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## EPR Dose Reconstruction of Two Kazakh Villages Near the Semipalatinsk Nuclear Test Site

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**Abstract.** Electron paramagnetic resonance (EPR) dose reconstruction has been performed on archived tooth samples from residents of two villages near the Semipalatinsk nuclear test site in Kazakhstan. The context of this work is a large multidisciplinary study of thyroid disease prevalence and radiation dose among long-term residents of villages near that nuclear test site, in which EPR is used for biosimetric validation of the gamma-ray component of dose reconstruction algorithms applied to the data for various villages whose residents were exposed to radioactive fallout during 1949–1962, the period of above-ground atomic bomb testing. The tooth samples, nine from the village of Kainar and 23 from the village of Znamenka, were extracted in 1964 and 1967, respectively, and stored indoors in closed boxes in Semipalatinsk. According to provided information, some time in the past, the teeth from Kainar were heated to 80°C for one day. Experiments carried out on 12 teeth from US sources to determine the effects of long-term storage and heat treatment found that EPR assay findings were not compromised for storage times less than 35 years and annealing at temperatures below 200°C. For tooth enamel samples prepared from molars and premolars the average reconstructed gamma dose was  $390 \pm 70$  mGy for Kainar residents and  $95 \pm 40$  mGy for Znamenka residents.

### 1 Introduction

The Semipalatinsk nuclear test site (SNTS), an area of 19000 km<sup>2</sup> in northeastern Kazakhstan, was the location for over 450 nuclear test explosions during 1949–1989 with a total explosive energy of 17.4 Mt TNT equivalent. The majority of tests conducted before 1963 were on the surface or in the atmosphere, as opposed to the mostly underground tests conducted after that date. Surface tests,

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in which the fireball interacted with the ground, were the main sources of radioactive fallout affecting areas downwind of the SNTS and were mainly to the east. It is estimated that 30000 to 40000 residents of nearby areas may be at risk of health effects related to radiation exposure from the tests. It is also estimated that the bulk of the radiation exposure to the population resulted from three tests, conducted in 1949, 1951, and 1953.

Determination of the radiation doses to residents of various areas downwind of the SNTS is important for estimating the likely health risks associated with exposure and for epidemiological analyses of radiation-related risks. Estimates of fallout deposition have been calculated from theoretical models on the basis of bomb characteristics (explosive power, location and altitude of detonation), the speed and trajectories of individual fallout plumes at different altitudes, wind and precipitation patterns, and measurements of radionuclides remaining in the soil at different times after detonation. Such models have been widely used to reconstruct fallout exposures from tests carried out by the United States, the former Soviet Union, and other countries. Considerable attention has been devoted to understanding and reconciling the different approaches used by Russian and American scientists, in particular, comparing methods used for dose reconstruction for areas downwind of the SNTS and the Nevada test site in the United States.

It is important that dose reconstruction models be validated by independent measurements. Biodosimetric methods on the basis of the measurements with tissues from exposed persons, such as electron paramagnetic resonance (EPR) assays of tooth enamel and fluorescent *in situ* hybridization (FISH) assays of chromosome aberrations in cultured lymphocytes, are the main methods in use today.

EPR biodosimetry is based on measurements of free radicals induced by ionizing radiation exposure in tooth enamel [6]. It has been successfully applied to dose reconstruction for the survivors of the atomic bombings of Hiroshima and Nagasaki [6, 7], victims of the Chernobyl reactor accident [6], Russian nuclear workers [8], residents exposed to radioactive discharges along the Techa River in the South Urals region of Russia [9] and, most recently, persons exposed to radiation from nuclear bomb tests at the Totskoye test site in Russia [10].

The EPR assessment of archival teeth collected from residents of two villages near the SNTS, Kainar and Znamenka, are reported. Tooth samples from other villages are presently being assayed, and it is intended that the results will be employed in a comprehensive validation of dose reconstruction models applied to several different villages in the vicinity of the SNTS.

An interesting aspect of the present analysis is that the Kainar teeth were subjected to heat treatment, whereas those from Znamenka were not, and additional experiments were required to evaluate the effects of heat treatment on EPR findings.

## 2 Materials and Methods

The teeth used in the present study were kindly provided by Dr. Boris I. Gusev from the Kazakh Scientific Research Institute of Radiation Medicine and Ecol-

ogy (IRME), Semipalatinsk. They were extracted for medical reasons from residents of two villages near the SNTS, Kainar and Znamenka. All teeth from Znamenka were extracted in 1967, whereas the teeth from Kainar were extracted in 1964. Between the date of extraction and April of 1999, the teeth were stored in a metal box at the IRME. The donors' ages ranged from 51 to 63 at the time of tooth extraction. According to Gusev, at some time during the storage period the teeth from Kainar were heated to about 80°C for about one day. The objective of this procedure was to reduce water content in the teeth for more accurate radiochemical determination of  $^{90}\text{Sr}$  content. The latter analysis was planned but not carried out (B. I. Gusev, pers. commun.). Unlike the teeth from Kainar, the teeth from Znamenka were never intentionally heated during their storage. According to information from Dr. Gusev, there were no medical X-ray examinations before extraction of the collected teeth from Kainar and Znamenka.

In order to examine any long-term storage and heating effects on EPR dosimetry for Kazakh teeth, two experiments were designed. The first experiment involved the *in vitro* heating of a tooth enamel sample prepared from a US tooth preirradiated to 14 Gy from a  $^{60}\text{Co}$  source. The sample was heated at 210°C for 31.5 h, while EPR spectra were recorded every 30 min. For the second experiment, a dose of 1 Gy was delivered to 12 US teeth simultaneously. Then, three groups (of three teeth each) were selected for annealing at 100, 200 and 380°C, respectively. The annealing time was 20 h each. The fourth group of three teeth was saved as a control and was not annealed. The teeth collected from the US population (extracted for medical reasons and collected under conditions of anonymity) were provided by the American Dental Association Health Foundation.

The EPR sample preparation procedure was similar to that used in ref. 12 and included the following steps.

1. Separation of the tooth roots from the crowns with a low-speed diamond saw.
2. 20 h ultrasonic treatment (Misonix, Inc.) of the tooth crowns placed into glass test tubes with 10 and 30% KOH aqueous solution for Semipalatinsk teeth and modern US teeth, respectively, at 70°C in glass test tube (the KOH solution was changed after 5 and 15 h of ultrasonic treatment).
3. Washing in an ultrasonic bath with distilled water.
4. Drying at 70°C for 10 h.
5. Crushing with pestle and mortar.
6. Sieving to a grain size of 0.1 to 0.5 mm.

This procedure effectively isolates the tooth enamel from dentin without the use of a dental drill and also significantly reduces the organic component of enamel. Minimizing the organic component is important because the endogenous EPR signal, attributed mainly to the organic component of enamel, is spectrally adjacent to the radiation-induced signal and obscures the measurement of the radiation response at doses below 400 mGy [13].

EPR measurements (ESP300E, Bruker) were made in the X band with a rectangular microwave cavity 4108TMH. The following experimental parameters were

used for spectral recording: magnetic field sweep, 5.0 mT; number of accumulated spectra, 64; microwave power, 25 mW; modulation frequency, 100 kHz; and modulation amplitude, 0.25 mT. Five EPR spectra were taken at each dose interval for each sample. The sample was shaken between each spectrum recording to randomize the enamel grains. In order to assess the dose, the peak-to-peak amplitude of the radiation-induced EPR signal was measured.

The sample mass for EPR measurements was typically about 100 mg. Some of the Kazakh teeth were severely damaged by caries, to the extent that there was not enough material for high-quality EPR measurements. The smallest sample mass used in this study was 30 mg. All teeth from Kainar had a dark brown color most probably caused by heating.

For four teeth from Znamenka and two from Kainar, a separate dose reconstruction was performed with enamel from the buccal and lingual surfaces of each tooth.

Calibration of the EPR radiation response was done by the additive dose method with a  $^{60}\text{Co}$  source. After irradiation the samples were annealed at 70°C for 12 h.

### 3 Results

Figure 1 shows the EPR spectrum evolution of a US tooth enamel sample irradiated to 14 Gy over a 31.5 h period of *in vitro* annealing at 210°C. Visible changes in the spectrum began to appear after 2 h of annealing. The final spectrum contained five heat-induced signals and exhibited a substantial decrease in the radiation-induced signal. The central heat-induced signal is superimposed on the native component of the EPR tooth enamel spectrum (Fig. 1).

The results of the second annealing experiment showed that annealing at temperatures below 200°C does not affect the reconstructed dose. Dose recon-

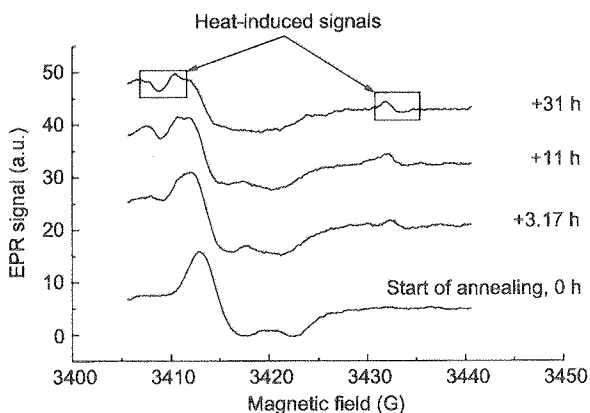


Fig. 1. Time evolution of the EPR spectrum for tooth enamel irradiated to 14 Gy and annealed at 210°C.

struction for tooth enamel samples prepared from the irradiated (1 Gy) teeth annealed at 100 and 200°C for 20 h gave practically the same absorbed dose (within 10%) as the control teeth irradiated to the same dose. The sample annealed at 380°C showed an intense signal that completely obscured the radiation-induced signal. The latter heat-induced signal has been observed previously by others [14–17], and the signal renders tooth enamel annealed at temperatures higher than 300°C unsuitable for dose reconstruction.

The results of the EPR dose reconstruction for Znamenka and Kainar residents are given in Tables 1 and 2, respectively. No significant difference in the dose reconstruction results was found for buccal and lingual sections evaluated separately (Table 1, numbers 16, 17, 18 and 20 and Table 2, numbers 7 and 8). Enamel samples prepared from the teeth of the Znamenka residents extracted in 1967 had a spectral shape similar to samples prepared from US teeth (Figs. 2 and 3). The radiation sensitivity (as indicated by the slope of the dose reconstruction line) of

**Table 1.** Tooth characteristics and reconstructed doses for Znamenka residents. In contrast to Kainar teeth, for Znamenka ones there was no heating procedure in prehistory.

Sample	Donor	Date of birth	Type of tooth	Date of tooth extraction	Reconstructed dose <sup>a</sup> (mGy)
1	1	04/25/1913	Molar	06/16/1967	190±75 <sup>b</sup>
2	2	09/14/1913	Molar	06/16/1967	165±25
3	3	03/13/1914	Molar	06/17/1967	155±25
4	4	08/07/1909	Canine	06/17/1967	150±25
5	5	10/01/1909	Canine	06/17/1967	175±40
6	6	06/07/1908	Molar	06/17/1967	65±40
7	7	06/20/1908	Premolar	06/17/1967	— <sup>c</sup>
8	8	08/23/1908	Molar	06/17/1967	70±40
9	9	10/12/1908	Molar	06/18/1967	75±30
10	10	08/26/1909	Molar	06/18/1967	75±30
11	11	09/25/1907	Canine	06/18/1967	120±45
12	12	10/14/1907	Canine	06/18/1967	120±45
13	13	09/23/1906	Molar	06/18/1967	60±25
14	14	03/17/1905	Premolar	06/19/1967	60±45
15	15	12/09/1905	Molar	06/19/1967	70±50
16	16	10/14/1906	Molar	06/20/1967	90±35 <sup>d</sup> , 90±40 <sup>e</sup>
17	17	01/14/1916	Molar	06/20/1967	50±25 <sup>d</sup> , 70±35 <sup>e</sup>
18	18	04/24/1916	Molar	06/20/1967	90±35 <sup>d</sup> , 105±25 <sup>e</sup>
19	19	08/12/1915	Premolar	06/20/1967	— <sup>f</sup>
20	20	02/14/1915	Molar	06/20/1967	80±45 <sup>d</sup> , 115±35 <sup>e</sup>
21	21	10/12/1915	Molar	06/20/1967	90±45

<sup>a</sup> Absorbed dose in tooth enamel.

<sup>b</sup> Small mass (30 mg).

<sup>c</sup> An unusually intensive nonradiogenic EPR signal that prevented dose reconstruction.

<sup>d</sup> Sample prepared from the lingual side of the tooth.

<sup>e</sup> Sample prepared from the buccal side of the tooth.

<sup>f</sup> Severe damage of the tooth by caries prevented the preparation of an enamel sample from it.

**Table 2.** Tooth characteristics and results of EPR doses reconstruction for Kainar residents. Year of all teeth extraction is 1964. In the sixties all teeth were heated to 70–80°C for about 1 day.

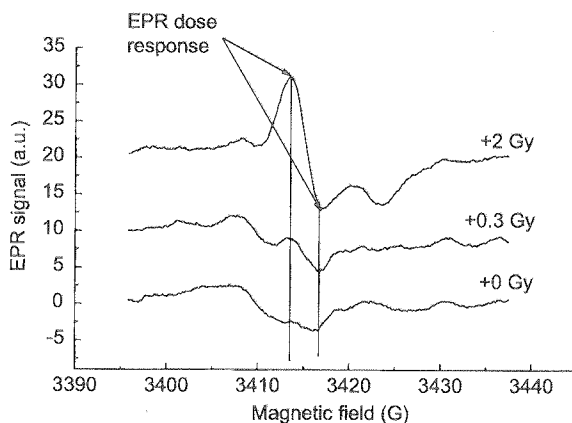
Sample	Donor	Type of tooth	Date of birth	Reconstructed dose <sup>a</sup> (mGy)
1	1	Molar	05/18/1901	420±95
2	2	Molar	05/19/1906	480±90
3	3	Canine	04/08/1905	480±90
4	4	Molar	01/06/1905	280±45
5	5	Premolar	03/27/1904	375±45
6	6	Premolar	12/09/1902	460±907
7	7	Premolar	01/15/1902	400±70 <sup>b</sup> , 400±35 <sup>c</sup>
8	8	Premolar	11/18/1904	290±70 <sup>b</sup> , 320±70 <sup>c</sup>
9	8	Canine	11/18/1904	520±70

<sup>a</sup> Absorbed dose in tooth enamel.

<sup>b</sup> Sample prepared from lingual side of the tooth.

<sup>c</sup> Sample prepared from buccal side of the tooth.

the Znamenka samples was similar to that obtained for the US samples. The EPR spectra of the samples prepared from Kainar residents' teeth (Fig. 4) appeared quite different from the spectra of unheated US and Znamenka samples. All Kainar samples show the presence of heat-induced signals in their spectra that are similar to a signal that appeared in the spectra of US tooth enamel during *in vitro* heating (Fig. 1). This heat-induced signal partly overlaps a distinguishable radiation-induced component in all Kainar sample spectra (Fig. 4). This circumstance lowers the accuracy of dose assessment for Kainar samples relative to the Znamenka samples. However, the doses reconstructed from the Kainar samples (280 to 520 mGy) are significantly higher than those from the Znamenka teeth (50 to 190 mGy).



**Fig. 2.** Evolution of the EPR spectrum with increasing radiation dose for a contemporary US tooth enamel sample.

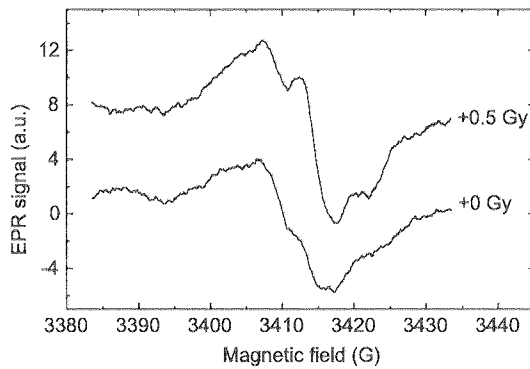


Fig. 3. Pre- and postirradiation (added dose of 0.5 Gy) EPR spectra for an enamel sample prepared from Znamenka tooth no. 4 (Table 1). The reconstructed dose is  $170 \pm 30$  mGy.

#### 4 Discussion

Our investigation on the impact of heat demonstrates the suitability of teeth previously heated up to  $200^\circ\text{C}$  (including Kainar teeth) for EPR dose reconstruction. This confirms earlier findings by Liidja et al. [17].

The most remarkable heat effect on EPR in tooth enamel is the appearance of five heat-induced signals in the spectrum. The central heat-induced signal is superimposed on the tooth enamel native signal (Fig. 1). There are a number of publications devoted to the study of heat effects on the EPR spectrum of irradiated tooth enamel (see, for example, refs. 14–18). It is known that at temperatures above  $285^\circ\text{C}$  the radiation-induced EPR signal in tooth enamel decays rapidly. However, some decrease in the peak-to-peak amplitude of this signal was observed with time when a sample was isothermally heated at  $150^\circ\text{C}$  [14]. A

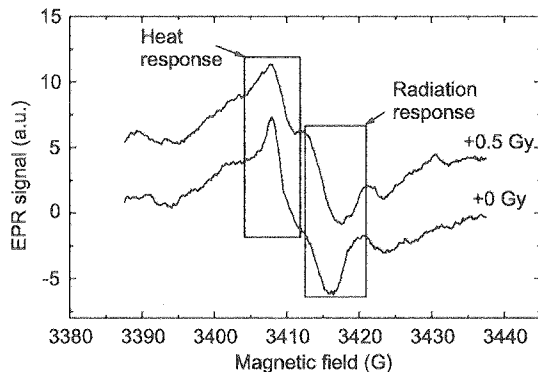


Fig. 4. Pre- and postirradiation (added dose of 0.5 Gy) EPR spectra of an enamel sample prepared from Kainar tooth no. 3 (Table 2). The reconstructed dose is  $550 \pm 150$  mGy.



fivefold increase in the peak-to-peak amplitude of the native (nondosimetric) component was found in tooth enamel samples prepared from US teeth when the annealing temperature was increased from 100 to 200°C in ref. 15. This result appears to contradict the finding described in earlier publications devoted to the study of fossil tooth enamel samples (see, for example, refs. 14, 16–18 and references therein), that the heating of tooth enamel develops a signal quintet centered on the top of the native signal at  $g = 2.0032$ . This quintet was attributed to the dimethyl radical [16] or alanine [18]. Our results obtained from chemically treated US teeth seem to be more similar to results for fossil teeth (see Figs. 1 and 2). The apparent contradiction may be explained by differences in the sample preparation procedures used in the present study compared to tooth enamel isolated from dentin by mechanical removal of the dentin with a dental drill [15]. It is quite possible that high-temperature ultrasonic treatment with KOH followed by 12 h of drying at 60°C could reduce the water content in modern US tooth enamel. This can make EPR properties of the chemically treated modern teeth more similar to the fossil samples. The higher water content of mechanically prepared samples could modify the EPR spectra [15]. In particular, the ability to resolve hyperfine structure could be affected at temperatures below 250°C, where a sharp reduction in the water content of tooth enamel occurs [19].

The issue of the effects of long-term storage on the EPR tooth enamel spectrum is an important subject for retrospective dosimetry. The archiving of dental tissues for extended periods prior to EPR measurement is not unusual. The mean life-time of radiation-induced radicals in fossil tooth enamel has been estimated as  $10^9$  years [14]. This estimation was based on the Arrhenius approximation of the temperature-dependent decay of radiation-induced radicals in fossil tooth enamel from a mammoth. We find no significant effect on the EPR spectrum of tooth enamel for teeth stored up to 35 years because spectra from the samples prepared from Znamenka teeth have the same appearance as those from the samples prepared from recently extracted teeth. To a certain extent, the effects of long-term storage on the EPR tooth enamel spectrum should be similar to those caused by low-temperature (<200°C) annealing, which, according to our results, does not damage the dosimetric information.

There were several canine teeth (7 of 30) among the investigated samples from Kainar and Znamenka population (Tables 1 and 2). As it is well known [7, 20], sunlight exposure can cause an overestimation in the dose measured from front teeth (incisors and canines). Unfortunately, all canine teeth in the present investigation were seriously damaged by caries. Therefore, it was not possible to conduct EPR measurements separately for the lingual and buccal parts of the tooth enamel as in ref. 7. Separate measurements are desirable because sunlight can potentially contribute to the EPR signal resulting in the dose overestimation with the buccal part of the tooth enamel. In general, the results of the dose reconstruction for both Kainar and Znamenka show that doses for the canine teeth are higher than for molars and premolars by about 100 mGy. For this reason only the data for molars and premolars were selected to estimate correctly the average reconstructed doses for Kainar and Znamenka. For the tooth enamel

samples prepared from Znamenka molars and premolars, the average reconstructed dose is  $95 \pm 40$  mGy, whereas for Kainar it is  $390 \pm 70$  mGy.

For the population that resided in the vicinity of SNTS, there are three possible main contributions to their exposure: medical X-ray exposure, natural radioactive background and accidental exposure from the nuclear tests. The limited penetration depth of medical diagnostic X-rays exposure produces a significant difference in the absorbed doses for the lingual and buccal tooth sections [21, 22]. Hence failure to find such a difference by separate analyses of lingual and buccal enamel from four teeth from Znamenka residents (Table 1) and two teeth from Kainar residents (Table 2) supports Gusev's information that those patients did not receive dental X-rays prior to extraction. Thus, we can limit our consideration to contributions from radioactive background and fallout exposure. Kainar residents were mainly affected by three nuclear tests, on September 24, 1951 (38 kt of TNT equivalent, average estimated gamma dose of about 246 mGy), October 5, 1954 (4 kt, 25 mGy) and August 2, 1955 (12 kt, 27 mGy) [1-4]. Published dose estimates for the Znamenka population are on the order of a few mGy [23]. Therefore, to some extent the Znamenka results can be used to estimate the background radiation for Kainar residents, so that the difference between average doses reconstructed for Kainar and Znamenka residents can be used to estimate the average radiation dose received by Kainar residents from nuclear tests at the SNTS. That difference is  $(390 \pm 70) - (95 \pm 40) = 295 \pm 80$  mGy. This is in excellent agreement with the previous dose assessments for the Kainar residents from the three nuclear tests to be 300.9 mGy [4].

## 5 Conclusions

The following conclusions can be drawn from the present study.

1. Heat-treated teeth can be used for EPR dose reconstruction if the heating temperature is below  $200^\circ\text{C}$ ; characteristic signals in the EPR spectrum produced by heat treatment allow one to determine if the sample was heated prior to measurement.

2. Long-term storage (up to 35 years in the present study) of teeth has no significant effect on the EPR dose reconstruction of tooth enamel.

3. The mean reconstructed dose for the Znamenka village, which is not believed to have had significant exposure from the SNTS, was found to be about  $90 \pm 40$  mGy, which is a reasonable value for cumulative exposure to background radiation at 50 to 65 years of age. Unlike the Znamenka teeth, the Kainar samples showed a strong radiation-induced signal. The estimated average gamma dose of Kainar residents from the SNTS is  $295 \pm 80$  mGy.

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