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Review

An overview of male reproductive studies of boron with an emphasis on studies of highly exposed Chinese workers

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ABSTRACT

Boron treatment of rats, mice, and dogs has been associated with testicular toxicity, characterized by inhibited spermiation at lower dose levels and a reduction in epididymal sperm count at higher dose levels. The no-adverse-effect level for reproductive effects in male rats is 17.5 mg B/kg bw/day. Earlier studies in human workers and populations have not identified adverse effects of boron exposure on fertility, but outcome measures in these studies were relatively insensitive, based mainly on family size and did not include an evaluation of semen end points. A recent study of nearly 1000 men working in boron (B) mining or processing in Liaoning province in northeast China has been published in several Chinese and a few English language papers. This study included individual assessment of boron exposure, interview data on reproductive experience and semen analysis. Employed men living in the same community and in a remote community were used as controls. Boron workers ($n = 75$) had a mean daily boron intake of 31.3 mg B/day, and a subset of 16 of these men, employed at a plant where there was heavy boron contamination of the water supply, had an estimated mean daily boron intake of 125 mg B/day. Estimates of mean daily boron intake in local community and remote background controls were 4.25 mg B/day and 1.40 mg/day, respectively. Reproductive outcomes in the wives of 945 boron workers were not significantly different from outcomes in the wives of 249 background control men after adjustment for potential confounders. There were no statistically significant differences in semen characteristics between exposure groups, including in the highly exposed subset, except that sperm Y:X ratio was reduced in boron workers. Within exposure groups the Y:X ratio did not correlate with the boron concentration in blood, semen and urine. In conclusion, while boron has been shown to adversely affect male reproduction in laboratory animals, there is no clear evidence of male reproductive effects attributable to boron in studies of highly exposed workers.

Contents

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1. Introduction

Boron, the fifth element in the periodic table, has widespread commercial uses. Male reproductive toxicity has been demonstrated in experimental animals exposed to boron compounds, and there is considerable interest in possible human reproductive effects of these chemicals. Although there appears to be considerable human exposure to boron compounds, epidemiological studies have not been sufficient for an evaluation of reproductive risk. A series of papers describing semen characteristics in a highly exposed group of mine and boron ore processing workers in China has provided a unique opportunity to enhance our understanding of possible boron effects on male reproduction.

The Chinese workers were studied by a group from the Beijing University of Science and Technology and the China National Environmental Monitoring Center in collaboration with the University of California at Los Angeles. This study has been described in articles in English and Chinese published between 2002 and 2008. Because of the importance of this effort and the use of a highly exposed population, a review panel reviewed and summarized the papers describing this study in order to make the study results available in a comprehensive format in the English language literature. Members of the review panel were selected based on their background in male reproductive health, epidemiology, and public health. Chinese papers were translated into English[2] and one member of the review panel was a bilingual native of China. Members of the original Chinese research team (Professor Wei Fusheng, Professor Wu Guoping, and Dr. Xing Xiaoru) were consulted by telephone, email, and in a face-to-face meeting in order to clarify questions about the research, and these researchers read this manuscript and confirmed its accuracy. The Chinese investigators were not asked to endorse our interpretation of the data.

Although published research papers provided most of the data presented here, additional information about study design and numbers of subjects was obtained from a book published in Chinese on the study [1] and from a report to the US National Institute for Occupational Safety and Health (NIOSH), which funded a portion of the research [2]. Published sources included papers in English [3–5] and Chinese [6–10]. Exposure information published by the Chinese investigators was also reviewed [11–18].

In the current paper, we will briefly review general information about boron and what is known about reproductive effects of boron in experimental animals and humans. We will then present the basic procedures used in the Chinese study, including recruitment of subjects, collection of samples, and estimation of exposure followed by presentation of results and comments for each of the major end points. We identified three categories of end points: semen analysis, reproductive outcome, and sperm Y:X ratio. Finally, we will present overall conclusions and we will identify data needs that would contribute to our understanding of possible effects of boron exposure on male reproduction in humans.

1.1. Chemical properties and uses

Boron (B) has an atomic weight of 10.81 with two isotopes, ^{10}B and ^{11}B, neither of which is radioactive. Because boron is electron-deficient, it has a strong affinity for electron donors such as oxygen, which explains the absence of boron in its elemental form in nature. Boron-containing minerals are almost all inorganic salts of boron and commercially important deposits are found in the United States, Turkey, South America, Russia, and China [19]. In addition, boron as borates or boric acid is ubiquitously present in soil, water, and food where its presence is due to its being an essential element for plant growth [20]. Boron has also been shown to be essential in some animals and is an important nutrient in

[2] Prof. Wei Fusheng of the China National Environmental Monitoring Center and the Chinese Academy of Engineering has agreed that interested individuals may request a copy of the translations for academic research purposes. Requests may be made to Prof. Wei via email weifsh@cae.cn or to the corresponding author of this review, Dr. Scialli via email ascialli@sciences.com.

humans [21,22]. In aqueous solution at low concentrations and low pH, borates convert to boric acid. It is in this form that boron is absorbed through mucous membranes, distributed to all body compartments, and excreted in urine.

In this review the word boron will be used generically to refer to boron chemically combined in minerals, dusts, or biological substances. In China, commercial production of boron chemicals is largely limited to boric acid $B(OH)_3$ and borax, the disodium tetraborate decahydrate, $Na_2B_4O_7 \cdot 10H_2O$. There, these compounds are produced from either the magnesium containing mineral szaibelylite or to a lesser extent the iron-magnesium containing mineral ludwigite. Because both minerals are poorly soluble in water, processing in China involves calcining of the ore. According to the China Mining Association, production of borax was over 200,000 tons and production of boric acid was over 10,000 tons in 1998. To convert mg B to mg boric acid multiply by 5.721; to convert mg B to mg borax decahydrate multiply by 8.818.

Woods [19] states that the earliest source of borax may have been the Tibetan lakes from which it was transported over the Himalayas to India. The Babylonians imported borax over 4000 years ago to be used as a flux for working gold. Boron was used by the ancient Egyptians in mummification, medicine, and metallurgy. In the United States today, the major uses of boron minerals and chemicals include manufacture of glass, especially glass fibers, ceramics, detergents and bleaches, alloys and metals, fire retardants, fertilizers and increasingly wood preservatives. The European Borates Association [23] listed EU uses as including glass (insulation fiberglass, textile fiberglass, borosilicate glass), ceramics, detergents (perborates), cleaning materials, cosmetics, flame retardants, fertilizers, wood preservatives, industrial fluids (metal-working, antifreeze, brake fluids, motor oil), metallurgy, and miscellaneous chemical formulations. The Chinese Mining Association [24] reported that borax and boric acid are used in the chemical industry, the light industry, in medicines, building materials, and other uses.

1.2. Previous literature

1.2.1. Experimental animal

Boric acid and borax have very low acute toxicity in experimental animal studies, and the LD_{50} in rats has been reported to be in the order of 3000–6000 mg/kg bodyweight (bw) when administered orally and greater than 1000 mg/kg bw when administered parenterally. However, in repeat dose toxicological studies in animals, the most sensitive endpoints are on the reproductive system where effects are seen at much lower dose levels. Effects are consistent across species, and testicular damage has been observed in both sub-chronic and chronic studies in three species: rats, mice, and dogs. Only the more important studies are summarized below.

The testicular effects tend to be similar in all three species, although most data come from rat studies. The effects are both dose and time dependent. The reproductive effects in rats at low doses and short time periods start with reversible inhibition of spermiation, the release of mature spermatozoa from the Sertoli cells into the lumen of the seminiferous tubules. Early effects are seen after 7 and 14 days dietary treatment with boric acid at daily doses around 61 and 38 mg B/kg bw respectively, and at a lower daily dose of 26 mg B/kg bw the effects take about 28 days to manifest [25,26]. Higher doses or longer treatment periods may lead progressively to reduced sperm count, necrosis of spermatocytes, degeneration of seminiferous tubules, and finally testicular atrophy with loss of germ cells.

These effects were first reported in 60–90-day repeated dose studies in rats given disodium tetraborate decahydrate in drinking water at doses equivalent to 25, 50, and 100 mg B/kg bw per day, in which testicular atrophy was observed at the highest dose level after 60 days treatment [27]. Subsequently, reduction in fertility in a three-generation study of boric acid and disodium tetraborate decahydrate was observed in rats at 58.5 mg B/kg bw/day with testicular atrophy (NOAEL 17.5 mg B/kg bw/day). Reduced female fertility was also observed but with no clear effect on the ovaries. Testicular atrophy was also seen at 6, 12, and 24 months in the top dose group only in a two-year repeated dose study of boric acid and disodium tetraborate decahydrate at the same dose levels of 5.9, 17.5, or 58.5 mg B/kg bw/day [27]. In male rats fed disodium tetraborate decahydrate for either 30 or 60 days at 60 or 130 mg B/kg bw/day (NOAEL, 30 mg B/kg bw/day), testis weight was reduced, testicular germ cells were depleted, fertility was reduced, and plasma FSH levels increased. Plasma LH and testosterone levels were unaffected [28]. As might be expected, while recovery from inhibition of spermiation or spermatocyte damage occurred at the lower dose levels, there was no recovery from testicular atrophy when the germ cells were lost. Ku and colleagues also reported that they found no detectable treatment-related changes in the testis in rats from consumption of 17.5 mg B/kg bw per day for up to 9 weeks [25].

Fewer data are available for mice and dogs, but the results confirm the findings in rats. In a continuous breeding study in mice of boric acid administered in the diet at levels equivalent to daily doses of 27, 111, or 220 mg B/kg bw, dose-related effects on the testis (on sperm-motility, morphology, and concentration and testicular atrophy) were noted in the mid and high dose groups; fertility was reduced at 111 mg B/kg bw/day and was absent at 220 mg B/kg bw/day. The NOAEL was 27 mg B/kg bw/day (154 mg boric acid/kg bw/day), although at this dose the motility of epididymal sperm was slightly affected without any effect on fertility [29]. These results in mice are consistent with those in rats.

Data in dogs come from very limited 90-day and 2 two-year feeding studies on boric acid and borax [27]. In the 90-day study, testis atrophy was observed at the top dose level equivalent to 33 mg B/kg bw (boric acid) or 38 mg B/Kg bw (disodium tetraborate decahydrate) daily but not at the next lower dose level of 4.4 mg B/kg bw. In the two-year studies, groups of four dogs were fed either boric acid or disodium tetraborate decahydrate at doses up to 10.2 mg B/kg bw/day (62.4 mg boric acid/kg bw/day) and 9.6 mg B/kg bw (84.7 mg disodium tetraborate decahydrate/kg bw/day) in the first part of the study and 39.5 mg B/kg bw/day (233.1 mg boric acid/kg bw/day) and 39 mg B/kg bw (373.2 mg disodium tetraborate decahydrate/kg bw/day) in the second part of the study. These numbers are slightly higher than in the published papers but have been recalculated using the data in the original reports. Only four male dogs per group were used in each study part, and animals were sacrificed at various time periods such that observations were reported on groups of only 1 or 2 animals. One boric acid-treated and one disodium tetraborate decahydrate-treated dog were allowed to recover for 3 weeks, and recovery was observed in both dogs. Testicular atrophy was observed at the highest dose levels in the treated animals but was also present in three out of four control dogs so that the significance of the effect in the treated animals is difficult to assess. The NOAEL was reported to be equivalent to 10.2 mg B/kg bw/day. It has been agreed by most regulatory authorities that these dog studies are inadequate for risk assessment (see for example discussion on page 86 of the IPCS monograph [30]), but the studies do confirm the effects seen in the other species.

In conclusion, adverse effects have been observed on the testis following administration of boric acid and borax in rats, mice, and dogs, with effects on sperm, spermiation, spermatocytes, and germ cells. The overall NOAEL is reported as 17.5 mg B/kg bw/day (equivalent to 100 mg boric acid/kg bw/day or 154 mg borax decahydrate/kg bw/day).

Table 1
US mine worker reproductive health and dust exposures.

Exposure category	1	2	3	4	5
Number of respondents	108	108	108	109	109
Range of dust exposures (mg/m^3)	<0.82	0.82–1.77	1.78–2.97	2.98–5.04	>5.04
Mean dust exposure (mg/m^3)	0.37	1.34	2.23	3.98	8.58
Estimated daily boron exposure (mg-boron/day)[a]	0.45	1.64	2.73	4.88	10.5
Observed births	94	108	93	114	120
Number of excess births (observed – expected)	+31.9	+3.8	-1.1	+3.8	+24.1
% Female offspring	55.3	56.5	51.6	50.9	49.2
% Male offspring	44.7	43.5	48.4	49.1	50.8
% Vasectomy	29	43	34	41	35

Data from Whorton et al. [32–34].

[a] Estimates based on mean dust exposure assumed 14% boron concentration in sodium borates, respiratory volume of 8.75 m^3 air/day and 100% absorption. Whorton et al. used these calculations to estimate occupational exposure for a subset of the study group but did not estimate daily boron exposure for these categories.

Although not strictly relevant to the current studies, developmental toxicity has also been reported in rats, mice, and rabbits following boric acid administration during the period of organogenesis. The rat has been shown to be the most sensitive species with initial effects being reduction of fetal body weight (which is reversible postnatally) and reduced size of the 13th ribs. At higher dose levels ≥28 mg B/kg bw, anomalies of the eyes, central nervous system, cardiovascular system, and axial skeleton are observed. The NOAEL for developmental effects in the rat is 9.6 mg B/kg bw based on reduced fetal body weight at 13.3 mg B/kg bw [30].

1.2.2. Human

A study from Russia was published on 28 workers exposed to workplace dust levels of borate, 4–8 times the maximum permissible limit of 10 mg/m^3 [31]. However, the effects were poorly defined and the reporting of the data was inadequate to permit proper analysis.

Fertility and sex ratio were investigated among 753 mine workers at U.S. Borax (now Rio Tinto Minerals) [32–34]. Reproductive data were obtained by questionnaire and telephone interview. Standardized Birth Ratio (SBR) based on U.S. population adjusted for maternal age, race, parity, and calendar year was used to assess biological fertility of the male employees. Excess numbers of male and female births were reported by the male boron workers. Five exposure categories were constructed to contain equivalent numbers of subjects, based on previous studies of exposures associated with job categories [35] (Table 1). There was no dose relationship between excess births and exposure category: the highest numbers of excess births were in the lowest and highest exposure categories. The excess numbers of births were statistically significant, indicating that fertility rates among US boron mine workers was not adversely affected. The standardized birth ratio is a crude measure of biological fertility, which in addition to male and female fecundity is strongly influenced by volitional factors and use of contraception [36]. Therefore, studies of fertility rates can only be expected to detect strong toxic actions as was seen in workers exposed to the nematocide dibromochloropropane (DBCP) [37].

Male to female ratio at birth was also assessed. Overall, boron workers fathered 52.7% female offspring, compared to the U.S. national average of 48.8%. However, the authors pointed out that the data did not indicate effects attributable to boron. Workers in the two lowest exposure categories had the highest percentage female offspring, while workers in the highest exposure category had virtually the same percentage (49.2%) as the national average.

Whorton et al. identified a subset of 42 subjects who had worked at high exposure jobs for more than 2 years. This group of workers was older than the general worker population and 48% had had a vasectomy. Their daily boron exposure was 28.4 mg [34]. The observed number of births for this group was near the expected number (SBR = 102) considering their entire work history, but was equivalent to other workers during the period when they were in high exposure jobs (SBR 115–121). Thus the fertility rate was not adversely affected when workers were most exposed to boron. The ratio of boys to girls was not reduced. The male to female ratio at birth was equivalent to the ratio for the rest of the participants.

Şayli et al. [38] and Tuccar et al. [39] evaluated the reproductive history of families living in regions of Turkey with varied boron concentrations in the environment. In addition, some of the subjects worked at borate mining and processing facilities in the high-boron region. Subjects were chosen for convenience and, thus, the study was not population based. Data were obtained about the fertility of the proband generation, their parents' generation, and their children's generation. Evidence of fertility was the birth of a living child. The authors reported no significant differences between the 1068 families in the high-boron region and the 610 families in the low-boron region. Lack of strict epidemiological study design and the use of fertility rate to measure fecundity detracted from the utility of these papers for an evaluation of human reproductive toxicity. Şayli et al. reported non-significant differences in sex ratio, with more females than males (52.73% female) in the boron-rich region than in the boron-poor region (48.86% female) [38].

Some years later, the human daily boron exposure in the same area was reported [40]. Daily boron exposure was estimated based on boron excretion in 24-h urine samples based on the assumption that urine boron excretion accounted for 85% of daily boron exposure. The boron exposure level was 6.77 mg/day for males living in the boron-rich region and 1.26 mg/day for the controls.

Çöl et al. [41] evaluated infertility rates, gender ratio at birth, numbers of stillbirths and spontaneous abortions, premature births, and infant mortality rates among the families of 799 boron-exposed workers at three production facilities in Turkey. Patterns were compared with national or regional values, and 642 production workers were compared with 157 office workers. No significant adverse effects were found. Infertility rates among the workers averaged 1.8%, at the low end of the Turkish national rate of 1.49–3.8%. Gender ratio was 1.12 (52.9% boys, 47.1% girls), greater than the Turkish national range of 1.05–1.08. When comparing the production workers (expected to have higher boron exposures) to office workers, the only significant differences were that average pregnancies and live births among production workers exceeded those of office workers. Çöl et al. therefore concluded that boron exposure to workers did not adversely affect any of the indicators of fertility or development studied, including gender ratio. The reported infertility rates are very low compared to usual general population infertility rates of ∼15%, and we question the reliability of this aspect of the report.

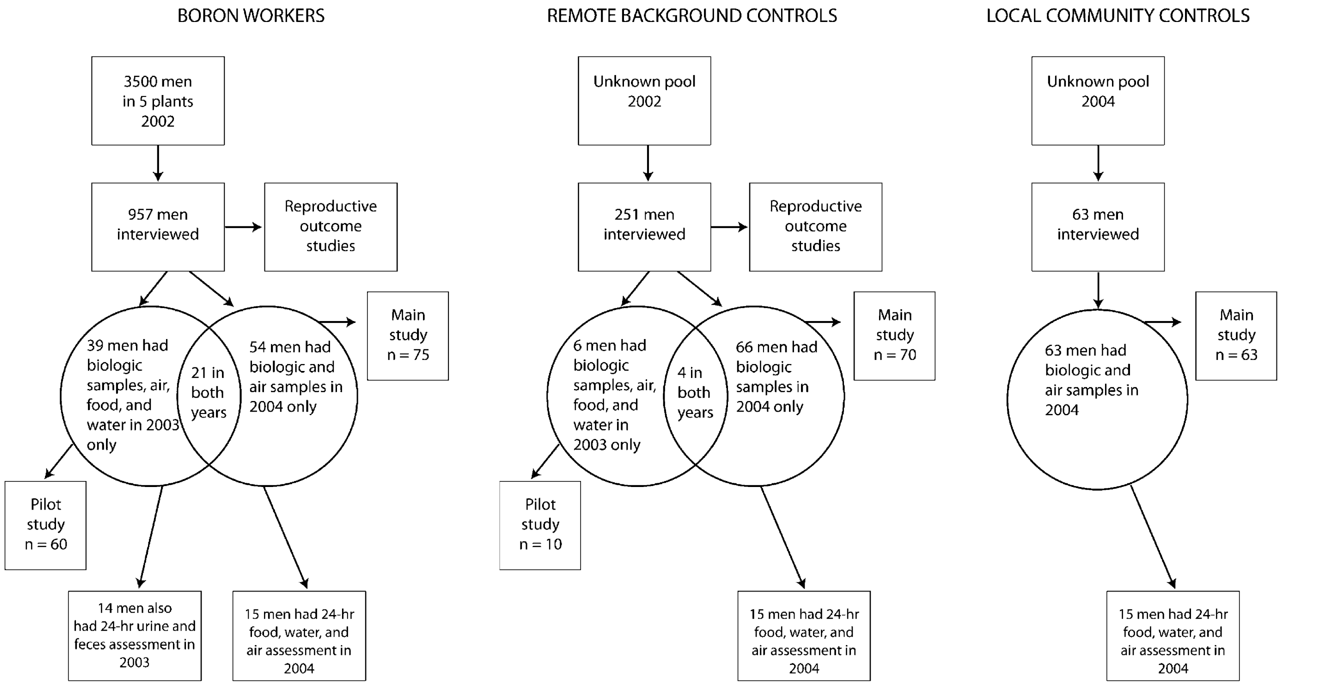

Fig. 1. Subjects and sampling procedures in the Chinese boron worker study. Biological samples in 2003 included two semen, blood, pre-shift urine, and post-shift urine samples. In 2004, biological samples included three semen and post-shift urine samples and one blood sample. The numbers represent men starting the study, taken from Wei and Robbins [65].

2. The Chinese boron workers study

2.1. Recruitment, collection of samples

The study involved male workers at one boron mine and four boron processing plants in Kuandian City in Liaoning province in northeast China. The five workplaces were selected based on the location, number of employees, and the presence and cooperation of an industrial hygienist at the site [2]. From the approximately 3500 men in the five workplaces, 957 men between 18 and 40 years of age agreed to complete an interview to provide demographic, exposure, reproductive, and general health information. Of the interviews, 945 were considered eligible. The interviews were used to select potential subjects for the study. Potential subjects were 25–35 years old, married, and without a history of contact with lead, mercury vapor, cypermethrin, folimat, carbofuran, parathion, acetochlor, atrazine, chlordecone, 2-dichlorophenoxyacetic acid, toluenediamine, dinitrotoluene, ethylene glycol, tetrachloroethylene, radioisotopes, electric welding, acetone, styrene, or other plastics. Men were excluded if they had had a hot bath within 3 months, X-ray studies of the inguinal region or low back, or a history of reproductive disorder or chronic disease. Excluded disorders were mumps orchitis, testicular injury, abnormal genitalia (e.g., cryptorchidism), prostate or genital surgery, testicular cancer, other urinary system diseases (such as urinary tract infection, bladder infection), sexually transmitted diseases including external genital infections, chlamydia, syphilis, gonorrhoea, genital herpes, and HIV/AIDS, and other diseases: tumors, heart disease, lung disease, diabetes, liver disease, kidney disease, or other chronic diseases. Boron workers were also evaluated with a physical examination including urologic examination. In addition to general physical examination, men were evaluated for body habitus, hair distribution, breast tissue size, the size, firmness, and location of testes, epididymides, and ductus deferens, and the presence of varicocele or hydrocele.

A comparison group of 251 men were recruited from Tiantuia Gu, an area 30 miles away from Kuandian City. Tiantuia Gu has low background boron exposure levels. The men were screened with the same questionnaire and physical examination used for the boron workers, resulting in 70 eligible subjects who agreed to submit biologic samples. This group was called the “background control” group in the original papers and will be called the “remote background control group” in this review. Later in the course of the series of studies, another comparison group was added consisting of 63 workers without occupational exposure to boron but drawn from the same community as the boron workers. This group was called the “community control” group in the original papers and will be called the “local community control” group here.

Fig. 1 gives the number of men recruited for each portion of the research. Data were collected in two phases. In 2003, a pilot study was performed that included 60 boron workers and 10 remote background control men. In 2004, the main study was performed, consisting of 75 boron workers, 21 of whom had also participated in 2003, 70 remote background controls, and 63 local community controls. A variety of environmental and biological samples were collected as indicated in the figure and summarized below. Not all end points were collected for all subjects, and Table 2 summarizes the number of men contributing data for each of the end points in each of the published papers.

Boron content of environmental and biological samples was measured using state of the art inductively coupled plasma mass spectrometry (ICP-MS) when boron concentration was very low and inductively coupled plasma atomic emission spectrometry (ICP-AES). The detection limits and relative standard deviation for boron in different media were: airborne particulates 0.01 μg/g ± 5.01%; food 0.0063 μg/g ± 0.63%; drinking water and urine by ICP-AES 0.0072 ng/ml ± 0.60%; drinking water and urine by ICP-MS 0.057 μg/ml ± 1.25%; blood and semen by ICP-AES 0.200 ng/ml ± 5%; blood and semen by ICP-MS 0.06 ng/ml ± 3.05% [12,42].

Table 2
Reports on boron workers in Liaoning province.

Citation	Interviewed populations		Study populations			Boron measurements			Semen/sperm endpoints							Fertility and pregnancy outcomes (paternal exposure)					
	Boron workers	Remote background controls[a]	Boron workers	Local community controls[b]	Remote background controls	Blood/plasma	Urine	Semen	Forward motility	Rapid progressive motility	Viability	Sperm density	Total sperm	Motility[d]	X:Y Ratio	Sex ratio newborns	Delayed pregnancy	Number of live births	Spontaneous abortion	Ectopic pregnancy	Still birth
Chang et al. [3]	936	251	936		251											×	×	×	×	×	×
Liu et al. [4]	957	251	60	9	–				×	×	×	×		×							
Liu et al. [8]			62	53	61		×		×	×		×	×	×							
Liu et al. [6]			62	53	61				×	×		×	×	×							
Liu et al. [7]			66[c]	61	68		×		×	×		×	×								
Liu et al. [9]	–	–	66	61	68				×	×		×									
Robbins et al. [5]	936	251	63	39	44	×	×	×							×						
Liu et al. [10]	957	251	945	249												×			×		

[a] Remote background controls are men living 30 miles away from the boron processing center and not exposed to high environmental boron levels.
[b] Local community controls are men living in the same community as the boron workers but not occupationally exposed to boron.
[c] The final number of men contributing biologic samples is given. The number of men recruited to give samples may have been larger.
[d] Motility endpoints included VSL = straight line velocity, VCL = curvilinear velocity, VAP = average path velocity, STR = straightness, LIN = linearity.

2.2. Exposure to boron and intake by inhalation and ingestion

2.2.1. Inhalation

Air sampling [18] was done in borate processing areas using a laser (light scattering) real-time dust monitor and an Anderson 9-stage cascade impactor. Both measurement devices work with good accuracy for particle size below 10 μm (PM_{10}). In addition personal measurements were performed using IOM inhalable dust sampler operating at 2 l/min. The difference in mass of a membrane filter before and after sampling was the mass of the particulates. The total airborne dust concentrations ranged from 0.3 to 33 mg/m^3. The concentration of boron in the dust collected in heavy dust areas ranged from 1.5 to 4.2%, values markedly below those to be expected in work areas heavily contaminated with borates. Workplace measurements of total airborne dust (<30 μm in aerodynamic diameter) and particulate matter by size ($PM_{2.5}$ and PM_{10}) as well as boron mass concentration in the total dust are presented in Tables 3 and 4.

The reported measurements of boron in dust may not be accurate. Under usual conditions, the makeup of dust in a workspace should reflect the product being handled. It would be expected that the finished product workshop would have a higher dust boron concentration than the raw material workshop, which should have a higher boron dust concentration than the underground mine. A comparison of the data of Whorton et al. (Table 1) with the Chinese data (Table 4) suggests that airborne dust exposure has been under-estimated, although it is possible that differences in processing, operations, material handling, and the ore itself can account for the apparent underestimation.

Daily boron exposure through airborne particulates was estimated initially by multiplying the boron mass concentration in airborne particulates (μg/m^3) by 5 (m^3) [12] and then in later papers by 10 (m^3) [5,16], which roughly corresponds to the amount of air inhaled during an 8 h working shift. If there are errors in the measurements of dust boron concentration, these errors would lead to erroneous estimations of boron exposure through air.

2.2.2. Ingestion

Ingestion was measured from the sum of boron intake from food and drink several times in slightly different subsets of the same worker populations using a duplicate plate method for food and drink in which all ingested food and drink were carefully measured and analyzed.

In order to estimate boron absorption from the gastrointestinal tract, 14 boron workers collected 24-h urine and fecal samples on two separate occasions [13]. For the first collection, boron urinary excretion averaged 8.22 mg B/24 h (based on an average of 1913 ml urine) and for the second collection, the average boron urinary concentration was 5.84 mg B/24 h (based on an average of 2058 ml urine). Fecal boron averaged 0.37 mg B/24 h for the first collection and 0.28 mg B/24 h for the second. Thus, gastrointestinal absorption of boron was measured at more than 94%, comparable to the findings of Jansen et al. [43].

From the data available [11,13,17], oral intake of boron by boron workers had by our calculation a weighted mean of 16.9 mg B/day, while the community comparison group's boron intake was 4.25 mg B/day. This difference is most likely due to the reported [2] 64% of workers who ate at the worksite with as many as one-third of workers eating directly in dusty working areas. The difference between the 4.24 mg B/day ingested by the community comparison controls and the mean 1.43 mg B/day ingested by the remote background controls may have been due to the elevated boron content of crops grown in the boron-rich soils surrounding Kuandian City where workers and community controls lived. Potatoes, maize, and legumes were found to increase incorporation of boron in response to elevated boron content of soil [44].

Table 3
Workplace measurements of total airborne dust (<30 μm in diameter) and particulate matter by particle size.

Workplace	Airborne dust fraction, mg/m^3			
	Total (mg/m^3)	PM_{10}	$PM_{2.5}$–PM_{10}	$PM_{2.5}$
Initial process workshop	28	17.4	15.7	1.76
Ball milling workshop	33	20.7	17.8	2.94
Packaging workshop	1.6	1.11	0.835	0.275
Inside factory area	0.3	0.214	0.168	0.0459
Leimeng milling workshop	23	13.0	11.8	1.20
County government offices	0.114	0.0763	0.0497	0.0266

Data expressed as arithmetic means, from Xing et al. [18].

Table 4
Total airborne dust and airborne boron concentration by sampler type.

Workplace	Total dust (mg/m^3)		Total dust boron concentration (μg/m^3)		
	Arithmetic mean	Range	Arithmetic mean	Range	Percent boron in total dust
IOM personal sampler[a]					
Underground mining	7.47	1.95–15.9	316	78.1–664	4.2
Raw material workshop	36.2	1.65–227.0	1111	65.6–9529	3.1
Finished product workshop	6.82	0.24–50.5	197	12.5–701	2.9
Managerial personnel	1.37	1.23–1.63	27.9	12.4–46.6	2.0
Remote background control	0.53	0.10–1.65	1.87	0.92–3.17	0.4
Anderson sampler[b]					
Ball milling workshop	28		425.6		
Leimeng milling workshop	23		669.1		
County government offices	0.114		0.152		

[a] Data from Xing et al. [14].
[b] Data from Xing et al. [18].

2.2.3. Total daily boron intake

Although initial papers took into account the concept that inhaled boron was either not biologically available or had limited bioavailability [12], some later papers [5,16] include the assumption that inhaled boron is completely absorbed. However, the absorption of boron in the alveolar region of the lung may indeed depend on particle size and mineral composition. One study found that the absorption rate in the form of boron magnesium ore was only 8%, whereas it was 74% for boric acid or borax [45]. Values for boron intake of boron workers, the local community comparison group, and remote background control are shown in Table 5.

2.2.4. Assessment of boron in biological samples

2.2.4.1. Twenty-four hour boron urine concentration. The amount of boron in a 24-h urine collection reflects the total intake of boron quite well and would probably be the best method to use. It is only valid if all urine samples during a 24-h period have been collected and no contamination has occurred. Both assumptions are often violated under field conditions. Twenty-four hour urine samples were collected in one study [13]. They were used to estimate the degree of gastrointestinal boron absorption. Post-shift boron urine concentration and 24-h urine concentration showed a high correlation coefficient of 0.95. The Chinese investigators therefore decided to use the post-shift boron urine concentration—standardized per gram creatinine to account for the state of urine dilution—as a substitute for the 24-h boron urine excretion [12].

2.2.4.2. Post-shift boron urine concentration. Sampling was carried out after the study participant took a shower after work and a clean 500-ml urine collector was used to collect the sample. Two 20-ml urine samples were removed, one for boron analysis and one for creatinine measurement.

The relationship between boron exposure and post-shift boron urine concentration ($R^2 = 0.72$) was described as: log(daily boron exposure) = 1.03912 + 0.908 × log(boron concentration of post-shift urine) [16].

This regression equation was applied to the 2004 post-shift boron concentrations obtained from 15 workers from each worker group in order to predict 24-h total boron intake [16]. These predicted total boron exposures were compared with actual measured 24-h boron intakes and were found to be significantly associated (Pearson correlation coefficient = 0.80). However, when actual total exposures for each group separately were compared with predicted total exposures, the Pearson correlation coefficient was 0.90 for borate workers ($P < 0.0001$), 0.48 for local community controls ($P = 0.008$), and 0.28 for remote background controls ($P = 0.60$).

2.2.4.3. Boron concentration in serum and semen. Blood samples were collected with the assistance of doctors in the local hospital. Semen was collected by masturbation into a clean, sterilized wide-mouth plastic sample vial by participants after washing their hands. Care was taken to avoid contamination with dust [12]. Values for mean boron intake and boron concentrations in urine, serum, and semen of boron workers, the community comparison group, and remote background control are shown in Table 6.

In summary, total airborne dust concentrations ranged from 0.3 to 33 mg/m^3 for boron workers. The concentration of boron in the dust collected in heavy dust areas ranged from 1.3 to 2.9%, percentages recognized by the Chinese investigators as low, and low also by comparison with other studies. Total daily boron intake for workers varied between reports from 11.84 to 31.3 mg B/day, although one paper reporting carefully collected samples for 15 workers measured 41.2 mg B/day. The average value for post-shift urine boron concentration was 14.7 mg B/g creatinine. The concentration in serum was 252 ng B/ml and the concentration in semen was 592 ng B/ml.

Table 5
Personal measurements and estimates of boron intake via inhalation and ingestion for boron workers and two control groups.

Sampling year	Mean boron [mg B/day]; measures of dispersion are (range) or ± SD											
	Boron workers				Local community controls				Remote background controls			
	Subjects	Oral	Inhalation	Total	Subjects	Oral	Inhalation	Total	Subjects	Oral	Inhalation	Total
2003 [13]	53	7.5	4.3	11.84 (1.07–74.4)					9	1.52	0.009	1.5 (0.48–4.24)
2003/2004 [12]	75	31.3	3.44	34.45 (1.60–469)	15	4.25	0.01	4.26	23	1.40	0.01	1.41 (0.42–3.6)
2003/2004 [11][a]	75			36.1 (2.83–354)	63			4.16	70			1.31 (0.16–2.72)
2004 [17]	66	8.00			15	4.25			23	1.40		
2004 [17][b]	6	219										
2003 [16]	60			37.0 ± 11.7					9			1.2 ± 1.1
2004 [16]	15			41.2 ± 37.4	15			4.3	15			2.3 ± 1.4
2003/2004 [1][a,c]	16			125 ± 89.1								

[a] Estimates based on regression of post-shift urine concentrations measured in 2003.
[b] Subset of workers, who drank canteen well water and dined in the canteen.
[c] Workers at the Pengxiang plant where drinking water was heavily contaminated with boron.

The papers on boron intake in the boron workers also provide some industrial hygiene information related to borate mining and ore processing. Such information may be the subject of a future paper.

3. Biological endpoints

The biological end points in the study included semen quality, reproductive outcomes, and sperm Y:X ratio. We here present the specific methods in evaluating the end points, the results, and our evaluation of the data.

3.1. Semen quality

3.1.1. Methods

Semen was collected by masturbation after at least 2 days of sexual abstinence and was examined within an hour of collection. Men with periods of abstinence exceeding 8 days were excluded from analysis. Collection occurred in a hospital setting after subjects had changed into a clean hospital gown. Two semen samples in 2003 or three semen samples in 2004 were collected separated by 20–30 days. Samples were analyzed using computer-assisted sperm analysis (CASA; IVOS, Hamilton Thorne) for motility end points. Sperm concentration was obtained during the 2003 pilot study using CASA and a counting chamber. When the two methods were compared, CASA was believed to be superior and was used for sperm concentration in the 2004 main study. Assessment of sperm morphology was not reported in the study papers, but it was reported in the Chinese book [1] that there were no significant effects of worker group status on sperm morphology or on the sperm chromatin structure assay, an assessment of chromatin denaturability. The book also gave results separately for 16 men who were employed in the Pengxiang processing plant, where drinking water was heavily contaminated with boron, stating that this highly exposed subgroup did not differ significantly from the other groups in semen end points.

The three semen samples collected in 2004 were found not to differ from one another within each subject, suggesting stability of sperm end points within one spermatogenic cycle and reliability of the instrumental method [9]. For at least some of the analyses, end points for the three samples were averaged for each subject, although most of the papers do not indicate that the results of the three samples per subject were averaged. For some analyses, subjects were grouped by whether their urine boron concentrations predicted boron intake above the WHO recommended limit of 13 mg B/day.

Statistical analyses in some papers used simple *t* tests and in other papers used linear regression or mixed models with adjustment for potential confounders such as ingestion of beans, abstinence period, and testicular volume. In some analyses the full information from the three semen samples were included in mixed models accounting for the interdependence between several samples from the same individual [9].

3.1.2. Results

In the preliminary study in 2003 on 60 boron workers and 10 remote background controls, a larger proportion of boron workers than remote background controls had semen samples not meeting WHO criteria for normal semen analysis results [4]. There were no control men who failed to meet the criteria. An unspecified number of boron-exposed workers failed to meet at least one criterion. Considering the criteria separately, 4/58 boron-exposed workers had <20 million sperm/ml, 26/58 failed to have ≥50% forwardly motile sperm, and 8/58 failed to have ≥25% rapidly progressive sperm. In pairwise testing of individual sperm parameters, there were statistically significant decrements in boron workers in percent sperm

Table 6
Mean boron concentrations in urine, serum, and semen. Measure of dispersion, when available: ±SD.

Group	Subjects, *n*	Daily boron intake, mg/day	Boron concentration		
			Urine, mg/g creatinine	Blood or serum, ng/ml	Semen, ng/ml
Boron workers [12]	75	31.3 (1.60–469)	14.7 (1.46–117)	252 (22.8–2482)	592 (59.7–4087)
Pengxiang plant [1][a]	16	125 ± 89.1[b]	63.3 ± 43.3	1558 ± 1041	1844 ± 1516
Local community controls [12]	15	4.25 (0.68–13.9)	4.49 (1.19–15.7)	114 (3.29–348)	281 (98.8–1111)
Remote background controls [12]	23	1.40 (0.42–3.60)	1.58 (0.66–3.02)	39.1 (8.20–72.1)	146 (11.0–411)

[a] The drinking water at the Pengxiang plant was heavily contaminated with boron.
[b] Estimated in Ref. [1].

with forward motility, viability, rapidly progressive sperm, average path velocity (VAP), and straight line velocity (VSL). The authors concluded that boron exposure had adverse effects on sperm viability and sperm motion endpoints.

In the main study in 2004, group membership (66 boron workers, 61 remote background controls, 68 local community controls) was not statistically associated with differences in sperm density, in the percent progressive sperm, in the percent rapidly progressive sperm, or in the proportion of subjects in each group failing to meet WHO criteria for normal semen analysis [7,9]. There were no differences by group (62 boron workers, 53 background controls, 61 community controls) in VSL, curvilinear velocity (VCL), VAP, straightness (STR), or linearity (LIN) [6]. Mean values for the exposure groups are given in Table 7.

When subjects were evaluated based on urinary boron concentration as members of a “high-boron” ($n = 28$) or “low-boron” ($n = 148$) group, or when analyzed by quartile of urinary boron concentration, there were no statistically significant relationships between creatinine-adjusted urinary boron concentration and sperm density, total sperm count, semen quantity, semen zinc, or motility endpoints [7,8]. The authors identified an increase in STR in the high-boron group compared to the low-boron group, but the magnitude of the difference (80.4 versus 77.8) and the lack of statistical significance ($P = 0.052$) do not suggest a meaningful effect of boron exposure. There was no statistically significant difference between high-boron and low-boron groups in the proportion of men failing to meet WHO criteria for normal semen analysis [8].

3.1.3. *Comments*

This series of papers presents an evaluation of a unique group of boron workers who have biological boron measures higher than previously reported in humans and is the first study to include analyses of semen characteristics. There is little detail on the recruitment of subjects, and therefore, the potential for selection bias is a concern. With these limitations, however, this study is reassuring that occupational boron exposure in the range of 34.4–41.2 mg B/day does not alter sperm count or motility. Although the preliminary study came to the conclusion that boron exposure may be associated with impaired semen quality, this conclusion was not supported by the larger and more complete main study.

The panel critically evaluated a number of issues relating to the validity of the semen findings and interpretation of the results.

3.1.3.1. Selection bias. A low participation rate in cross-sectional semen studies is a general concern [46,47]. If the willingness to provide semen samples is motivated by the experience of subfertility to a different degree among exposed and unexposed, risk estimates may be biased in either direction. There were altogether about 3500 workers employed at the five boron mines and processing plants selected for the study [3]. Neglecting consideration of the selection of participants from the source population into the questionnaire study, the crude participation rate in the semen study among boron workers was 70/957 = 7.3% and among background control subjects, the crude semen study participation rate was 70/251 = 27.8%. The source population for the group of community control workers was not provided, and therefore the corresponding crude participation rate cannot be computed. The researchers approached 75 men in the boron worker group, 63 men in the local community control group, and 70 men in the remote background group with a request to participate in the semen sampling portion of the study. Of these men, 93.75% completed the study. Although we do not

Table 7
Semen endpoints from the Chinese boron workers study.

End point	Boron workers			Controls	
	All, $n = 66$	Not Pengxiang[a], $n = 50$	Pengxiang, $n = 16$	Local community	Remote background
Sperm					
Density, $\times 10^6$ ml^{-1}	71.1 ± 40.5	72.0 ± 45.5	68.2 ± 18.4	65.0 ± 38.8	72.8 ± 42.2
Total count, $\times 10^6$	218 ± 124	215 ± 137	229 ± 67.5	202 ± 115	222 ± 126
Forward progression, %	39.9 ± 13.8	38.8 ± 14.3	43.5 ± 12.1	39.3 ± 16.4	38.0 ± 17.6
Rapid forward progression, %	34.8 ± 12.4	33.8 ± 12.8	37.9 ± 10.9	34.7 ± 14.8	33.1 ± 15.8
Motility, %	51.7 ± 17.8	50.0 ± 18.7	56.8 ± 14	49.6 ± 20.7	49.6 ± 22.1
Velocity average path, μm/s	57.1 ± 5.7	57.3 ± 6.0	56.5 ± 4.9	55.6 ± 11.4	55.3 ± 12.7
Velocity straight line, μm/s	46.4 ± 5.1	46.2 ± 5.3	47.0 ± 4.5	45.2 ± 9.7	45.6 ± 10.3
Velocity, curvilinear, μm/s	87.8 ± 12.2	88.5 ± 12.4	85.7 ± 11.6	85.9 ± 18.6	84.6 ± 21.1
Straightness, %	79.4 ± 5.3	78.6 ± 5.7	81.7 ± 3.3	78.3 ± 11.4	77.5 ± 14.8
Linearity, %	53.6 ± 6.2	52.9 ± 6.2	55.8 ± 5.5	52.4 ± 9.4	52.4 ± 11.0
Semen mass, g	3.3 ± 1.4	3.13 ± 1.3	3.78 ± 1.6	3.2 ± 1.4	3.3 ± 1.5
Percent not meeting WHO criteria					
Sperm density	3.0	4	0	6.6	2.9
Total sperm count	4.6	6	0	4.9	1.5
Forward progression	77.3	80	68.7	72.1	73.5
Rapid forward progression	22.7	26.0	12.5	24.6	30.9

Data presented as mean ± standard deviation, from Wei and Robbins [1].
[a] Pengxiang processing plant workers were potentially exposed to drinking water heavily contaminated with boron.

know the basis on which the researches selected men for participation, it does not appear that the men selected themselves. Thus, there are no direct indications that findings were biased by skewed selection of men providing semen samples.

3.1.3.2. Misclassification bias. Subjects were dichotomized as having high and low exposure based on an estimation of whether daily boron intake was above or below 13 mg/day. The estimation was based on urinary boron/creatinine ratio, but different criteria were used for boron workers and for men not exposed to boron. Specifically, a urinary concentration of 7.08 mg B/g creatinine was used in boron workers and a urinary concentration of 9.47 mg B/g creatinine was used in non-boron workers [8]. We did not identify an appropriate rationale for the different criteria, which may have served to misclassify "low" exposed boron workers in the high group or "high" exposed non-boron workers in the low group. The effect of such misclassification would be expected to bias the results towards the null.

3.1.3.3. Over-controlling. In some of the analyses, semen parameters were adjusted for testicular volume. Because testicular volume is closely related to sperm concentration and count, adjusting semen results for testicular volume may have obscured an effect of group status or urinary boron concentration on semen characteristics.

3.1.3.4. Laboratory methods. Sperm counting and measurement of motility were based upon well-described semi-automated procedures (CASA, computer assisted sperm analysis). CASA was originally developed for analyses of sperm motility and may be less reliable for counting sperm. Comprehensive and adequate quality assurance programs involving external standards consisting of semen samples with high and low sperm counts were implemented. The reported average values for sperm count and motility are within the expected range. It is highly reassuring and indicative of good data quality that well known and strong associations as, for instance, the relation between period of abstinence and sperm count [48] were demonstrated with the given data [6,7]. The same applies to the relation between testicular volume and sperm count.

3.1.3.5. Confounding. The period of sexual abstinence is by far the strongest known determinant of sperm count in humans. This crucial factor was taken account of by requesting at least 2 days of abstinence before a semen sample was collected, but sperm counts increase with period of abstinence up to at least 7 days. In some cases, men with an abstinence period exceeding 7 days were excluded. Moreover, the statistical analyses accounted for differences in abstinence period between groups although distributions in the study groups were not given. Confounding related to period of abstinence in this study seems unlikely.

Some analyses of odds risk ratio were adjusted by a number of determinants, including factors defining ineligibility (age, exposure to pesticides). The incorporation of exclusion criteria into the analyses appears contradictory to the study design.

Time from collection of the sample to analysis and temperature conditions are strong determinants of sperm motility. Samples were sent for testing within 30 min after collection and analyzed within 60 min, which is adequate. However, details on the actual distribution of time from collection to analysis and information about handling of outliers were not given. Several other factors such as seasonal variation and smoking habits may also interfere with sperm counts, but severe bias is less likely.

3.1.3.6. Exposure contrast. The study design ensured a high contrast of boron exposure between study groups. Post-shift creatinine-adjusted boron concentrations in urine varied by more than an order of magnitude across study groups and the study took advantage of a local as well as a remote reference population to account for several life style and environmental exposures. The highest recorded intake of boron in this study population (7.8 mg B/kg bw/day [17]) was almost 50% of the NOAEL for male reproductive toxicity in rats (17.5 mg B/kg bw/day [27]). It is reassuring that 16 men who worked at the Pengxiang processing plant did not demonstrate decrements in semen quality in spite of a mean estimated boron intake of 125 mg/day, more than nine times higher than that of other boron workers and almost 100 times higher than the boron intake of remote background controls.

3.1.3.7. Statistical power. Considering the lack of statistically significant associations, the power of the study becomes an important issue. The authors performed power calculations to arrive at study group sizes of 60–70 men, but details were not given. The authors do not state how large a difference in sperm counts between groups could have been detected with confidence and whether those differences are meaningful. According to our calculations, the number of men per exposure group needed to detect a 25% difference would be 65 for sperm concentration but 110 for sperm count [46]. Collection of several semen samples per man is not expected to improve statistical power substantially.

In conclusion, the panel did not identify any flaws that are likely to bias the findings, but the review revealed some limitations of study design, analysis, and reporting that call for cautious interpretation of the results.

3.2. Reproductive success in men

3.2.1. Methods

Interviews on the reproductive experience of men and their wives were conducted in 957 boron workers and 251 remote background controls. After exclusion of some men for unstated reasons, responses from 945 boron workers and 249 control men were analyzed [10]. End points included spontaneous abortion and delayed pregnancy, defined as failure to become pregnant for 1 year or more. Data were analyzed using stepwise logistic regression evaluating smoking, ethanol use, pesticide exposure, diseases, X-ray exposure, age, nationality, education level, religion, and wearing a mask at work. Age and pesticide exposure were the only factors remaining in the final model. The pregnancy or failure to become pregnant within one year appeared to be the statistical unit of analysis.

A second paper, published in English, used information from 936 boron workers and 251 remote background controls [3]. End points included delayed pregnancy, multiple births, spontaneous abortion, induced abortion, stillbirth, ectopic pregnancy, more boys than girls, number of pregnancies fathered, and number of live births fathered. Statistical analysis was performed using univariate methods with multiple logistic regression reported for some but not all comparisons.

3.2.2. Results

In the first paper [10], the unadjusted odds ratio for an association between male boron exposure at work and spontaneous abortion was 1.79 (95% confidence interval 1.00–3.19). The adjusted odds ratio was 1.66 (95% confidence interval 0.88–3.13). The unadjusted odds ratio for delayed pregnancy was 1.69 (95% confidence interval 0.75–3.81), and there was essentially no change with adjustment for potential confounders. There were too few ectopic pregnancies and congenital developmental disorders for analysis. Sex ratio among the 326 children fathered by the control group was 118.79 and sex ratio for the 1043 children fathered by the boron workers was 109.44. These sex ratios were not significantly different. We converted the sex ratios to numbers of boys and girls. In the boron-exposed group, there were 545 boys and 498 girls. In the background control group, there were 177 boys and 149 girls ($P = 0.53$, Fisher exact test). The authors concluded that although there were no statistically significant differences in any endpoint between the groups, there was a higher prevalence of miscarriage and delayed pregnancy and a lower sex ratio in the boron workers.

In the second paper [3], men in the two groups were reported to be of similar age, but according to a data table analyzed by us using chi-square, the boron exposed group was less highly educated, more likely to be Hanzu, and less likely to be married than the control group. The two groups were comparable in the proportion smoking, but considering passive smoking, more boron-exposed men (97%) than control men (93%) were exposed to tobacco smoke. A similar proportion of men in each group drank alcohol, but the control group drank an average of twice as much wine as the boron worker group.

Boron workers fathered a mean ± SD of 1.98 ± 1.08 pregnancies compared with 2.11 ± 1.10 pregnancies fathered by men in the comparison group. This difference was not statistically significant at $P = 0.064$ on univariate analysis according to the authors and $P = 0.1$ when analyzed by us using the Student *t* test. Boron workers fathered 1.26 ± 0.61 pregnancies resulting in a live birth compared to 1.35 ± 0.65 pregnancies in control men, a difference reported to be significant at $P = 0.028$ by the authors ($P = 0.046$ by us). Adjustment for potential confounders was not reported for these comparisons.

Delay in pregnancy was identified in 9.42% of the 828 boron workers and 4.63% of the 238 control men who had been married at least a year. This difference was statistically significant by univariate analysis ($P = 0.018$) but not after adjustment for age, educational level, race, smoking, ethanol use, and soybean intake ($P = 0.11$). Induced abortion was reported by 39% of boron workers and 47% the control men, a difference that was statistically significant at $P = 0.03$. Wives of boron workers gave birth to 52.45% boys and wives in the control group gave birth to 54.35% boys. There was no significant difference in these proportions. Excluding men who had an equal number of boys and girls, men with more boys than girls were similar in both groups (56% of boron workers and 60% of control men, $P = 0.234$). The authors concluded that men exposed to boron had a decrease in live births and, in spite of the lack of statistical significance, a greater likelihood of delayed pregnancy and a deficit of boy children.

3.2.3. Comments

These papers provide no evidence that boron exposure alters male reproductive capacity, although the authors have concluded otherwise. The study was not designed in accordance with up-to-date standards for epidemiological studies of infertility and spontaneous abortion, and was beset with numerous pitfalls [47,49]. One important limitation of the work is the use of information from men in an assessment of the reproductive outcome of their wives. In western cultures, women are considered more reliable reporters of their reproductive outcomes. Although we do not have comparable information on the reliability of reporting by Chinese men, the unexpected low prevalence of infertility and spontaneous abortion in the reference group indicate low reliability of the outcome data. The delayed pregnancy end point showed a statistically significant difference between groups that disappeared on multivariate analysis, suggesting that confounding could explain the apparent difference. The report of fewer live births in boron workers than controls was based on univariate analysis; adjustment for potential confounders was not reported. The small difference between the groups (0.09 live births per subject) and the lack of adjustment for potential confounders or for multiple comparisons detract from the reliability of the reported difference. The authors' conclusions that boron workers have an increase in miscarriage and a deficit in boy children are not supported by the data presented in these papers. In addition, the reliability of sex ratio data may be severely compromised in societies such as China where selective abortion of female fetuses is practiced, although we do not have information on the prevalence of this practice in Liaoning province at the time of this study. The review panel found that these reports do not add reliable data on male reproductive success associated with boron exposure in humans.

3.3. Y:X ratio

3.3.1. Methods

Men were selected by an unspecified method for biologic sampling from those completing interviews on their reproductive experience [5]. Boron concentrations were determined in blood, urine, and semen samples from 63 boron workers, 39 local community controls, and 44 remote background controls (men from the low-boron region) using inductively coupled mass spectrometry and atomic emission spectrometry. A convenience sample of 15 men/group kept 24-h food and beverage diaries and had workplace inhaled dust monitoring. The numbers of X- and Y-bearing spermatozoa were evaluated using fluorescence in situ hybridization for the X and Y chromosome. Men with more boys than girls were compared in the three groups using a *t*-test with Tukey correction for multiple comparisons. Univariate linear regression models were constructed to test the predictive value for Y:X ratio of semen concentration, total motile cells, sperm morphology, days of abstinence, boron concentration in biological fluids, total daily boron exposure, diet, years of marriage, medications, chronic diseases, exposure to known reproductive toxicants, and history of reproductive problems. Multiple linear regression was used to evaluate the effect of potential confounders on the Y:X ratio. The final model included age, smoking, alcohol, education, and pesticide exposure.

3.3.2. Results

There were no significant differences between men in the three groups in age, education level, alcohol exposure, cigarette smoking, pesticide exposure, or spontaneous abortion in a spouse. Elective termination of a spouse's pregnancy was reported not to be different between the groups; however, chi-square analysis performed by us showed an overall *P*-value of 0.007 attributable to a higher elective abortion rate (50%) in the remote background control group than in the community control group (28.2%). There was a statistically significant difference in the percentage of men with more boys than girls across the three groups (overall $P = 0.03$), which by our analysis was attributable to more boys than girls in 76.7% of remote background controls compared to 42.3% of community controls. There were more boys than girls in 57.7% of boron workers, which by our analysis using Fisher exact test was not different from the rates in either control.

Blood, semen, and post-shift urine concentrations and estimated boron intakes were highest in the boron workers, intermediate in the local community control men, and lowest in the remote background control men. Y:X ratio was lowest in the boron workers (mean ± SD: 0.93 ± 0.03), intermediate in the local community control men (0.96 ± 0.04), and highest in the remote background control men (0.99 ± 0.03). Linear regression adjusting for age, smoking, alcohol use, education, and pesticide exposure confirmed an association between boron worker status and community control status and a decrease in Y:X ratio compared to background control men. Linear regression showed a significant relationship of the log boron concentration in each biologic fluid and Y:X ratio, although the direction of the relationship was not stated. Within each exposure group, there was no significant relationship between biological fluid boron and Y:X ratio.

3.3.3. Comments

The relationship between Y:X ratio in sperm and boron exposure group is interesting. The reported finding that Y:X ratio is significantly related to boron in urine, blood, and semen in the entire sample is also interesting, but the lack of relationship of biological sample boron concentration with Y:X ratio within exposure groups suggests that Y:X ratio is associated with an aspect of group status other than body burden of boron. An analysis of Y:X ratio as a function of biological sample boron concentration with adjustment for exposure group is warranted.

The premise that boron exposure is associated with a decreased sex ratio (fewer boys than expected) has not been supported by the data reviewed in the papers on the Liaoning workers. No statistically significant decrement in male births was identified in boron workers. The use of "men with more boys than girls" is not a standard metric for evaluating sex ratio; however, it offers the advantage of using the man as the experimental unit. The larger number of men with "more boys than girls" in the background control group may be explicable by the larger number of men reporting elective abortions in their spouses' pregnancies. The proportion of boys was 76.7% in the remote background control group and 42.3% in the community control group. The proportion of boys fathered by boron workers was higher, 57.7%, than in the local community controls, 42.3%. However, these data neither strengthen nor weakens the hypothesis that boron exposure has impact on the sex ratio, because any purported investigation of sex ratio at birth requires consideration of the selective abortion of female fetuses, which has been widely practiced in China and to which has been attributed an exceptionally high national ratio of male:female births [50–52].

The Y:X ratio was 0.99 in the remote background control, 0.96 in the close community control group and 0.93 in the boron workers. This indication of declining Y:X ratio with increasing exposure to boron is not linear: the difference in Y:X ratio across the three regions was of similar magnitude while exposure levels differed twofold between the local community and remote background controls but fivefold between boron workers and local community control men. This apparent non-linearity between boron exposure and Y:X ratio is, however, not a strong argument against a causal relationship, because the relationships might represent a genuine non-linear dose–response curve or might be a random phenomenon.

An issue not addressed in the study report or in any other source available to us was the manner in which men were selected for this study. The number of men participating in this paper was lower than the number providing biological samples in any of the other reports from this group. This dropout, which is differential (higher in the reference groups) is not accounted for. There is concern that the selection process may have introduced bias affecting the results. Thus, the proportion of Y-chromosomes in the reference group was 0.99/1.99 = 49.7%, but the expected value, according to the thus-far largest environmental study of Y:X ratio, is above 50–51% [53]. In addition, pesticide exposure was reported in previous papers from this group to disqualify a man from participation, yet 13–32% of men in the Y:X ratio study reported pesticide exposure. Information on how men were recruited into the three groups in the Y:X study would be welcome. It remains speculative how selection of men might systematically change the ratio of Y:X bearing sperm since strong determinants of Y:X ratio have not yet been identified.

Sex ratio and possibly Y:X ratio has been suggested as a sensitive indicator of environmental impact on human reproductive health, but in fact the evidence that reproductive toxicants interfere with the sex ratio is weak. For example, DBCP is a powerful male reproductive toxicant that can produce complete sterility and is considered an example of a toxicant that changes the secondary sex ratio. This claim is, however, based upon one small study [54]. Similarly, while positive studies may have been reported [55,56], there is no compelling evidence that established reproductive hazards such as tobacco smoking, ionizing radiation, or inorganic lead have any impact on the sex ratio of offspring [57–59]. A relationship of sex ratio or sperm Y:X to poor reproductive health outcomes has not been documented.

The earliest sign of reproductive toxicity of boron in rodents is delayed release of Step 19 spermatids (inhibited spermiation). At higher exposure levels, there is a reduction in epididymal sperm concentration and testicular atrophy (reviewed by Moore et al. [60]). These effects are observed consistently in several species (mice, rats, and dogs). The expected effect of boron in men would thus be a reduction in sperm count and, perhaps after more prolonged or higher exposures, reduced motility and percentage normal sperm forms. However, there are no experimental animal data to indicate that exposure to boron changes the sex ratio. A secondary review of data in a three-generation rat study [27], a continuous breeding study in mice [29], rat developmental toxicity studies [61,62], and a rabbit developmental toxicity study [63] provide no indication of a boron-related decrease in male:female ratio (Jay Murray, personal communication, 2009). The three-generation rat study and the continuous breeding study involved exposure of both parents, whereas the developmental toxicity studies involved only maternal exposure.

In conclusion, there is a need to corroborate or refute the unexpected finding of reduced proportion Y-bearing sperm among men exposed to boron. Consequences for human reproductive health, if any, are unknown.

4. Overall conclusions and data needs

The panel agreed on the following conclusions regarding the male reproductive toxicity study of boron in Liaoning province:

4.1. Exposure

(a) For the purpose of comparing boron exposures between groups, creatinine-adjusted urine boron concentration may be reliable.
(b) As an estimate of total daily boron exposure, urine boron concentration did not appear to be reliable. Measurement of boron in food, water, and dust appeared more reliable, although air exposure seems to have been under-estimated. Thus it appears that the percentage of boron in the air samples was lower than expected. It is possible that the sampling procedure resulted in retention of unmeasured boron-containing dust in the sampling device.
(c) Urine boron concentration would be expected to be increased after periods of high-boron exposure during a work shift; however, in this study, first morning urine boron concentrations were the highest and the concentrations were similar throughout the day.
(d) Use of post-shift urine boron concentration to estimate 24-h boron intake appears to have been unreliable; use of 24-h measurements of food, water, and air boron exposure are more reliable. In the Chinese studies the relationship between urine concentration and blood concentration is close to the relationship in the study by Culver et al. [64], as pointed out by Xing et al. [16]. But if the relationship of post-shift urine concentration to total boron intake identified by Culver et al. is used to estimate total boron intake based on the Chinese urinary concentration, an underestimation of Chinese total daily boron intake of more than 7 mg B/day would result.

4.2. Semen analysis

(a) The data do not indicate that boron exposure under the conditions described impairs testicular function with respect to sperm concentration, motility, morphology, or chromatin denaturability.
(b) The methods used to assess these endpoints were standard methods that appeared to have been reliably performed, and the data analysis accounted for the most important potential confounders as, for example, period of sexual abstinence.
(c) There are questions about the selection of subjects for semen evaluation and whether bias may have been introduced in subject selection. Without additional information, we cannot predict whether the biases, if present, would have influenced the results towards or away from the null hypothesis.
(d) The study size of some 65 men in each exposure group allow, according to our computations, the detection of a 25% difference in sperm concentration between groups at the 5% significance level with about 80% statistical power. The power is not expected to be substantially increased by analyses of several samples per man and the power to detect differences in total count is lower. Moreover, the study power to detect a doubling of risk of failure to meet WHO criteria for normal semen analysis at the 5% significance level is also about 80%. Altogether, the panel believes the statistical power of analyses based on these end points was adequate.

4.3. Reproductive success

(a) The methods used were not adequate to address the question of whether men exposed occupationally to boron have different reproductive experience than men not so exposed.
(b) Evaluation of sex ratio in these reports did not show a significant effect of boron exposure; however, the assessment of sex ratio in China is unlikely to be reliable.

4.4. Sperm Y:X ratio

(a) There were differences in Y:X ratio across the three groups defined by boron exposure.
(b) Y:X ratio appeared to be more related to group membership than to boron exposure.
(c) The within-subject variability of Y:X ratio and possible determinants of Y:X ratio are unknown except for possible minuscule effects of age, calendar time, and race.
(d) Y:X ratio is not known to be associated with impaired semen quality, reproductive success, or offspring health.

4.5. Data needs

The panel considered the following data needs and research priorities regarding the Chinese study as well as research on human reproductive toxicity in general:

(a) Comprehensive data are needed to evaluate occupational and environmental exposure such as:
  - description of industrial processes, work tasks, canteen conditions, and other worker facilities—photographs would be useful;
  - dietary, water, and accurate air exposure;
  - measures of exposure of men in the packaging operation;
  - better explanation of urine measurement (it was difficult to know what was done);
  - bioavailability of inhaled borate;
  - mapping of exposure levels in people not working in boron mining and processing (e.g., downstream users);
  - development and validation of standard methods for describing total exposure.
(b) With regard to semen analysis:
  - clarification of recruitment and selection of men providing semen and other biological samples and computation of participation rates would help an evaluation of possible selection bias;
  - reanalysis of endpoints according to biological measures of boron exposure to avoid misclassification bias;
  - although findings of the semen study are reassuring that boron does not cause impairment of testicular function in occupations conferring high exposure to boron, findings need independent replication.
(c) With regard to reproductive outcomes:
  To obtain a full account of boron's reproductive toxicity in humans there is a need to supplement studies of biological markers of male reproductive function with functional studies of couple fertility and pregnancy failures; the latter is in particular of interest following female exposure during gestation. If prospective studies are not feasible, the panel recommends time-to-pregnancy studies of recent pregnancies with women as the information sources. Follow-up studies of pregnancy outcomes verified by medical records using standard design should also be given priority.
(d) With regard to Y:X chromosome ratio in spermatozoa:
  - We wonder whether a Y:X ratio of sperm chromosomes that is deviant from the normal 50:50 is a genuine stable biological reality in some men. Studies examining within-subject variability have never been performed and might shed light on this question. A relationship between Y:X sperm chromosome ratio and offspring sex has never been demonstrated but might be explored in the Chinese dataset.
  - An analysis of Y:X ratio as a function of boron concentration in biological fluids with adjustment for exposure group membership would help to establish if boron exposure is an independent determinant of Y:X ratio. A replication study is definitely needed to corroborate or refute the observed association between Y:X ratio and boron exposure group, but given the lack of evidence that disturbances in Y:X ratio represents a health problem, the priority of a replication study can be questioned.

4.6. Overall conclusion

Although boron has been shown to adversely affect male reproduction in rats treated with 58.5 mg B/kg bw/day with a NOAEL of 17.5 mg B/kg bw/day, there is no clear evidence of male reproductive effects attributable to boron in this study of highly exposed workers. Boron exposures in these workers was not as high as the no-effect level in the rat studies; however, it is reassuring that these highly exposed workers did not show clear evidence of reproductive toxicity.

Conflict of interest

Dwight Culver is a medical Consultant to U.S. Borax, a component company of Rio Tinto Minerals. Frank M. Sullivan has been a Consultant to U.S. Borax, Borax Europe, and Rio Tinto Minerals for many years and was involved in design and interpretation of animal studies on reproductive toxicity of borates and in the interpretation of human studies on reproductive toxicity of borates.

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