.12	0.34 ± 0.09[b]	0.40 ± 0.11[b]
Kidneys	3.08 ± 0.34	1.92 ± 0.32[b]	1.89 ± 0.33[b]
Adrenals	0.046 ± 0.006	0.039 ± 0.008	0.037 ± 0.009[b]
Testes	3.50 ± 0.26	0.79 ± 0.17[b]	0.83 ± 0.11[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

TABLE 3

CHANGES OF BODY WEIGHT (g) AND ORGAN WEIGHTS (g) FOR FEMALE RATS RECEIVING DIETARY BORAX OR BORIC ACID (1750 PPM AS BORON EQUIVALENT) FOR 90 DAYS[a]

	Control	Borax	Boric acid
Body	247 ± 21	222 ± 28[b]	216 ± 28[b]
Brain	1.91 ± 0.09	1.91 ± 0.13	1.91 ± 0.17
Thyroids	0.015 ± 0.003	0.015 ± 0.003	0.018 ± 0.006
Liver	7.90 ± 1.20	6.40 ± 1.46[b]	6.38 ± 1.16[b]
Spleen	0.52 ± 0.12	0.39 ± 0.11[b]	0.38 ± 0.06[b]
Kidneys	1.88 ± 0.15	1.75 ± 0.28	1.73 ± 0.19
Adrenals	0.05 ± 0.01	0.040 ± 0.009[b]	0.047 ± 0.007
Ovaries	0.124 ± 0.02	0.071 ± 0.025[b]	0.090 ± 0.030[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

body weight ratio at 175 ppm and a decrease in liver/body weight ratio at 525 ppm. Both boron compounds, when fed to rats at 1750 ppm, caused increases in brain, thyroids and adrenal/body weight ratios in the males (Table 4). In addition to these

TABLE 4

CHANGES OF ORGAN WEIGHT: BODY WEIGHT RATIOS (%) FOR MALE RATS RECEIVING DIETARY BORAX OR BORIC ACID (1750 PPM AS BORON EQUIVALENT) FOR 90 DAYS[a]

	Control	Borax	Boric acid
Brain	0.447 ± 0.003	0.81 ± 0.20[c]	0.75 ± 0.11[c]
Thyroids	0.814 ± 0.002	0.007 ± 0.002[c]	0.006 ± 0.002[c]
Liver	3.54 ± 0.27	2.96 ± 0.23[b]	2.77 ± 0.34[b]
Spleen	0.17 ± 0.02	0.14 ± 0.02	0.15 ± 0.04
Kidneys	0.65 ± 0.08	0.81 ± 0.08[c]	0.71 ± 0.09
Adrenals	0.010 ± 0.001	0.015 ± 0.002[c]	0.014 ± 0.003[c]
Testes	0.73 ± 0.06	0.34 ± 0.04[b]	0.32 ± 0.07[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.
[c] Significantly higher than controls, $p < 0.05$.

findings, borax also increased kidney/body weight ratio. There was an increase in brain/body weight ratios in female rats receiving either borax or boric acid at 1750 ppm boron content (Table 5). Furthermore, borax decreased ovaries/body weight ratio; boric acid increased thyroids and decreased liver/body weight ratios.

There were no changes of organ/brain weight ratios in the rats fed either borax or boric acid at 52.5, 175 and 525 ppm as boron equivalent. Both borax and boric acid at 1750 ppm boron content caused decreases in liver, spleen, kidneys and testes/brain weight ratios in male rats (Table 6). Female rats receiving boron compounds at the

TABLE 5

CHANGES OF ORGAN WEIGHT: BODY WEIGHT RATIOS (%) FOR FEMALE RATS RECEIVING DIETARY BORAX OR BORIC ACID (1750 PPM AS BORON EQUIVALENT FOR 90 DAYS)[a]

	Control	Borax	Boric acid
Brain	0.78 ± 0.07	0.90 ± 0.12[c]	0.90 ± 0.14[c]
Thyroids	0.006 ± 0.001	0.007 ± 0.001	0.008 ± 0.003[c]
Liver	3.19 ± 0.21	3.06 ± 0.26	2.95 ± 0.22[b]
Spleen	0.20 ± 0.03	0.18 ± 0.03	0.18 ± 0.04
Kidneys	0.75 ± 0.05	0.79 ± 0.06	0.81 ± 0.05
Adrenals	0.022 ± 0.002	0.019 ± 0.005	0.022 ± 0.002
Ovaries	0.05 ± 0.01	0.034 ± 0.010[b]	0.042 ± 0.013

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.
[c] Significantly higher than control, $p < 0.05$.

TABLE 6

ORGAN: BRAIN WEIGHT RATIOS (%) FOR MALE RATS RECEIVING DIETARY BORAX OR BORIC ACID (1750 PPM AS BORON EQUIVALENT) FOR 90 DAYS[a]

	Control	Borax	Boric acid
Thyroids	0.88 ± 0.22	0.81 ± 0.16	0.79 ± 0.21
Liver	795 ± 68	378 ± 72[b]	373 ± 58[b]
Spleen	37.0 ± 5.0	18.0 ± 4.0[b]	20.0 ± 5.0[b]
Kidneys	146 ± 20	103 ± 16[b]	95.2 ± 11.1[b]
Adrenals	2.2 ± 0.3	2.1 ± 0.4	1.9 ± 0.4
Testes	165 ± 14	42.4 ± 7.7[b]	42.4 ± 5.8[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

same dose levels had lower liver, spleen and ovaries/brain weight ratios (Table 7). Furthermore, borax at 1750 ppm boron content also caused a decrease in adrenals/brain weight ratio in female rats.

Necropsies performed on the animals that died (one each from 52.5 and 1750 ppm boron levels of borax and all rats at 5250 ppm boron level of borax and boric acid) showed congestion of liver and kidneys, bright red lungs and in several animals a swollen appearance of the brain, small gonads and a thickened pancreas.

TABLE 7

ORGAN: BRAIN WEIGHT RATIOS (%) FOR FEMALE RATS RECEIVING DIETARY BORAX OR BORIC ACID (1750 PPM AS BORON EQUIVALENT) FOR 90 DAYS[a]

	Control	Borax	Boric acid
Thyroids	0.76 ± 0.15	0.76 ± 0.13	0.95 ± 0.29
Liver	414 ± 61	334 ± 67[b]	338 ± 69[b]
Spleen	27 ± 7	20 ± 5[b]	20 ± 5[b]
Kidneys	98.7 ± 8.3	91.3 ± 10.6	91.4 ± 12.6
Adrenals	2.8 ± 0.3	2.1 ± 0.4[b]	2.5 ± 0.4
Ovaries	6.49 ± 1.90	3.7 ± 1.3[b]	4.7 ± 1.5[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels were comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

Microscopic examination of the tissues revealed complete atrophy of testes in all males fed either borax or boric acid at 1750 ppm as boron equivalent, partial atrophy in 4 males at 525 ppm of borax and in 1 at 525 ppm of boric acid. Spermatogenic arrest was found in 1 male at 525 ppm of borax. The adrenals of the majority of the males and several females at 1750 ppm boron equivalent of borax revealed a slight to moderate increase in lipid content and the size of the cells in the zona reticularis; the adrenals of 4 males at 1750 ppm boron content of boric acid had similar changes but to a lesser degree.

Dogs. Dogs, with one exception, fed both borax and boric acid at 17.5, 175 and 1750 ppm as boron equivalent were essentially normal in appearance, behavior, elimination, body weights and food consumption. One male dog at 1750 ppm level of boron as borax died of diarrhea on day 68 of the study and showed congested kidneys and severe congestion of the mucosa of small and large intestines. Hematologic, biochemical and urine values were within normal limits except for 2 male and 3 female dogs in the high borax level group (1750 ppm boron content). These animals had decreased packed cell volume and hemoglobin values during the study.

The spleen/body weight ratio in male dogs at 17.5 ppm level of boron as borax was significantly lower than that of the controls. At 175 ppm boron content, as boric acid, a decrease in testes/body weight ratio was observed. Both borax and boric acid caused significant decreases in thyroids and testes/body weight ratios in dogs at 1750 ppm boron content (Table 8). Other organs including spleen, liver, kidneys and adrenals were found to be within normal limits. Neither borax nor boric acid at 17.5 and 175 ppm boron content levels produced any changes in organ weights and organ/body weight ratios in female dogs. Increases in brain/body weight ratio and liver/body weight ratio occurred in dogs fed 1750 ppm boron content levels of borax and boric acid, respectively.

No histologic alterations were seen in dogs fed 175 ppm (or less) of boron in boric acid. Both borax and boric acid at the 1750 ppm boron level produced severe testicular atrophy in all male dogs. Degeneration of the spermatogenic epithelium was generally complete. Red blood cell destruction, as indicated by the presence of hemosiderin in the reticular cells of the liver and spleen and the proximal tubules of the kidney, was somewhat greater in the animals that received borax than in those that received boric

TABLE 8

MEAN BODY WEIGHTS (kg), ORGAN WEIGHTS (g) AND ORGAN: BODY WEIGHT RATIOS (%) FOR MALE AND FEMALE DOGS RECEIVING DIETARY BORAX OR BORIC ACID (1750 PPM AS BORON EQUIVALENT) FOR 90 DAYS[a]

	Control		Borax		Boric acid	
	Weight	Ratio	Weight	Ratio	Weight	Ratio
Male						
Body weight	8.5 ± 1.9		9.3 ± 0.8		8.6 ± 1.0	
Thyroids	0.77 ± 0.14	0.009 ± 0.001	0.59 ± 0.13	0.006 ± 0.001[b]	0.48 ± 0.18	0.006 ± 0.002[b]
Testes	1.72 ± 3.3	0.20 ± 0.03	9.6 ± 3.4	0.10 ± 0.03[b]	10.5 ± 1.5	0.12 ± 0.02[b]
Female						
Body weight	6.2 ± 2.0		7.7 ± 1.2		9.0 ± 2.3	
Brain	68.7 ± 7.1	1.1 ± 0.4	80.3 ± 3.1[c]	1.10 ± 0.15	72.3 ± 2.7	0.85 ± 0.23
Liver	190.0 ± 47.0	2.8 ± 0.5	257 ± 47	3.3 ± 0.5	345 ± 49	4.1 ± 1.2[c]

[a] All numbers are expressed as mean ± SD for four male and five female dogs. All lower levels are comparable to controls.
[b] Significantly lower than control at $p < 0.05$
[c] Significantly higher than control at $p < 0.05$.

acid. The thyroid gland of the borax treated males presented a slightly greater proportion of solid epithelial nests and minute follicles than was found in the control animals, In the adrenal gland, the zona reticularis was consistently increased in width in borax fed dogs and only in boric acid treated female dogs. The high level of boric acid (1750 ppm boron content) also increased the width of the zona glomerulosa in the adrenals of the female dogs. The zona fasciculata was, in general, somewhat decreased in width. The thyroids of the two females were infiltrated by lymphoid tissue, and one was rather markedly atrophied.

Chronic Oral Toxicity Study

Rats. The appearance and behavior of the rats fed both borax and boric acid at 117 and 350 ppm as boron equivalent in the diets were generally comparable with those of the controls. The following gross signs were observed among the rats at the highest level (1170 ppm boron content): coarse hair coats, scaly tails, a hunched position, swelling and desquamation of the pads of the paws, abnormally long toenails, shrunken appearance of the scrotum of the males, inflamed eyelids and bloody discharge of the eyes. Onset of these signs was at the beginning of the second month. They became more frequent and pronounced by the end of the first year, but remained relatively unchanged during the second year.

Both borax and boric acid at 1170 ppm as boron equivalent lowered food consumption during the first 13 wk and suppressed growth in rats throughout the 2 yr study.

Low packed cell volume and hemoglobin values found at many intervals during the study are considered to be significant in male and female rats fed borax at 1170 ppm as boron equivalent, and in female rats which received the same level of boric acid. Biochemical values and urine analyses were found to be within normal limits in rats which received different levels of both boron compounds.

There were no histologic alterations in the organs of rats fed either borax or boric acid at 117 and 350 ppm levels as boron equivalent for 2 yr. Atrophic testes were found in all males receiving 1170 ppm boron in both borax and boric acid at 6, 12 and 24 mo. Microscopic examination revealed atrophied seminiferous epithelium and decreased tubular size in the testes. The testes weights and testes/body weight ratios were significantly lower, whereas the brain and thyroid/body weight ratios were significantly higher than those of the controls.

Dogs. There were no remarkable changes of appearance, behavior, appetite and elimination in dogs fed either borax or boric acid at 58, 117 and 350 ppm as boron equivalent during the 2 yr study. There were no apparent effects on body weight or food consumption. The necropsy findings, organ weights and organ/body ratios were comparable to those of the controls. The hematologic and biochemical values from borax fed dogs were within normal limits.

Since neither borax nor boric acid at low levels (58, 117 and 350 ppm as boron equivalent) produced any testicular change, additional groups of dogs were given boron compounds at 1170 ppm. In this second dog study, ingestion of both borax and boric acid at 1170 ppm as boron equivalent did not produce any adverse effects except the testicular changes. The severe degree of testicular atrophy and spermatogenic arrest found in two test dogs sacrificed at 26 wk appeared to be attributable to ingestion of borax. Boric acid also caused testicular degeneration in dogs. Microscopic examina-

tions of tissues from boric acid treated dogs revealed spermatogenic arrest and atrophy of the seminiferous epithelium of the tubules. Since the testicular changes occurred in dogs following the treatment with both boron compounds at high dose levels (i.e., 1170 ppm as boron equivalent), the study was then terminated at 38 wk. In two dogs placed on control food for 25 days after eating the boric acid diet for 38 wk, weight of the testes and the testes/body weight ratio were comparable to control values. Microscopically, the seminiferous tubules were lined by thick, generally moderately active, spermatogenic epithelium in one of the dogs. Based on only one animal, the testicular degeneration appears to be of a reversible nature, and spermatogenesis appears to take place within a rather short time after withdrawal of boric acid.

Reproduction Study

There were no adverse effects on the reproduction of rats receiving a diet containing either borax or boric acid at 117 and 350 ppm as boron equivalent. Litter size, weights of progeny and appearance were normal compared with those of the controls. The overall fertility indices for the two test compounds at levels of 177 and 350 ppm boron were significantly higher than those of the controls (Tables 9 and 10). Higher lactation

TABLE 9

REPRODUCTION DATA FOR RATS RECEIVING DIETARY BORAX AT 117 AND 350 PPM AS BORON EQUIVALENT

Index	Control	117	350	Control	117	350
		P1–F1A			P1–F1B	
Fertility index[a]	62.5	68.8	75.0	60.0	62.5	75.0
Lactation index[b]	56.3	63.6	82.3[d]	58.8	60.0	74.2[d]
Live birth index[c]	98.4	98.4	100.0	99.1	99.2	99.4
		P2–F2A			P2–F2B	
Fertility index[a]	81.3	81.3	100.0	80.0	75.0	93.8
Lactation index[b]	48.3	79.8[d]	82.7[d]	92.1	93.2	95.5
Live birth index[c]	97.8	99.4	96.9	98.6	92.4	98.8
		P3–F3A			P3–F3B	
Fertility index[a]	68.8	87.5	100.0[d]	68.8	87.5	100.0[d]
Lactation index[b]	91.5	81.1	79.1[e]	89.7	91.8	95.9
Live birth index[c]	100.0	100.0	99.4	100.0	100.0	100.0

[a] Fertility index = number of pregnancies/number of matings ×100.
[b] Lactation index = number weaned/number left to nurse ×100.
[c] Live birth index = number of pups born alive/number of pups born ×100.
[d] Significantly higher than control.
[e] Significantly lower than control.

indices also occurred in rats fed at the same levels of boric acid and in rats fed 350 ppm boron in borax. Live birth indices were within normal limits in the test groups. No gross abnormalities were observed in the organs examined from either parents or weanlings. The high level test groups fed both borax and boric acid at 1170 ppm as boron equivalent were found to be sterile. An attempt to obtain litters by mating the

treated females with the males fed only the basal diet was not successful. Microscopic examination revealed the lack of viable sperm in the atrophied testes of all males at the 1170 ppm boron equivalent level of both borax and boric acid. Evidence was also found of decreased ovulation in the majority of the ovaries examined from the same level females sacrificed following the reproduction study.

TABLE 10

REPRODUCTION DATA FOR RATS RECEIVING BORIC ACID AT 117 AND 350 PPM AS BORON EQUIVALENT

Index	Control	117	350	Control	117	350
		P1–F1A			P1–F1B	
Fertility index[a]	62.5	87.5	81.3	60.0	87.5	75.0
Lactation index[b]	56.3[d]	96.2	70.3[d]	58.8	85.6[d]	80.0[d]
Live birth index[c]	98.4	96.0	97.2	99.1	99.4	100.0
		P2–F2A			P2–F2B	
Fertility index[a]	81.3	93.8	93.8	80.0	93.8	93.8
Lactation index[b]	48.3	79.2[d]	83.1[d]	92.1	81.0	98.0
Live birth index[c]	97.8	100.0	99.4	98.6	99.4	97.9
		P3–F3A			P3–F3B	
Fertility index[a]	68.8	100.0	87.5	68.8	93.8	93.8
Lactation index[b]	91.5	82.5	86.5	89.7	86.7	87.9
Live birth index[c]	100.0	99.5	97.9	100.0	99.0	98.8

[a] Fertility index = number of pregnancies/number of matings ×100.
[b] Lactation index = number weaned/number left to nurse ×100.
[c] Live birth index = number of pups born alive/number of pups born ×100.
[d] Significantly higher than control.

DISCUSSION

The acute LD50 values for both borax and boric acid obtained from the present study confirm those that have been reported in the literature (Pfeiffer *et al.*, 1945). These values indicate that borax and boric acid are relatively nontoxic by the po route.

It is important to note that the dermal effects seen during the subchronic and chronic rat studies closely parallel those described in human intoxication as reported by Pfeiffer and Jenney (1950), Goldbloom and Goldbloom (1953) and Valdes-Dapena and Arey (1962). The violet-red color of the skin of treated dogs (Pffeiffer and Jenney, 1950) was not seen in the present acute or long term studies in dogs.

In previous subchronic or chronic studies, alterations of organs have been described including kidney, central nervous system, liver and skin (Valdes-Dapena and Arey, 1962). Fisher (1951) and Valdes-Dapena and Arey (1962) have reported intracytoplasmic inclusions in the pancreas due to boric acid poisoning. In the present studies, microscopic pathology was limited to the testes which showed atrophy or degenerative changes. This finding is essentially in agreement with that of Nguyen Phy Lich as described by Ploquin (1967). Nguyen Phy Lich described testicular damage in the rat at a dietary level of 2 g/kg of diet as boric acid (350 ppm as boron equivalent), while 1 g/kg (175 ppm

as boron equivalent) was tolerated. In the present study, borax was tolerated at 350 ppm as boron equivalent in rats and dogs, while boric acid at the same boron equivalent level produced no effects in either species. Higher levels of borax or boric acid produced testicular alterations.

Differences in the study design do not reveal why such a discrepancy should exist, but it may be due to: (1) a higher level of boron in the basic diet in the French study. According to Ploquin (1967) the basic human diet in France averages 36.0 ppm boron, whereas the American diet contains about 8 ppm. (2) A higher level of polyhydroxy compounds were used in this study. According to Ploquin (1967) polyhydroxy moiety inactivates the boron compounds.

Other toxic effects produced by both boron compounds at higher levels were growth suppression and decreased food utilization efficiency. These effects complicate the interpretation of organ weight and organ/body weight ratio findings. For example, inconsistent changes of organ weights and organ/body weight ratios were observed in rats fed both boron compounds at 175 ppm boron (or less) for 90 days, while at 525 ppm boron levels the organ weights and organ/body weight ratios were found to be within normal limits. Another inconsistency can be demonstrated here in the 2 yr rat study, in which changes of organ weights and organ/body weight ratios occurred in rats fed at 350 ppm boron levels (or less); however, there were no gross or histologic alterations in these organs. These examples led to the general conclusion that these organ weight or ratio findings were more a result of the body weight change than effect of the compounds directly on the organs.

In the present study, it was found that the organ/brain weight ratios were better indices for organ damage than that of organ/body weight ratios. In 90 day rat studies, there were no changes in organ/brain weight ratios in rats at 525 ppm boron (or less); however, the ratios decreased uniformly in both male and female rats at 1750 ppm as boron in both compounds. These findings were in good agreement with histologic reports.

In rat reproduction studies, doses of 1170 ppm borax or boric acid as boron equivalent were found to interfere with reproduction, while doses of 350 ppm boron in borax and boric acid had no adverse effect on fertility, lactation, litter size, weight or appearance. Ploquin (1967), reporting the work of Nguyen Phy Lich, indicated that boric acid at 350 ppm as boron equivalent produced stillbirth or death 3 or 4 days postpartum. Ploquin relates this to the earlier work of Caujolle et al. (1962) and the teratogenic effect observed by Ridgeway and Karnofsky (1952) and Landauer (1953) using the chick embryo technique. The controversy over the validity of the latter technique for evaluation of teratogenicity may make such a correlation undesirable even when the evidence by two techniques points in the same direction.

The results obtained from these studies indicate that there is a reasonable margin of safety between the toxic dose in animals and the levels of boron which may occur as incidental residues from the use of borax and boric acid in agriculture and industry. The toxicologic effects and levels which can be tolerated in the case of borax and boric acid are markedly similar when doses are compared on a boron equivalent basis. In these studies, 350 ppm boron equivalent in the chronic exposure of rats and dogs as well as in the reproduction study is without adverse effect and can be described as the no effect level.

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