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Methods to Assess Reproductive Effects of Environmental Chemicals: Studies of Cadmium and Boron Administered Orally

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Results of a U.S.S.R.–U.S. cooperative laboratory effort to improve and validate experimental techniques used to assess subtle reproductive effects in male laboratory animals are reported. The present studies attempted to evaluate the reproductive toxicity of cadmium as cadmium chloride and boron as borax ($Na_2B_4O_7$) and to investigate the mechanism of toxicity in the rat following acute and subchronic oral exposure. *In vitro* cell separation techniques, *in vivo* serial mating tests, and plasma assays for hormones were utilized. Effects on the seminal vesicle and prostate were evaluated with chemical and enzyme assays. Clinical chemistry was monitored routinely. Acute oral doses, expressed as boron were 45, 150, and 450 mg/kg while doses for cadmium equivalent were 3.25, 12.5, and 25 mg/kg. Rats were also allowed free access to drinking water containing either boron (0.3, 1.0, and 6.0 mg/l.) or cadmium (0.001, and 0.1 mg/l.) for 90 days. Randomly selected animals were studied following 30, 60, and 90 days of treatment. These initial studies, utilizing a variety of methods to assess the reproductive toxicity of environmental substances in male animals, suggest that cadmium and boron at the concentrations and dose regimens tested are without significant reproductive toxicity.

Our U.S.S.R.–U.S. cooperative laboratory effort seeks to improve and validate experimental techniques used to assess subtle reproductive effects in laboratory animals. In our laboratory, we routinely use the velocity sedimentation cell separation technique to determine cellular uptake of environmental chemicals and to determine the effect on environmental substances on the incorporation of thymidine, uridine, and L-leucine by specific spermatogenic cell types (*1*). *In vivo* serial mating studies assess reproductive function. A variety of other experimental techniques are also to be tested and evaluated jointly. It was agreed that the acute and subchronic oral exposure (in drinking water) of selected environmental chemicals would be studied in the U.S. by *in vitro* cell separation techniques, *in vivo* serial mating tests, and plasma assays for hormones. Effects on the seminal vesicle and prostate would be evaluated with chemical and enzyme assays. Soviet scientists agreed to study the male reproductive effects of the same environmental chemicals using techniques common to their laboratories. Cadmium and boron were selected for initial study.

Health effects of environmental cadmium are a continuing concern in the United States. Cadmium concentrations in both natural and drinking water are usually less than 1 ppb. Concentrations of cadmium greater than 0.01 mg/l. constitute grounds for rejection of a water supply for drinking. Cadmium is sometimes taken up in grains, such as wheat, depending on the cadmium concentration of the soil. The use of sludge may increase the cadmium uptake in food grains severalfold. Cadmium

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in foodstuffs is generally less than 0.05 ppm. In marine organisms, fish meat contains very small amounts of cadmium, whereas internal organs of fish and shellfish may contain appreciable amounts. Daily uptake of cadmium in nonpolluted areas is estimated to be 50 μg with a possible regional variation from 30 to 70 μg (*2*).

Numerous studies of cadmium-induced toxicities have been reported in the past, although most involve parental administration. In the pregnant rat and mouse, cadmium (2.24–4.48 mg/kg) causes rapid placental necroses and fetal death. Cadmium also damages the sensory ganglia in both male and female rats and produces a transient disturbance in liver function in the same dosage range. A single subcutaneous or intramuscular dose of cadmium can induce sarcoma at the injection site and interstitial cell tumors of the testis (*3*).

The toxic effect of cadmium on the testes has been reported to be very selective, and this fact is well documented. Doses as low as 1.12–2.24 mg/kg of cadmium can cause testicular damage without pathological changes of other organs. A single large injection of 10 mg/kg of cadmium leads to the selective destruction of rodent testes. Two theories are generally offered for the mechanism of cadmium-induced testicular lesions: a circulatory failure due to vascular damage, and a direct action of cadmium on spermatogenic cells which can be reversed by zinc. Doses of cadmium without histopathologic effects have a direct action on germinal epithelium (*3*). Cadmium metabolism and toxicity have been reviewed recently by Nordberg (*4*).

In the United States, much less concern surrounds the possible health hazards associated with boron compounds. These chemicals are not highly toxic and therefore not considered an industrial problem. Boron is used in medicine as sodium borate, boric acid, or borax, which is also a common cleaner. Accidental poisoning due to boric acid and oral ingestion of borates or boric acid have been reported. The fatal dose of orally ingested boric acid for an adult is somewhat more than 15 or 20 g and for an infant 5–6 g. Weir and Fisher (*5*) have studied the chronic toxicity of borax and boric acid in laboratory species and confirm the low order of toxicity for these chemicals. Boron can affect the central nervous system. Boron poisoning causes depression of the circulation, persistent vomiting, and diarrhea, followed by shock and coma. Boric acid intoxication can arise from absorbing toxic quantities from ointments applied to burned areas or to open wounds, but it is not absorbed from intact skin.

Soviet scientists have been concerned with the long-term use of drinking water with a high boron content. Bokina has reported gastrointestinal tract functional effects of boron in drinking water (*6*). The United States Public Health Service does not list boron in its 1962 chemical standards for drinking water. In contrast to the boron compounds discussed, decaborane has been used as a rocket propellant and for various industrial purposes and is toxic to exposed workers. Signs and symptoms of human intoxication are in general referable to the central nervous system.

The present studies attempt to evaluate the reproductive toxicity of cadmium (cadmium chloride) and boron (borax, $Na_2B_4O_7$) and to investigate the mechanism of toxicity in the rat. Both acute and subchronic oral administration were studied. The velocity sedimentation cell separation technique was utilized to identify the spermatogenic cell types which incorporate these substances. Both *in vivo* and *in vitro* effects of cadmium and boron on the uptake of tritiated thymidine, uridine, and L-leucine by selected spermatogenic cells were investigated as an indication of alternation of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and/or protein syntheses. *In vivo* effects on fertility were determined using the serial mating techniques.

The effects of subchronic exposure to drinking water containing either cadmium or boron on endocrine function and levels of selected enzymes were also studied. Fructose, zinc, and acid phosphatase levels were assayed in the prostate. Plasma levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) were also determined.

Acute Studies

Single oral doses of cadmium chloride and borax were administered to adult male Sprague-Dawley rats to determine reproductive effects during serial mating studies. Acute doses, expressed as boron, were 45, 150, and 450 mg/kg while doses for cadmium equivalent were 6.25, 12.5, and 25 mg/kg.

In vivo Assessment of Fertility

Serial mating assesses the biological functionality of sperm cells and produces fertility patterns which are inversely related in time to the phase of spermatogenesis damaged by the chemical tested. For instance, effects on spermatozoa appear

first, and those due to interference with spermatogonia appear last. The successful application of this method depends upon the fact that spermatogenesis proceeds continuously without regard to frequency of mating. Thus, the relationship between a chemical effect on fertility and the type of spermatogenic stage affected can be readily estimated. For these experiments, the serial mating technique of Jackson et al. (7) was utilized. Serial mating studies for control and experimental groups were performed concurrently. Male rats were acclimated to the animal room and breeding conditions for at least 4 weeks before they were used. They were housed in a quiet room maintained at a temperature of 72 ± 1°F with constant air circulation. Animals were treated on day 0 with cadmium chloride or borax. Treatment groups of 10 male rats were used. After treatment, each male rat was housed singly with a virgin female for a period of 7 days. During each 7-day period, female animals were examined daily for vaginal plugs to ensure that the treatment did not interfere with ejaculation and mating capability. After 7 days the female rats were removed from the males and replaced with virgin females. These breeding studies are usually terminated 70 days after chemical treatment. Nine days after the end of the breeding period, when a female could be approximately 9–16 days pregnant, the rats were sacrificed, uteri and fetuses were examined, and the number of reabsorptions and viable fetuses were recorded. The fetuses were examined grossly before fixing representative samples in neutral formalin solutions for future study.

Fertility profiles were drawn from these data in which the ordinate expresses the percentage of the males determined to be fertile as indicated by pregnant females. Females that had three or more viable fetuses were considered pregnant. The abscissa indicates the days after drug treatment. The vertical lines indicate the cell type that was present at the time of treatment. The relative duration of each main type of spermatogenic stage is as follows: spermatogonia, 14 days; spermatocytes, 21 days; spermatids, 21 days; spermatozoa, 14 days. The more mature the cell type at the time of treatment, the earlier it appears in the ejaculate as mature sperm (see Figs. 6 and 7).

In addition, morphologic evidence of testicular damage was obtained by sacrificing rats on days 1 and 7 and for six succeeding 7-day intervals; the testes were removed, and after fixation in Bouin's solution and routine histological processing, examined.

Velocity Sedimentation Cell Separation Studies

Major spermatogenic cell types were separated by velocity sedimentation by using a modified method of Lam et al. as previously described by Lee and Dixon (1). Attempts were made to determine the uptake of cadmium by individual spermatogenic cell types and to assess the effects of these chemicals on the incorporation of thymidine, uridine, and L-leucine by selected spermatogenic cell types.

The methodology is presented diagrammatically in Figure 1. A is the sedimentation chamber, B is the gradient maker, and C is a small intermediate vessel. The sedimentation chamber is a cylindrical cavity with a conical angle of 30° to the horizontal and made from a block of Lucite. Testicular cells are prepared by removing the tunica albuginea from the testes under a stereomicroscope and cutting the testicular tissue into short segments with an array of stainless steel razor blades spaced 0.2 mm apart. This tissue mince is washed with Spinners solution containing 0.5% bovine serum albumin. Subsequently, the cells are suspended by gently pipetting the suspension up and down. Following this, the heavy tissue segments are allowed time to settle, and the supernatant is filtered through lens paper to remove cell aggregates.

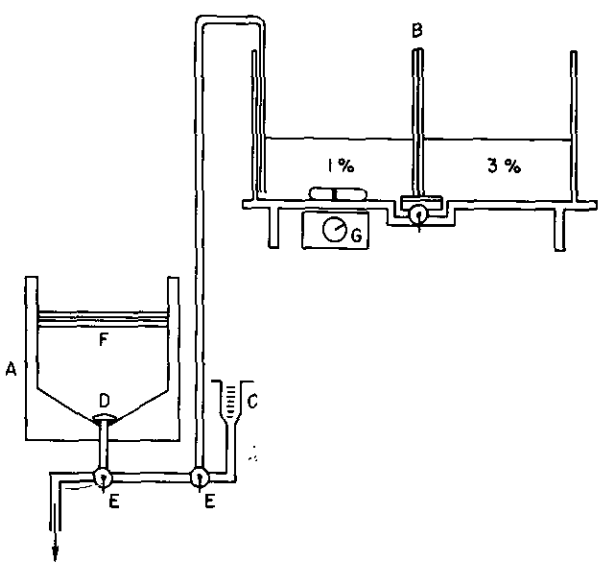

FIGURE 1. Schematic diagram of velocity sedimentation apparatus: (A) sedimentation chamber; (B) gradient maker,; (C) disposable syringe; (D) baffle; (E) three-way valve; (F) cell band after loading; (G) magnetic stirrer.

Aliquots of cell suspensions containing 2×10^7 cells are loaded into the sample vessel C and 720 ml of a 1–3% linear gradient of bovine serum albumin in Spinners solution is developed through the bottom of the chamber. At the end of 4 hr of sedimentation, the chamber is drained from bottom at the rate of 10 ml min. One hundred thirty-five individual fractions are collected with a fraction collector. Each fraction corresponds to a sedimentation distance of 0.76 mm. The chamber size is such that 1 mm of vertical distance contains 7.2 ml of gradient fluid.

Figure 2 presents the relative cell number in each of the fraction collected. The primary cell types indicated were identified by time sequence studies of thymidine incorporation and histologic identification.

Methods for preparation of the cell suspensions, preparation of buffers and gradients, cell separation procedures, cell counting and identification, size analysis of cell suspension, and determination of radioactivity have also been described (*1*). Single-label isotope counting was utilized in these studies with the required consideration for purity of labeled compound, specific activity, quenching and other aspects of liquid scintillation spectrometry.

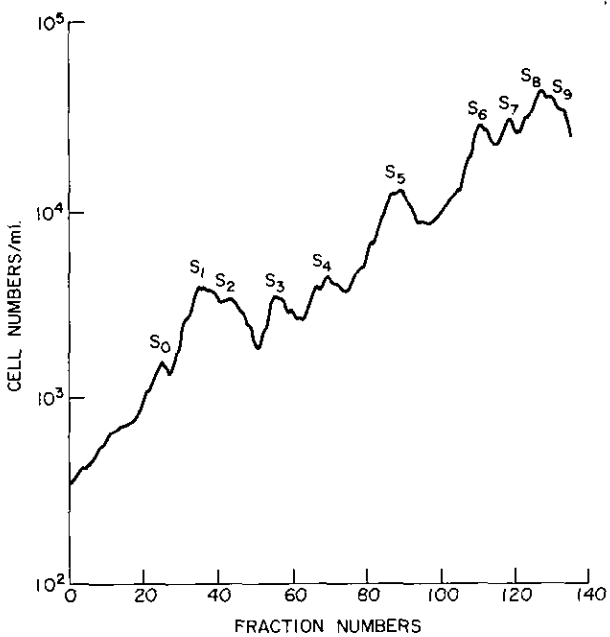

FIGURE 2. Sedimentation pattern of mouse spermatogenic cells. Identification of spermatogenic cells: (S_0) unidentified; (S_1) diplotene; (S_2) pachytene; (S_3) zygotene; (S_4) secondary spermatocytes; (S_5) round spermatids; (S_6) spermatogonia; (S_7) early elongated spermatids; (S_8) late elongated spermatids; (S_9) spermatozoa.

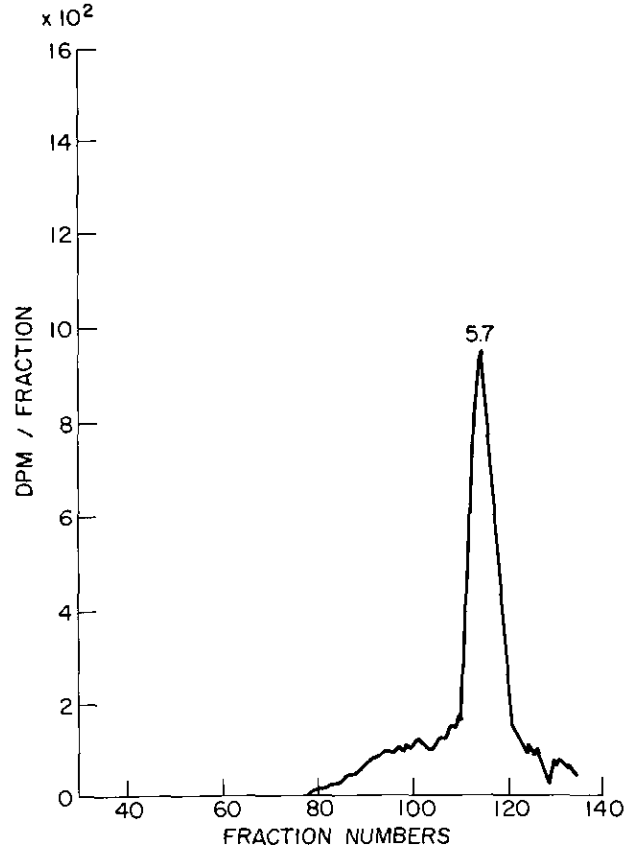

FIGURE 3. Pattern of radioactivity in mouse spermatogenic cells following tritiated thymidine administration. Each mouse received 6.6 μCi/g.

Isotopically labeled chemicals were tritiated thymidine (thymidine-methyl-*T*, specific activity, 19.3 Ci/mmole), uridine (uridine-*G*-*T*, specific activity, 30 Ci/mmole), and L-leucine (L-leucine-4,5-*T*, specific activity, 22 Ci/mmole). Carrier-free ^{109}Cd was used for cellular uptake and autoradiographic studies.

Figure 3 demonstrates the cellular incorporation of radioactive thymidine 1 hr after intraperitoneal injection of the nucleoside. The fraction with greatest activity was identified as spermatogonia which sediments at a rate of 5.7 mm/hr.

Figure 4 demonstrates that tritium-labeled uridine appeared in the cell fraction sedimenting at 3.0 mm/hr when cells are isolated 1 hr after injection of the substrate. This peak of activity represents early elongated spermatids.

Figure 5 indicates the cell types that incorporate tritium labeled leucine 1 hr after intraperitoneal administration of the amino acid. The 2.2 mm/hr fraction contained late stages of spermatids and

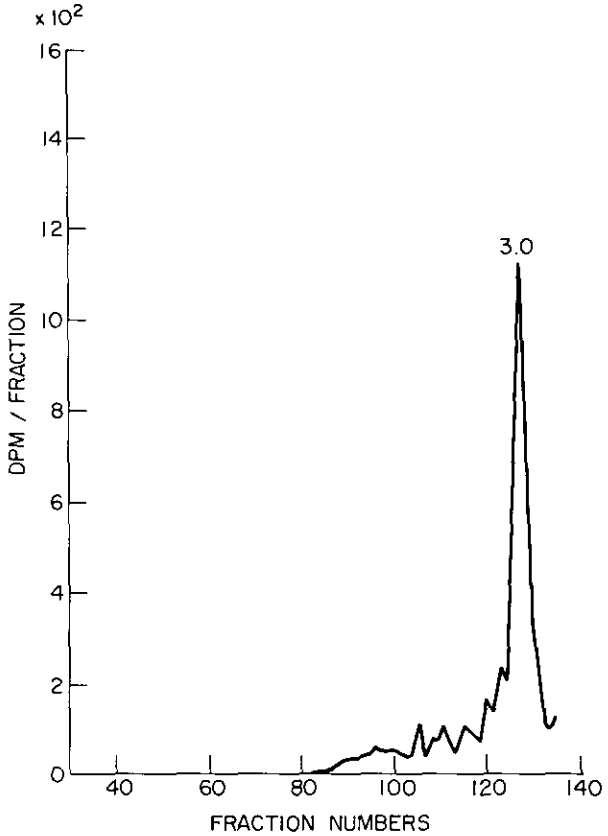

FIGURE 4. Pattern of radioactivity in mouse spermatogenic cells following tritiated uridine administration. Each mouse received 6.6 μCi/g.

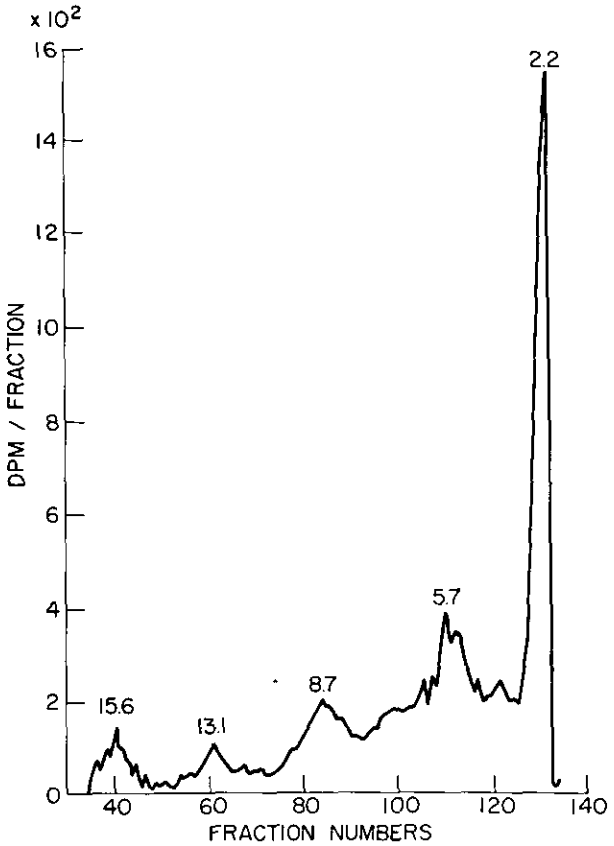

FIGURE 5. Pattern of radioactivity in mouse spermatogenic cells following tritiated L-leucine administration. Each mouse received 6.6 μCi/g.

some spermatozoa; the 5.7 mm/hr fraction represented spermatogonia; the 8.7 mm/hr fraction contained early spermatids; the 13.1 mm/hr fractions were secondary spermatocytes, and the 15.6 mm/hr fraction was identified as diplotene and pachytene cells.

The effects of environmental chemicals on testicular function are usually assessed both *in vitro* and *in vivo*. Rats were sacrificed by cervical dislocation, and the testes were removed, washed in Spinner's salt solution and placed in a Petri dish on ice. The tunica albuginea was removed under a stereomicroscope, and the bundle of seminiferous tubules was carefully teased apart for *in vitro* studies. Incubation studies, with the test substance, are carried out in polyethylene screw cap vials using a metabolic shaker. *In vivo* studies usually involve the administration of the chemical directly to the test species.

The *in vivo* effects of cadmium and boron on the uptake of tritiated thymidine, uridine and L-leucine into spermatogonia, early elongated spermatids, and late spermatids, respectively, were determined.

Results

Figures 6 and 7 present fertility profiles for cadmium- and boron-treated rats. Three oral acute doses were tested. No significant effects on male fertility were apparent at any of the doses tested for either chemical.

Figure 8 demonstrates the relative uptake of cadmium by spermatogenic cell types. The major

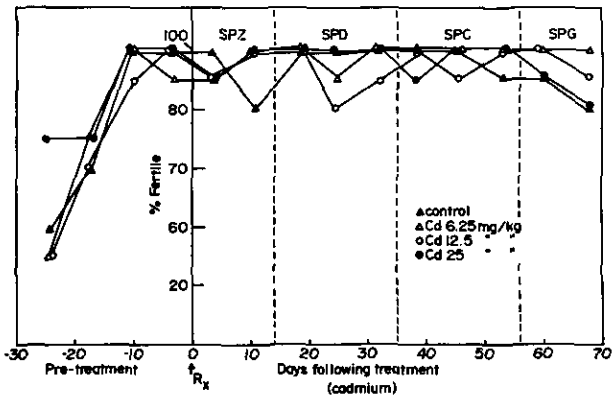

FIGURE 6. *In vivo* fertility profile for control and for cadmium-treated male rats; (SPZ) spermatozoa; (SPD) spermatids; (SPC) spermatocytes; (SPG) spermatogonia.

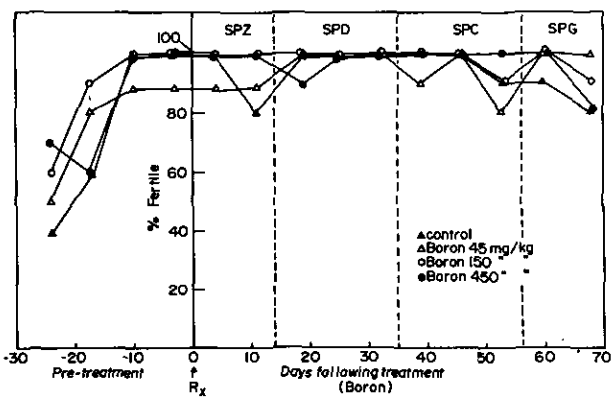

FIGURE 7. *In vivo* fertility profile for control and boron-treated male rats: (SPZ) spermatozoa; (SPD) spermatids; (SPC) spermatocytes; (SPG) spermatogonia.

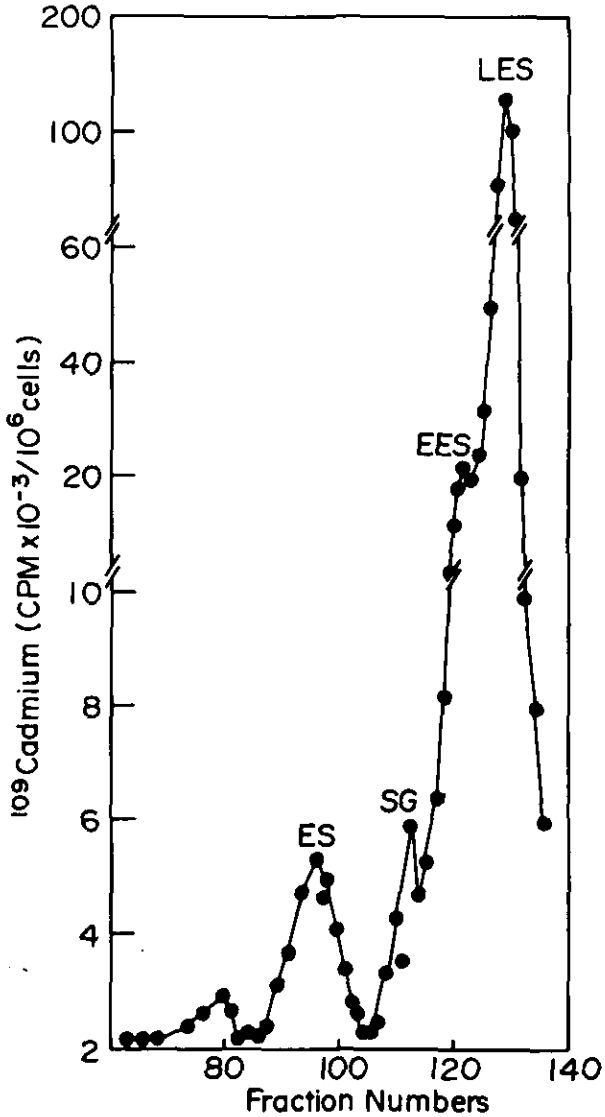

FIGURE 8. Radioactive profile of spermatogenic cell types which incorporate ^{109}Cd. The following abbreviations are used: (LES) late elongated spermatids; (EES) early elongated spermatids; (SG) spermatogonia; (ES) early spermatids.

portion of cadmium was incorporated into spermatogenic cells sedimenting at the rate of 2.2 mm/hr which are the late elongated spermatids. Radioactive cadmium was also incorporated into early elongated spermatids sedimenting at the rate of 3.0 mm/hr and into spermatogonia and early spermatids.

No significant effect of either cadmium or boron on the incorporation of thymidine by spermatogonia, uridine by early elongated spermatids, or L-leucine by late elongated spermatids was demonstrated at the test doses.

Subchronic Studies

Rats were allowed free access to drinking water containing either borax or cadmium chloride. Borax is 11.3% boron and cadmium chloride is 61% cadmium. Treatment groups were exposed to the concentrations of boron of 0.3, 1.0, or 6.0 mg/l. or to cadmium at levels of 0.001, 0.01, or 0.1 mg/l. If

Table 1. Blood chemistry 30 days following cadmium or boron exposure.

Dose, mg/l.	Sodium, meq/l.	Potassium, meq/l.	Chloride, meq/l.	CO_2, meq/l.	Total protein, g/100 ml	Albumin, g/100 ml
Control	140 ± 0.6	4.4 ± 0.4	103 ± 0.6	30.2 ± 1.0	5.6 ± 0.7	2.4 ± 0.2
Cadmium						
0.001	140 ± 0.6	4.0 ± 0.4	108 ± 1.2	27.6 ± 0.4[a]	5.9 ± 0.3	2.3 ± 0.1
0.01	144 ± 1.5	4.9 ± 0.4	105 ± 0.9	29.2 ± 1.0	5.6 ± 0.2	2.2 ± 0.1
0.1	143 ± 1.5	4.7 ± 0.3	105 ± 0.5	29.3 ± 1.0	5.8 ± 0.2	2.3 ± 0.2
Boron						
0.3	151 ± 0.8	5.0 ± 0.2	104 ±0.6	29.1 ± 1.0	5.8 ± 0.1	2.2 ± 0.1
1	148 ± 0.8	5.1 ± 0.6	104 ± 0.6	29.1 ± 1.0	5.9 ± 0.1	2.3 ± 0.1
6	147 ± 0.8	5.1 ± 0.3	105 ± 1.7	28.7 ± 1.6	5.9 ± 0.4	2.4 ± 0.1

[a]$p < 0.05$.

Table 2. Blood chemistry 30 days following cadmium or boron exposure.

Dose, mg/l.	Ca^{2+}, mg/100 ml	Alkaline phosphatase, KAU	Bilirubin, mg/100 ml	BUN, mg/100 ml	Glucose, mg/100 ml	SGOT, KU	SGPT, KU
Control	9.9 ± 0.2	94.7 ± 19.6	1.43 ± 0.11	19.5 ± 1.8	185 ± 13.2	141 ± 16.6	30 ± 7.5
Cadmium							
0.001	10.3 ± 0.2	94.3 ± 20.1	1.44 ± 0.05	20.0 ± 2.0	175 ± 8.5	149 ± 10.2	36 ± 4.6
0.01	9.8 ± 0.2	111.0 ± 16.0	1.38 ± 0.03	18.7 ± 0.6	158 ± 5.8[a]	186 ± 28.0	36 ± 1.7
0.1	10.2 ± 0.2	94.5 ± 4.2	1.40 ± 0.12	20.3 ± 1.1	173 ± 10.4	180 ± 38.8	36 ± 6.0
Boron							
0.3	9.7 ± 0.1	99.4 ± 10.4	1.33 ± 0.05	19.5 ± 1.3	181 ± 2.7	201 ± 17.8[a]	40 ± 5.5
1	9.7 ± 0.1	102.1 ± 6.6	1.25 ± 0.04	19.3 ± 2.1	197 ± 9.8	209 ± 48.0	41 ± 5.6
6	9.7 ± 0.2	106.2 ± 24.9	1.28 ± 0.04	19.7 ± 2.1	183 ± 12.0	202 ± 24.0[a]	38 ± 2.3

[a]$p < 0.05$.

Table 3. Testis weight following cadmium or boron treatment.

Dose, mg/l.	Testis weight, g		
	30 days exposure	60 days exposure	90 days exposure
Control	1.30 ± 0.24	1.70 ± 0.12	1.78 ± 0.12
Cadmium			
0.001	1.57 ± 0.06	1.84 ± 0.16	1.76 ± 0.07
0.01	1.65 ± 0.07[a]	1.63 ±0.15	1.63 ± 0.06
0.1	1.34 ± 0.33	1.66 ± 0.09	1.68 ± 0.16
Boron			
0.3	1.74 ± 0.07[a]	1.68 ± 0.08	1.64 ± 0.67
1	1.65 ± 0.07[a]	1.47 ± 0.11	1.66 ± 0.49
6	1.67 ± 0.05[a]	1.54 ± 0.10	1.71 ± 0.10

[a]$p < 0.05$.

Table 4. Prostate weight following cadmium or boron ingestion.

Dose, mg/l.	Prostate weight, g		
	30 days exposure	60 days exposure	90 days exposure
Control	0.60 ± 0.06	0.61 ± 0.08	0.60 ± 0.12
Cadmium			
0.001	0.43 ± 0.02[a]	0.55 ± 0.06	0.42 ± 0.02[a]
0.01	0.56 ± 0.15	0.61 ± 0.07	0.52 ± 0.06
0.1	0.44 ± 0.10[a]	0.62 ± 0.08	0.58 ± 0.02
Boron			
0.3	0.45 ± 0.07[a]	0.54 ± 0.12	0.61 ± 0.01
1	0.54 ± 0.01	0.50 ± 0.12	0.62 ± 0.06
6	0.39 ± 0.13[a]	0.54 ± 0.07	0.71 ± 0.03

[a]$p < 0.05$.

it is assumed that rats drink an average of 35 ml of water daily, the maximum dose for boron can be estimated as 840 and that for cadmium as 14 μg/kg-day. However, only about 1% of cadmium is absorbed. Animals were exposed for 90 days, and randomly selected groups were studied following 30, 60, and 90 days of treatment. These test doses and experimental design were selected during conferences with our Soviet co-workers.

Animals were evaluated for toxicity by using various techniques. Body weights were recorded as well as the weight of the testis, prostate, and seminal vesicles. Clinical chemistry included serum determinations for sodium, potassium, chloride, carbon dioxide, total proteins, albumin, calcium, alkaline phosphatase, total bilirubin, blood urea nitrogen (BUN), glucose and serum glutamic–oxalic transaminase (SGOT) and serum glutamic – pyruvate transaminase (SGPT). Fructose levels in the seminal vesicles were also determined, as were zinc and acid phosphatase levels in the prostate. Testes were fixed in Bouins solution, washed, dehydrated, and embedded in paraffin for histological studies.

FSH and LH plasma levels following 30, 60, and 90 days of cadmium or boron treatment are presented in Figure 9. Neither cadmium nor boron treatment affected FSH or LH in plasma. Although LH levels tended to increase with exposure time in both treatment groups, these effects were not statistically significant. Subchronic tests failed to reveal any reproductive effects or biologically significant change in clinical serum chemistry or weight of the body, testis, prostate, or seminal vesicles. Fructose, zinc, and acid phosphatase levels in the prostate were unaltered. These data are presented in Tables 1-7. Forced breeding studies failed to reveal any effects on male fertility following any of these treatment periods. Animals refuse to drink

Table 5. Seminal vesicle weight following cadmium or boron ingestion.

Dose, mg/l.	Seminal vesicle weight, g		
	30 days exposure	60 days exposure	90 days exposure
Control	0.64 ± 0.04	0.60 ± 0.10	0.60 ± 0.06
Cadmium			
0.001	0.55 ± 0.02[a]	0.63 ± 0.01	0.48 ± 0.05[a]
0.01	0.55 ± 0.10	0.68 ± 0.01	0.44 ± 0.03[a]
0.1	0.41 ± 0.11[a]	0.63 ± 0.08	0.48 ± 0.06[a]
Boron			
0.3	0.46 ± 0.05[a]	0.71 ± 0.07	0.46 ± 0.06[a]
1	0.49 ± 0.03[a]	0.58 ± 0.15	0.45 ± 0.05[a]
6	0.49 ± 0.07[a]	0.51 ± 0.02	0.53 ± 0.05

[a] $p < 0.05$.

Table 6. Effect of boron on acid phosphatase, fructose, and zinc levels of the prostate of the rat.

Boron dose, mg/l.	Acid phosphatase, μmole thymolphthalein/g-hr			Fructose μmole/g wet wt.			Zinc, ppm/g wet wt. (after 30 days)
	After 30 days	After 60 days	After 90 days	After 30 days	After 60 days	After 90 days	
Control	923 ± 332	1012 ± 218	869 ± 147	2.15 ± 0.8	1.80 ± 0.4	2.28 ± 0.2	165.4 ± 8
0.3	834 ± 216	969 ± 160	1119 ± 126	0.86 ± 0.3	2.14 ± 0.1	2.82 ± 0.2	206.0 ± 77
1	858 ± 193	890 ± 97	1099 ± 115	1.50 ± 0.5	2.12 ± 0.6	2.82 ± 0.4	175.8 ± 49
6	855 ± 94	1040 ± 157	980 ± 106	1.67 ± 0.6	1.83 ± 0.8	3.02 ± 0.1	210.7 ± 20

Table 7. Effect of cadmium on acid phosphatase, fructose, and zinc levels of the prostate of the rat.

Cadmium dose, mg/l.	Acid phosphatase, μmole thymolphthalein/g-hr			Fructose, μmole/g wet wt.			Zinc, ppm/g wet wt. (after 30 days)
	After 30 days	After 60 days	After 90 days	After 30 days	After 60 days	After 90 days	
Control	923 ± 332	1012 ± 218	869 ± 147	2.15 ± 0.8	1.80 ± 0.4	2.28 ± 0.2	165 ± 8
0.001	873 ± 228	814 ± 110	1094 ± 80	1.16 ± 0.2	1.84 ± 0.4	2.43 ± 0.5	183 ± 9
0.01	871 ± 21	791 ± 97	1275 ± 110	0.90 ± 0.1[a]	2.20 ± 0.8	2.20 ± 0.2	170 ± 29
0.1	731 ± 91	910 ± 279	1058 ± 255	1.08 ± 0.2[a]	2.30 ± 0.1	2.24 ± 0.7	184 ± 39

[a] $p < 0.05$.

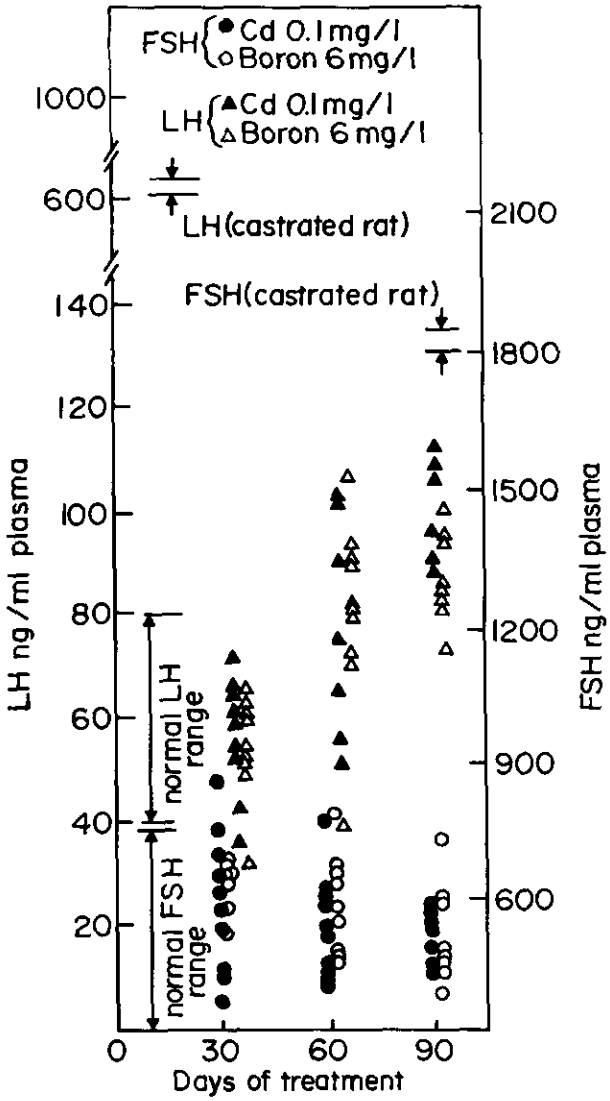

FIGURE 9. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) plasma levels are presented for individual rats following 30, 60, and 90 days of treatment with either cadmium or boron in the drinking water. FSH and LH ranges for normal and castrated rats are also presented. Normal FSH and LH plasma levels are 618 ± 110 ng/ml of plasma and 60 ± 10 ng/ml of plasma, respectively.

water containing concentrations of cadmium or boron significantly greater than those tested.

Our initial results utilizing a variety of methods to assess the reproductive toxicity of environmental substances in male animals suggest that cadmium and boron at the concentrations tested are without significant reproductive toxicity in the male when administered as a single oral dose or subchronically in the drinking water. Weir and Fisher (5) have demonstrated that doses of borax or boric acid as high as 350 ppm boron in the diet produced no adverse effects on reproductive function. However, these same investigators reported that doses of boron of 1170 ppm produced sterility in test animals.

Hopefully, conferences during these meetings will provide time to compare the results of our joint experiments and select other environmental chemicals for future study.

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