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Nuclear Instruments and Methods in Physics Research B 184 (2001) 219–228

NIM B
Beam Interactions with Materials & Atoms

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Electron paramagnetic resonance (EPR) biodosimetry

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Received 2 January 2001; received in revised form 29 March 2001

Abstract

Radiation-induced electron paramagnetic resonance (EPR) signals were first reported by Gordy et al. [Proc. Natl. Acad. Sci. USA 41 (1955) 983]. The application of EPR spectroscopy to ionizing radiation dosimetry was later proposed by Brady et al. [Health Phys. 15 (1968) 43]. Since that time EPR dosimetry has been applied to accident and epidemiologic dose reconstruction, radiation therapy, food irradiation, quality assurance programs and archaeological dating. Materials that have been studied include bone, tooth enamel, alanine and quartz. This review paper presents the fundamentals and applications of EPR biodosimetry. Detailed information regarding sample collection and preparation, EPR measurements, dose reconstruction, and data analysis and interpretation will be reviewed for tooth enamel. Examples of EPR biodosimetry application in accidental overexposures, radiopharmaceutical dose assessment and retrospective epidemiologic studies will also be presented. © 2001 Elsevier Science B.V. All rights reserved.

PACS: 87.64.H
Keywords: EPR biophysics; Ionizing radiation; Biodosimetry; Bone; Tooth

1. Introduction

In 1921 Stern and Gerlach showed that an atom with a net electron magnetic moment can take up only discrete orientations in a magnetic field [1]. In 1925 Uhlenbeck and Goudsmit proposed a new intrinsic property for the electron in addition to mass and charge [1]. In order to explain the behavior of alkali atom spectra in a magnetic field they suggested that the electron must have a magnetic moment and they linked this with electron spin.

Electron paramagnetic resonance (EPR), also know as electron spin resonance (ESR) can be defined as resonant absorption of microwave energy in paramagnetic species by transition of the spin of an unpaired electron from one energy level to the next in the presence of a strong magnetic field. The first EPR experiment was conducted in 1944 when Zavoisky [2] detected a peak in the paramagnetic absorption from $CuCl_2 \cdot 2H_2O$.

In the absence of an applied magnetic field, unpaired electrons of paramagnetic species can occupy either of two spin states ($m_s =$

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0168-583X/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0168-583X(01)00614-0

$+1/2, -1/2$). However, in the presence of a strong magnetic field, one of these two states becomes more energetically favored. A greater number of spins are found in the lower state. Simultaneous application of electromagnetic radiation of appropriate frequency (typically >2 GHz) corresponding to the energy difference between spin states cause a spin–flip transition to the higher energy state [1]. Absorption of the applied electromagnetic radiation is detected by an EPR spectrometer, and after appropriate amplification, is displayed as the first or second derivative of the absorption curve with respect to the applied magnetic field (Fig. 1). Absorption resonance spectra are characterized by their shape, width, intensity, and spectroscopic splitting factor, or *g*-factor.

In 1955, Gordy et al. [3] were the first to publish data on EPR signals in irradiated skull bone. Ionizing radiation interacts with mineralized tissues to produce dose-dependent concentrations of long-lived paramagnetic centers. As a result, the tissue is the dosimeter, and the calibration can be regarded as absolute.

Brady et al. [4] suggested using EPR dosimetry and the additive re-irradiation method to obtain dose estimates from accidental overexposures. EPR dosimetry of irradiated mineralized tissue was proposed and validated by Desrosiers et al. [5,6] as a quantitative method to measure the absorbed dose from bone-seeking radiopharmaceuticals. Desrosiers [7] and Schauer et al. [8] applied this method to the dosimetry of accidental radiation overexposures in San Salvador ^{60}Co and Gaithersburg, MD (3 MeV electrons), respectively.

The present paper builds on a previous publication by Desrosiers and Romanyukha [9]; revisions have been made to reflect the current issues and state-of-the-art.

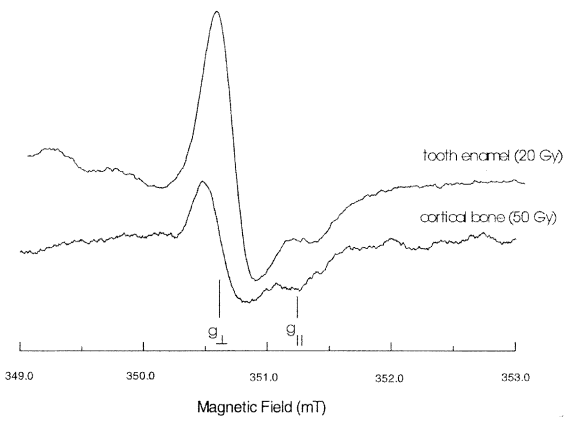

Fig. 1. First derivative of the absorption curve (arbitrary units) with respect to the applied magnetic field (mT) for a human femur (50 Gy) and bovine tooth enamel (20 Gy) irradiated with ^{60}Co gamma rays. The signal of interest, $g_\perp$ (2.0018) is derived from the hydroxyapatite in bone or teeth.

2. EPR fundamentals

EPR is a non-destructive method sensitive to materials containing unpaired electrons (i.e., produced by the absorption of ionizing radiation). When paramagnetic materials are placed in a strong magnetic field, the absorption of applied microwave energy effects electron spin–flip transitions. The intensity of these transitions is proportional to the number of unpaired spins in the material, which is proportional to the absorbed dose. In addition, by varying the magnetic field, radical centers with different structures and environments are spectroscopically resolvable.

The method of retrospective EPR dosimetry using calcified tissues (bone, enamel, dentin) is based on the measurement of radiation-induced radicals in hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$. During the mineralization process of biological hydroxyapatites, carbonate ions are incorporated into the crystalline lattice substituting for both phosphate and hydroxyl ions. Upon absorption of ionizing energy by the hydroxyapatite crystal, the carbonate ions capture free electrons in the crystal matrix to form free-radical centers [10]. The dose-dependent formation of carbonate radical centers can be quantified through the use of EPR.

Hydroxyapatite constitutes 95–97% of tooth enamel, 70–75% of dentin, and 60–70% of bones. The predominance of hydroxyapatite along with its high degree of crystallinity makes tooth enamel the most suitable material for retrospective dosimetry. Human tooth enamel is a calcified tissue with several special features. Acellular in its adult state, tooth enamel is composed of hydroxyapatite

crystallites, which can be up to several hundred nanometers in length. The concentration of radiation-induced radicals, and hence the intensity of the EPR signal, increases proportionally with the absorbed dose from about 100 mGy to above 10 kGy. There are no known dose rate effects. The carbonate radical center is extraordinarily stable with a calculated lifetime at 25°C of 10^7 years [11]. Free-radical centers in tooth enamel are produced by a wide variety of ionizing radiations, including X-rays, gammas, betas, alphas, protons, and heavy ions [12–22]. Unfortunately, there is no published information on neutron interactions with tooth enamel.

3. EPR dosimetry essentials

The process of EPR dose reconstruction consists of several important steps:

- Sample collection.
- Sample preparation.
- EPR measurements.
- Dose reconstruction.
- Interpretation of results.

These steps are shown in greater detail in Fig. 2. Discussed below are several critical points for each step, which must either be carefully considered when applying the method, or are in need of clarification by further studies.

It should be noted that although the EPR properties of bone and dentin are very similar to those of enamel, they differ in the procedure of sample preparation [15,23]. The procedure described here is relevant only to tooth enamel.

4. Tooth sample collection

There are two critical points to consider when collecting samples for EPR dosimetry, the health and location of the tooth. Not all collected teeth are equally suitable for retrospective EPR dosimetry. Typically teeth are extracted for medical reasons. For some dental diseases the mineral content and carbonate concentration can be changed considerably [24]. Therefore, only the sound (healthy) part of tooth enamel should be selected for analysis. With regard to the suitability of the teeth depending on their location in the mouth, adult human teeth (altogether 32) can be separated into four groups: molars, premolars, canines and incisors. A special molar subgroup is presented by wisdom teeth. All these types of teeth are distinguished by shape, thickness of enamel layer, position in the mouth, growth period, and geometry. In the case of internal exposure there is also a difference in metabolism for the various types of teeth. Moreover, sunlight can also give an appreciable contribution to the measured dose in tooth enamel for the front teeth [14,19]. The contribution is reportedly as large as an equivalent of 200 mGy, however, definitive quantitative studies of these photochemical/photophysical effects have yet to be performed. In the interim, the preferred sites for sample collection are molars and premo-

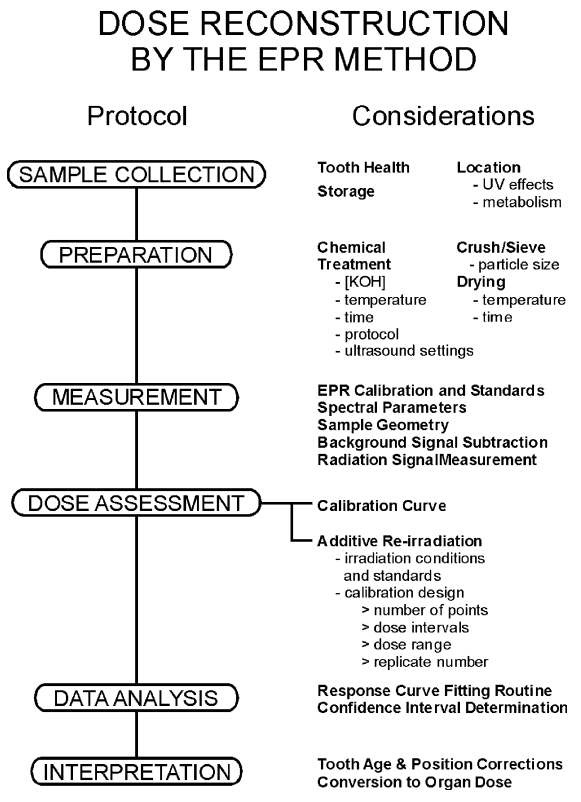

Fig. 2. Schematic of the EPR protocol for retrospective dose assessment. Protocol steps and associated considerations are cited.

lars, and for incisors and canines to use the inner side of tooth enamel.

5. Tooth sample preparation

The main aim of this phase of the procedure is to physically separate dentin from tooth enamel and remove any remaining organic material from the hydroxyapatite. It is necessary to thoroughly remove the organic material because its presence contributes a broad EPR signal, which can obscure measurement of the radiation-induced hydroxyapatite signal. For doses above 1 Gy, the organic contribution to the spectral intensity is relatively small and its contribution can either be removed artificially through software manipulations, or even ignored. However, the relative contributions of the two signals to the overall intensity reverse with decreasing dose such that the organic signal can totally or partially mask measurement of the weaker hydroxyapatite signal.

The following cautionary notes should be considered when applying one or more of the following types of sample treatment.

- When using dental drills and saws to mechanically separate tooth enamel from dentin, one is strongly cautioned not to overheat the sample since it can produce an interfering EPR signal [25]. The separation of enamel from dentin can also be achieved by the gravitation method based on the difference in their densities [24].
- The application of UV light to visually differentiate between enamel and dentin is also inadvisable because UV light produces an EPR signal in the hydroxyapatite that has EPR parameters very similar to the radiation-induced signal [14,19].
- Since the organic fraction of tooth enamel is bound very tightly, extensive ultrasonic treatment with a KOH-concentrated solution should be applied to remove the organic component [15,17].
- In order to minimize effects that arise from the orientation dependence of the EPR spectral intensity on the externally applied magnetic field, the tooth enamel should be crushed to small grains (0.3–0.5 mm). The precision of the EPR measurement will also be improved if the range of the grain size is kept to a minimum [26].

6. EPR measurements

Typical EPR recording conditions for tooth enamel measurements can be found in Chumak et al. [27]. The EPR spectrum of tooth enamel is usually interpreted in terms of two main components. The first is a broad background signal that is largely due to the presence of organic radicals. However, a correlation with defects in the crystal structure of hydroxyapatite cannot be discounted; this signal can be reduced by chemical treatment of the samples, but a weak background signal is always observable. The second component in the tooth enamel EPR spectrum is radiation induced. Details of the spectroscopic parameters and studies on the origin of this signal can be found elsewhere [10].

For absorbed doses lower than 200 mGy the broad background signal obscures the radiation-induced signal. Therefore, it is necessary to subtract the broad signal from the total spectrum. Historically, two different approaches have been used for the solution of this problem: computer simulation of background or radiation-induced signals [28–30] and the subtraction of a background spectrum from a representative pool of non-irradiated tooth samples [31]. An alternative approach based on signal-selective microwave saturation has also been introduced [32]. The microwave power dependencies on the EPR intensity differ for the two main components of the spectrum. In this latter approach, the resultant EPR intensity is derived from the difference of two spectra recorded at two different microwave powers for the same tooth enamel sample. This results in a factor of 10 improvement in the resolution of EPR spectrum, a reduction of the signal-to-noise after subtraction by a factor of 2, and a reduction in the minimum detectable dose to about 100 mGy.

Recently, an important advancement has led to a breakthrough in the minimum detectable dose. The sensitivity of EPR instruments has been increased by one order of magnitude due to new

designs of their critical components [33]. This new advancement was employed to test its applicability to EPR tooth enamel retrospective dosimetry. These tests determined 29 mGy to be the lower limit of detection for EPR tooth dosimetry [34]. Additional incremental improvements in this limit are projected.

7. EPR dose reconstruction

Two methods have been used to assess the absorbed dose of irradiated enamel by EPR: additive re-irradiation and the use of a calibration curve. In the additive re-irradiation method, the sample is incrementally irradiated to construct a response curve specific to the sample in question (Fig. 3). The method typically requires 4–5 additional dose increments (see [35] for guidelines). The other method uses a "universal" calibration curve (EPR signal intensity versus absorbed dose) generated using a large blended sample pool of enamel material designed to average the sample-to-sample variances. For doses greater than a few hundred mGy, the variation in EPR signal intensity from sample to sample for tooth enamel is ≈10% (Desrosiers, unpublished results). Dose reconstruction using the "universal" calibration curve

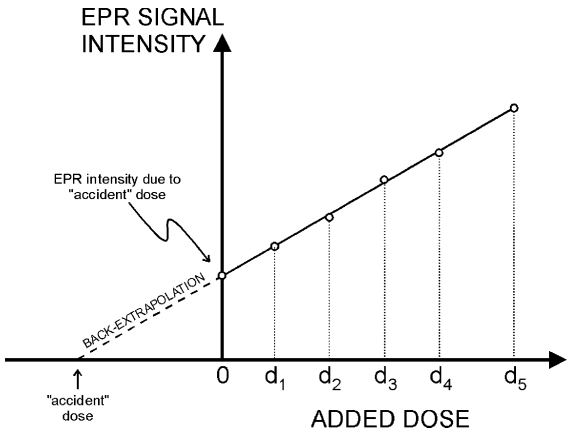

Fig. 3. The additive dose method for dose reconstruction is based on the re-irradiation (d_1–d_5) of a tooth sample to obtain a sample-specific dose response curve, which is used to back-extrapolate to the absorbed dose value.

method is much less time-consuming and is non-destructive. However, the method and its associated uncertainties have not been fully validated.

The expansion of EPR dosimetry into the area of retrospective dose assessment requires a clear understanding of the energy response characteristics of bone and tooth. If dose to bone or tooth enamel is the desired quantity, then with proper application of the relevant dosimetric quantities, the system is absolute. An absolute system is one in which the tissue of interest is also the dosimeter. However, since the desired quantity in most epidemiologic studies is tissue dose, bone and tooth enamel serve as relative response dosimeters. An integral part of any relative response dosimetry system is the dosimeter response as a function of incident energy.

The early works aimed at measuring the energy response of hydroxyapatite were controversial. In 1974, Stachowicz et al. [36] reported an increase of up to a factor of 2 with decreasing photon energy, for a given absorbed. They compared ^{60}Co gamma rays (average photon energy of 1250 keV) with 250 kV (0.4 Sn + 0.2 Cu + 1.0 Al) X-rays, and reported that the EPR signal intensity from the lower-energy radiation was approximately a factor of 2 higher than the ^{60}Co value. The authors attributed this observation to differences in initial stopping powers. In 1993, Copeland et al. [37] studied ovine cortical bone and reported that an increased signal intensity was observed at lower photon energies (160 kV, half-value layer (HVL) = 0.5 mm Cu) when compared to ^{60}Co, but of a much lower magnitude than Stachowicz et al. observed. These data introduced potential complications. If the EPR signal intensity was energy dependent, it could limit the application of this dosimetry method.

Later in 1993, Schauer et al. [38] reported that these previous EPR energy dependence results were due to errors in the dose estimated by the ionization method, rather than an energy dependence per se. This finding was based on a detailed review of the experimental design and methods. Specifically, Copeland et al. [37] used the ICRU10b [39] composition of compact bone, rather than the more current ICRU44 [12] composition in the conversion of exposure-to-

absorbed-dose, and that an equivalent photon energy derived from HVL measurements was used in place of spectrum-averaging [40]. The findings of Schauer et al. were later confirmed by de Oliveira et al. [41] for synthetic and biological hydroxyapatite.

The use of bone or tooth enamel as a relative response dosimeter to assess photon dose to soft tissue requires knowledge of:

1. photon energy at the sample location;
2. photon energy response characteristics of bone and tooth enamel.

The relative response approach has a well-established history in the use of "non-tissue equivalent" dosimeters (i.e., film and solid-state thermoluminescent dosimeters) to assess personnel dose. These conventional dosimetry systems employ multiple dosimeters and filters to perform crude photon spectrometry. An algorithm is used to determine the incident photon energy (typically by ratios of responses) and then an appropriate response function correction factor is applied.

Fig. 4 is a plot of the response (relative to air) for International Commission on Radiation Units and Measurements (ICRU) 44 soft tissue and some common TL and EPR dosimeters as a function of incident photon energy. The relative response of lithium fluoride (LiF) closely resembles the relative response of soft tissue and, therefore the correction factors are small. Conversely, the relative response of bone and enamel to soft tissue can be as large as 6.9 and 10.9, respectively. These increased responses represent the extremes of the corrections that may need to be applied when these materials are used to assess dose to soft tissue at photon energies less than 0.1 MeV.

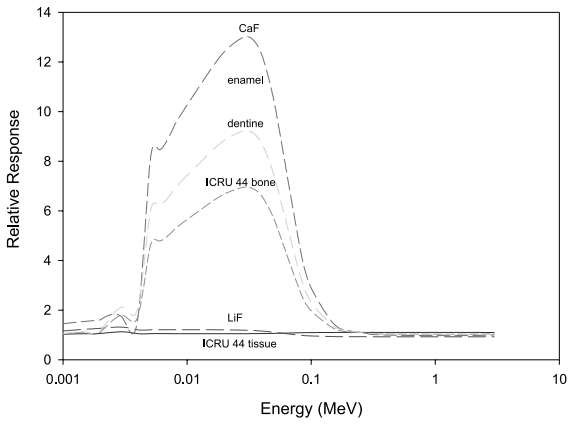

Fig. 4. Relative response curves for ICRU 44 soft tissue and some common TL and EPR dosimeters relative to air as a function of incident photon energy.

Actual correction factors will depend on the photon energy at the sample location. In the case of tooth enamel dosimetry, photon energy approximations may be made by comparing the anterior and posterior responses of teeth. However, this represents a very crude approximation and a priori knowledge of the photon energy is preferred.

Clearly, it is important to define the medium of interest and to perform all dose calculations accordingly. Future publications on this subject are encouraged to define the medium and to provide detailed descriptions of the experimental design. Large sources of error can arise from the selection of the X-ray irradiation geometry and the factors used to calculate the X-ray dose; some guidelines are given in the paper by Schauer et al. [42]. It should be noted that for bone tissue, one should refer to Schauer et al. [22].

It has been shown that (for a given sample mass) the EPR signal intensity decreases as the irradiation energy of X-rays [22] and electrons [42] decreases. This is expected, however, since at lower energies the penetrating ability of the ionizing radiation decreases. Thus, the EPR signal accurately maps the radiation energy deposition. Two-dimensional spatial EPR images dramatically demonstrate this effect (for electrons) [42].

8. Interpretation of EPR results

The main problem of the interpretation of EPR dose reconstruction in tooth enamel is how to relate the results to absorbed doses measured or calculated according to regulations and models of ICRU and ICRP. An advantage and disadvantage of EPR dosimetry with enamel is that one determines cumulative lifetime dose, that combines dose components from natural radioactive background, medical, occupational, accidental and

other sources of exposure [15]. Reconstruction of dose for internal exposure is complicated by absence of necessary models for conversion of dose absorbed by tooth enamel to the dose absorbed, for example, by skeleton or bone marrow. In the latter case, a comparative EPR study for different calcified tissues would produce important information about relation of doses absorbed in tooth enamel, dentin and bones and concentration of the radionuclide.

9. Attributes of the EPR tooth dosimetry method

The EPR-tooth dosimetry method is characterized by the following attributes:

- The dose dependence ranges from ≈30 mGy to ≈100 kGy.
- Although interfering signals (e.g., produced by UV) can occur, the EPR signal is specific to ionizing radiation.
- Rapid estimates are possible, with higher accuracy/precision conformation available within a few days.
- The lifetime of the radiation-induced EPR signal far exceeds the human life span.
- Determination of partial-body exposures is possible.
- The method is applicable to fractionated and chronic exposures.
- Most radiation qualities are covered by the method.
- Absorbed doses due to internal emitters are measurable.
- The method is invasive (although there are efforts to develop new, non-invasive methods) [43].
- The measurement method is capable of being transferred from experts to the technical staff.

10. EPR applications

At present the three main areas where the EPR biodosimetry with calcified tissues has been successfully applied are medical radiation therapy; dose reconstruction for relatively small radiological accidents for health and safety reasons; large-scale dose reconstruction for epidemiological investigations.

The applicability of EPR at medical therapy dose levels (approximately 6–60 Gy) was demonstrated for internally administered radiopharmaceuticals [5,6,44]. Dose maps were generated using EPR for bone tissue treated with beta-emitters for bone marrow ablation. Measured results yielded good agreement with calculations performed according to the medical internal radiation dose (MIRD) schema. MIRD is the most widely accepted method for estimating the internal radiation dose from radionuclides used in nuclear medicine.

Some small-scale radiological accident reconstructions have used bone tissue to assess the absorbed dose of exposed individuals [7,8,29]. Schauer et al. [8] performed a dose reconstruction using conventional dosimetry systems (alanine and radiochromic dye). The dose rate data obtained from these measurements, combined with the total dose results obtained from EPR analysis of amputated bones provided a detailed view of the relevant dosimetry aspects of the overexposure.

Early work on the use of tooth enamel to assess the dose of Japanese A-bomb survivors is detailed by Ikeya [45]. The Chernobyl accident renewed the interest in developing dose reconstruction methods. Applicability of EPR retrospective dosimetry to dose reconstruction after the Chernobyl accident both for the non-occupational population of the contaminated areas and the clean-up workers (or "liquidators") has been extensively considered in the literature [28,32,46,47]. Another case of overexposure of large groups of population occurred because of environmental releases into the Techa river from the Russian industrial nuclear facility called Mayak in the Southern Urals [16–18,21,48,49]. These studies were not only able to reconstruct the total dose, but obtained separate assessments for three different components (background, internal and external exposures) of the total lifetime accumulated dose. More recent investigations of the Mayak incident demonstrated the importance of introducing corrections to the determined dose. Since these teeth had a large dose contribution from internally deposited strontium-90, it was found that the tooth position and its

associated growth period had strong influences on the assessed dose [48]. A set of correction factors was introduced to account for this effect. Also, since the teeth were irradiated from internal emitters, the tooth geometry also influenced the dose. Monte Carlo methods were used to further correct the assessed dose [49].

In this most recent study of teeth from Techa residents [48], some ultra-high doses were measured; moreover, in some cases adjacent teeth differed by a factor of ten. These ultra-high doses were determined to be an anomaly attributed to enamel that was being formed during the period of release. These teeth incorporated strontium-90 directly into the enamel. This observation unveils an added feature of EPR biodosimetry. Since enamel tissue formed during radionuclide releases exhibit anomalously high doses, one can target specific teeth from specific residents to use this feature to identify the time of release. This allows investigators to avoid having to unnecessarily measure large numbers of tooth samples, but to select and measure a subset of the biological samples to confirm the timing of the radiological incident.

EPR dosimetry can be applied to large populations for epidemiological studies and assessment of risk coefficients. The key issue for these studies is to properly develop the main principals of design and to discuss the methods of analysis for the resulting data. The design of a wide-scale EPR dose reconstruction study should contain the following elements:

1. preliminary evaluation of the primary sources contributing to the dose received by members of the population of interest;
2. selection of different groups from the population based on the dominant dose contribution;
3. comparative analysis of the results of dose reconstruction for selected groups with additional dosimetric information;
4. identification and quantitation of the different sources contributing to the overall dose.

11. Current and future tasks

The International Atomic Energy Agency has stated that attention should be directed to the development of established, harmonized methodologies and appropriate calibration information for retrospective dosimetry techniques [50,51]. The plan below is offered as a guide to advance EPR biodosimetry as a reliable method for epidemiological studies of exposed populations:

- Dental tissue dose assessment: enamel and dentin can be used to assess radiation absorbed doses from external and internal sources.
  - Develop and validate standard protocols: validation and standardization of protocols will lead to reliable data for epidemiological studies that are universally accepted.
    - Enamel dosimetry: a protocol specific to healthy enamel tissue containing optimized steps and parameters, with a comprehensive uncertainty budget.
    - Dentin dosimetry: a protocol specific to healthy dentin tissue containing optimized steps and parameters, with a comprehensive uncertainty budget.
    - Protocol variances: develop special adaptations in cases where measurements must be made on dental tissues exposed to known interfering influences.
      - Sunlight or UV exposure: interfering EPR signals from light exposure should be minimized by preparation techniques or correction factors.
      - Dose inhomogeneity: develop models and correction factors for dental tissues that have a non-uniform dose deposition.
      - Mixed-field exposure: develop models and correction factors for dental tissues that occur with mixed exposures (α, β, γ, X).
      - Diseased tissue: preparation procedures for selecting and harvesting healthy tissue, and models and correction factors for measurements made on diseased tissues.
- Conversion methods for dental tissue dose to whole-body/organ dose: models for translating the dental tissue doses to whole body dose or doses in various tissues and organs (e.g., bone and marrow).

- Protocols for bone dose assessment: adapt protocols to direct measurements on bone tissue.

Completion of this plan is a daunting task requiring collaboration among several laboratories. This will require open, detailed reporting of experimental design, and their associated parameters. These efforts should culminate in the adoption of standardized methods achieved by consensus among the world experts. This was also a conclusion of the recently published second international intercomparison on EPR tooth dosimetry [52]. A cooperative approach will foster confidence in this powerful tool for radioepidemiology studies.

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