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RADIATION DOSE DETERMINATION BY USING NAILS WITH ESR BIODOSIMETRY TECHNIQUE

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ESR BİYODOZİMETRİ TEKNİĞİ İLE TIRNAK KULLANILARAK RADYASYON DOZUNUN BELİRLENMESİ

Abstract:

In recent years, the number of Electron Spin Resonance (ESR) studies on nails have increased as a mean of rapid and accurate biodosimetry. The most important outcome of these studies is the identification of a stable radiation-induced signal (RIS5) component in nails reported by the French Radioprotection and Nuclear Safety Institute (IRSN). The other important result is the dose determination protocol publicated in 2016 proposed by Sholom and McKeever funding from Dartmouth ESR center, with American National Institute of Health (NIH) funding. In this study, the results of three approaches on nail dosimetry were presented: two of them were based on the described protocols of IRSN and Dartmouth and the other used the classical dose addition method. Nail samples were collected from a donor irradiated with ^{137}Cs gamma rays (0.5 kGy/h). ESR measurements were carried out using a Bruker e-scan X-band ESR spectrometer. Samples irradiated up to the doses of 25, 30, 85 and 168 Gy were used to construct the added dose-response curves in steps of 5 and 10 Gy. The reported intensities of RIS5 and center field signal (near g = 2.004) were derived from peak-to-peak distance of the ESR signal (Figures 1 and 4). To test last protocol published in 2016 (Darthmouth protocol), nail samples were irradiated by Co-60 gamma rays at 2 and 5 Gy as accidental radiation doses. The ESR signal intensities were recorded to use in formula given in proposed protocol. By using the first protocol, differences between dose saturation points

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of exposed and unexposed samples which determine the accident doses were found as 15 Gy for the first group and 10 Gy for the second group (Figures 2 and 3). On the other hand, by the second protocol, the ESR signal intensity increased with increasing added doses. The experimentally measured ESR signal intensity values fitted well by polynomial function and the extrapolated doses were calculated as 13.89 and 22.19 Gy for 10 and 15 Gy accident doses, respectively (Figures 5 and 6). Lastly, according to Dartmouth center protocol, the mean accident doses were calculated as 2.18 and 4.5 Gy for 2 and 5 Gy accident doses, respectively. In summary, IRSN protocol evaluating the RIS5 component and Dartmouth protocol were found to be successful methods for the evaluation of dose to nails exposed high-doses. However, classical approach using center field ESR signal given extrapolated doses in error of 38.9 and 47.9 percent was found to be unsuccessful for dose assessment by nails. Further studies should be planned to test the IRSN and Dartmouth approaches for lower accident doses on more samples from different individuals, taking attention to get results in short time.

Özet:

Son yıllarda, hızlı ve doğru biyodozimetri tekniği olarak tırnaklar üzerinde yapılan Elektron Spin Rezonans (ESR) çalışmalarının sayısı artmıştır. Bu çalışmaların en önemli sonucu, Fransız Radyasyondan Korunma ve Nükleer Güvenlik Enstitüsü (IRSN) tarafından tırnaklarda oluşan kararlı radyasyona bağlı ESR sinyalinin (RIS5) tanımlanmasıdır. Bir diğer önemli sonuç, Dartmouth ESR Merkezi aracılığıyla Amerikan Ulusal Sağlık Enstitüsü (NIH) tarafından desteklenen Sholom ve McKeever tarafından 2016'da yayımlanan çalışmada verilen doz belirleme protokolüdür. Bu çalışmada, tırnak dozimetrisi konusunda önerilen üç yaklaşımın sonuçları sunulmuştur: ikisi yukarıda anlatılan IRSN ve Dartmouth protokollerine dayanmaktadır, diğerinde ise klasik eklemeli doz yöntemi kullanılmıştır. Toplanan tırnak numuneleri Cs-137 gama kaynağında (0.5 kGy / saat) ışınlanmıştır. ESR ölçümleri, Bruker e-scan X-band EPR spektrometresi kullanılarak gerçekleştirilmiştir. 5 ve 10 Gy'lik adımlarla eklemeli doz-cevap eğrilerini oluşturmak için 25, 30, 85 ve 168 Gy dozlarına kadar ışınlanan örnekler kullanılmıştır. RIS5 ve merkez alan (yaklaşık g = 2.004) ESR sinyal şiddetleri, ~~EPR~~ sinyalinin tepeden-tepeye yüksekliği ölçülerek belirlenmiştir (Şekil 1 ve 4). 2016 yılında

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yayınlanan son protokolü test etmek için ise, tırnak örnekleri Co-60 gama kaynağında 2 ve 5 Gy kaza dozlarında ışınlanmıştır. ESR sinyal şiddetleri, bu protokolde verilen formülde kullanılmak üzere kaydedilmiştir. İlk protokolde, ışınlanmış ve ışınlanmamış örneklerin doyum noktaları arasındaki fark, birinci grup için 15 Gy ve ikinci grup için 10 Gy olarak bulunmuştur (Şekil 2 ve 3). Diğer yandan, ikinci protokolde ESR sinyal yoğunluğu eklenen doza bağlı olarak artmıştır. Deneysel olarak ölçülen ESR sinyal şiddeti değerleri en iyi polinom fonksiyonuna fit edilmiş ve dozlar, 10 ile 15 Gy kaza dozları için, sırasıyla, 13.89 ve 22.19 Gy olarak hesaplanmıştır (Şekil 5 ve 6). Son olarak, Dartmouth Merkezi protokolüne göre, 2 ve 5 Gy kaza dozlarında ışınlanmış örnekler için sırasıyla dozlar 2.18 ve 4.5 Gy olarak hesaplanmıştır. Özetle, RIS5 ESR sinyal şiddeti değişimini kullanan IRSN ve Dartmouth protokollerinin yüksek dozlara maruz kalmış tırnak örneklerinde kaza dozunun değerlendirilmesinde başarılı yöntemler olduğu görülmüşken, merkez alan ESR sinyal şiddeti değişimini kullanan klasik yaklaşımın yüzde 38.9 ve yüzde 47.9 hata ile kaza dozları vermesi sebebiyle bu yöntemin başarılı olmadığı tespit edilmiştir. IRSN ve Dartmouth yaklaşımlarının farklı bireylerden daha fazla örnek üzerinde daha düşük kaza dozları kullanılarak test edilmesi ve bu testlerin de kısa süre içerisinde yanıt verebilmesi yönünde ileri çalışmalar planlanmalıdır.

Keywords: Nails, biodosimetry, accident doses, ESR spectroscopy
Anahtar Kelimeler: Tırnak, biyodozimetri, kaza dozları, ESR spektroskopi

1.Introduction

Biodosimetry has the potential to provide the information needed from individuals with unplanned exposure to ionizing radiation in a way that enables the medical response system to function effectively. There are two different but potentially complementary types of biodosimetry. Biologically-based parameters have limitations because of inherent differences in response to damage in individuals; prior or concurrent pathophysiologically-based processes; and changes over time in an individual's response to radiation after the exposure. Physically-based biodosimetry has the advantage of measuring dose at a spesific body location and is not affected by biological responses to the radiation exposure. In ESR

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technique which is physically based biodosimetry, the signal intensity is directly proportional to the amount of free radical generated by ionizing radiation.

ESR spectroscopy is a versatile and key tool for dose assessment after a severe radiological accident using biological materials collected from victims (bones and tooth enamel) or other materials irradiated during the accident (sugar, glass from personal items, etc.). Use of human nails was considered several decades ago for radiation accident dosimetry (Brady, Aarestad & Swartz, 1968; Dalgarno & McClymont 1989; Symons, Chandra & Wyatt, 1995) and for triage in case of large scale events (Trompier et al., 2007). They were suggest to use of IR-induced signal in nails as the basis of biodosimeter. Nails are easy to collect and can possibly give an estimation of the dose distribution when nails from each finger or toe can be analyzed separately. More recently, extensive efforts have been made to understand the free radical mechanism in nails and establish ESR nail dosimetry with different approaches (Marciniak, Ciesielski & Prawdzik-Dampc, 2014; Romanyukha et al. 2007a, 2010, 2014; Reyes et al. 2008, 2009, 2012, Trompier et al. 2007, 2009, Black & Swarts, 2010; Wilcox et al. 2010, He et al. 2011). Different ESR signals; radiation-induced signals (RIS), mechanically-induced signals (MIS) and background (BKG) identified in nails are reported in the studies mentioned above and summarized by Trompier et al. 2014a. A major finding in a subset of these studies (Marciniak, Ciesielski & Prawdzik-Dampc, 2014; Romanyukha et al.,2014; Trompier et al 2014a) was the identification of a stable RIS, labeled as RIS5, which is described in more detail by Trompier et al 2014a. This new finding has made the possibility of a human nail dosimetry method practical. Based on that work, a new protocol of ESR nail dosimetry is proposed. This protocol is designed to identify and estimate a high dose of ionizing radiation to fingers, which is currently the common case in localized accidental exposures to ionizing radiation. The protocol has been applied by the Radioprotection and Nuclear Safety Institute (IRSN) for the analysis of nail samples collected from different victims of four high dose accidental radiological exposures that occurred between 2008 and 2012 (Trompier et al. 2014b, Romanyukha et al. 2014). In the latest case (Chilca accident), nail dosimetry was performed in the early management phase which allowed identification of those fingers with the highest dose before the appearance of any clinical signs (IAEA 2012). The data have been decisive in the management of the victims and open the possibility to use

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human nail ESR dosimetry in radiological accidents and to assist casualties before they become symptomatic. Recent comparison with doses estimated by means of ESR bone dosimetry has also shown the reliability of this newly developed approach (Trompier et al. 2013).

All the components of the MIS and RIS, except one (the RIS5), can be eliminated by humidification of nails. Due to its stability, the only component that can be used for dosimetric application is the RIS5. Due to its weak intensity and its similarity to the BKG, a dedicated protocol remains to be established. For high-dose application, which is of major interest regarding the number of accident cases with localized high dose radiation exposure to the hands, a new approach in dosimetry using the RIS5 component has been developed and applied. This approach is based on the dose saturation behavior of the RIS5 as described by Trompier et al. 2014a.

A new procedure was proposed for ESR dosimetry using finger or toe nails by the study of Sholom and McKeever 2016. They developed a procedure of isolation and seperate observation of the RIS, MIS and BG signals using a combination of water soaking, irradiation and additional clipping for multiple nail samples. They proposed storage of samples in vacuum for all time between sample clipping and ESR measurements. It exploits the stability of the RIS if the samples are kept in a vacuum after nail harvesting.

Within the scope of this study, we have compared the IRSN dose calibration method against a simple dose additive method for calculating “accidental” doses ranging from 10-25 Gy dose based on RIS5 measurements acquired by ex vivo by X-band ESR in nail clipping samples collected from a single donor. Both methods use an additive serial irradiation approach to calculate a dose from the the RIS5 formed from an “accidental” dose but differ in the endpoints used to calibrate the dose. The IRSN method uses the differential in the dose saturation points between the exposed and unexposed nail clippings to calculate the accidental dose whereas the classical dose additive method calculates the dose based on an extrapolation from a simple dose-response function. In addition, we presented here the results of dose recovery tests using the protocol proposed by Sholom and McKeever. We present here the results of the comparative testing and provide recommendations for further advances in the dose calibration methodology.

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2.Materials and Methods

The ESR experiments were carried out in two different times. Nail samples were collected from the same donor for each experiment. First, two approaches were tested for 15 Gy accident dose and then, they were tested for 10 Gy. In the first run, the samples were divided into two groups: one was for non-exposed and the other was exposed to an accident dose of 15 Gy checked by alanine dosimeter, cut into small pieces, humidified in distilled water for about 10 min, and dried on a towel in air for about 1 day (in the dark). To control mass changes in the nail samples to insure that significant mass changes due to water loss/gain is not affecting the dose-response results, the samples in each experiment were weighed (~ 24 mg) before and after each measurement. In the second run, all steps above were repeated with an accidental dose of 10 Gy in a second set of nails from the same donor. In the IRSN approach, when adding new dose, the process of humidification and drying were repeated before each set of measurements. In the classical one; these process were performed only before the first measurement to avoid MIS. The samples were irradiated with ^{137}Cs gamma rays with an air kerma rate of 0.5 kGy/h at the Sarayköy Establishment of the Turkish Atomic Energy Authority in Ankara. ESR measurements were carried out using a Bruker e-scan X-band ESR spectrometer. A microwave power of 1 mW was adjusted during the experiment according to power studies of BKG, MIS and RIS signals (not presented). The samples irradiated up to dose of 25, 30, 85 and 168 Gy were used to construct the added dose-response curves in steps of 5 or 10 Gy. The reported intensities of RIS5 and center field ESR signals (near g = 2.004) were derived from peak-to-peak distance of the ESR signals whose spectrums can be seen in Figures 1 and 4. To test last protocol published in 2016, nail samples were irradiated by Co-60 gamma rays at 2 and 5 Gy as accident doses. The ESR signal intensities were recorded to use in formula given in proposed protocol whose spectrums can be seen in Figure 7. In this protocol, the following steps were applied to nail samples; 1.nail harvesting (clipping), 2.storage in a vacuum desicator, 3.first ESR measurement (ESR Dx due to unknown dose Dx plus MIS and BG), record time tc since nail harvesting; 4. in order to remove RIS and MIS and minimize BG, soak nails for 1 hr in water, plus 1 hour drying in vacuum; 5. Divide samples into two; give one a dose of 10 Gy; other 0 Gy (unirradiated);

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6.make extra cut in each sample; 7.measure ESR signal (10 Gy) and (0 Gy) at time tc after extra clipping; 8.calculate unknown dose Dx by using: (Sholom and McKeever, 2016)

$$Dx = 10x(ESR(Dx) - ESR(0\ Gy)) / (ESR(10\ Gy) - ESR(0\ Gy))$$

3. Results

The ESR spectra obtained from different approaches in two runs are shown in Figures 1 and 4, IRSN and classical approaches, respectively.

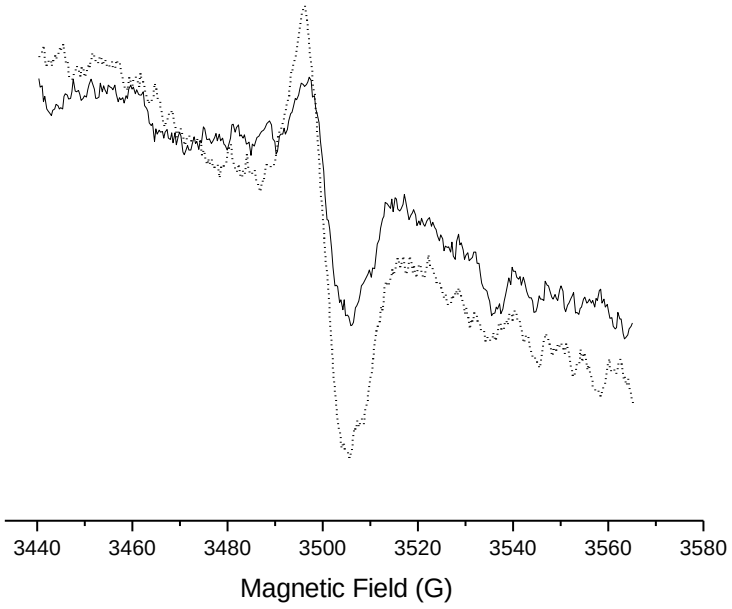

Figure 1. EPR spectra of fingernails at 10 Gy added dose after irradiated 15 Gy (solid line) and 10 Gy (dash line) before the cycle of post irradiation started.

Evaluation of the RIS5 intensity regarding multiple irradiation doses for two groups; the first group was not irradiated while the second groups were irradiated at 10 or 15 Gy dose prior to the cycle of post-irradiation started are shown in Figures 2 and 3. It seems that the unexposed nails saturate at radiation dose levels about 15 and 20 Gy. The differences between saturation dose levels of exposed and unexposed nails were determined at accident doses as 15 and 10 Gy.

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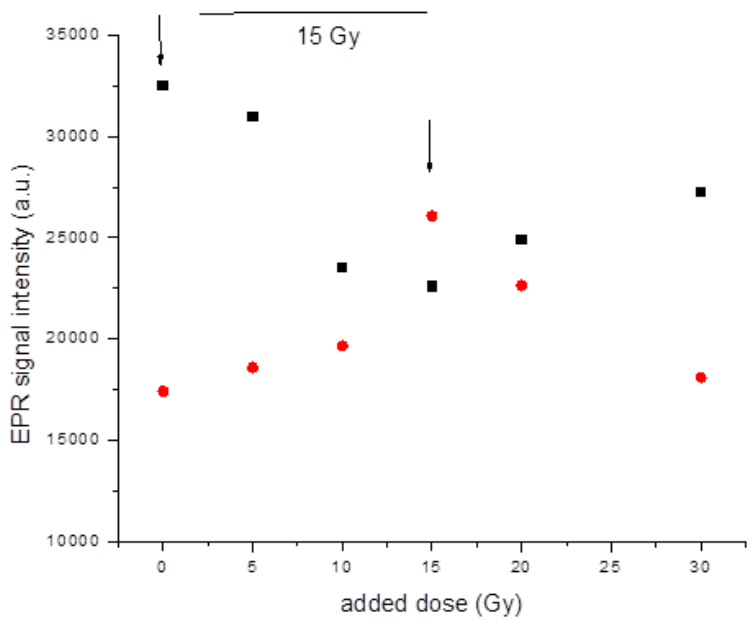

Figure 2. Evolution of the RIS5 intensity regarding multiple irradiation doses for two samples: (●) unexposed sample (■) exposed to 15 Gy sample.

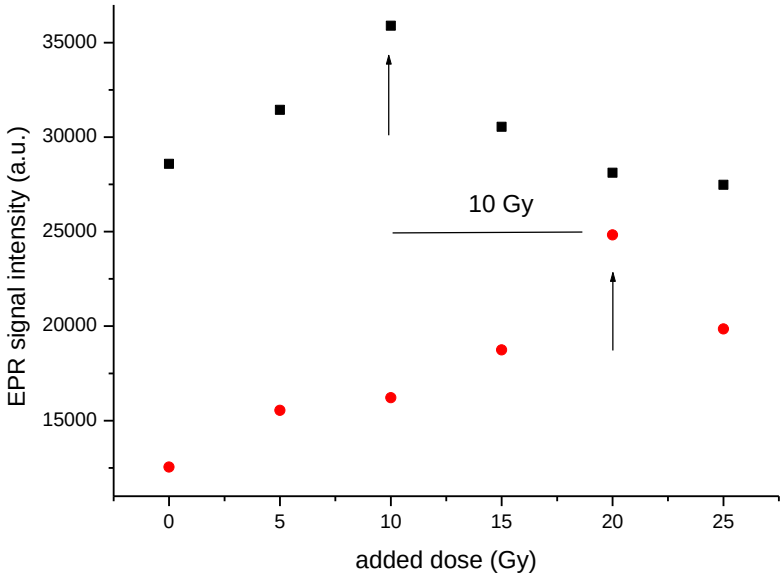

Figure 3. Evolution of the RIS5 intensity regarding multiple irradiation doses for two samples: (●) unexposed sample (■) exposed to 10 Gy sample.

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In classical additive dose method, the dose-response curves for the exposed samples are shown in Figures 5 and 6. The experimentally measured EPR signal intensity values were fitted well by a second order polynomial function type given as $y = aD^2+bD+c$. The parameter b represents the radiation yield of the radicals responsible from the RIS5 and the parameter c represents the radical concentration (signal intensity) at zero additive dose. The term aD^2 is known as the correction factor in ESR/dosimetry. The parameter values of –0.003±0.0005, 1.18± 0.11 and 28.00±4.08 for a, b and c, respectively, were calculated from fitting procedure with the correlation coefficient of $r^2 = 0.99$ for the sample exposed to 15 Gy accident dose (Figure 5). The same calculations were repeated for the sample exposed to 10 Gy accident dose. The parameter values of –0.25±0.05, 46.60±5.60 and 696.76±115.80 for a,b and c, respectively, were calculated with the correlation coefficient of $r^2 = 0.99$ (Figure 6). The extrapolated doses (ED) were calculated to be 13.89 and 22.19, respectively, for the sample exposed to 10 Gy and 15 Gy accident doses.

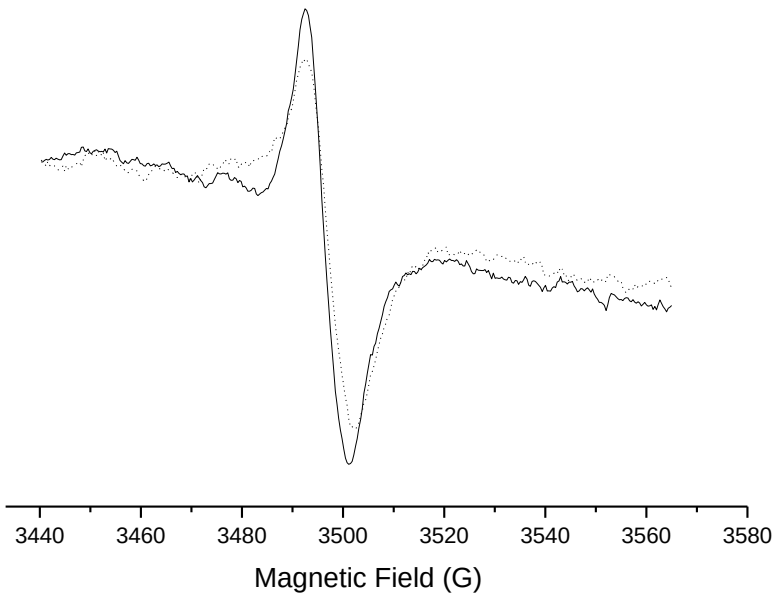

Figure 4. ESR spectra of fingernails at 40 Gy added dose after irradiated 15 Gy (solid line) and 10 Gy (dot line) before the cycle of post irradiation started.

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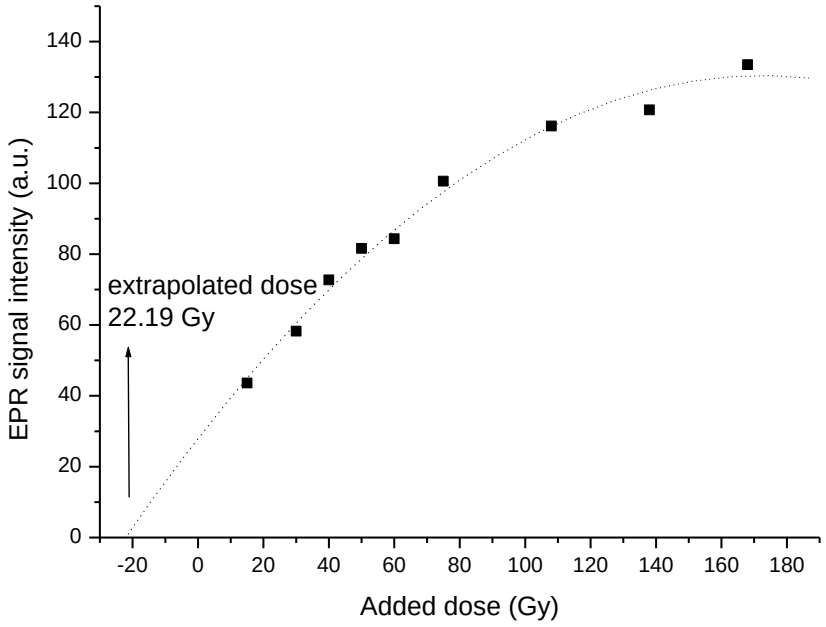

Figure 5. Additive dose-response curve for the sample exposed to 15 Gy accident dose.

(■) Experimental data, (…) Calculated data.

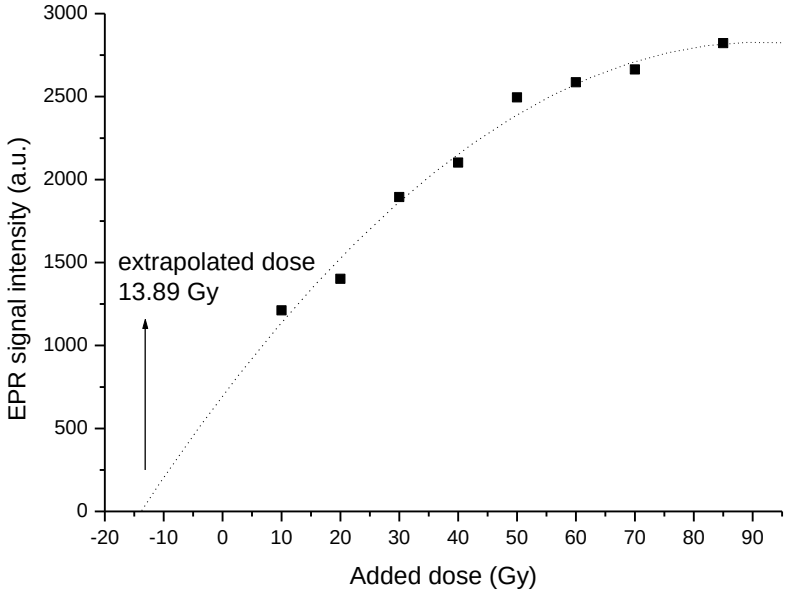

Figure 6. Additive dose-response curve for the sample exposed to 10 Gy accident dose.

(■) Experimental data, (…) Calculated data.

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Lastly, using the protocol outlined above (Dartmouth), the results of dose recovery test were shown in Table 1. The mean accident doses were calculated as 2.18 and 4.5 Gy for the samples exposed to 2 and 5 Gy accident doses, respectively.

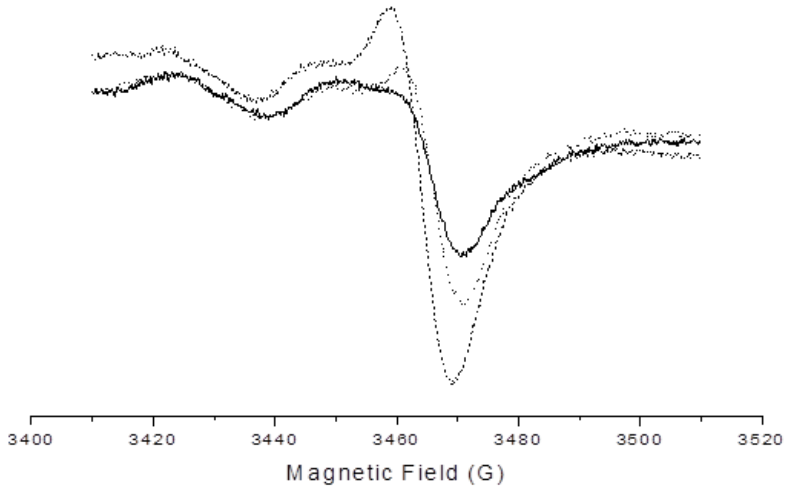

Figure 7. ESR spectra of fingernails at 10 Gy (dot line) and 0 Gy (solid line) added dose after irradiated 2 Gy (dash line)

Table 1. Results of dose reconstruction for samples exposure to 2 and 5 Gy and meausred at different times after exposure.

Dose, Gy	Fading time, days	Reconstructed dose, Gy	Mean,Gy	Mean all days,Gy
2	0,125	2.0;2.07;2.96;3.06;1.78	2.37	2.18
	1	2.51; 2.1; 1.8; 2.95; 1.5	2.17	
	5	2.77; 2.0; 1.0; 4.0; 3.85	2.72	
	7	1.87; 1.55; 1.4; 1.07; 1.3	1.44	
5	0,125	5.2;.4,3; 4.7; 5.8; 4.1	4.82	4.50
	1	4.5; 5.2; 3.9; 5.7; 4.4	4.61	
	3	5.7; 4.2; 5.5; 3.9; 3.3	4.52	
	7	3.8; 4.2; 4.3; 3.5; 4.5	4.06	

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4. Discussion

Recently, a new international standard on ESR retrospective dosimetry from ionizing radiation has been published (ISO/FDIS 13304-1 2013). It covers in vitro nail dosimetry using nail clippings measured principally at X-band, but higher frequencies are also being considered: in vivo nail dosimetry with the measurements made at X-band on the intact finger or toe in addition to in vitro tooth enamel, in vivo tooth dosimetry and in vitro measurements of bone. In this sense, nail dosimetry research like this work provides the development of such standards.

In the present study, we tested three approaches on nail dosimetry; one based on the described protocol by Trompier et al. 2014a and the other used the classical additive dose method and the last based on the described protocol by Sholom and McKeever 2016. Since the aim is to investigate performance and limit of the protocols, the samples for two different "accident doses" were collected from the same donor. Fingernails exhibit some variability (mainly, but not only, on the dose saturation). Therefore, we have eliminated this variability on the results.

The results obtained from the IRSN approach showed that the dose saturation for the unexposed sample of first group was reached after having added dose of 15 Gy, whereas for exposed, the signal intensity decreased already for the first post-irradiation (Figure 2). It seems that the nails used have a dose saturation point about 15 Gy. That was why the signal intensity of sample pre-irradiated at 15 Gy decreased after the first dose addition, after it reaches a level for which even with added new dose, the intensity did not change any more. In the second run of IRSN approach, the difference between dose saturation points of exposed and unexposed samples was found at 10 Gy which was the same with the accident dose applied before post-irradiation. Thus, the accident doses given to the samples before starting post-irradiation were reached by applying the new approach to the nail samples. The post-irradiation in step of 5 Gy can be the reason of successful result. If the step was adopted smaller than 5 Gy, the dose saturation point can be determined more accurately, then the results can be devoted from accident doses. Moreover, the presented data indicate the saturation dose at approximately 15 Gy with 30 % lower ESR signal amplitude at dose saturation of the originally unexposed sample in comparison to the exposed sample. These are

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different from the results presented by Trompier et al. 2014a, in which ESR signal amplitude at dose saturation was approximately identical of the exposed and unexposed sample and dose saturation occurred at about 40 Gy. However, in other studies of the same authors supported the present results where signal amplitude of exposed sample at saturation dose was higher as 50 % than unexposed one (Trompier et al. 2014b). Romanyukya et al. 2014 reported a 25 % higher signal amplitude at same point for unexposed sample compare to exposed one and dose saturation occurred at about 27 Gy, which are different than thoses mentioned above. Also, it is reported that the dose saturation was seen at about 28-55 Gy in water treated nail samples (Trompier et al. 2014a), so the saturation dose found in the present study is out of range from that study. Thus, yet, in the related studies, there is no consistency on saturation dose points and on the rate of ESR signal amplitude of exposed and unexposed nail samples at that points. However, as for the nails of a same person, the RIS5 signal is supposed to saturate at an identical value of dose whatever the finger (Trompier et al. 2014a), so it can be deduced that our work supports this by observing saturation dose points above 15 Gy in the first run, and at 20 Gy in the second run for unexposed nail samples. It means that nail samples used in this work saturated at about 20 Gy.

This new approach in dosimetry is dedicated to the estimation of high doses (>10 Gy) and its applications can be found in Trompier et al. 2013 and Romanyukha et al. 2013. In order to determine lower doses by nail dosimetry, it is needed to differentiate RIS5 from BKG which has been discussed very well by Trompier et al. 2013. One way of differentiation of low doses from higher is to apply additional dose to RIS5 signal. Any increase in its intensity will indicate that the dose under evaluation is below the saturation dose. For this reason, to know the saturation dose exactly is important on which the dose estimation is based.

In classical approach, the ESR signal intensities increased with increasing added dose as seen in Figures 5 and 6. The experimentally measured ESR signal intensity values (y) were fitted well by a second order polynomial function. The extrapolated doses and calculated doses from fitting procedure were found to be 38.9 and 47.9 percent deviated from actual accident dose values of 10 and 15 Gy, respectively. In this approach, the nail samples were not soaked in water after each new irradiation except the first irradiation (data was not presented in Figures 5 and 6). It means that the slopes of the curves were determined by the

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signal intensity variation on BKG, RIS5 and other unstable RIS signals (RIS 1-4, Trompier et al. 2014a) and would contribute to the supposed larger RIS signal amplitude which can be a source of error and uncertainty. Thus, classical dose additive method was found unsuited for dose reconstruction.

In the Dartmouth protocol, the ESR signal intensities were recorded for unknown dose (Dx), plus MIS and BG, for 0 Gy and for 10 Gy. How to calculate the unknown dose was summarized in Figure 8 of the study of Sholom and McKeever 2016. The proposed dosimetry technique was applied to the samples irradiated in the laboratory in order to simulate the accident doses by accounting for other ESR signals (MIS and BG), which overlap with RIS. Our results were demonstrated that the method has the ability to recover the doses in the region 0-5 Gy with a mean estimate of ~0.35 Gy. In that study, the authors reconstructed the doses in the region 0-10 Gy with a mean estimate of uncertainity of ~0.5 Gy over the entire dose range tested (Sholom & McKeever 2016).

Consequently, this work was aimed to compare the protocols to measure radiation dose in the nail sample from individuals with unplanned exposure to ionizing radiation. IRSN protocol using the RIS5 component and Dartmouth protocol were found to be successful methods for the evaluation of dose to fingernails exposed high-doses. However, classical approach using center field ESR signal given extrapolated doses in error of 38.9 and 47.9 percent was found to be unsuccessful. On the other hand, IRSN method adds valuable time to the sample analysis due to the need for successive soaking/drying of the nail samples during the serial irradiation spectral acquisition sequence. This makes the method much less amenable to conducting retrospective dose estimation in a mass radiological exposure event. Further studies should be planned to test the IRSN and Dartmouth approaches for lower accident doses on more samples from different individuals, taking attention to get results in short time.

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Declaration of interest

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The authors report no conflicts of interest.

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