Retrospective Dosimetry on Human Nails using X-band EPR Spectroscopy.

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Abstract:

After a nuclear accident it is important to soon after exposure determine individual doses to the victims. Electron paramagnetic resonance (EPR) spectroscopy provides a mean to determine absorbed doses by quantification of free radicals induced by ionizing radiation. Among body materials tooth enamel and bone biopsies have been used for dose determinations by means of EPR, but the collection of these samples are invasive. Finger nails containing keratin are easy to collect. Keratin has a high yield for creation of free radicals by ionizing radiation. Unfortunately free radicals are also easily induced by cutting, causing a mechanically induced EPR signal. Beside the radiation induced signal (RIS) and the mechanically induced signal (MIS) a quite high background signal (BKS) is present.

In a preliminary study the background signal was obtained for nails from 10 donors. For comparison both toe and fingernails were collected from two donors. A preparing procedure was developed including water preparation of cut pieces of nails. The mechanical stress was studied by repeated cutting of fingernails just before the measurements. Finally dose response curves for both mixed samples from different donors and from one individual were obtained.

Preliminary results showed that the background signal from untreated nails varies greatly between individuals making it impossible to establish a robust averaged BKS. However a comparison between toe and finger nails from the same individuals showed a striking similarity. The mechanically induced signal decayed within 24 hours and the water treatment reduced the BKS significantly. Both BKS and MIS increase with additional cuts of a whole dry nail. The dose response curves obtained for irradiated samples 4 days after water treatment were found to be linear up to 30 Gy.

Key Words; EPR, retrospective dosimetry, finger and toe nails, background, mechanical stress.

1. Introduction

After a nuclear accident fast determinations of individual doses are important e.g. for radiological triage and for dose reconstruction. There are a number of different types of dosimetry for this purpose. One of them is electron paramagnetic resonance, EPR, spectroscopy others are optically stimulated luminescence, OSL, (Bøtter-Jensen and Murray, 1996, deWitt *et al* 2010) thermo luminescence, TL and biodosimetry using cytogenic, transcriptomic and proteomic technologies (Fenech 2011).

These methods are applied to both body materials and also materials in the close vicinity to the victims e. g. from pockets and clothing. Physical methods for dose determinations are chosen depending on available materials, the needed accuracy and also how fast the doses must be established.

Among body materials EPR dosimetry of tooth enamel has been an established method for retrospective determinations for a long time (Fattibene and Callens 2010). Till now most in-

vitro determinations are performed when high accuracy is needed. The disadvantage is that a whole tooth or a significant part of the enamel (Gómez *et al* 2011) have to be extracted.
Much more convenient is to use finger and toe nails for retrospective dose determinations. The nails are mainly composed of alpha keratin, a protein of alpha helical peptid chains, coiled and strengthened by S-S bridges formed from cysteine groups. Chandra and Symons (1987) also found that radicals were formed when nails are cut. Symons *et al* (1995) made a thorough evaluation of the possible mechanisms in radical formation and assumed that the signal induced of cutting; the background signal and the radiation induced signal overlap and make dose determinations difficult, but still possible. Since then significant progress in nail dosimetry has been achieved, especially regarding the preparation of the cut nails to decrease the background signal. (Romanyukha *et al* 2007, Reyes *et al* 2008, Trompier *et al* 2009, Romanyukha *et al* 2011)

Three types of EPR signals can be identified in a finger nail spectrum, the background signal (BKS), the mechanically induced signal (MIS) and the radiation induced signal (RIS). These signals overlap with each other in the spectrum. The MIS however shows a doublet signal which can therefore be distinguished from the singlet signal formed by BKS and RIS. Regarding the effects of cutting the "sponge theory" interprets the fractured fingernails as a deformed sponge where the MIS and BKS are associated with the stress of the deformations. The MIS is assumed to be caused by the elastic deformation in the S-S bonds whereas BKS might be caused by the plastic deformation of the peptide helix in alpha keratin. The elastic deformation does not need additional energy to restore whereas the plastic deformation needs some energy to restore the original shape. After absorbing water, just like a sponge, the fingernail tends to restore its original shape and the stress will be largely reduced which might explain the reduction of MIS and BKS. The MIS completely disappears without water treatment within 24 hours after cutting, while the BKS could not be totally extinguished by the solution treatment. (Reyes *et al* 2008, Trompiér *et al*. 2009).

As found earlier the background signal is almost the same as the RIS and shows such a high degree of variation between individuals that it is difficult to subtract a general background at dose determinations. The radiation induced signal, however, decay within about 100 days depending on storing conditions, but it is unrealistic to wait for that to subtract the remaining BKS (Symons at al. 1995).

The BKS saturates at a rather low microwave power of about 2 mW while the MIS do not saturate even at 100 mW. This makes it possible to use two different power settings to isolate the MIS from the background signal (Reyes *et al*. 2008).

By using a higher microwave frequency, i. e. Q-band, Romanyukha *et al* (2011) found more details in the MIS and also that probably the radiation induced signal consists of two components for untreated samples.

It is known from previously published investigations that the dose response for untreated finger nails is linear up to at least 100 Gy (Symons *et al* 1995, Romanyukha 2007) and that after chemical or water treatment the dose response is exponential with saturation at 8-10 Gy. (Rhomanyukha *et al*. 2007, Reyes *et al* 2011).

The radiation induced signal fading is found to depend on the storage temperature; with almost no fading after 20 minutes post irradiation at -4^{o}C, while at room temperature the signal has decreased to 50% in less than 24 hours. The decay of the RIS is also highly dependent on the

water content; there is almost no decay for dry samples while with a water content of 3%, 50% of the signal is lost after 60 hours (Trompier *et al*. 2009).

The aim of the present investigation was to develop a method for preparation of finger and toe nails for retrospective determination of radiation doses. Further to investigate the BKS from finger and toe nails from the same person for the possibility of obtaining an individual background signal for dose determinations at accidental hand exposure.

2. Material and Methods.

2.1 Investigation of the BKS

Pieces of fingernails (4-7 mg weight) were collected from ten donors. All samples were analysed by means of EPR spectrometry to obtain the BKS signal

Out of them 5 pieces were soaked into water for 10 min followed by 5 min drying in room temperature. This procedure was repeated 3 times with the BKS recorded after each round. Every sample was measured five times and the sample tube was taken out of the cavity between each measurement.

The water absorption was determined from the difference in mass before and after the treatment.

2.2 Investigation of the MIS

Four pieces of dry fingernails were collected from 4 individuals respectively. Each piece was equally cut into 2 pieces and further into 4 pieces and finally into 8 pieces. EPR signals were recorded immediately after each round of cutting.

2.3 Comparison between toe- and fingernails

Two pieces of finger nails and two pieces of toe nails, all dry, were collected from 2 individuals respectively. The BKS from all four pieces were recorded.

2.4 Determination of the dose response

Samples were selected from different donors, washed in a soap solution to remove the dirt on the surface and all whole nails were then cut into small pieces and the masses were determined. Then two rounds of water treatments were applied to extinguish the MIS followed by EPR analysis for recording the BKS.

Four days later 6 mixed samples from 8 different donors and 6 samples from one specific donor were irradiated to 6 different doses between 2 and 30 Gy, followed by EPR analysis.

2.5 Irradiation

For determination of the dose response the irradiations were performed using a 6 MV linear accelerator Varian Clinic 600 C/D at the Radiation Treatment Department of the University Hospital, Linköping.

The samples were wrapped with paper and placed in a circle (15 cm diameter) on a 6 cm thick PMMA plate to ensure full backscatter and covered with a 1cm bolus of plastic to achieve charged particle equilibrium. The radiation field size was 20x20 cm^2 (Fig. 4). The distance from the samples to the radiation source was 100cm. All samples received doses additively in order to shorten the irradiation time and the samples were removed one by one from the PMMA plate as the new dose was given. The final irradiated doses were 2 Gy, 5 Gy, 10 Gy,

15 Gy, 20 Gy, 30 Gy. The given doses were determined by means of an ion chamber calibrated to give dose in water.

2.6 EPR measurements and spectrometer settings

The EPR measurements were performed at room temperature using a JES-FR30EX EPR spectrometry (JEOL). The spectrometer settings are shown in Table 1

Table I Spectrometer settings for the experiments described in sections

Settings	Experiment sect 2.1 and 2.4	Experiment sect 2.2	Experiment sect 2.3
Microwave power	4mW	4 mW	4 mW
Sweep time	20 s	20 s	20s
Modulation amplitude	0,63 mT	0,79 mT	0,63 mT
Amp gain	200	500	400
No of sweeps	10	10	80
Reference sample position	530 a. u.	450 a. u.	430 a.u.

For experiments 2.1, 2.2, and 2.4 the sample was placed in a test tube with outer diameter 5 mm, for experiment sect 2.3 a test tube with outer diameter 3 mm with the nail samples in the center of the cavity and a Mn^{2+}/MgO reference was used as a spectrometer stability control

2.7 Spectrum analysis

The acquired spectra were corrected for the base line slope by the subtraction of a linearly fitted base line. The field sweep was centered between the third (g = 2.034) and fourth Mn^{2+} lines. The third line served as the reference, as the spectra were normalized to the magnetic field value of that line. For the purpose of noise reduction the spectra were thereafter smoothed, by averaging each point in the spectrum over the nearest N points, with N ≈ 3–6% of the total number of measurement points. The dose response signal was then determined by reading out a peak-to-peak amplitude from the spectrum. Data analysis was performed in MATLAB (The MathWorks Inc.).

2.8 Statistical methods

The least square regression was used to analyze the dose response curve. The uncertainty for measurements was determined as one standard deviation from the mean value of repeated measurements.

3 Results

3.1 Investigation of the BKS

The intensity of the background signal from 10 individuals showed a more than 2-fold variation. Figure 1 shows the fluctuation both before and after normalization with the mass.

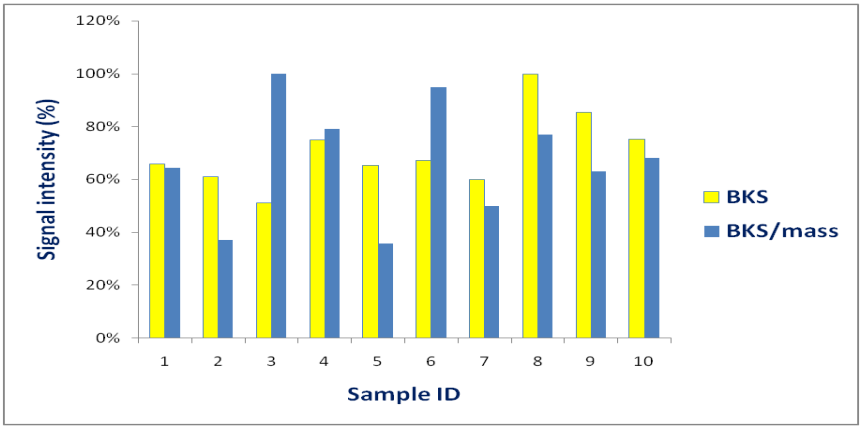

Fig. 1 Intensity of the BKS and BKS divided by mass and normalized to 100%.

After the 1st round of water treatment the signal intensity decreased between 36% and 57%. The amplitude of the signal was further reduced after the 2nd and 3rd rounds of treatment (Fig. 2). The difference between individuals also seems to decrease after water treatment. The water absorption, measured as the mass difference before and after water treatment, reaches the maximum for each sample (12% - 22%) after the 2nd round of treatment, shown in Table 2.

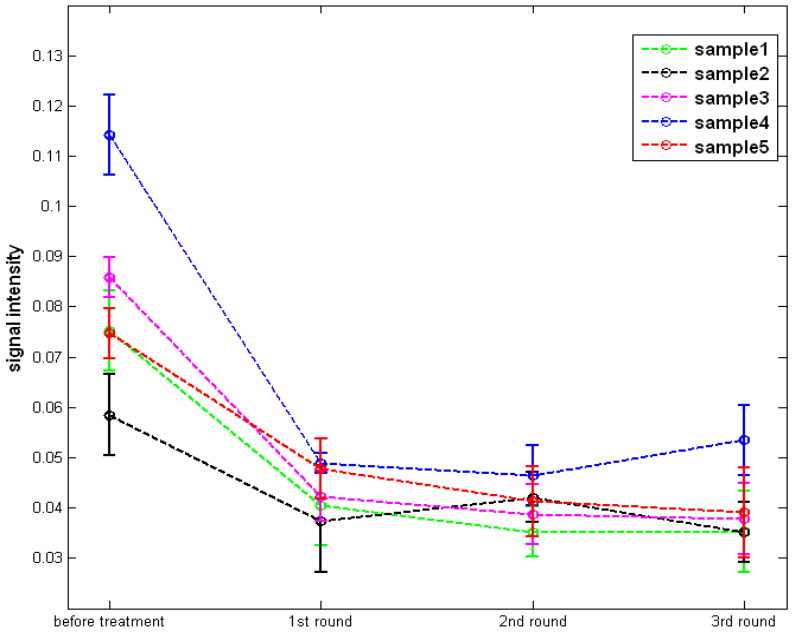

Fig. 2 The reduction of the signal amplitude after repeated treatments with water. The error bars are one SD of the mean from 5 measurements.

Table 2. Water absorption, (Mass after treatment – mass before treatment) / (mass before treatment) x 100 % after each round of treatment.

sample ID	first round	second round	third round
s1	12%	16%	14%
s2	4%	22%	18%
s3	6%	14%	1%
s4	9%	15%	12%
s5	6%	12%	8%

3.2 Investigation of the MIS.

The signal amplitudes from 4 samples indicate a trend of increasing amplitude with the number of cuttings. As shown in Figure 3 the spectra broaden and a minor signal (at the arrow) was observed after the1st cutting, which showed a slight growth along the following cuttings.

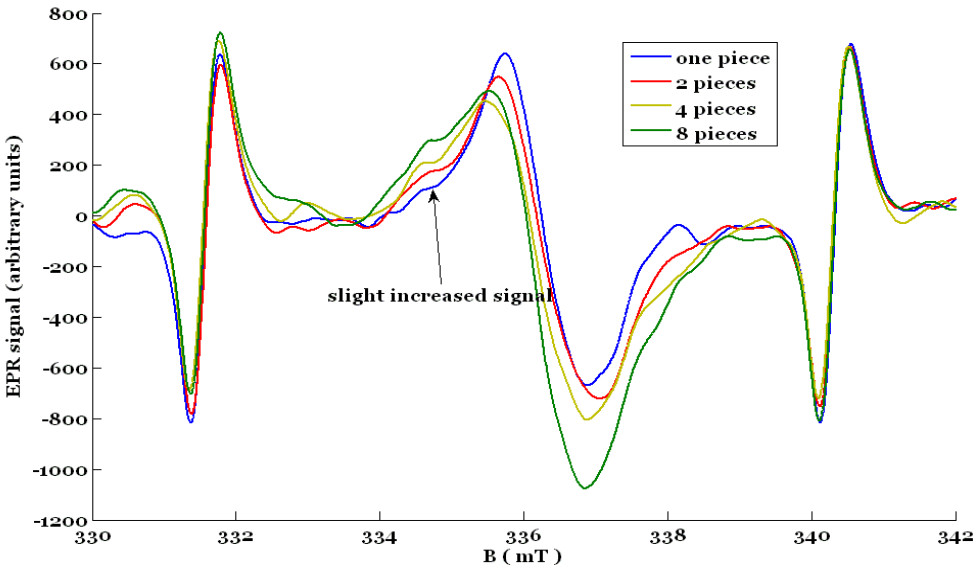

Fig. 3 EPR spectrum evolution with consecutive cuttings, the arrow shows a minor signal appearing after cutting. Together with the BKS and MIS the reference signals from Mn^{2+}/MgO are shown.

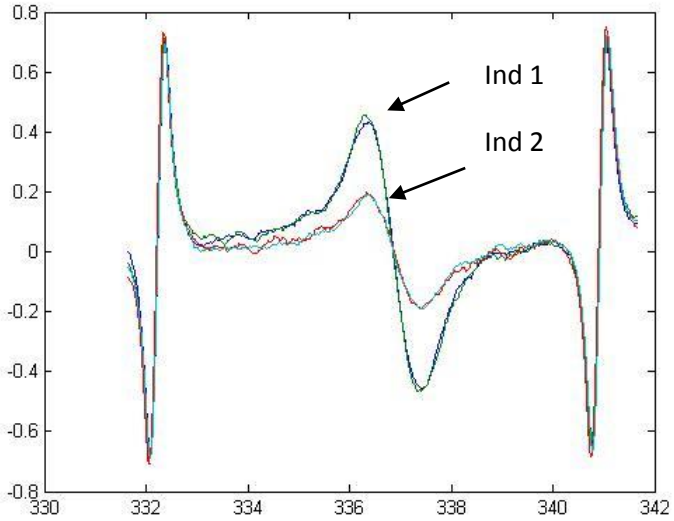

Fig. 4 EPR signals from toe and fingernails for individual 1 and individual 2 respectively.

3.3 Comparison between toe and fingernails

Spectra were recorded for dry finger- and toe nails from two individuals (shown in figure 4). The samples were dry nails cut several days before measurements why the signal includes only BKS. The signals are normalized regarding small differences in mass and the spectra normalized to the Mn reference peaks. The signals from finger- and toe nails from the same individual are very similar, while the signals from nails from different individuals in this case differ significantly, see sect 3.1.

3.4 Determination of the dose response.

In figure 5 the dose response is shown for the total signal from a mixed sample from 8 different donors. The samples are washed and dried to eliminate the MKS but some remaining BKS are present in the total intensity. The irradiation and post radiation analysis is performed 4 days after the water treatment. The dose response is linear with the square of the correlation factor $r^2 = 0.978$. The error bars are 1SD of the average value for 3 measurements. The total intensity is given in arbitrary units and the dose in Gy denote dose in water.

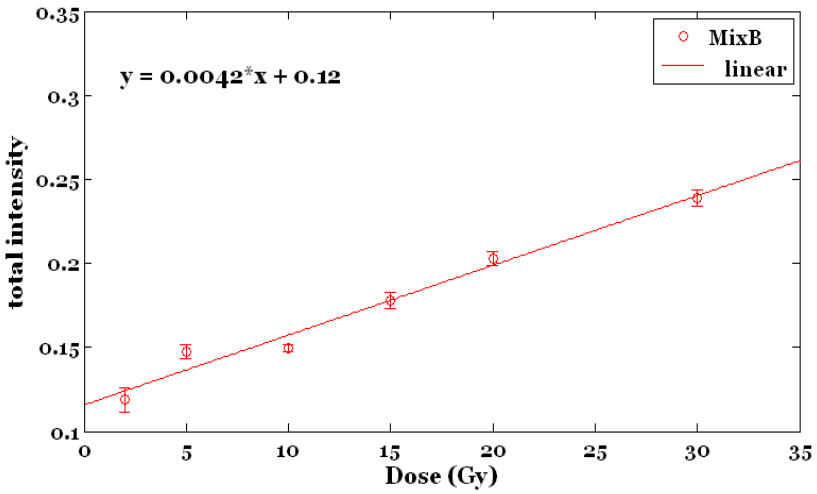

Fig. 5 Dose response for the total intensity for one mixed set of samples, Mix B. Error bars are 1 SD of the mean of 3 measurements. Total intensity is given in arbitrary units and the dose in Gy denote dose in water.

In this experiment it was possible to obtain the BKS for the individual nails and in Fig 6 is shown the dose response for irradiated fingernails from one individual with the BKS subtracted, thus the remaining signal should include mainly the RIS. The best fit to the experimental points is a linear function with r^2=0.982. The error bars are 1 SD calculated as the combined uncertainty from the mean value of three measurements of the total intensity and the mean value from three measurements of the BKS.

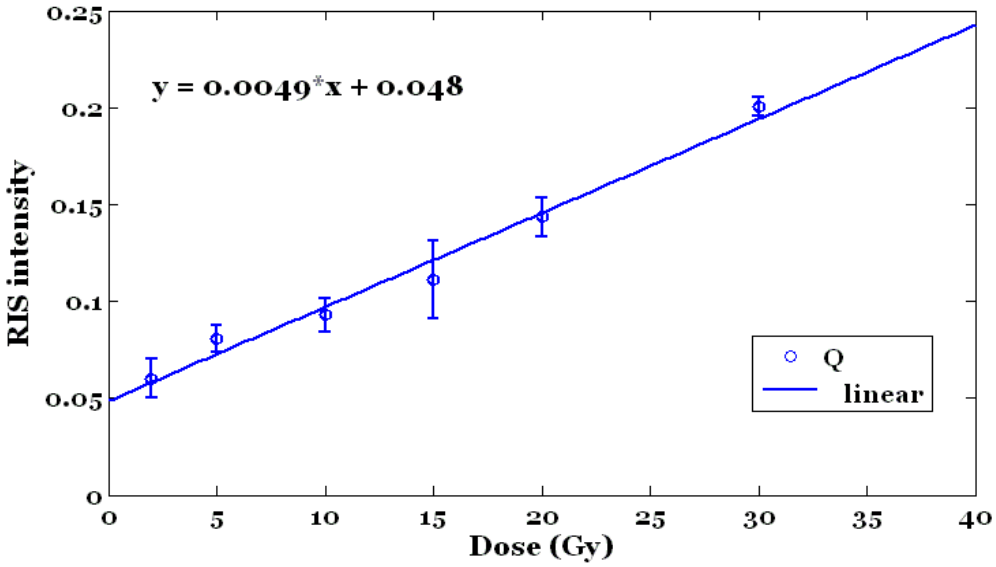

Fig. 6 Dose response for fingernails from one individual with the BKS subtracted from the total signal giving mainly the radiation induced response. The error bars are 1 SD for the combined errors from the total signal and the BKS. RIS intensity is given in arbitrary units and the dose in Gy refer to dose in water.

4 Discussion

In accordance with earlier investigations (Reyes *et al* 2008) a large variation in background signal is shown between different individuals. Fingernails are biological material and even with cautious control of the mass the organic composition of fingernails vary between individuals. For instance, the change of carbon content is age-related whereas the diverse of sulphur content is gender-related. Usually female nails have a higher content of sulphur than nails from males (Dittmar *et al*. 2008). Other facts such as protein content, various thickness, dirt on the surface etc. could affect the background signals and make them heterogeneous.

Our results showed that repeated water treatments efficiently removed about 50% of the original BKS in accordance with other findings that both water and chemical treatments could largely reduce the signal (Romanyoukha *et al*. 2007, Reyes *et al*. 2008). We also found that the spread in BKS intensities among different individuals decreased. This show the importance of water treatment of cut nails before measurements. The present study shows that the BKS and MIS decrease after only one treatment while the water absorption reaches a maximum after 2 or 3 rounds of treatment (Table 2). It has to be further investigated how the absorbed water content affects the dose response and the fading of the RIS in irradiated nails.

As reported by Trompiér *et al*. (2009) the signal decreases to 50% within 60 hours for a water content of 3%. This indicates that one round of water treatment might be optimal.

After repeated cutting of dry finger nails the signal composed of BKS and MIS changed in intensity and shape. The signal broadens and a slight indication of a doublet structure appeared. If a lower modulation amplitude had been used probably the characteristic shape of the doublet MIS had been seen.

At a real incidence the cut nail samples will probably not be measured until most of the MIS has decayed. It is easier to cut soft nails for instance after some time in a water bath and in further experiments the MIS and BKS will be studied when nails are cut after irradiation and a water treatment of some minutes. The hypothesis is that the BKS and MIS are kept low without affecting the response for the RIS. However, the radiation induced signal will be carefully studied especially regarding the fading. This will be complementary to the thorough investigation by Reyes *et al*. (2009) including more than 80 fingernail samples collected and treated in different ways for studying the influence of stress on the RIS.

Scenarios of nuclear accidents may include situations where people handle radioactive material with their bare hands, irradiating the hands more than the rest of the body. In such cases our present results with the similarity between BKS from toe nails and finger nails from the same individual indicate that the EPR signal from the toe nails could be used as background for subtraction from the total signal from the finger nails to obtain the radiation dose. Reyes *et al*. (2009) could however not prove that the intra-individual variability was significantly smaller than the inter-individual variability of the EPR radiation dose.

The dose response was found to be linear both for samples from one donor with the BKS subtracted and for the total signal from mixed samples from 8 donors in the dose interval 2-30 Gy. The samples were analyzed 4 days after the water treatment of the samples. This might explain the linear response up to 30 Gy contrary to earlier results showing an exponential relationship between signal intensity and absorbed dose, with saturation around 10 Gy (Reyes *et al*. 2008, Romanyokha *et al*. 2007 and Reyes *et al* 2009). In these cases, however, the exponential response curve is obtained for freshly water treated nails and a linear response is found for dry, stressed samples (Reyes *et al*. 2009). We may assume that the nails in the present study were dry and partly stressed after the water treatment.

5 Conclusion

EPR dosimetry for retrospective dose determinations using finger and toe nails is promising. The advantage is that the collection of samples is almost non-invasive, the nail dosimetry is sensitive and that the sample preparation is fairly simple. However still remaining is a better explanation for the background signal and the mechanically induced signal and their behavior after water treatment and drying

In this preliminary study some promising results will lead to further investigations as

- Examination of the possibility to use the BKS from the toe nails as background at dose determinations from the fingernails of the same individual.
- Examination of the response and fading characteristics of the radiation induced signal in nails cut after water treatment.

- Examination of the dose response curves obtained after different time intervals after irradiation, cutting and water treatment.

This will hopefully result in a protocol for cutting treatment and analysis of finger and toe nails after accidental irradiation for the purpose of retrospective dose determinations.

6. Acknowledgement

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7. References:

Bötter-Jensen L and Murray A S, 2002, Optically stimulated luminescence in retrospective dosimetry, Radiation Protection dosimetry **101**,no 1-4, pp 309-3014.

Chandra H and Symons M C R, 1987, Sulphur Radicals formed by cutting α-keratin, Nature **328,** pp 833,834.

deWitt R, Klein D M, Wukihara E G, Simon S L, and McKeever, S W S, 2010, Optically stimulated luminescence (OSL) of human tooth enamel and its potential use in post-radiation exposure triage. Health Physics **98**, pp 432-439.

Dittmar M, Dindorf W and Banerjee A. 2008. Organic Elemental Composition in Fingernail Plates Varies between Sexes and Changes with Increasing Age in Healthy Humans .Gerontology **54**, pp 100-105.

Fattibene P and Callens F, (2010) EPR dosimetry with tooth enamel: a review. Applied Radiation and Isotopes **68,** (11), pp 2033-2116.

Fenech M, 2011, Current status, new frontiers and challenges in radiation biodosimetry using cytogenic, transcriptomic and proteomic technologies, Radiation Measurements **46**, pp 737-741

Goméz J A, Kinoshita Leonor S J, Belmonte G C and Baffa O, 2011, Retrospective biodosimetry with small tooth enamel samples using K-band and X-Band. Radiation Measurements **46**, pp 754-759.

Reyes R A, Romanyukha A, Trompier F, Mitchell C A Clairand I, De T, Benevides L A and Schwartz H M, 2008, Electron paramagnetic resonance in human fingernails: the sponge implication, Radiat. Environ. Biophys. **47**, pp 515-526

Reyes R A, Romanyukha. A, Olsen C, Trompiér F, Benevides L A, 2009, Electron paramagnetic resonance in irradiated fingernails: variability of dose dependence and possibilities of initial dose assessment. Radiation Environment Biophysics **48**, pp 295-310.

Romanyukha A, Trompier F, LeBlanc B, Calas C, Clairand I, Mitchell C A, Smirniotopoulos J G, Schwarz H M, 2007, EPR dosimetry in chemically treated fingernails, Radiation Measurements **42**, pp 1110-1113.

Romanyukha A, Trompier F, Reyes R A and Melanson M, 2011, EPR measurements of fingernails in Q-band, Radiation Measurements **46,** pp 888-892.

Symons M C R, Chandra H and Wyatt J L, 1995, Electron paramagnetic resonance spectra of irradiated finger nails: A bossible measure of accidental exposure, Radiation Protection Dosimetry **58**, 1, pp 11-15.

Trompier F, Romanyukha, A, Kornak L, Calas C, LeBlanc B, Mitchell C A, Schwarz H M and Clairand I, 2009, Electron paramagnetic resonance radiation dosimetry in fingernails, Radiation Measurements, **44**, pp 6-10.