

# DETERMINATION OF THE AVERAGE NATIVE BACKGROUND AND THE LIGHT-INDUCED EPR SIGNALS AND THEIR VARIATION IN THE TEETH ENAMEL BASED ON LARGE-SCALE SURVEY OF THE POPULATION

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The aim of the study is to determine the average intensity and variation of the native background signal amplitude (NSA) and of the solar light-induced signal amplitude (LSA) in electron paramagnetic resonance (EPR) spectra of tooth enamel for different kinds of teeth and different groups of people. These values are necessary for determination of the intensity of the radiation-induced signal amplitude (RSA) by subtraction of the expected NSA and LSA from the total signal amplitude measured in L-band for *in vivo* EPR dosimetry. Variation of these signals should be taken into account when estimating the uncertainty of the estimated RSA. A new analysis of several hundred EPR spectra that were measured earlier at X-band in a large-scale examination of the population of the Central Russia was performed. Based on this analysis, the average values and the variation (standard deviation, SD) of the amplitude of the NSA for the teeth from different positions, as well as LSA in outer enamel of the front teeth for different population groups, were determined. To convert data acquired at X-band to values corresponding to the conditions of measurement at L-band, the experimental dependencies of the intensities of the RSA, LSA and NSA on the m.w. power, measured at both X and L-band, were analysed. For the two central upper incisors, which are mainly used in *in vivo* dosimetry, the mean LSA annual rate induced only in the outer side enamel and its variation were obtained as  $10 \pm 2$  (SD = 8) mGy y<sup>-1</sup>, the same for X- and L-bands (results are presented as the mean  $\pm$  error of mean). Mean NSA in enamel and its variation for the upper incisors was calculated at  $2.0 \pm 0.2$  (SD = 0.5) Gy, relative to the calibrated RSA dose–response to gamma radiation measured under non-power saturation conditions at X-band. Assuming the same value for L-band under non-power saturating conditions, then for *in vivo* measurements at L-band at 25 mW (power saturation conditions), a mean NSA and its variation correspond to  $4.0 \pm 0.4$  (SD = 1.0) Gy.

## INTRODUCTION

Currently, *in vivo* radiation dosimetry based on electron paramagnetic resonance (EPR) measurements of tooth enamel spectra can be achieved at L-band (1.2 GHz). Radiation dose absorbed in the tooth is determined by measuring the intensity of the stable radiation-induced signal (RS) in the enamel, and then calibrating the signal intensity against a known dose–response. EPR spectrometers have been designed for making routine measurements of the RS amplitude (RSA) in irradiated enamel with an uncertainty in the signal amplitude corresponding to a dose of  $\sim 0.5$  Gy<sup>(1)</sup>. In the EPR spectrum of irradiated enamel, the RS is overlapped with a native background signal (NS). The NS amplitude (NSA) on average corresponds to RSA in irradiated enamel that is equivalent to that expected from a dose of  $\sim 1$ – $2$  Gy, according to measurements conducted at X-band (10 GHz) accounting for microwave (m.w.) power saturation of the sample<sup>(2, 3)</sup>. At L-band, the RS and NS are fully overlapped and cannot be

differentiated due to similar linewidth and position in the spectrum.

To facilitate throughput in making *in vivo* measurements and minimise sampling variation of the resonator, the spectrometer is designed for making *in vivo* measurements on the outer enamel of the incisor teeth. However, exposure of the outer enamel of the incisors to the ultraviolet component of sunlight results in a solar light-induced signal (LS) formed in the outer surface of enamel of these teeth. This LS almost fully coincides with the RS in shape and position in the L-band spectrum. The amplitude in this signal may vary by age, geographical region, level of sunlight exposure, behavioural differences (such as working outdoors), and ranges from 0 up to about a 2–3 Gy dose equivalent RS amplitude<sup>(4, 5)</sup>.

When measurements are made of the overall EPR signal in the enamel of a presumably irradiated individual by L-band, the contributions of the NSA and LS amplitude (LSA) to the measured RSA are

unknown One approach, at present, to determining that the intensity of the RS measured *in vivo* is to measure the total amplitude of the signal, and then subtract the population-averaged NSA and LSA determined from individuals with similar known confounders, such as living in the same area and same approximate age. However, the deviation of individual NSA and LSA values from the population-averaged values contributes to the uncertainty in the estimate of the radiation dose calculated from the RSA. To use this approach, it is necessary to know the average NSA and LSA and their variation within a representative population.

The aim of this work is to determine the average values and variation of the NSA and LSA based on analysis of EPR spectra measured at X-band. For this investigation, an analysis was performed on EPR spectra measured for enamel samples collected from a large number of individuals in the Central Russia region representing different demographic groups (age, gender and geographic area) during a wide-scale dosimetric survey of population to reveal the radiation effects of the Chernobyl accident<sup>(6)</sup>. The NSA and LSA determined from these spectra measured at X-band need to be converted to corresponding measurement conditions at L-band *in vivo*. For this reason, theoretical and experimental analysis of the dependencies of these signals on the registration parameters (m.w. frequency and power, modulation amplitude, etc.) at X- and L-bands are required.

The LSA has the same m.w. power saturation behaviour as the RSA<sup>(7)</sup>. Therefore, there was no need to make corrections for the differential power saturation behaviour of these two signals. The NS, however, has a differing saturation behaviour from the LSA and RSA. This necessitates the need to convert the amplitude of this signal measured under conditions of the X-band experiment to one that is appropriate for the operating conditions at L-band, including formulating a correction for the differential power saturation behaviour between the X- and L-bands EPR experiments.

## MATERIALS AND METHODS

EPR (X-band) spectra acquired from measurements on several hundred tooth enamel samples collected from the population of the Central Russia (Kaluga, Bryansk and Tula regions) were used in the current analysis. Results of the dose estimate calculations from these samples are presented in previous publications<sup>(4, 6)</sup>. These earlier measured spectra were used for determination of RSA and NSA in the present work.

Spectral measurements on these samples were performed on an *ESP-300 E (Bruker)* spectrometer at X-band in a standard cavity ST4102 with  $Q = 1600$ – $2500$  (loaded–unloaded) at 10 mW power and a

modulation amplitude of 0.3 mT. Measurements were performed mainly within 1 y from sample collection.

RSA and NSA were determined using spectra processing features provided from spectrometer operational software after operator-controlled fitting of the amplitude, linewidth and spectral position of the simulated NS. Conversion of RSA and NSA to dose units was performed in comparison to the calibrated RSA dose–response that was generated from serial irradiations of teeth in a Co-60 gamma source. Details of measurement, spectral processing and calibration are described elsewhere<sup>(6)</sup>.

RSA, expressed in dose units, Arsd, was calculated from Equation (1):

$$\text{Arsd (mGy)} = \frac{(\text{Ars-epr } K)}{(\text{Aref } m)} - \text{Bias}, \quad (1)$$

where Ars-epr (a.u.) is the maximum amplitude of the RS;  $K = (240 \pm 20)$  mGy·g is the dose–response calibration coefficient from the serial gamma-irradiation of teeth relative to tissue kerma; Aref (a.u.) is the reference MnO signal amplitude;  $m$  (g) is the sample mass.

The bias value ( $72 \pm 11$  mGy) was estimated based on the dependence of RSA on the enamel age for the posterior teeth as determined in the control populations within territories unaffected by radiation exposure from the Chernobyl accident<sup>(4, 6)</sup>. This bias value is specific to a spectra processing procedure used, and it originated from a difference between the line shape of the reference NS used for subtraction during spectra processing and the average NS for different teeth<sup>(8)</sup>. It is assumed that on average, the NS have the same line shape in different teeth (although this has not been experimentally verified); therefore, the same bias value was used for anterior teeth.

To compare with RSA, maximum amplitudes of NS were converted to dose units, Ansd, in comparison to the calibrated RSA dose–response as described above using the same calibration coefficient,  $K$ , and then dividing by a factor of 2, as shown in Equation (2):

$$\text{Ansd (a.u.)} = \frac{(\text{Ans-epr } K)}{(\text{Aref } m)}, \quad (2)$$

where Ans-epr (a.u.) is the maximum NSA. These values were determined from spectra processed after acquiring the EPR measurements. Results were recorded to database files and then used for the present analysis.

Statistical analysis of data and fitting of model functions to experimental results was performed using a non-linear least-square method provided in the *OriginLab (Microcal)* program.

Results of the statistical analysis, in general, are expressed as the mean, standard error of the mean (SE) and standard deviation (SD) using the following format:

$$(\text{mean} \pm \text{SE}, \text{SD} = \text{standard deviation})$$

$$\text{units } (n = \text{number of values})$$

SD is defined here as the root mean square of the differences between individual values and the mean value and characterises the scatter in the data.

## RESULTS AND DISCUSSION

### Determination of the average LSA and its variation based on wide-scale measurements in X-band

Examination of results of the RSA measurements performed on teeth samples acquired from subjects in the Central Russia region found that RSA values obtained from the front (anterior) teeth (Palmer notation of numbering: Positions 1–4, i.e. central incisor through the first bicuspid) are much higher in comparison with back (posterior) teeth (Positions 5–8, i.e. the second bicuspid through third molar)<sup>(4)</sup>. This effect was attributed to solar UV-induced paramagnetic centres formed in front teeth from exposure to the sunlight.

During the early stages of the wide dosimetric survey (prior to 1996), the effect of solar exposure on front teeth had not yet been determined, and all measurements were performed on samples prepared from mixed enamel from inner and outer sides of teeth. In the present work, for determination of the LSA, we used spectra acquired from the front teeth, which were obtained from samples prepared from the mixture of enamel of both sides of teeth. We have selected results of RSA estimation for the front teeth (Positions 1–3) calculated from teeth

collected from individuals in low radioactivity contaminated territories of Bryansk region (Klintsy city and district), and from control individuals in uncontaminated territories of Kaluga region. For teeth collected in the low radioactive contaminated territories of the Bryansk region, the additional average contribution to RSA due to radioactive contamination is estimated at  $30 \pm 10$  mGy, based on the data presented by Skvortsov *et al.*<sup>(6)</sup>. This contribution was subtracted from the RSA values measured in teeth collected from these territories. For control uncontaminated territories, no such correction was performed.

The annual rate of RSA accumulation was determined by dividing the measured RSA by enamel age. Enamel age is determined by subtraction of the age of enamel formation from the chronological age of the individual. Age of enamel formation for teeth in Positions 1–3 is accepted as 6 y<sup>(3)</sup>. The dependencies of RSA and the annual rate of RSA accumulation on chronological age for incisors and canine teeth in Positions 1–3 are presented in Figure 1.

Since the mixed enamel from both sides of teeth was used for measurements of RSA, it is assumed that, on average, equal amounts of enamel from both sides of tooth are contained in the samples. Therefore, contribution of the solar illumination to the annual increase of LSA in the outer enamel, LSA rate, is considered to be twice that for the samples with pooled enamel prepared from both sides of tooth. In addition, the contribution of natural background radiation to RSA should be subtracted from the RSA rate:

$$\text{LSA rate} = \frac{(\text{RSA rate} - \text{RSA nat.rate})}{2}, \quad (3)$$

where RSA nat.rate ( $0.80 \pm 0.28$  mGy y<sup>-1</sup>) is the natural background radiation contribution to the

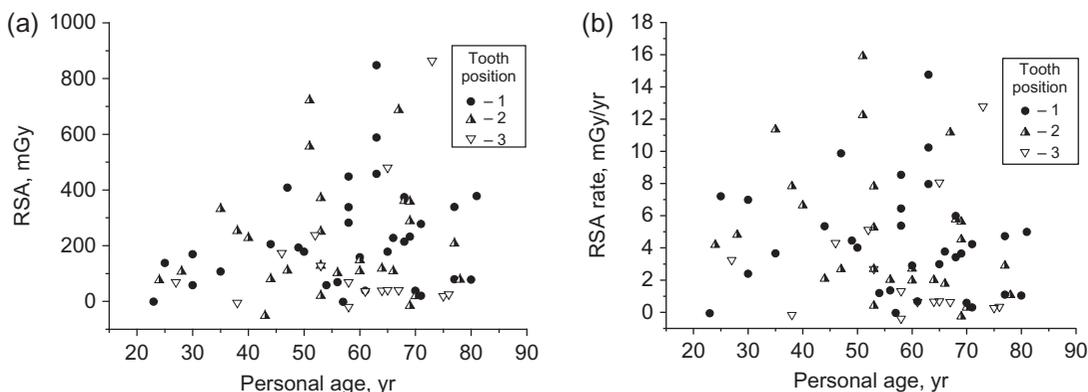


Figure 1. Dependencies of RSA (a) and of the annual rate of RSA accumulation (b) on chronological age for front teeth at different positions.

RSA as calculated from the slope of the RSA measured in posterior teeth as a function of age<sup>(6)</sup>.

Since enamel composition and external irradiation conditions are similar for anterior and posterior teeth, the same natural background radiation contribution was used for different teeth.

Mean values of RSA and LSA rate for teeth with different positions were estimated, and they are presented below.

Teeth positions 1–3; Quarters = 1–4 (upper and lower 1st and 2nd incisors, and canines—all front teeth):

Mean RSA rate =  
 $(5.75 \pm 0.45, SD = 3.94) \text{ mGy y}^{-1} (n = 76)$ ,  
 Mean LSA rate =  
 $(9.90 \pm 1.10, SD = 8.00) \text{ mGy y}^{-1} (n = 76)$ .

The two upper first incisors are mainly used in the L-band *in vivo* dosimetry. Therefore, LSA rate for these teeth was estimated separately since this case is the most important for the dosimetry application:

Teeth position 1; Quarters = 1, 2 (two upper 1st incisors):

Mean RSA rate =  
 $(6.04 \pm 0.82, SD = 3.76) \text{ mGy y}^{-1} (n = 21)$ ,  
 Mean LSA rate =  
 $(10.5 \pm 1.8, SD = 7.6) \text{ mGy y}^{-1} (n = 21)$ .

These values are not significantly different from the results for all front teeth.

**Determination of the average NS and its variation based on wide-scale measurements in X-band**

A large number of spectra measured in samples collected from the Central Russia region for the purpose of examining the radiation exposures from the Chernobyl accident were used for NSA determination. Below we present the results of NSA determination based on results from the analysis of these spectra. Dependencies of NSA on different teeth characteristics are presented for samples collected

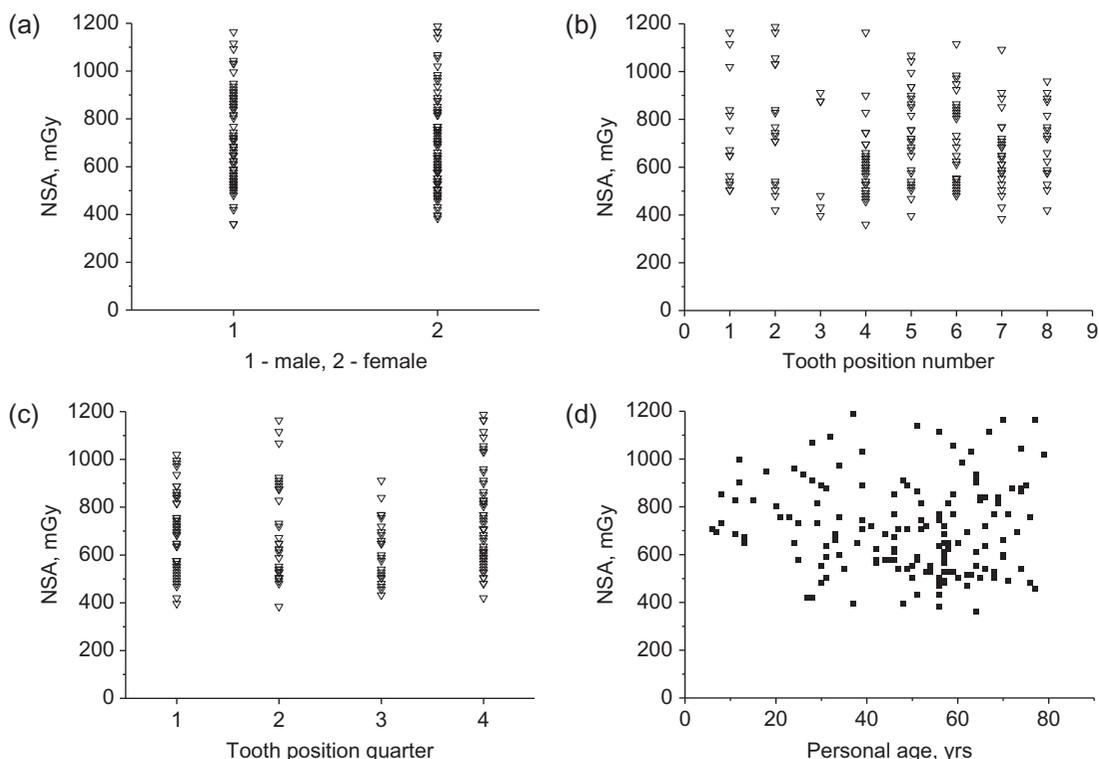


Figure 2. Dependencies of the NSA maximum expressed in dose units on the different teeth characteristics for the control territories of Kaluga region: (a) male/female; (b) tooth position number; (c) tooth position quarter (1, 2: upper jaw; 3, 4: lower jaw); (d) personal age.

from the control territory of Kaluga region as shown in Figure 2.

Average values of the NS peak–peak amplitude (in a.u.) for all teeth with different positions for groups of samples collected in different territories and for male/female groups are presented in Table 1. A comparison of the NSA from the different territories using Student's *t*-test found no significant ( $p > 0.05$ ) difference in the values. Still, differences in the mean values of NSA between some territories might be considered significant in some instances if measurement variations from the EPR instrumentation were to be considered. There is likely to be some variations in the measurements due to differences in instrument operating conditions (i.e.  $Q$  of the resonator, setting of the reference sample signal amplitude, etc.) that may have resulted due to variations in the measurement time periods for samples obtained from different territories. However, information on these instrumental errors was not available.

As follows from the data in Table 1, for all teeth, the following value is obtained for the mean NSA as measured at a power of 10 mW within a standard Bruker cavity at X-band:

$$\text{Mean NSA} = (0.75 \pm 0.10, \text{ SD} = 0.20) \text{ Gy } (n = 768).$$

For the two first upper incisors (tooth number positions 1 and 2, Quarters 1 and 2), which are mainly used for the L-band *in vivo* dosimetry:

$$\text{Mean NSA} = (0.72 \pm 0.05, \text{ SD} = 0.20) \text{ Gy } (n = 20).$$

This value is not significantly different from the mean value for all teeth.

### Conversion of the NSA measured at X-band under non-m.w. power saturation conditions for L-band application

#### *Accounting for the effects of different m.w. frequency at X- and L-bands and modulation amplitude*

At X-band, RS is described by an axially symmetric signal consisting of *xy*- and *z*-components with fixed *g*-factors, and linewidth of each *g*-factor component of 0.4 mT, for a total linewidth of the RS signal of ~0.8 mT. At L-band, the difference between the *g*-factor components of the RS is reduced due to the lower frequency. Therefore, a nearly symmetric singlet line is observed with a linewidth of 0.4 mT. Similarly, the linewidth of the NS at X-band is ~0.8 mT, but is also reduced to ~0.4 mT at L-band. Effects connected with heterogeneous broadening (a combination of unresolvable lines with different *g*-factors) of this line probably cause such a reduction linewidth when going from X-band to L-band.

The differences in the line shapes of the RS and NS at X- and L-bands are expected to have an effect on the conversion of NSA to dose units. However, in the present work, these potential effects are not taken into account. Estimation of these effects on dose calculation is a subject of a separate investigation.

When the same modulation amplitude is used to make measurements at X- and L-bands, and does not exceed an amplitude of 0.4 mT, no effects of overmodulation are expected and, therefore, no correction for linewidth differences at X- and L-bands originating from the modulation is needed.

**Table 1. Results of NSA determination from an average of all teeth positions from individuals representing different territories of Central Russia, presented as separate and combined gender groupings.**

Terr.	Male				Female				Male + female			
	Mean	SE	SD	<i>n</i>	Mean	SE	SD	<i>n</i>	Mean	SE	SD	<i>n</i>
1-Ctr	733	23	205	77	706	23	217	91	715	17	214	168
2-Klim	763	41	204	25	866	55	216	15	809	32	210	40
3-Kr	760	37	232	38	739	26	191	53	748	22	208	91
4-Zl	811	22	167	61	851	26	182	48	828	17	174	109
All 1–4	—	—	—	—	—	—	—	—	760	17	204	407
5-Uzl	708	20	232	129	750	19	228	134	730	14	229	263
6-Uzlr	790	25	173	46	715	28	192	51	751	19	186	97
All 5–6	736	18	237	175	740	17	218	181	738	12	228	356
All 1–6	—	—	—	—	—	—	—	—	750	12	216	763

Mean NSA amplitude (maximum amplitude, in mGy); SE, standard error of mean (mGy); SD, standard deviation from the mean (mGy); *n*, number of samples; Terr., territory notations: control territory of Kaluga region (1-Ctr), different districts of Bryansk region (2-Klim, 3-Kr and 4-Zl) and Tula region (5-Uzl and 6-Uzlr).

Accounting for the m.w. power saturation effects at X- and L-bands

$$Y(P) = A \times P^{1/2} \times S(P), \tag{4}$$

Special analysis was performed to convert the mean value of the NSA measured at X-band to measurement conditions at L-band. Local m.w. power distribution in the sample should be taken into account, if measurements are performed under conditions of m. w. power saturation. Dependencies for NSA and RSA at X-band on m.w. power for a pooled sample (prepared from a mixture of different teeth) irradiated to a dose of 2.0 Gy are presented in Figure 3a. These data are taken from our previous publication<sup>(9)</sup>. Power dependencies for NSA and RSA at L-band for a whole tooth irradiated at the same dose are presented in Figure 3b. These data are taken from the work of Iwasaki *et al.*<sup>(10)</sup>. In this work, data for RSA are presented for a sample irradiated in dose 30 Gy. The RSA is divided by a factor of 15 to obtain an RSA that is equivalent to a 2 Gy irradiated tooth sample. Both groups of power dependencies are measured at the same modulation amplitude of 0.3 mT.

where  $A$  is the amplitude parameter corresponding to non-power saturation conditions;  $P$  is the m.w. power augment;  $S(P)$  is the saturation factor.

In general cases of homogeneously and heterogeneously broadened lines, the saturation factor may be described by Equation (5):

$$S(P) = \left( 1 + \frac{P}{P_{\text{sat}}} \right)^{-b}, \tag{5}$$

where  $P_{\text{sat}}$  is the m.w. power saturation parameter; and  $b$  is the exponential saturation parameter.

According to the theory of magnetic relaxation<sup>(11)</sup>, for the homogeneously broadened line (described by a pure Lorentzian line shape),  $b = 3/2$ . For the heterogeneously broadened line (Gaussian or combination of Gaussian and Lorentzian line shapes) the parameter,  $b$ , is smaller.

Conversion of the estimated mean of the NSA from X- to L-bands is performed in two steps. In the first step, NSA determined at X-band and expressed in dose units relative to the calibrated RSA dose-response to gamma radiation is converted to non-power saturation conditions. At the second step, the obtained value is converted to the corresponding conditions of power saturation when measured at L-band.

Implementing the power saturation model, we found that the dependencies of RSA and NSA at X-band, and RSA at L-band, on m.w. power are well fitted by Equation (4) with saturation factor described by Equation (5), as shown in Figure 3. The dependence of NSA on m.w. power at L-band could not be fitted with the use of this equation. This dependence is well fitted by a second-order polynomial in the range from 0 to 5 mW<sup>1/2</sup> (corresponding to 0–25 mW) as shown in Figure 3b. Therefore, the saturation factor is described by linear dependence relatively to  $P^{1/2}$

A general type of equation which may be used for analysis of power dependencies of derivative signal amplitude, similar to that proposed in<sup>(11)</sup> is

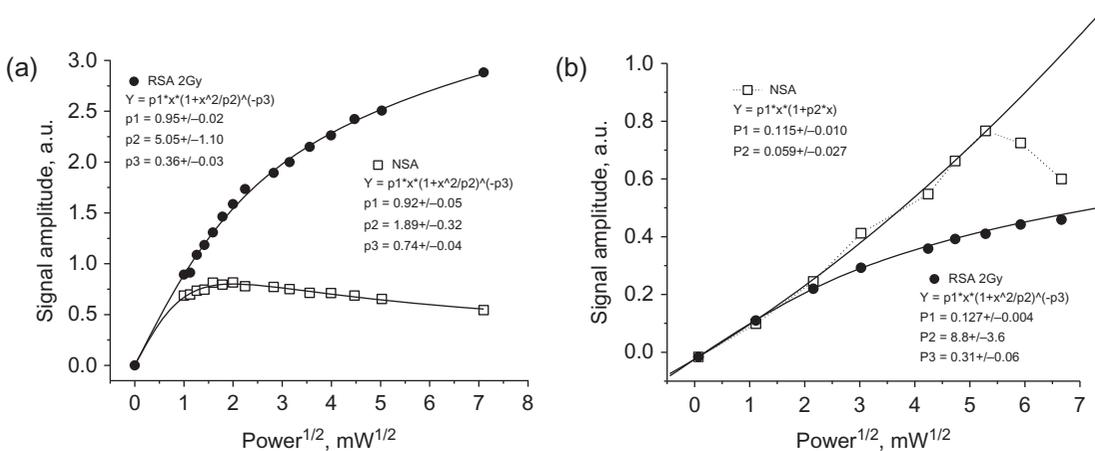


Figure 3. M.w. power dependencies of RSA and NSA measured at X-band with a standard resonator (a) and at L-band with a loop resonator. (b) Data are taken from previous publications for X-band<sup>(9)</sup> and L-band<sup>(10)</sup>. Values for RSA are scaled to a 2 Gy radiation dose. Both data sets were obtained at 0.3 mT modulation amplitude. The solid lines represent the model fitting to the data. The fit model functions are shown in the plot panels along with the fitted parameters.

$$S(P) = (1 + p2 \times P^{1/2}), \quad (6)$$

where  $p2$  is the fitted parameter.

Dependencies of saturation factors on the m.w. power described in Equations (5) and (6) with estimated parameters are presented in Figure 4.

Conversion of the NSA as expressed in dose units according to the calibrated RSA measured at X-band under conditions of power saturation,  $NSD_X(P_X)$ , to conditions without saturation,  $NSDX(0)$ , may be performed using Equation (7):

$$NSD_X(0) = NSD_X(P_X) \cdot R_X(P_X), \quad (7)$$

where  $R_X(P_X) = S_{RX}(P_X)/S_{NX}(P_X)$  which is the correction coefficient for saturation of NSD, defined as a ratio of saturation factors for RSA and NSA  $S_{RX}(P_X)$  and  $S_{NX}(P_X)$ , respectively, calculated from measurements taken at power  $P_X$ .

Conversion of the NSA expressed in dose units at L-band under conditions without power saturation  $NSD_L(0)$ , to conditions of measurement at power  $P_L$ ,  $NSD_L(P_L)$ , may be performed using Equation (8):

$$NSD_L(P_L) = NSD_L(0)/R_L(P_L), \quad (8)$$

where  $R_L(P_L) = S_{RL}(P_L)/S_{NL}(P_L)$  which is the correction coefficient for saturation at L-band at power  $P_L$ , calculated as the ratio of the saturation factors for RSA and NSA for measurements conducted at L-band. The dependencies of the correction coefficients on power saturation for X- and L-bands

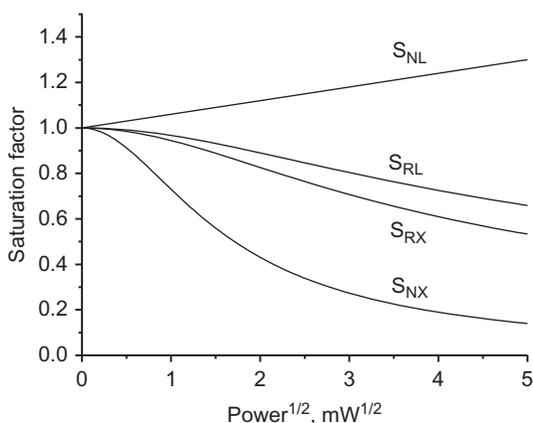


Figure 4. Dependencies of functions describing the saturation factors for NSA and RSA on m.w. power at X- and L-bands (denoted by S with indexes N and R for NSA and RSA, respectively, and X and L for X-band and L-band, respectively).

derived from the fitted saturation functions are presented in Figure 5.

It can be seen from the plots in Figures 3 and 4 that the power saturation behaviour of the NSA at L-band is anomalous and cannot be described by common spin relaxation theory. Increase of the saturation factor for NSA at L-band below 25 mW power may be due to effects of spatial distribution of m.w. field strength in a tooth from the loop of the resonator. At different distances from the loop, there is a differential distribution of saturation states of the signal in enamel as the m.w. power decreases with distance from the resonator. In addition, contribution to NS may be not only from the enamel, but also from layers of dentin underneath the enamel that may result in a heterogeneity in the saturation behaviour of NSA. It may be expected that increases in the m.w. power at the resonator will result in earlier power saturation of the NS in the enamel as compared to the NS in dentin, which will effectively see a weaker m.w. field strength due the greater distance from the resonator. The overall effect is a greater contribution of the NS in dentin with increasing m.w. power to the overall NSA as compared to the NS in enamel. The sharp decrease of NSA at m.w. power over 25 mW at L-band may be due to saturation of the signal within the whole enamel layer.

To check these suppositions, it is necessary to perform a theoretical analysis of the NS formation in a whole tooth under conditions of real m.w. field strength distribution in the tooth near the L-band loop resonator. Such a distribution may be obtained by means of a m.w. field simulation method<sup>(12)</sup>, which includes taking into account

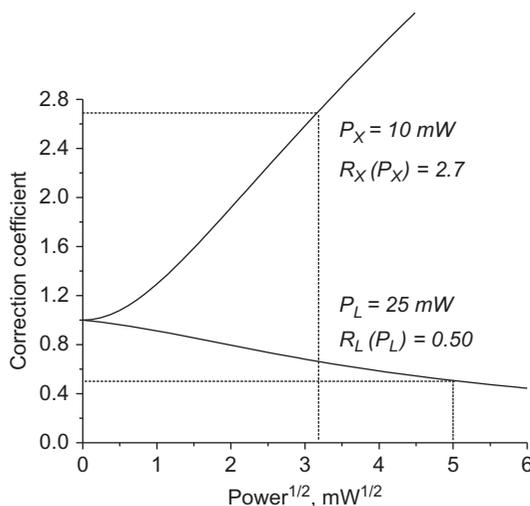


Figure 5. Dependencies of the correction coefficients on the m.w. power for X- and L-bands ( $R_X$  and  $R_L$ , respectively).

saturation effects within the enamel and dentin<sup>(12)</sup>. If it is assumed that  $NSD_L(0) = NSD_X(0)$ , then the relationships described in Equations (7) and (8) may be used to convert the values of NSD measured at X-band to the respective values measured at L-band. In this case, conversion of the NSA expressed in dose units measured at X-band at power  $P_X(NSD_X(P_X))$  to conditions of measurement at L-band at power  $P_L(NSD_L(P_L))$  may be derived directly from the value of  $NSD_X(P_X)$  using of the following equation:

$$NSD_L(P_L) = \frac{NSD_X(P_X) \cdot R_X(P_X)}{R_L(P_L)}, \quad (9)$$

where the indexes X and L refer to conditions of measurement at X- and L-bands.

#### *Estimation of the mean NSD and its variation in L-band based on results obtained in X-band*

Finally, we perform calculations based on the estimated value of mean NSA expressed in dose units measured at X-band at 10 mW power where the mean  $NSD_X(P_X) = (0.72 \pm 0.05, SD = 0.20)$  Gy.

As it follows from Figure 5, for X-band at 10 mW  $R_{XS}(P_X) = 2.7$  and for L-band at 25 mW  $R_{LS}(P_L) = 0.50$ , then:

$$\begin{aligned} \text{MeanNSD}_X(0) &= \text{MeanNSD}_X(P_X = 10 \text{ mW}) \cdot 2.7 \\ &= (1.94 \pm 0.14, SD = 0.54) \text{ Gy}, \end{aligned}$$

which follows that

$$\begin{aligned} \text{MeanNSD}_L(P_L = 25 \text{ mW}) &= (1.94 \pm 0.14, SD = 0.54)/0.50 \\ &= (3.88 \pm 0.28, SD = 1.1) \text{ Gy}. \end{aligned}$$

#### *Accounting for the mean LSA and NSA in the estimation of RSA at L-band*

The mean values of LSA and NSA obtained for the upper incisors may be directly subtracted from the total EPR signal amplitude measured at L-band prior to calculating a dose estimation from the RSA in *in vivo* EPR dosimetry. Their variation should be taken into account as a source of error in dose calculations. To illustrate, for an individual with a chronological age of 46 y (enamel age 40 y), the value of LSA in outer enamel of incisors is expected to be equivalent to  $0.40 \pm 0.06$  Gy with  $SD = 0.30$  Gy. In the case of measurements conducted at L-band *in vivo*, this value of 0.40 Gy should be subtracted from the measured signal amplitude, and the amplitude variation of 0.30 Gy should be accounted for as the additional contribution to the error in the RSA estimate. For example, if the total measured signal is  $6.0 \pm 0.5$  Gy, it is

necessary to subtract 0.4 Gy to make a correction for the LSA. The resulting signal will be 5.6 Gy with an uncertainty of  $(0.5^2 + 0.3^2)^{1/2} = 0.6$  Gy. This value includes both the RSA and NSA.

To obtain the pure RSA, it is necessary to subtract the mean NSA estimated from the given conditions of the L-band measurements. As obtained in the present work, the average NSA expressed in dose units corresponding to measurements conducted at L-band at 25 mW is estimated as  $3.9 \pm 0.3$  (SD = 1.1) Gy. After subtraction of this value, the resulting RSA is 1.7 Gy with an uncertainty of  $(0.6^2 + 1.1^2)^{1/2} = 1.2$  Gy. Errors in the average values for LSA and NSA are neglected in these calculations of the uncertainties since they are much smaller in comparison to their SDs.

#### *Possible contribution of dentin to NSA in L-band*

In deriving the estimation of NSA for L-band from X-band measurements, we assumed that under non-power-saturation conditions, these signals will have the same amplitudes. However, when measurements are conducted on a whole tooth at L-band, not only the enamel but the dentin placed below the enamel will contribute to the NSA. NSA in the dentin is several times greater in comparison to enamel at the same measurement conditions<sup>(3)</sup>. However, the m.w. field strength in dentin is less than in enamel, because it is at a greater distance from the resonator loop. It was shown by modelling<sup>(12)</sup>, at a distance of from 0.5 to 1.5 mm (corresponding to about the thickness of the enamel layer), that the signal from irradiated enamel in tooth is reduced by approximately a factor of 2 because of reducing field strength. Therefore, NSA from dentin should be reduced because of the greater distance from the resonator. In addition, when making measurements on the living tooth, dentin is in a moistened state, so we would expect a relatively high dielectric loss and relatively high conductivity in the dentin. This may cause further weakening of the m.w. field inside the dentin due to the high dielectric loss, effectively preventing the penetration of the field into the dentin and eliminating the contribution of the dentin NS to the NSA.

In direct L-band measurements of teeth at 25 mW, a value for the NSA corresponding to ~2–3 Gy was obtained<sup>(10)</sup>. This value is less than the mean value of NSA = 3.9 Gy obtained in the present work at L-band. The smaller value observed in this publication could have resulted from properties of the tooth samples used in their measurement.

#### *Contribution of solar UV-induced signal to NSA*

It should be noted that under the influence of the UV component of solar light on the front teeth, a

signal is formed that is similar to the NS and also overlaps with the RS in irradiated teeth<sup>(7, 13–15)</sup>. In experiments with artificial UV-irradiation of teeth it was shown<sup>(14)</sup> that this NS-like signal produced in teeth decreased by ~30% within 1–2 d after UV-exposure, and then nearly stabilised. It is expected that this NS-like solar induced signal will result in a variable contribution to the amplitude of the total measured *in vivo* signal, especially if *in vivo* measurements are made within several days after exposure of teeth to the sun.

Contribution of the solar-induced NS-like signal may be estimated based on results of experiments on exposure of enamel samples to the sun in the summer time in Japan<sup>(7)</sup>. It is shown that this NS-like signal is stabilised after ~15 d of sunlight exposure at an amplitude that is about half of the NSA. The rate of increase of this signal in the initial period of irradiation is ~10% per day of the equilibrium level and, once stabilised, does not change for up to at least 2.5 y based on studies of stored samples. Accounting for the signal decay rate in the initial period after sunlight exposure and rate of its formation from this exposure, it can be expected that the contribution of the signal even in measurements acquired immediately after exposure to the sun will not exceed >10–20% of the NSA.

## CONCLUSION

A statistical analysis was performed on the parameters of the EPR spectra acquired in the X-band measurements of tooth enamel samples obtained earlier from a large-scale survey of the population of Central Russia. From the analysis, estimates of the mean amplitude and variations of the LSA in front teeth and NSA measured at X-band were obtained.

Also, based on the analysis of the dependencies of RSA and NSA on m.w. power at X- and L-bands, a method for converting the NSA, expressed in terms of dose units, obtained at X-band to L-band conditions was derived which takes into account the m.w. power saturation effects on the signal amplitudes.

Average values and the variation of the LSA and NSA for L-band based on the data obtained at X-band are estimated, taking into account m.w. field saturation effects. These values can be directly subtracted from the amplitude of the total signal measured at L-band to provide a corrected estimate of the RSA. The variation of the NSA and LSA values should be taken into account when evaluating the accuracy of the RSA and subsequent dose estimations based on the RSA.

It should be noted that the difference in the line shapes of RS acquired at L- and X-bands, as well as the possible effects of spatial distribution of m.w. field in the whole tooth on the NSA registered at L-band, were not taken into account in the

conversion of the X-band based NSA values to L-band. This may affect the results of the evaluation NSA expressed in terms of the dose units obtained by the conversion of data from X-band to L-band. To assess the impact of these effects on the results of conversion of NSA from X- to L-band, it is necessary to conduct additional studies.

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## REFERENCES

- Williams, B. B., Flood, A. B., Salikhov, I., Kobayashi, K., Dong, R., Rychert, K., Du, G., Schreiber, W. and Swartz, H. M. *In vivo EPR tooth dosimetry for triage after a radiation event involving large populations*. Radiat. Environ. Biophys. **53**, 335–346 (2014).
- Skvortsov, V. G., Ivannikov, A. I. and Eichhoff, U. *Assessment of individual accumulated irradiation doses using EPR spectroscopy of tooth enamel*. J. Molec. Struct. **347**, 321–329 (1995).
- International Atomic Energy Agency. *Use of electron paramagnetic resonance dosimetry with tooth enamel for retrospective dose assessment*. Report of a Coordinated Research Project. IAEA-TECDOC-1331. (Vienna: IAEA) (2002).
- Ivannikov, A. I., Skvortsov, V. G., Stepanenko, V. F., Tikunov, D. D., Romanyukha, A. A. and Wieser, A. *Wide scale EPR retrospective dosimetry: results and problems*. Radiat. Prot. Dosim. **71**, 175–180 (1997).
- Nakamura, N., Miyazawa, C., Akiyama, M., Sawada, S. and Awa, A. A. *A close correlation between electron spin resonance (ESR) dosimetry from tooth enamel and cytogenetic dosimetry from lymphocytes of Hiroshima atomic-bomb survivors*. Int. J. Radiat. Biol. **73**, 619–627 (1998).
- Skvortsov, V. G., Ivannikov, A. I., Stepanenko, V. F., Tsyb, A. F., Khamidova, L. H., Kondrashov, A. E. and Tikunov, D. D. *Application of EPR retrospective dosimetry for large-scale accidental situations*. Appl. Radiat. Isot. **52**, 1275–1282 (2000).
- Jiao, L., Takada, J., Endo, S., Tanaka, K., Zhang, W., Ivannikov, A. and Hoshi, M. *Effects of sunlight exposure on the human tooth enamel ESR spectra used for dose reconstruction*. J. Radiat. Res. **48**, 21–29 (2007).
- Ivannikov, A. I., Skvortsov, V. G., Stepanenko, V. F., Tsyb, A. F., Khamidova, L. G. and Tikunov, D. D. *Tooth enamel EPR dosimetry: sources of errors and their correction*. Appl. Radiat. Isot. **52**, 1291–1296 (2000).
- Ivannikov, A. I., Trompier, F., Gaillard-Lecanu, E., Skvortsov, V. G. and Stepanenko, V. F. *Optimisation of recording conditions for the EPR signal used in dental enamel dosimetry*. Radiat. Prot. Dosim. **101**(1–4), 531–538 (2002).
- Iwasaki, A., Walczak, T., Grinberg, O. and Swartz, H. M. *Differentiation of the observed low frequency (1200 MHz)*

- EPR signals in whole human teeth in vivo measurements of EPR signals in whole human teeth.* Appl. Radiat. Isot. **62**(2), 133–139 (2005).
11. Poole, C. H. *Electron Spin Resonance. A Comprehensive Treatise on Experimental Techniques.* (New York: Interscience Publishers Wiley and Sons) (1967).
  12. Pollock, J. D., Williams, B. B., Sidabras, J. W., Grinberg, O., Salikhov, I., Lesniewski, P., Kmiec, M. and Swartz, H. M. *Surface loop resonator design for in vivo EPR tooth dosimetry using finite element analysis.* Health Phys. **98**(2), 339–344 (2010).
  13. Nilsson, J., Lund, E. and Lund, A. *The effects of UV-irradiation on the ESR-dosimetry of tooth enamel.* Appl. Radiat. Isot. **54**, 131–139 (2001).
  14. El-Faramawy, N. A. *Comparison of gamma- and UV-light-induced EPR spectra of enamel from deciduous molar teeth.* Appl. Radiat. Isot. **62**(2), 191–195 (2005).
  15. Sholom, S., Desrosier, M., Chumak, V., Luckyanov, N., Simon, S. L. and Bouvile, A. *UV effects in tooth enamel and their possible application in EPR dosimetry with front teeth.* Health Phys. **98**(2), 360–368 (2010).