A BRIEF AND CRITICAL REVIEW OF ENDEMIC HYDROFLUOROSIS IN RAJASTHAN, INDIA

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ABSTRACT: Almost all the drinking water sources, in the rural areas of the 33 districts of Rajasthan, the largest state in India, are contaminated with varying amounts of the fluoride ion (F). Most of the sources, from groundwater, bore-wells, and hand-pumps, contain F beyond the country standard for India set by the Bureau of Indian Standards, a requirement (acceptable limit) of 1.0 ppm (mg/L) or a permissible limit in the absence of an alternate source of 1.5 ppm, or the World Health Organization "desirable" upper limit of 1.5 ppm. No extensive epidemiological studies on water-borne hydrofluorosis (chronic fluorosis) have been conducted in many of these endemic high drinking water F level districts. However, many reports on endemic hydrofluorosis in villagers and their domesticated animals are available from southern Rajasthan and in the present review these are critically reviewed and areas for possible future research are identified. The review considers the sources of F exposure, the distribution of F in potable water, F tolerance and the development of hydrofluorosis, the clinical features of chronic F toxicity, factors affecting the severity of F toxicity, biomarkers for chronic F poisoning, and the mitigation and prevention of hydrofluorosis. These factors may be useful in the framing and implementation of health policies at the state level for the mitigation and control of endemic hydrofluorosis.

Keywords: Domestic animals; Fluoride; Groundwater; Humans; Hydrofluorosis; Rajasthan, India.

INTRODUCTION

Almost all the drinking sources, in the rural areas of the 33 districts of Rajasthan, the largest state in india, are contaminated with varying amounts of the fluoride ion (F). Most of the sources, from groundwater, bore-wells, and hand-pumps, contain F beyond the country standard for India, a requirement (acceptable limit) of 1.0 ppm (mg/L) or a permissible limit in the absence of an alternate source of 1.5 ppm,[1-6] or the WHO "desirable" upper limit of 1.5 ppm.[7-9] No extensive epidemiological studies on water-borne hydrofluorosis (chronic fluorosis) have been conducted in many of these endemic high drinking water F level districts. However, many reports on endemic hydrofluorosis in villagers and their domesticated animals are available from southern Rajasthan and in the present review these are critically reviewed and areas for possible future research are identified. The review considers the distribution of F in potable water sources at the district level, the prevalence of dental and skeletal fluorosis in relation to F concentrations, the irreversibility of chronic fluorosis, the susceptibility to chronic fluorosis, and the bio-indicators of chronic F poisoning. These factors may be useful in the framing and implementation of health policies at the state level for the mitigation and control of endemic hydrofluorosis.

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RAJASTHAN

Rajasthan is the largest state in India and lies between latitude 23º30' and 30º11' N and longitude 69º29' and 78º17' E. The area of Rajasthan is 342,239 km^2 and it comprises 10.4% of the area of the country. The state is eco-geographically divided by the Aravalli mountain range into two regions: (i) the Thar Desert region in the west, and (ii) the forest belt in the east. At present, Rajasthan has a total number of 33 districts of which 12 districts are located in the western desert region. The human population of Rajasthan in 2015 was 73.53 million and the domestic animal population in 2012 was 57.73 million.

SOURCES OF F EXPOSURE

The principal sources of F for man and animals are: drinking water, vegetation and crops grown on fluorotic soils, certain edible marine animals, F-rich phosphate feed supplements, mineral mixtures, medicines, cosmetics, dust in the air, and certain F-emitting industrial processes.[9,10] The first two F sources, drinking water and plants grown on fluorotic soils, are natural and generally responsible for endemic fluorosis while the remaining sources are anthropogenic and are restricted to particular locations. In the state of Rajasthan, the main F source for the development of hydrofluorosis (chronic F intoxication or chronic fluorosis caused by F in drinking water) in both man and domestic animals is groundwater.

In recent years in Rajasthan, another source of F exposure has been industrial F emissions. A number of coal-burning and industrial activities[11] now discharge F, in both gaseous and particulate/dust forms, into the surrounding environment. This industrial F pollution is an important cause of occupational F exposure in many districts. F emitted by industry contaminates not only the surrounding soil, air, and water but also the vegetation, crops, and many other biotic communities on which people, domestic animals, and wild animals are generally dependent for food. The long-term of inhalation or ingestion of industrial F also causes mild to severe toxic effects in the form of industrial[12] and neighbourhood fluorosis.[13]

DISTRIBUTION OF F IN POTABLE WATER

Currently, in Rajasthan, the water supply for drinking, cooking, and other domestic uses in urban areas is mainly from fresh water sources, including large reservoirs, ponds, dams, rivers, and streams, which are mostly free from F contamination. Up until 1986, in the rural areas of Rajasthan, there were only a limited number of bore-wells or tube-wells fitted with hand-pumps which provided drinking water. A bore-well is a deep narrow well that is drilled into the ground, has a pipe fitted as a casing in the upper part of the borehole, and is typically equipped with a pump to draw the water to the surface. However, in 1986 a programme to eradicate a human nematode, the guinea-worm (*Dracunculus medinensis*) was commenced[14,15] and numerous bore-wells or tube-wells with hand-pumps were dug in the villages, even in remote areas of Rajasthan. Simultaneously, all the traditional drinking water sources step-wells, in which the water is reached by descending a series of steps, and open-wells, dug wells in which the groundwater is visible, were closed down as part of the strategy to break the life-cycle of the guinea-worm.

Based on the available scientific reports, almost every hand-pump and bore-well located in the rural areas of Rajasthan are contaminated with F and are not safe for either human or animal health. Most of these water sources have a F level beyond the country standard for India, a requirement (acceptable limit) of 1.0 ppm (mg/L) or a permissible limit in the absence of an alternate source of 1.5 ppm,[1-6] or the WHO "desirable" upper limit of 1.5 ppm.[7-9] In addition, the Bureau of Indian Standards has stated that the "lesser the fluoride the better, as fluoride is injurious to health."[1-4] In the first revision of the *Indian Standard Drinking Water—Specification*, in 2003,[5] the word "requirement" is followed in parentheses by "desirable limit" while in the second revision, in 2012,[6] the requirement for the limit of 1 ppm has been strengthened by using description in parentheses of "acceptable limit" instead of "desirable limit."

Recently, the data on the F concentration (ppm) in the drinking water sources in all of the 33 districts of Rajasthan were reviewed.[16] The highest amount of F in drinking water was observed in Nagaur (90.0 ppm) district followed by Churu (32.0 ppm), Sri Ganganagar (28.2 ppm), Jaipur (28.1 ppm), Bhilwara (24.0 ppm), Jodhpur (22.0 ppm), Udaipur (21.6 ppm), Bikaner (20.0 ppm), Barmer (19.6 ppm), Bharatpur (18.4 ppm), Pali (18.3 ppm), Ajmer (17.6 ppm), Sirohi (16.0 ppm), Tonk (15.8 ppm), Sikar (15.0 ppm), Dausa (14.9 ppm), Jalore (14.2 ppm), Jaisalmer (12.0 ppm), Jhunjhunu (12.0 ppm), Dungarpur (10.8 ppm), Sawai Madhopur (10.0 ppm), Alwar (9.9 ppm), Hanumangarh (8.5 ppm), Bundi (6.8 ppm), Chittorgarh (6.6 ppm), Dholpur (4.9 ppm), Kota (4.8 ppm), Pratapgarh (4.7 ppm), Banswara (4.6 ppm), Karauli (4.5 ppm), Rajsamand (4.5 ppm), Baran (2.0 ppm), and Jhalawar (1.5 ppm) district (Figures 1 and 2).[16]

Image courtesy of Wikipedia; https://en.wikipedia.org/wiki/Rajasthan

Figure 1. Location of the state of Rajasthan in India.

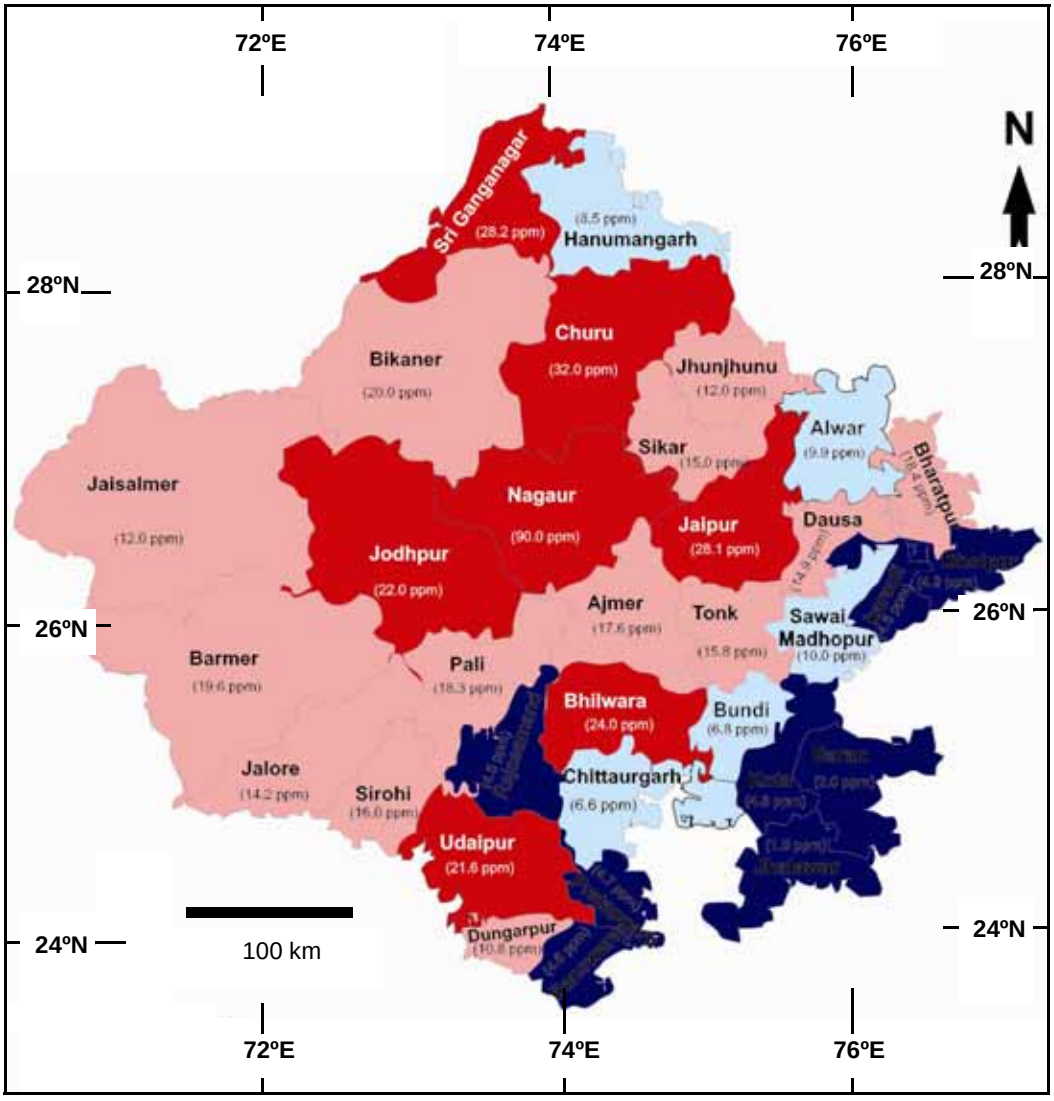


Figure 2. Map of Rajasthan showing the district-wise F distribution in groundwater sources. The ranges for the F concentrations (ppm) in the districts are: deep-blue: 1.5–5.0; light-blue 5.1–10.0; light-red: 10.1–20.0; and deep-red: >20.0.[16]

The occurrence of anomalously high F concentrations in the groundwaters of all 33 districts of Rajasthan is not anthropogenic in origin but is a natural phenomenon and due to the presence of a heavy deposition of F-bearing minerals in the host rocks and sediments. The important F-bearing rocks and minerals contain a mean F in the range of 180–31,000 ppm and include granites, gneisses, mica, schists, limestone, sand stone, phosphorite, shales, clays, acidic igneous rocks, basalts, and alluvium. Their chemical properties like decomposition, dissociation, dissolution, and interaction with water are considered to be the main cause of F in groundwater. The distribution of F in the various regions is also influenced by the regional physiography, climate, and hydrogeological conditions.[16]

F TOLERANCE AND THE DEVELOPMENT OF HYDROFLUOROSIS

In 1973, a World Health Organization (WHO) report, *Trace elements in human nutrition*, noted that fluorine had emerged as an essential nutrient in only the last 3–4 years, was now considered to be one of 14 essential trace elements, and to be one of 7 trace elements of special significance.[17] In 1983, Wheeler and Fell noted that the necessity of F in biological functions was still doubted, probably because the amount required was so small that a deficiency could not be produced, under even the most careful laboratory condition using trace element sterile isolators.[18] In 1996, a WHO report, *Trace elements in human nutrition and health*,[19] considered fluoride in the section on "Potentially toxic elements, some possibly with essential functions" and commented that "Although fluoride should probably be regarded as essential, there is no evidence so far from human studies that overt clinical signs of fluoride deficiency exist. No specifically diagnostic clinical or biochemical parameters have been related to fluoride inadequacy. The Expert Consultation was therefore unable to specify a minimum desirable intake. However, in view of the toxicity associated with excessive fluoride ingestion from a variety of sources, recommendations for maximum safe intakes are required. For this purpose, dental mottling may be taken as definitive evidence of toxicity." They recommended that "Total intakes at 1, 2, and 3 years of age should, if possible, be limited to 0.5, 1.0, and 1.5 mg/day respectively, with not more than 75% in the form of highly soluble fluorides of drinking water." They noted that "Adult intakes exceeding 5 mg of fluoride per day from all sources probably pose a significant risk of skeletal fluorosis." In 2011, the opinion of a Scientific Committee on Health and Environmental Risks (SCHER) of the European Commission, European Union, was that although F has an anticaries effect when applied topically to the teeth, fluorine is not an essential trace element and is not necessary for the development of healthy teeth and bones.[20]

More soluble F compounds are relatively more toxic and are easily absorbed in the digestive tract of animals. In general, inorganic forms of F are more toxic than its organic forms.[10] If drinking water contains F beyond the threshold value of 1 or 1.5 ppm, [1-9,21] then its consumption for a prolonged period will cause serious damage to both hard (teeth and bones) and soft tissues (organs). Based on these recommendations, in Rajasthan, the drinking groundwater of the rural areas of all 33 districts is highly toxic and not safe for human or animal health. Because Rajasthan has a persistently warm climate, the author suggests that the optimal F concentration in drinking water should remain below 1.0 ppm. Physiologically, people perspire more in hot weather and consequently drink more water. More debate and scientific studies are needed to reset the permissible upper limit for F in drinking water in India.

In India, hydrofluorosis was recognized and reported for the first time in people from the Nellore district of the Madras Presidency of south India.[22,23] In Rajasthan, the first case of hydrofluorosis was reported from Jobner of the Jaipur district in 1959[24] whereas, in domestic animals, hydrofluorosis was first reported from the Dungarpur district in 1996.[25] At present, based on the available reports, out of 33 endemic high drinking water F districts, hydrofluorosis has been reported in only 14 districts viz. Ajmer, Alwar, Banswara, Bharatpur, Bhilwara, Bikaner, Dungarpur,

Jaipur, Jalor, Jhunjhunu, Jodhpur, Nagaur, Sirohi, and Udaipur.[26-60] In only five districts, namely Banswara, Bikaner, Dungarpur, Sirohi, and Udaipur districts, has endemic hydrofluorosis been reported in diverse species of domestic animals, including cattle (*Bos taurus*), buffaloes (*Bubalus bubalis*), horses (*Equus caballus*), donkeys (*Equus africanus asinus*), camels (*Camelus dromedarius*), sheep (*Ovis aries*), and goats (*Capra hircus*). The prevalence of dental and skeletal fluorosis in people and domestic animals in relation to the F concentration in the drinking water is shown in Tables 1–3 and the endemic hydrofluorosis districts are shown in Figure 3. In southern Rajasthan (Banswara, Dungarpur, and Udaipur districts), studies have been carried out on F distribution[29, 61-63] and endemic osteo-dental fluorosis in different human populations[64] and different species of domestic animals.[65,66] The first description of the occurrence anywhere of hydrofluorosis in donkeys and the first description of the occurrence in India of hydrofluorosis in horses were made in 2010.[43] However, more district level epidemiological studies on hydrofluorosis are needed to assess the current status of chronic F poisoning in Rajasthan.

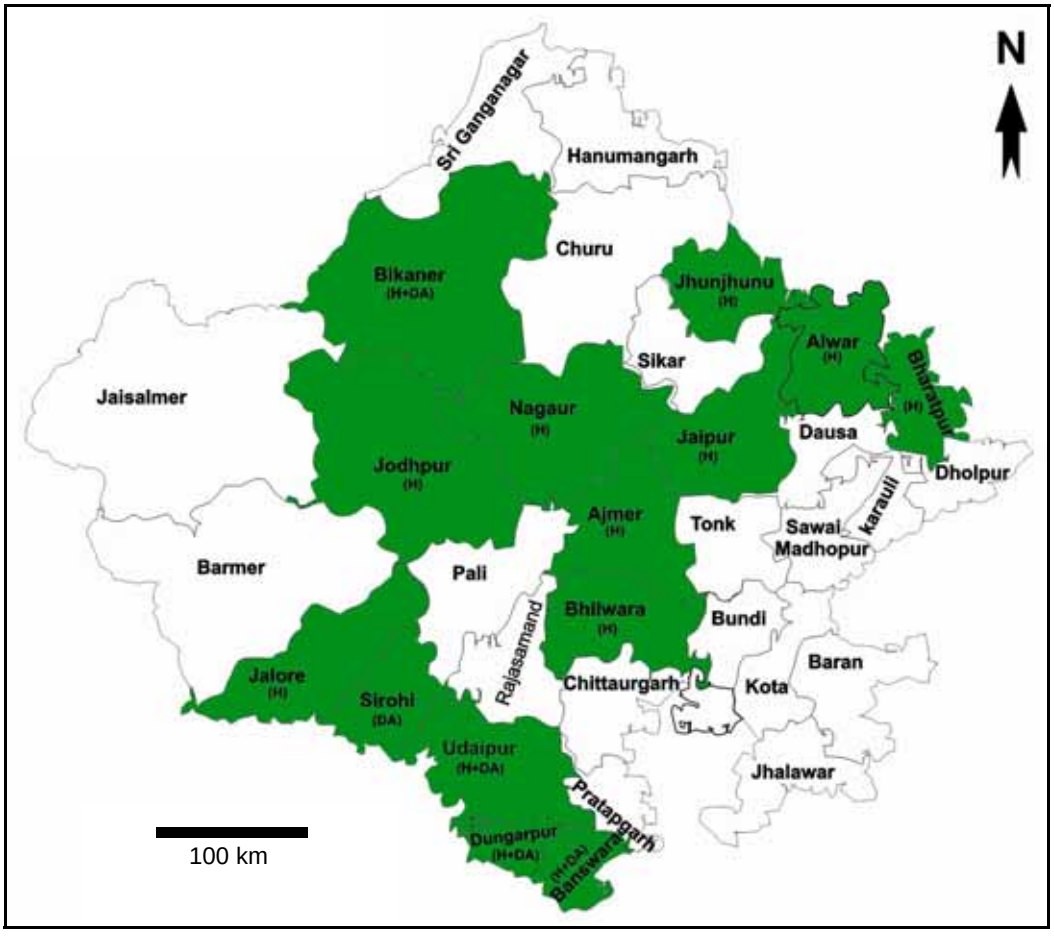


Figure 3. Map of Rajasthan showing where endemic hydrofluorosis was studied in humans (H) and domestic animals (DA) in 14 districts (indicated in green).

Table 1. Prevalence (%) of dental fluorosis (DF) and skeletal fluorosis (SF) in people and domestic animals in relation to the F concentration (ppm) in drinking groundwater sources reported from various districts of Rajasthan (India). (NE=not estimated; – = not determined; Animal species: cattle [*Bos taurus*], buffaloes [*Bubalus bubalis*], sheep [*Ovis aries*], goats [*Capra hircus*], horses [*Equus caballus*], donkeys [*Equus africanus asinus*], and camels [*Camelus dromedarius*]).

	District	Range of F in ground water (ppm)	Subjects studied	Sample size (n)	DF (%)	SF (%)	Reference
1	Ajmer	7.6–8.1	Adults	98	43.0	48.0	26
	Ajmer	13.9–14.3	Adults	357	83.5	26.9	27
2	Alwar	NE	Children	173	53.0	–	28
3	Banswara	0.2–5.5	Children	978	31.9	–	29
	Banswara	0.2–5.5	Adults	1350	33.4	10.2	29
	Banswara	0.2–5.5	Calves	22	13.6	–	29
	Banswara	0.2–5.5	Cattle	102	6.8	2.5	29
	Banswara	0.2–5.5	Buffaloes	54	7.4	2.7	29
	Banswara	1.2–4.6	Cattle	598	–	5.4	30
	Banswara	1.2–4.6	Buffaloes	75	–	12.0	30
	Banswara	1.2–4.6	Children	521	36.5	–	31
	Banswara	1.2–4.6	Adults	826	41.0	19.1	31
4	Bharatpur	NE	Children	700	54.5	–	32
5	Bhilwara	0.4–13.0	Adults	4252	76.9	47.5	33
6	Bikaner	1.5–2.5	Calves	24	41.7	–	34
	Bikaner	1.5–2.5	Cows	75	37.3	40.0	34
	Bikaner	1.6–2.2	Cattle	85	21.8	8.2	35
	Bikaner	1.5–2.5	Adults	60	36.7	22.4	36
7	Dungarpur	1.7–6.1	Children	2217	73.0	–	37
	Dungarpur	1.7–6.1	Adults	2497	82.9	32.5	37
	Dungarpur	1.7–6.1	Cattle	1521	66.2	35.8	37
	Dungarpur	1.7–6.1	Buffaloes	471	67.5	37.5	37
	Dungarpur	1.4–6.0	Cattle	901	59.3	35.6	25
	Dungarpur	1.4–6.0	Buffaloes	122	48.3	26.2	25
	Dungarpur	1.2–5.2	Adults+children	1272	93.5	–	38
	Dungarpur	0.3–10.8	Children	1953	62.7	–	39
	Dungarpur	0.3–10.8	Adults	1955	69.7	–	39
	Dungarpur	1.1–4.1	Cattle	538	–	9.6	30
	Dungarpur	1.1–4.1	Buffaloes	101	–	13.8	30

Table 2. Prevalence (%) of dental fluorosis (DF) and skeletal fluorosis (SF) in people and domestic animals in relation to the F concentration (ppm) in drinking groundwater sources reported from various districts of Rajasthan (India). (NE=not estimated; – = not determined; Animal species: cattle [*Bos taurus*], buffaloes [*Bubalus bubalis*], sheep [*Ovis aries*], goats [*Capra hircus*], horses [*Equus caballus*], donkeys [*Equus africanus asinus*], and camels [*Camelus dromedarius*]).

	District	Range of F in ground water (ppm)	Subjects studied	Sample size (n)	DF (%)	SF (%)	Reference
7	Dungapur	1.3–6.7	Children (Tribal)	1827	84.9	–	40
	Dungapur	1.3–6.7	Adults (Tribal)	1811	84.0	32.7	40
	Dungapur	1.3–6.7	Calves	280	89.6	–	40
	Dungapur	1.3–6.7	Bovines	1237	88.9	37.8	40
	Dungapur	1.2–8.9	Children	444	93.2	2.9	41
	Dungapur	1.2–8.9	Adults	1418	91.7	26.2	41
	Dungapur	0.8–7.6	Calves	326	67.7	–	42
	Dungapur	0.8–7.6	Cattle	1127	2.6	36.0	42
	Dungapur	0.8–7.6	Buffaloes	414	74.6	36.7	42
	Dungapur	0.8–7.6	Goats	516	–	–	42
	Dungapur	0.8–7.6	Sheep	486	–	–	42
	Dungapur	0.8–7.6	Lambs	236	–	–	42
	Dungapur	1.4–3.3	Horses	14	78.7	78.7	43
	Dungapur	1.4–3.3	Donkeys	9	100.0	100.0	43
	Dungapur	1.4–3.3	Foals	2	100.0	–	43
	Dungapur	1.4–3.3	Camels	18	44.4	16.7	44
	Dungapur	1.5–4.4	Cattle	836	50.0	42.7	45
	Dungapur	1.5–4.4	Buffaloes	131	70.2	64.1	45
	Dungapur	1.5–4.4	Goats	158	32.9	29.1	45
	Dungapur	1.5–4.4	Sheep	67	28.3	25.7	45
	Dungapur	1.5–4.4	Horses	11	81.8	81.8	45
	Dungapur	1.5–4.4	Donkeys	7	71.4	71.4	45
	Dungapur	1.5–4.4	Tribals	996	73.8	29.5	46
8	Jaipur	1.2–15.0	Adults+children	221	82.3	–	47
	Jaipur	–	Children	3200	34.5	–	48
	Jaipur	NE	Patients	477	89.0	–	49
9	Jalor	3.56–4.07	Adults	658	94.9	–	50
10	Jhunjhunu	0.3–2.7	Children	401	68.2	–	51
11	Jodhpur	0.7–1.2	Children	1810	18.9	–	52
12	Nagaur	NE	Adults	244	95.9	–	53
		0.2–14.6	Adults	–	93.1	–	54
		0.5–8.5	Adults	1136	69.4	–	45

Table 3. Prevalence (%) of dental fluorosis (DF) and skeletal fluorosis (SF) in people and domestic animals in relation to the F concentration (ppm) in drinking groundwater sources reported from various districts of Rajasthan (India). (NE=not estimated; – = not determined; Animal species: cattle [*Bos taurus*], buffaloes [*Bubalus bubalis*], sheep [*Ovis aries*], goats [*Capra hircus*], horses [*Equus caballus*], donkeys [*Equus africanus asinus*], and camels [*Camelus dromedarius*]).

	District	Range of F in ground-water (ppm)	Subjects studied	Sample size (n)	DF (%)	SF (%)	Reference
13	Sirohi	3.0–12.0	Bovines	260	59.2	31.5	56
	Sirohi	3.0–12.0	Flocks	405	7.4	1.7	56
14	Udaipur	0.3–7.0	Children	1167	49.7	–	57
	Udaipur	0.3–7.0	Adults	1396	58.2	16.4	57
	Udaipur	0.3–7.0	Calves	143	39.1	–	57
	Udaipur	0.3–7.0	Cattle	839	26.5	14.6	57
	Udaipur	0.3–7.0	Buffaloes	318	41.8	16.6	57
	Udaipur	0.2–4.6	Cattle	548	–	7.4	30
	Udaipur	0.2–4.6	Buffaloes	114	–	15.1	30
	Udaipur	0.2–5.1	Children	543	41.6	–	41
	Udaipur	0.2–5.1	Adults	755	52.2	21.5	41
	Udaipur	NE	Children	1587	36.7	–	58
	Udaipur	0.0–1.0	Calves	75	42.6	–	59
	Udaipur	0.0–1.0	Cattle	172	30.8	–	59
	Udaipur	0.0–1.0	Buffaloes	116	34.4	–	59
	Udaipur	0.8–4.1	Children	–	69.8	–	60

THE CLINICAL FEATURES OF CHRONIC F TOXICOSIS

Chronic F exposure through the ingestion of drinking water with a high F level will result in its accumulation, predominantly in hard tissues such as teeth and bones, and cause diverse adverse changes that appear in the form of dental mottling (dental fluorosis) and bone deformities (skeletal fluorosis) in both humans[1] and animals.[2] Dental fluorosis in humans only occurs with F ingestion while the teeth are still developing, up to approximately the age of 6 yr. Besides these maladies, there are also reports, in humans as well as in domestic and laboratory animals, of non-skeletal fluorosis or toxic effects of chronic F exposure in soft tissues or organs including gastrointestinal discomforts, neurological disorders, impaired endocrine and reproductive functions, teratogenic effects, renal effects, genotoxic effects, apoptosis, and excitotoxicity.[67]

F toxicity in teeth (dental fluorosis): In both humans and animals, the earliest visible pathognomic sign or manifestation of chronic F poisoning is dental mottling (dental fluorosis) It is the most recognizable and sensitive index. Dental fluorosis, which is irreversible, is rampant in Rajasthan. In general, dental fluorosis is characterized by horizontal staining on the enamel of the teeth in humans (Figure 4), as well as in immature (Figure 5) and mature (Figure 6) domestic animals, which is

white or light to deep brownish in colour, bilateral, striated, and condensed or diffuse.[46,65,66]

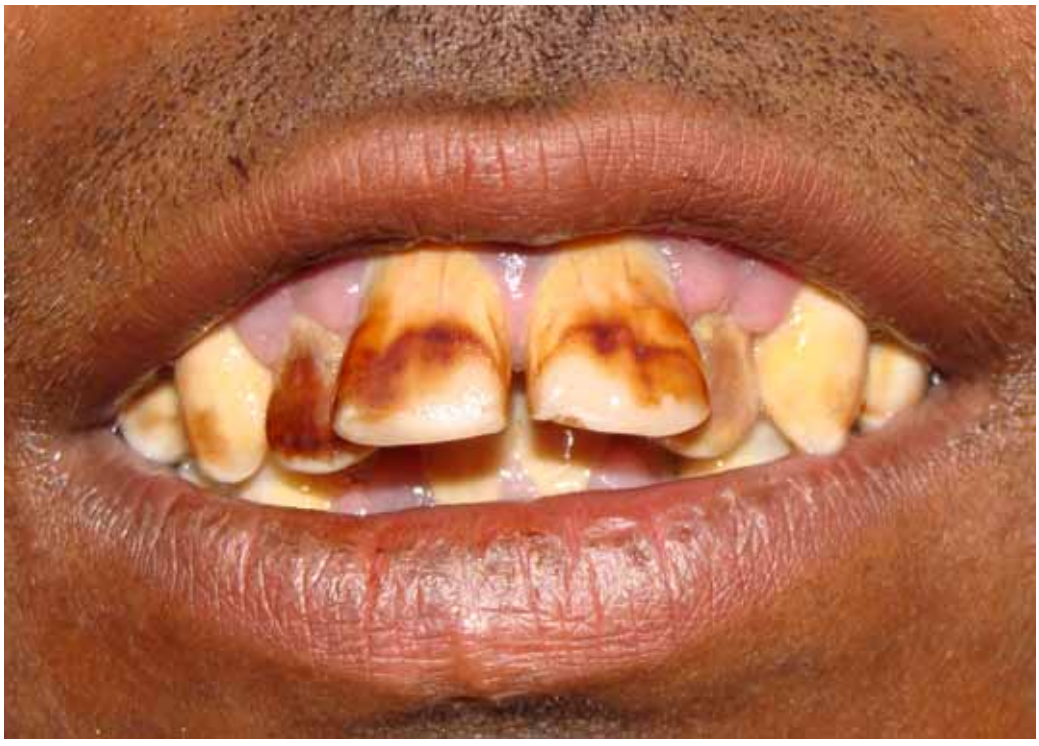


Figure 4. A severe form dental fluorosis characterised with striated and horizontally deep brownish staining on the upper anterior teeth in a young individual.

Figure 5. A severe form dental fluorosis characterised with striated and horizontal deep brownish staining on the anterior teeth of a cattle calf.

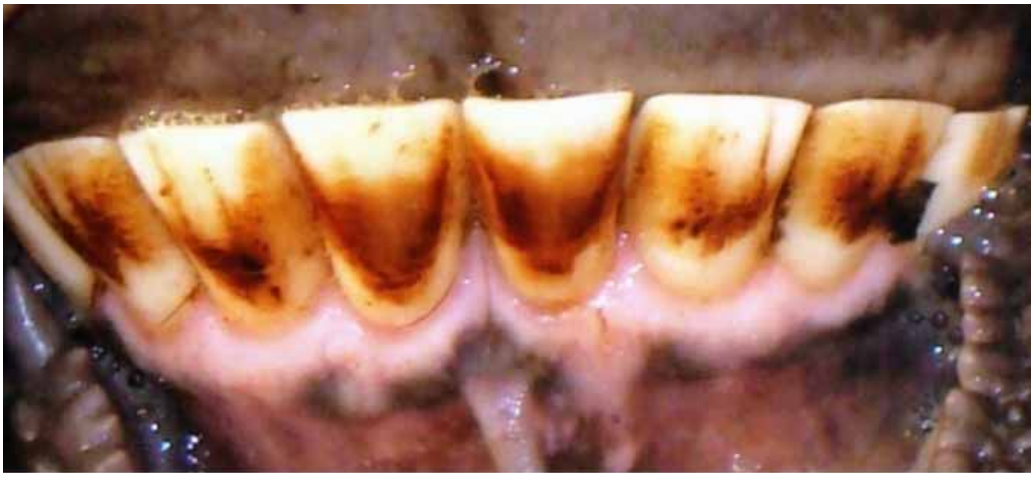


Figure 6. A severe form of dental fluorosis in a mature buffalo with excessive attrition, irregular wearing, and deep brownish yellow staining in the lower anterior teeth.

In some cases, dental fluorosis is also seen in the form of white or light to deep brownish spots, patches, and fine dots or granules on the enamel surface of the anterior teeth. In severe dental fluorosis, pronounced loss of the tooth-supporting alveolar bone occurs with recession and swelling of the gingival tissues, and excessive abrasion or irregular wearing of the teeth. In a recent study, conducted in the villages of the desert district, Bikaner, where the drinking water contained F in the range of 1.5–2.5 ppm, the teeth of calves showed two different forms of staining.[34] Some of the calves had blackish staining on their anterior teeth instead of the usual brownish-yellow. The reason for this difference in staining is not yet clear.

In domestic animals, dental disfigurement is also important because it reduces their life-span. When these dental lesions become severe enough to cause difficulty in grazing and mastication, the animals die at a young age from hunger and cachexia.[1] The death of animals at an early age has economic consequences for the herdsmen. It is interesting to note that, despite these economic losses, the herdsmen/villagers and many veterinarians in the rural areas of Rajasthan are unaware of the aetiology and consequences of dental fluorosis.

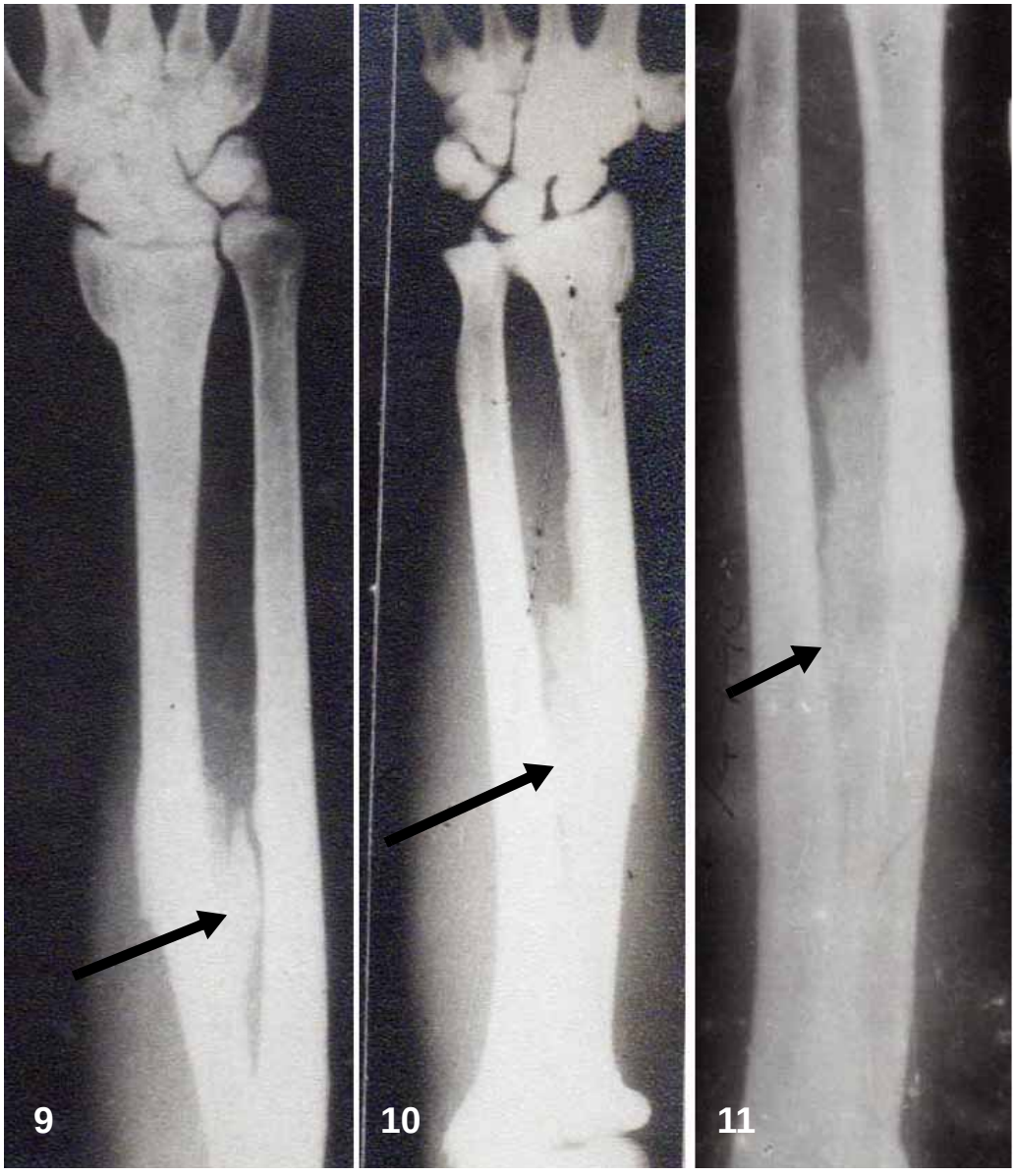
Toxicity in bones (skeletal fluorosis): It is well known that skeletal fluorosis is more dangerous than dental fluorosis, very painful, and highly significant since it diminishes the mobility of fluorotic humans and animals at a very early age by producing varying changes in the bones such as periosteal exostosis, osteosclerosis, osteoporosis, and osteophytosis.[40, 41] These changes appear clinically in the form of vague aches and pains in the body and joints which are associated with rigidity, lameness, stunted growth, palpable bony lesions, and a snapping sound in the feet during walking.[42-44] The excess accumulation of F in muscles also diminishes or restricts movements and the condition leads to crippling in humans and lameness in domestic animals. Neurological complications such as paraplegia and quadriplegia,

(Figure 7) and the syndromes of genu-valgum or genu-varum (Figure 8) can result in human beings from severe skeletal fluorosis.

Figure 7. Cases of severe skeletal fluorosis showing common deformities: kyphosis, invalidism, paraplegia, quadriplegia, and the genu-varum syndrome.

Figure 8. Cases of severe skeletal fluorosis showing scissor-shaped legs (genu varum).

An X-ray of the radius and ulna bones can be helpful in the diagnosis of skeletal fluorosis due to the early appearance of calcification in the interosseous membrane between these two bones (Figures 9–11).[68-70]



Figures 9–11. Radiographs of the radius and ulna bones of the forearms of fluorosed subjects showing calcification of the interosseous membrane (indicated by arrows), a diagnostic feature of skeletal fluorosis.

Well recognized signs of fluorosis in fluorosed animals reared in Rajasthan include intermittent lameness, swollen joints, debility, wasting of body muscles, bony exostosis or lesions in the mandibles, ribs, metacarpal and metatarsal regions, and increased mortality (Figure 12).

Figure 12. Emaciated <2-year-old cow calf afflicted with severe skeletal fluorosis. Note the wasting of the body muscles and bulging lesions on the legs and ribs.

In Rajasthan, none of the human fluorotic subjects showed evidence of the genu-valgum syndrome (knock-knee) or hypothyroidism. The genu-valgum syndrome has been found in endemic high drinking water F areas where the consumption of jowar (sorghum) as food is more prevalent[1] but, interestingly, it has not so far been detected or reported in the desert and semi-desert districts of Rajasthan where the staple diet of the people is jowar-bajra (sorghum-pearl millet). In some southern states (Andhra Pradesh, Karnataka, and Tamil Nadu) and the middle region of India (Madhya Pradesh), where skeletal fluorosis is hyperendemic, some cases of genu-valgum deformity have also been reported.[71,72] The way in which F causes these deformities

is still mysterious and uncertain. The relationship between F intoxication and thyroid function is also poorly understood, although an association of fluorotoxicosis with endemic goiter has been reported from several countries.[73] In India, some workers have failed to find evidence of goiter in areas of endemic fluorosis[74,75] while others have observed it.[76] In Rajasthan, none of fluorotic individuals were found to have evidence of goiter.[67]

Toxicity in soft tissues (non-skeletal fluorosis): F exposure for a prolonged period through the drinking of water with a high F level may also cause harmful toxic effects in the soft tissues or organs of both humans and animals. All the manifestations of F toxicity in the various organ systems, besides the teeth and bones, are referred to as non-skeletal fluorosis. In endemic high drinking water F provinces of Rajasthan, the most common health complaints and signs in humans and animals resulting from chronic F intoxication include gastrointestinal discomforts (nausea/loss of appetite, gas formation or bloating, pain in the stomach/colic, constipation, intermittent diarrhea, headache, etc.), frequent tendency to urinate (polyuria)/itching in the genital area, excessive thirst (polydipsia), extreme weakness/muscle weakness, allergic reactions, asthma, bronchitis with violent cough, nasal irritation, irregular reproductive cycles, abortion, and still birth.[46,66,77] These complaints are

reversible after the withdrawal of the F exposure. [78] However, to demonstrate an aetiological relationship between these symptoms and signs and F toxicity more scientific research studies are needed, in both humans and domestic animals.

Regular F exposure for a long-time also causes neurological complications in several ways besides the development of paraplegia and quadriplegia. Recently, studies conducted in Rajasthan[79] revealed that children living in endemic high drinking water F areas have many neurological ailments such as headache, insomnia, lethargy, polyuria, and polydipsia. In Rajasthan, chronic F exposure also affects higher cognitive functioning and reduces the intelligence quotient (IQ) levels, cognition, memory, and learning ability in children.[80,81] In fact, F is responsible for impairing central nervous system function.[82] However, the exact mechanism involved at the molecular level is, as yet, not clear. In recent studies,[83-85] a significant relationship was detected between the presence of mild, moderate, and severe dental fluorosis and impaired cognition in children. These studies indicate that dental fluorosis can be regarded as a marker for F-induced cognitive impairment.[86] However, more such studies are needed to reach a final conclusion on F-induced impaired cognition in children.

It has long been suspected that F may contribute to the formation of kidney stones. A study conducted in endemic fluorosis areas of southern Rajasthan,[87] suggested that a high intake of F provoked nephrolithiasis in tribal populations. The data suggested that F *in vivo* may behave as a mild promoter of urinary stone formation by leading to the excretion of insoluble calcium fluoride, increasing oxalate excretion, and mildly increasing the oxidative burden. Nevertheless, more such studies are needed to allow unanimous acceptance of the role of F in kidney stone formation, especially in Rajasthan where the drinking water contains a high amount of F.

FACTORS AFFECTING THE SEVERITY OF F TOXICITY

The variations in the prevalence and severity of fluorosis in humans and animals living in either the same or different geographical locations and having similar concentrations of F in their drinking water can be attributed to a number of factors such as the duration of F exposure, the concentration of F, the frequency of the F intake, age, sex, other chemical constituents in the drinking water, habits, nutrition, other food constituents, and environmental factors, as well as individual variations in susceptibility, biological response, tolerance, and genetics.[88-94] The prevalence and severity of osteo-dental fluorosis has been studied in Rajasthan with respect to F concentration, age, sex, other chemical constituents in the drinking water, nutritional status, food constituents, and these findings may be helpful in the mitigation and prevention of endemic hydrofluorosis.[88-94]

BIOMARKERS FOR CHRONIC F POISONING

A biomarker or biological marker generally refers to a measurable characteristic which may be used as an indicator of some biological state or condition. Biomarkers for chronic fluorotoxicosis should have a greater susceptibility to F exposure and thus give an early sign of F intoxication. Recently, a study was conducted in 2,747 mature and 887 immature domestic animals belonging to different animal species reared in endemic high drinking water F areas of Rajasthan.[95] The animals included bovines, equines, flocks, and camelids. Among the animals, the immature ones were found more susceptible to F toxicity. The presence of dental fluorosis in bovine calves was found to be an ideal biomarker as it appeared early when F poisoning was present.[95]

In both humans and animals, the F content in biological samples (e.g., milk, urine, blood serum, nails, teeth, etc.) is a better biomarker for F intoxication than the presence of morbidity and mortality.[96] At present, the level of F in the urine gives the best indication of the presence of chronic F poisoning.[96]

MITIGATION AND THE PREVENTION OF HYDROFLUOROSIS

Endemic hydrofluorosis can be prevented or minimized by using alternative water sources or low F drinking water, which may be produced by the defluoridation of high F drinking water, by improving the nutritional status of the population at risk, and by generating public awareness.

Defluoridation of water can be done by adopting appropriate defluoridation techniques at both the domestic and the community levels. Although several defluoridation techniques are available, one of them, the Nalgonda defluoridation technique is simple, effective, and low-cost.[97] In many endemic high drinking water F states, including Rajasthan, this technique has been adopted at the domestic and community level for defluoridation of high F potable groundwater. Although this technique is affordable and gives good results, its success rate at the community level is still very poor. In many places it has failed totally at the community level due to lack of public participation, a lack of responsibility for its supervision, and a lack of proper monitoring and maintenance. In Rajasthan, the hand-pumps and bore-wells in several villages are also attached to a defluoridation unit which is under the supervision of the Public Health and Engineering Department of the Rajasthan

Government. However, this initiative has not been particularly successful. Instead of these efforts involving using and maintaining a defluoridation unit, the harvesting and conserving of rainwater is a better method for obtaining low F water (<1 ppm) on a regular basis. Another option is to supply treated surface water to villages.

CONCLUSIONS

The drinking groundwater sources in rural areas of all 33 districts of Rajasthan are contaminated with F and most of them have F beyond the Bureau of Indian Standards requirement (acceptable limit) of 1.0 ppm or the permissible limit in the absence of an alternate source of 1.5 ppm.[5,6] The ingestion of such water for a long-time is not safe for health and causes hydrofluorosis in both humans and animals. Therefore, the provision of a regular supply of low F water (<1 ppm) is highly needed in all the villages of Rajasthan with endemic high-F drinking water. More epidemiological studies on hydrofluorosis in humans and animals in each district of Rajasthan are needed for the proper assessment of the extent of this health problem in Rajasthan.

It is well established that excessive F consumption leads to accelerated haematological degeneration leading to red cell death and anaemia.[98] In the endemic high drinking water F Rajasthan state, lethal red cell genetic disorders such as sickle cell anaemia (Hb-SS), α- and β-thalaseamia and glucose-6-phosphate dehydrogenase (G-6-PD) enzyme deficiency are also endemic and many people, belonging to different ethnic groups, suffer from these genetic diseases.[99-108] Therefore, more studies are required to reveal the effect of F in subjects with genetic red cell disorders.

The information in the present review on the distribution of high-F drinking water at the district level in Rajasthan and on the prevalence of dental and skeletal fluorosis in relation to the drinking water F concentrations in different endemic high-F districts of Rajasthan may be useful in helping frame health policy at the state level for the mitigation of hydrofluorosis.

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REFERENCES

1 Susheela AK. A treatise on fluorosis. 1st ed. Delhi: Fluorosis Research and Rural Development Foundation; 2001. p. 19.
2 Susheela AK. A treatise on fluorosis. Revised 2nd ed. Delhi: Fluorosis Research and Rural Development Foundation; 2003. p. 15.
3 Susheela AK. A treatise on fluorosis. revised 3rd ed. Delhi: Fluorosis Research and Rural Development Foundation; 2007. pp. 15-6.
4 Susheela AK, Desai VK, Gnanasundaram N, Nagesh KS, Dutta S. In: Susheela AK, editor. Prevention and control of fluorosis in India. New Delhi: Rajiv Ghandi National Drinking Water Mission, Ministry of Rural Development; 1993. p. 25.
5 Bureau of Indian Standards. Indian standard drinking water—specification. 1st revision, incorporating amendment no 1. New Delhi: Bureau of Indian Standards; 2003. p. 2.

6 Bureau of Indian Standards. Indian standard drinking water—specification. 2nd revision. New Delhi: Bureau of Indian Standards; 2012. p. 2.
7 WHO. Fluoride in drinking-water: background document for development of WHO Guidelines for drinking-water quality. WHO/SDE/WSH/03.04.96, English only. Geneva: WHO; 2004. Available from: 1.http://www.who.int/water_sanitation_health/dwq/chemicals/fluoride.pdf
8 WHO. Guidelines for drinking-water quality. 3rd ed incorporating the first and second addenda. Vol 1. Recommendations. Geneva: Word Health Organization; 2008. p. 376.
9 Adler P, Armstrong WD, Bell ME, Bhussry BR, Büttner W, Cremer H-D, et al. Fluorides and human health. World Health Organization Monograph Series No. 59. Geneva: World Health Organization; 1970.
10 Swarup D, Dwivedi SK. Environmental pollution and effect of lead and fluoride on animal health. New Delhi: Indian Council of Agricultural Research; 2002. pp. 68-106.
11 Choubisa SL, Choubisa D. Status of industrial fluoride pollution and its diverse adverse health effects in man and domestic animals in India. Environ Sci Pollut Res 2016;23(8):7244-54.
12 Choubisa SL. Industrial fluorosis in domestic goats *(Capra hircus*), Rajasthan, India. Fluoride 2015;48(2):105-12.
13 Choubisa SL, Choubisa D. Neighbourhood fluorosis in people residing in the vicinity of superphosphate fertilizer plants near Udaipur city of Rajasthan (India). Environ Monit Assess 2015;187(8):497. DOI: 10.1007/s10661-015-4723-z.
14 Choubisa SL. Guinea worm (*Dracunculus medinensis*) in Rajasthan, India: a case report. J Parasit Dis 2002;26(2):105-6.
15 Choubisa SL. Verma R, Choubisa L. Dracunculiasis in tribal region of Rajasthan (India): a case report. J Parasit Dis 2010;34(2):94-6.
16. Choubisa SL. Fluoride distribution in drinking groundwater in Rajasthan, India. Curr Sci 2018;114(9):1851-7.
17 WHO. Trace elements in human nutrition: a report of a WHO expert committee. World Health Organization Technical Report Series No. 532. Geneva: World Health Organization; 1973. pp. 6 and 60.
18 Wheeler SM, Fell LR, Fluoride in cattle nutrition. Nut Abs Rw B 1983;53:741-63.
19 WHO. Trace elements in human nutrition and health. Geneva: World Health Organization; 1996. p.192.
20 Scientific Committee on Health and Environmental Risks (SCHER). Opinion of critical review of any new evidence on the hazard profile, health effects, and human exposure to fluoride and the fluoridating agents of drinking water. Brussels, Belgium: Directorate General for Health and Consumers, European Commission; 2011 May 16. pp. 2-4.
21 Indian Council of Medical Research. Manual of standards of quality for drinking water supplies. Special report series No. 44, New Delhi: Indian Council of Medical Research; 1974.
22 Shortt HE, McRobbert GR, Barnard TW, Mannadi Nayer AS. Endemic fluorosis in the Madras Presidency. Indian J Med Res 1937-38;25:553-8.
23 Shortt HE, Pandit CG, Raghavachari TNS. Endemic fluorosis in Nellore district of south India. Indian Med Gez 1937;72:396-8.
24 Kalsiwal RM, Soloman SK. Fluorosis in a case report. J Assoc Physic India 1959;7:56-9.
25 Choubisa SL, Pandya H, Choubisa DK, Sharma OP, Bhatt SK, Khan IA. Osteo-dental fluorosis in bovines of tribal region in Dungarpur (Rajasthan). J Environ Biol 1996;17(2):85-92.
26 Mathure GM, Tamboli B, Mathure RH, Ray AK, Mathure GC, Goyal OP. Preliminary epidemiological investigation of fluorosis in Surajpura and Pratappura villages in Sarwa tehsil, Ajmer district (Rajasthan). Indian J Prevent Soc Med 1976;7:90-4.
27 Mathure AP, Tamboli BL, Mathure GM, Goyal OP, Lalla SK. Prevalence of fluorosis in Surajpura and Pratappura villages, district Ajmer (Rajasthan). Indian J Med Res 1980; 71:57-67.
28 Gupta RS, Meena VR, Pasha ST, Grover SS, Sharma M. Fluorosis in rural areas of Alwar district. J Commun Dis 2003;35(1):48.
29 Choubisa SL, Fluoride distribution and fluorosis in some villages of Banswara district of Rajasthan. Indian J Environ Health 1997;39(4):281-8.
30 Choubisa SL. Some observations on endemic fluorosis in domestic animals of southern Rajasthan (India). Vet Res Commun 1999;23(7):457-65.
31 Choubisa SL. Endemic fluorosis in southern Rajasthan (India). Fluoride 2001;34(1):61-70.

32 Dubey HV, Ingle NA, Gupta R, Charania Z, Ingle E, Sharma I. Prevalence of dental fluorosis among 12-15 years school children of Bharatpur city: a cross-sectional study. J Indian Assoc Pub Health Dent 2015;13(4):405-9.
33 Hussain J, Hussain I, Sharma KC. Fluoride and health hazards: community perception in a fluorotic area of central Rajasthan (India). Environ Monit Assess 2010;162:1-14.
34 Choubisa SL, Modasiya V, Bahura CK, Sheikh Z. Toxicity of fluoride in cattle of the Indian Thar Desert, Rajasthan, India. Fluoride 2012;45(4):371-6.
35 Modasiya V, Bohra DL, Daiya GS, Bahura CK. Observation of fluorosis in domestic animals of the Indian Thar Desert, Rajasthan, India. Int J Adv Res 2014;2(4):1137-43.
36 Modasiya V, Bohra DL, Bahura C K. Implication of fluoride toxicity on human health in a village of Indian Thar Desert, Rajasthan, India. Asian J Biochemic Pharmac Res 2014; 4(4): 52-9.
37 Choubisa SL, Sompura K, Bhatt SK, Choubisa DK, Pandya H, Joshi SC.et al., Prevalence of fluorosis in some villages of Dungarpur district of Rajasthan. Indian J Environ Health 1996;38(2):119-26.
38 Choubisa SL, Sompura K. Dental fluorosis in tribal villages of Dungarpur district (Rajasthan). Poll Res 1996;15(1):45-7.
39 Choubisa SL, Choubisa DK, Joshi SC, Choubisa L. Fluorosis in some tribal villages of Dungarpur district of Rajasthan, India. Fluoride 1997;30(4):223-8.
40 Choubisa SL. Chronic fluoride intoxication (fluorosis) in tribes and their domestic animals. Int J Environ Stud 1999;56(5):703-16.
41 Choubisa SL, Choubisa L, Choubisa DK. Endemic fluorosis in Rajasthan. Indian J Environ Hlth 2001;43(4):177-89.
42 Choubisa SL. Fluoridated ground water and its toxic effects on domesticated animals residing in rural tribal areas of Rajasthan (India). Int J Environ Stud 2007;64(2):151-9.
43 Choubisa SL. Osteo-dental fluorosis in domestic horses and donkeys in Rajasthan, India. Fluoride 2010;43(1):5-12.
44 Choubisa S L. Fluorosis in dromedary camels in Rajasthan, India. Fluoride 2010;43(3):194-9.
45 Choubisa SL, Mishra GV, Sheikh Z, Bhardwaj B, Mali P, Jaroli VJ. Toxic effects of fluoride in domestic animals. Adv Pharmacol Toxicol 2011;12(2):29-37.
46 Choubisa SL. Fluoride in drinking water and its toxicosis in tribals, Rajasthan, India. Proc Natl Acad Sci India Sect B Biol Sci 2012;82(2):325-30.
47 Kathuria AK, Jain AK, Thergaonkar VP, Bhargava VN. Fluorosis survey and preliminary report on urine analysis of fluorotic patients. Indian J Environ Health 1974;16:222-32.
48 Gupta R, Sharma A, Gaur K, Zafer A, Manohar RK. Dental fluorosis status in school children of Jaipur (Raj), India. IOSR J Dent Med Sci 2013;8(4):51-4.
49 Agrawal M, Agrawal S, Adyanthaya BR, Gupta HL, Bhargava N, Rastogi R. Prevalence and severity of dental fluorosis among patients visiting a dental college in Jaipur, Rajasthan. Indian J Res Pharm Biotech 2014;2(4):1339-44.
50 Dagli Rushabh J, Kumar TS, Mathur A, Jain M, Dhanni C, Balasubhramanya G, et al. Can improve nutrition reduce the risk of dental fluorosis? An epidemiological survey in Jalore, Rajasthan, India [abstract]. Fluoride 2008;41(3):235.
51 Thergaonkar VP, Bhargava RK. Water quality and incidence of fluorosis in Jhunjhunu of Rajasthan: preliminary observations. Indian J Environ Health 1974;16(2):168-80.
52 Solanki J, Dundappa J, Babu NK. Prevalence of dental fluorosis in school children of Jodhpur city. Indian J Dent Adv 2011;3(3):1-4.
53 Mogra R, Sharma S. Prevalence of fluorosis among the families of village Degana (distt. Nagaur), Rajasthan (India). J Environ Sci Eng 2009;51(4):273-6.
54 Gautam R, Bharadwaj N. Dental fluorosis – a case study from Newa tehsil in Nagaur district, Rajasthan (India). Environmentalist 2011;31:401-6.
55 Arif M, Husain I, Hussain J, Kumar S. Assessment of fluoride level in groundwater and prevalence of dental fluorosis in Didwana block of Nagaur district, central Rajasthan, India. Int J Occup Environ Med 2013;4:178-84.
56 Choubisa SL, Mishra GV. Fluoride toxicosis in bovines and flocks of desert environment. Int Pharmacol Biol Sci 2013;7(3):35-40.
57 Choubisa SL. Fluorosis in some tribal villages of Udaipur district (Rajasthan). J Environ Biol 1998;19(4):341-52.

58 Dhar V, Jain A, Van DTK, Kohli A. Prevalence of gingival diseases, malocclusion and fluorosis in school-going children of rural areas in Udaipur district. J Indian Soc Pedodont Prevent Dent 2007;25(2):103-5.
59 Choubisa SL. Dental fluorosis in domestic animals. Curr Sci 2008;95(12):1674-5.
60 Sarvaiya BU, Bhayya D, Arora R, Mehta DN. Prevalence of dental fluorosis in relation with different fluoride levels in drinking water among school going children in Sarada tehsil of Udaipur district, Rajasthan. J Indian Soci Pedodont Prevent Dent 2012;30(4):317-22.
61 Choubisa SL, Sompura K, Choubisa DK, Pandya H, Bhatt SK, Sharma OP, et al. Fluoride content in domestic water sources of Dungarpur district of Rajasthan. Indian J Environ Health 1995;37(3):154-60.
62 Choubisa SL, Sompura K, Bhatt SK, Choubisa DK, Pandya H, Sharma OP. Fluoride in drinking water sources of Udaipur district of Rajasthan. Indian J Environ Health 1996; 38(4):286-91.
63 Choubisa SL. An epidemiological study on endemic fluorosis in tribal areas of southern Rajasthan [a technical report]. New Delhi, India: The Ministry of Environment and Forests; 1996. pp. 1-56.
64 Choubisa SL, Choubisa L, Sompura K, Choubisa D. Fluorosis in subjects belonging to different ethnic groups of Rajasthan. J Commun Dis 2007;39(3):171-7.
65 Choubisa SL. Fluorotoxicosis in diverse species of domestic animals inhabiting areas with high fluoride in drinking waters of Rajasthan, India. Proc Natl Acad Sci India Sect B Biol Sci. 2013;83(3):317-21.
66 Choubisa SL. Fluoride toxicosis in immature herbivorous domestic animals living in low fluoride water endemic areas of Rajasthan, India: an observational survey. Fluoride 2013;46(1):19-24.
67 Choubisa SL. Status of fluorosis in animals. Proc Natl Acad Sci India Sect B Biol Sci 2012;82(3):331-9.
68 Choubisa SL, Verma R. Skeletal fluorosis in bone injury case. J Environ Biol 1996;17(1):17-20.
69 Choubisa SL. Radiological skeletal changes due to chronic fluoride intoxication in Udaipur district (Rajasthan). Poll Res 1996;15(3):227-9.
70 Choubisa SL. Toxic effects of fluoride on human bones. Adv Pharmacol Toxicol 2012;13(1):9-13.
71 Krishnamachari KAVR, Krishnaswamy K. An epidemiological study of the syndrome of genu-valgum among residents of endemic areas for fluorosis in Andhra Pradesh. Indian J Med Res 1974;62(2):1415-23.
72 Chakama T, Singh SB, Godbole S, Tiwari RS. Endemic fluorosis with genu-valgum syndrome in a village of district Mandla, Madhya Pradesh. Indian Pediat 1997;34:32-6.
73 Wilson DC. Fluorine in etiology of endemic goitre. Lancet 1941;1:211-7.
74 Teotia SPS, Teotia M. Endemic fluorosis in India: a challenging national health. J Assoc Physic India 1984;32(4):347-52.
75 Jolly SS, Singh VP, Sharma R, Ralhan SM, Sandhu SS. Endocrine aspect of endemic fluorosis. Fluoride 1974;7(4):208-19.
76 Desai VK, Saxena DK, Bhavsar BS, Kantharia SL. Epidemiological study of dental fluorosis in tribals residing near fluorspar mines. Fluoride 1988;21:137-41.
77 Sharma JD, Jain P, Sohu D. Gastric discomforts from fluoride in drinking water in Sanganer tehsil, Rajasthan, India. Fluoride 2009;42(4):286-91.
78 Susheela AK, Mondal NK, Singh A. Exposure to fluoride in smelter workers in a primary aluminum industry in India. Int J Occup Environ Med 2013;4:61-72.
79 Sharma JD, Sohu D, Jain P. Prevalence of neurological manifestations in a human population exposed to fluoride in drinking water. Fluoride 2009;42(2):127-32.
80 Singh VP, Chauhan DS, Tripathi S, Kumar S, Gaur V, Tiwari M, et al. A correlation between serum vitamin, acetylcholinesterase activity and IQ in children with excessive endemic fluoride exposure in Rajasthan, India. Int Res J Medical Sci 2013;1(3):12-6.
81 Singh VP, Chauhan DS, Tripathi S. Acetylcholinesterase activity in fluorosis adversely affects mental well-being: an experimental study in rural Rajasthan. European Acad Res 2014;2(4);5857-69.
82 Guo Z, HeY, Zhua Q. Research on the neurobehavioral function of workers occupationally exposed to fluoride. Industrial Health and Occupational Diseases 2001;27(6):346-8. [in Chinese]. Translated by Julian Brooke and published with the concurrence of Industrial Health and Occupational Diseases in Fluoride 2008;41(2):152-5.
83 Sudhir KM, Chandu GN, Prashant GM, Subba Reddy VV. Effect of fluoride exposure on Intelligence Quotient (IQ) among 13-15 year old school children of known endemic area of fluorosis, Nalgonda District, Andhra Pradesh. J Indian Assoc Public Health Dent 2009;13:88-94.

84 Shivaprakash PK, Ohri K, Noorani H. Relation between dental fluorosis and intelligence quotient in school children of Bagalkot district. J Indian Soc Pedod Prev Dent 2011;29:117-20.
85 Khan SA, Singh RK, Navit S, Chadha D, Johri N, Navit P, et al. Relationship between dental fluorosis and intelligence quotient of school going children in and around Lucknow district: a cross-sectional study. J Clin Diagn Res 2015;9(11):10-15.
86 Spittle B. Dental fluorosis as a marker for fluoride-induced cognitive impairment [editorial]. Fluoride 2016;49(1):3-4.
87 Singh PP, Barjatiya MK, Dhing S, Bhatnagar R, Kothari S, Dhar V. Evidence suggesting that high intake of fluoride provokes nephrolithiasis in tribal populations. Urol Res 2001;29(4):238-44.
88 Choubisa SL, Choubisa L, Choubisa D. Osteo-dental fluorosis in relation to nutritional status, living habits and occupation in rural tribal areas of Rajasthan, India. Fluoride 2009;42(3):210-5.
89 Choubisa SL, Choubisa L, Choubisa D. Osteo-dental fluorosis in relation to age and sex in tribal districts of Rajasthan, India. J Environ Sci Engg 2010;52(3):199-204.
90 Choubisa SL, Mishra GV, Sheikh Z, Bhardwaj B, Mali P, Jaroli VJ. Food, fluoride, and fluorosis in domestic ruminants in the Dungarpur district of Rajasthan, India. Fluoride 2011;44(2):70-6.
91 Choubisa SL. Osteo-dental fluorosis in relation to chemical constituents of drinking waters. J Environ Sci Engg 2012;54(1):153-8.
92 Choubisa SL, Choubisa L, Choubisa D. Reversibility of natural dental fluorosis. Int J Pharmacol Biol Sci 2011;5(2):89-93.
93 Choubisa SL. Natural amelioration of fluoride toxicity (Fluorosis) in goats and sheep. Curr Sci 2010;99(10):1331-2.
94 Choubisa SL. Why desert camels are least afflicted with osteo-dental fluorosis? Curr Sci 2013;105(12):1671-2.
95 Choubisa SL. Bovine calves as ideal bio-indicators for fluoridated drinking water and endemic osteo-dental fluorosis. Environ Monit Assess 2014;186(7):4493-8.
96 Swarup D, Dey S, Patra RC, Dwivedi SK, Lali S. Clinico- epidemiological observations of industrial bovine fluorosis in India. Indian J Anim Sci 2001;1(12):1111-5.
97 Bulusu KR, Nawlakhe WG, Patil AR, Karthikeyan G. In: Bulusu KR, Biswas SK, editors. Prevention and control of fluorosis: water quality and defluoridation techniques. Volume II. New Delhi, India: Rajiv Gandhi National Drinking Water Mission, Ministry of Rural Development; 1993. pp.31-58.
98 Pornprasert S, Wanachantararak P, Kantawong F, Chamnanprai CK, Pienthai N, Yanola J, et al. Excessive fluoride consumption increases haematological alteration in subjects with iron deficiency, thalassaemia, and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. Environ Geochem Health 2016, doi: 10, 1007/s10653-016-9845-x.
99 Choubisa SL, Choubisa L, Pande S, Bhatt DK. Abnormal haemoglobins, thalassaemia and G-6-PD deficiency in Suthar community of southern Rajasthan. Indian J Anthrop Hum Genet 1987;14(3):9-16.
100 Choubisa SL. Abnormal haemoglobins, thalassaemia and G-6-PD deficiency in school children belonging to scheduled castes and tribes of Rajasthan, India. Indian J Anthrop Hum Genet 1988;14(3):31-40.
101 Choubisa SL. Distribution of Hb-Bart's (α-thalassaemia) in various population of Dungarpur district of Rajasthan (India). Indian J Anthrop Hum Gent 1990;6(1&2):43-8.
102 Choubisa SL. Abnormal haemoglobins, thalassaemia and G-6-PD enzyme deficiency in Rajasthan (Western-India). Haematologia 1991;24(3):153-65.
103 Choubisa SL, Choubisa DK, Choubisa L. Erythrocyte genetic disorders in inhabitants of Aravali hilly environment. Indian J Anthrop Hum Genet 2004;23(2):145-59.
104 Choubisa SL. Haemoglobin-C gene in India? Curr Sci 2010;99(7):860.
105 Choubisa SL. Sickle cell haemoglobin, thalassaemia and G-6-PD enzyme deficiency genes in Garasiya tribe inhabited malaria endemic areas of Sirohi district, Rajasthan (India). J Commun Dis 2009;41(1):13-8.
106 Choubisa SL, Choubisa DK. Erythrocyte glucose-6-phosphate dehydrogenase deficiency in senile cataracts. Man in India 1994;74(3):267-70.
107 Choubisa SL. Erythrocyte genetic disorders in subject belonging to general castes and other backward castes of Aravali hilly region of Rajasthan (India). Indian J Anthrop Hum Genet 2003;22(1):33-43.
108 Choubisa SL, Choubisa L. Erythrocyte mutant genes in inhabitants of arid environment of Western-Rajasthan (India). Indian J Anthrop Hum Genet 2006;25(1): 45-50.