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# Potential effects of ionizing radiation on the evidentiary value of DNA, latent fingerprints, hair, and fibers: A comprehensive review and new results

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## Potential effects of ionizing radiation on the evidentiary value of DNA, latent fingerprints, hair, and fibers: A comprehensive review and new results



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#### ABSTRACT

An extensive literature review and new post-irradiation experimental results are presented of genotyping blood stains and hair, and physical examinations of latent fingerprints, hairs, and fibers. Results indicate that successful development of nuclear short tandem repeat (STR) and mitochondrial DNA sequence profiles from human blood and hair evidence is possible—up to a point—following exposure to gamma, neutron, beta, and alpha radiation at several levels that would most likely be present at this type of crime scene (i.e., a "dirty bomb," etc.). Commencing at gamma radiation levels between 90 and 900 kGy, DNA analysis using conventional DNA techniques was unsuccessful. In general, irradiation negatively affected the quality of latent fingerprints. All four radiation types degraded most fingerprint samples at all doses; nevertheless, many fingerprints remained of value for potential use in comparison. Although variable from one hair to another, microscopic changes observed for all types and levels of irradiation could potentially result in false exclusions. Negligible microscopic changes were observed in papers and fibers (used as substrates for fingerprints and bloodstains) up to 90 kGy gamma, but fluorescence of fibers began to change above that dose. Paper and fibers, as well as plastic evidence enclosures, became extremely brittle leading to breakage after a gamma dose of 900 kGy.

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#### 1. Introduction

The use of radioactive materials for terrorism is of great concern to our national security. When crimes occur that involve radioactive material, the evidentiary value of samples that have been exposed to radioactivity needs to be tested and forensically examined to ensure results of these validated forensic topic areas remain suitable for intelligence gathering and/or criminal prosecution. This paper provides an extensive review of previous studies on materials of forensic interest as well as new data. Both the

https://doi.org/10.1016/j.forsciint.2018.01.012 0379-0738/Published by Elsevier B.V. newly acquired and previously reported results are described below and summarized in Table 1.

The production of a device containing radiological dispersal technology (RDD or "dirty bomb") for criminal purposes is of significant concern to law enforcement officials [1–7]. Between 1993 and 2013, there were 2477 confirmed incidents of unauthorized possession, theft, loss, or other unauthorized activities or events involving nuclear and other radioactive material [8]. A 2006 undercover investigation by the U.S. Government Accountability Office revealed that shortcomings in the Nuclear Regulatory Commission licensing process allowed investigators to procure radioactive materials in quantities that would be sufficient to construct an RDD [9]. Unfortunately, vulnerabilities remain [10]. Consideration must be taken as to how the overall scene and individual items exposed to radioactivity from such a crime could

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#### Table 1

Reported effects of ionizing radiation on materials of evidentiary interest.

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<sup>a</sup> Dose was that used for sterilization of U.S. Mail; value inferred from [34].

be processed in situ or decontaminated [11–15]. The intersection of forensic and radiological sciences must be characterized well in advance of any attempt to bring forth such testimony in a criminal proceeding. In traditional forensics, some of the most probative evidentiary material comes from DNA (mitochondrial and nuclear) and latent fingerprints obtained from a crime scene. The probative value associated with the characterization of the radioactive material and nuclear forensics has been thoroughly covered elsewhere [16-19]. In both research and development and commercial applications involving the radiological sciences the management of contamination and radiation exposure is of the utmost concern. Where forensic evidence and radiation exposure meet, the concerns for evidence stability and material functionality become an additional priority. Physical effects of radiation may include yellowing, embrittlement, and increase in temperature with resultant distortion of some materials [20-22]. Tables of radiation tolerance for various materials are available [23-27].

In the current empirical study, a multifaceted approach was taken to determine the effects of radiation on representative items such as would be obtained from crimes of a radiological nature. Research was conducted to determine whether DNA, latent fingerprints, and hairs would maintain stability and evidentiary value after exposure to various sources of radioactivity. Also exposed were the paper index cards used for mounting hairs for exposure, photocopy paper on which latent fingerprints were deposited, and blood-stained cotton fibers. The extent and type of radiation exposure that evidence can sustain while still yielding DNA profiles, latent fingerprints of value for comparison or identification, and comparison of hairs and fibers are important information to support collection activities at a crime scene. These data will allow personnel to make a more informed decision as to whether the hazards faced by evidence collection personnel outweigh the benefits gained by collecting the evidence. Hair, blood stains, and latent print samples were irradiated by an array of sources at Savannah River National Laboratory (SRNL), with each sample receiving four separate irradiation treatments. Alpha, beta, gamma, and neutron irradiation treatments were defined and conducted. Post-irradiation testing at the FBI Laboratory involved initial visual inspection and traditional forensic examinations.

#### 2. Background

#### 2.1. Types of ionizing radiation

Gamma rays are produced by radioactive emission from an element such as cobalt-60 (<sup>60</sup>Co). X-rays are the same as gamma rays, except that they are generated by directing an electron beam at an X-ray converter target. Gamma rays are highly energetic and highly penetrating compared to X-rays which are lower in energy. Since exposure to gamma rays produces no significant temperature change, they are used to sterilize thermosensitive items such as solid-phase drugs [28]. They are also extensively used at varying dosage levels in commercial processing of polymers, including fibers [24] and for food processing [29,30].

Beta particles are free electrons that travel several feet in air and can penetrate the skin. Beta particles are emitted by certain radioactive nuclei, such as potassium-40. Particles generated by an electron gun (e-beam) are the same species as beta particles and are also used for sterilization of food and medical products, although their penetration depth ( $\sim$ 3 cm) is much less than gamma rays [31–33]. The U.S. Postal Service sterilizes mail using e-beam irradiation of 56 kGy [34]. The chain scissioning that beta irradiation induces is also used to intentionally crosslink or degrade polymers [35,36].

Alpha particles are emitted from the nucleus of several unstable elements by radioactive decay. They are identical to a helium nucleus and have a charge of +2. Alpha particles have a very short range (<0.1 mm in tissue) due to their relatively large mass (>7000 times that of the beta particle).

Neutrons are highly energetic and highly penetrating particles. Exposure of certain material to neutrons can result in the production of radionuclides. The effect of thermal neutrons (energy < 0.5 eV) on organic materials of forensic interest has received scant study. The bulk of the literature on the effects of neutron irradiation focuses either on health [37-39] or on structural and engineering materials of importance to the aerospace and nuclear power industries [40,41]. Related information may be gleaned from the literature on neutron activation analysis (NAA). For NAA, a steady-state neutron flux of 10<sup>11</sup>- $10^{13}$  neutrons cm<sup>-2</sup> s<sup>-1</sup> is typical [42,43] but physical modification of the specimen is of little concern, since the goal is limited to accurate trace element analysis. During exposure, the sample may experience temperatures of 70-90 °C [44]. One NAA procedure notes that, after a flux on the order of  $10^{18}$  neutrons cm<sup>-2</sup>, most organic samples (hair, paint, etc.) are so damaged that they must be dissolved before counting [45]. Embrittlement of hair, even disintegration into powder, has been noted during NAA [46], although not of particular concern when elemental analysis is the only goal.

#### 2.2. Potential effects of radiation on evidence: DNA

lonizing radiation damages materials by breaking chemical bonds and by forming reactive free radicals. The result is cross linking and/or chain scission. The damaging effects are often used advantageously for sterilization of food and medical products, as well as in a multitude of commercial applications, particularly involving polymers [47]. A dose of 25 kGy is generally accepted to provide a sterility assurance level of 10<sup>-6</sup> [33,48].<sup>2</sup> DNA is susceptible to lesions at numerous locations, with base damage and single-strand breaks most common [49]. Singleand double-strand breaks occur at random locations [50,51], and free radicals promote oxidation at various sites [49,52,53] and to lesser extent, crosslinking [54].

Previous studies report considerable variation in the radiation doses required either to initiate, or to cause complete degradation of, biomolecules and typical substrates (Table 1). Such variation in results is not unexpected by comparison to the complexity of radiation biology demonstrated by decades of research involving live cells and model systems. Even if the complexity of bio-repair mechanisms is not considered (being largely irrelevant in postexposure evidence recovery), there are multiple factors that affect degree of chemical change and dose response, including: degree of molecular hydration; hierarchy of molecules to lesions; hierarchy of sites within a molecule to lesions; scavenging, quenching, or sensitizing effects of other molecular species; ambient gas concentration.

Radiation doses have been investigated with the goal to reduce DNA contamination for PCR. A gamma dose of 4 kGy was reported sufficient to eliminate DNA contamination [55]. In another study conducted using 56 kGy [56], with respect to the 10 loci of the UK National DNA Database, 40% resulted in a full profile, 30% yielding at least four loci, and 30% less than four. Due to the typeable DNA remaining, both gamma and beta irradiation were judged less effective than ethylene oxide for decontamination. In another decontamination study DNA recovery from blood and paper was approximately 5% after a gamma dose of 5 kGy (no DNA typing was reported) [57]. The range of gamma doses used was nominally 0.5, 1, 2, 3, 5, and 10 kGy. Bacillus thuringiensis spores were effectively decontaminated from paper, glass, and plastic after a gamma dose of >3 kGy. Following a gamma dose of 10 kGy to inactivate suspected HIV contamination, there was no detrimental effect on subsequent serological examination of liquid or dried blood, semen, or saliva [58].

Abbondante [59] studied the effect of alpha and gamma irradiation on nuclear short tandem repeat (STR) DNA profiling of blood, saliva, bone, and genomic human control samples. With gamma irradiation, degradation was first observed at 1 kGy, but full profiles were obtained up to at least 10 kGy. DNA profiles were partially lost after 100 kGy and disappeared after 500 kGy. Alpha irradiation induced degradation of DNA profiles at 66 kGy. Because alpha particles are readily absorbed, the threshold of DNA destruction was matrix dependent. For both, the higher molecular weight loci were progressively lost as dosage increased. There was suggestion of possible reduction in extraction yield as the interval between irradiation and analysis increased from one day to four weeks. In contrast to the Abbondante study, complete degradation of DNA extracts on FTA paper was reported to occur at a dose of 91 kGy gamma, albeit with a concomitant temperature exposure of 50 °C for 20–30 min [59]. Another study reported loss of longer STR amplicons commencing after gamma exposure of only 0.2 kGy, noting greater vulnerability of certain tissues [60].

A baccalaureate thesis investigated the possibility of using a commercial DNA repair kit to mitigate the effects of irradiation [61]. Using gamma irradiation doses of 1–250 kGy, DNA concentration declined steadily (by approximately 3 orders of magnitude), as did allele counts. Progressive dropout of longer alleles began at 10 kGy; after 100 kGy only three of the 21 alleles remained detectable. Genotypes improved with use of the repair kit, although non-reportable and non-detectable alleles remained. In

 $<sup>^2</sup>$  For reference, a whole-body dose of 0.6–1 Gy is fatal 100% of the time for humans. The radiation levels in the worst areas of the Chernobyl site are estimated at 200 Gy  $h^{-1}$ .

a study of the effect of X-ray screening associated with shipping of DNA samples, use of a preservative with extracted DNA on 903 paper was noted to have a possible salutary, but definitely not detrimental, effect on subsequent typing [62].

Radiation-induced degradation of DNA in solution is several orders of magnitude greater than in the solid state [63]. This "indirect" effect is attributable to the production of highly reactive free radicals generated by water hydrolysis [28,49,53,64,65]. Thus, if crime scene blood was irradiated while in a liquid state and is collected after having dried, its degradation may exceed expectations based on experiments using blood or saliva stains. Fragment size-dependent degradation following gamma irradiation was observed for aqueous DNA, reporting degradation with a dose of 1 kGy [65].

Complete nuclear and mitochondrial DNA profiles were successfully developed from saliva on envelopes that were exposed to 29 and 52 kGy from an e-beam [66]. Differential amplification of the shorter loci indicated that some degradation of larger fragments had occurred. Using beta (e-beam) irradiation of 50 kGy, 70% gave full profiles, 27% yielded at least four loci, and 3% no profile, relative to the 10 loci of the UK National DNA Database [56]. To study the effects of e-beam irradiation of U.S. Mail, buccal swabs were exposed to 56 kGy [67]. No effect was noted on single nucleotide polymorphisms or "SNPs" (112–359 base pairs, bp), but the yield and quality of longer STRs was reduced.

#### 2.3. Potential effects of radiation on evidence: fingerprints

For successful development of latent fingerprints, irradiation effects on amino acids, proteins, and lipids are of interest [68]. Free amino acids and those in proteins are highly susceptible to oxidation leading to various chemical modifications including peptide bond cleavage [69]. Certain sites are more susceptible to scission along the polypeptide chain [70]. Main-chain cleavage is the major reaction mode in the radiolysis of peptides, resulting in mixed di-amino acid derivatives not normally found in plants or animals [71]. In a study of insulin in solution, the amino acids leucine, lysine, and arginine were destroyed with a gamma dose of 40 kGy, with amino acids cysteine, tyrosine, phenylalanine, proline, and histidine also being very radio sensitive [72]. Although fatty acid composition of saturated fats was unaffected by 2-10 kGy, that gamma dose destroyed 98% of the composition of unsaturated fats, the latter destruction increasing with storage time and temperature [73].

One study exposed fingerprints on porous and non-porous substrates to high gamma doses ranging from 1 to 1000 kGy [74]. Ridge definition was preserved on glass and aluminum substrates. On polyethylene, polystyrene, and paper, ridge detail was preserved up to 100 kGy, after which there was progressive deterioration. Glass and paper were susceptible to discoloration that adversely affected print contrast. Radiation damage of plastics adversely affected dye uptake by the fingerprints. Unfortunately, individual fingerprints were not split into irradiated/untreated halves, so that the ability to draw definitive conclusions is limited. A subsequent study [57] found no adverse effect on recovery of fingerprints on porous and non-porous materials for gamma doses up to 40 kGy (the highest dose tested). One study even showed that, for fingerprints less than 2 weeks old, there was an increase in characteristic points of about 50% after a low (unspecified) gamma dose [75]. Ristova et al. [76] reported a salutary effect of low-dose irradiation on the effectiveness of cyanoacylate fuming of aged sebaceous fingerprints. Irradiation by approximately 10 Gy gamma,  $2 \times 10^5$  neutrons cm<sup>-2</sup>, or  $0.2 \text{ W/m}^2$  UV resulted in a 20–30% increase in average minutiae count of fingerprints developed 2-16 days after deposition (lack of full particulars precludes accurate estimation of X-ray exposure).

Another study focused on the effects of 56 kGy e-beam irradiation, used to sterilize the U.S. Mail, specifically on the ability to visualize latent fingerprints using a variety of visualization reagents on porous and non-porous substrates [77]. They reported significant degradation of quantity and quality of friction ridge detail for 14 standard development reagents. Only physical developer and multi-metal deposition produced results comparable to untreated fingerprints. Variations were noted among substrate type and donors.

#### 2.4. Potential effects of radiation on evidence: fibers and hair

Depolymerization and oxidation of cotton-cellulose begins at a 15 kGy gamma dose [78]. Weakening of natural fibers begins to be noticeable above 15 kGy gamma, increasing progressively with dose [79–81]. Surface damage of cotton fibers, including cracks attributed to loss of interfibrillar bonding, was reported after a gamma dose of 100 kGy, becoming more severe with increasing dose [78,81,82]. In terms of mechanical properties, however, the effects of gamma irradiation of 25 and 50 kGy on cotton product durability "may be somewhere between significant and catastrophic" [81,p. 206]. Gamma and e-beam irradiation of equivalent energies produce equivalent changes in physical properties of fibers [83]; thermal neutrons and gamma rays are also equivalent in their effects [84–86]. Though all are cellulose-based fibers, acetate, rayon, and cotton, in decreasing order of stability, are susceptible to radiation damage [25,84].

Radiation induces the formation of carbonyl and carboxyl groups [78.83.87], both of which indicate chain cleavage. Beginning at 25 kGy gamma irradiation, and steadily increasing with dose, there were significant changes in the FTIR absorbance spectra of cotton-cellulose fibers, with a notable increase in the characteristic carbonyl region, 1730–1750 cm<sup>-1</sup> [78]. Contrastingly, Van der Sluijs and Church [81] saw no change in FTIR carbonyl absorbance with 74 kGy gamma. Takács et al. [78] ascribed observed increasing absorbance in the spectral regions that are representative of O—H stretching [88] to increased intermolecular hydrogen bonding at the expense of intramolecular hydrogen bridges. Molecular degradation is further supported by reports of radiation-induced depolymerization and lower molecular weight [78,81,83] and decreased paracrystalline regularity [81,82]. For neutron exposure of cotton, onset of fibrillation has been reported at a dose of  $10^{11}$  neutrons cm<sup>-2</sup> [89].

Fading, discoloration, and changes in microscopic appearance were reported for synthetic fibers irradiated by gamma >10 kGy and natural fibers >50 kGy [25,89,90]. Forensic examination was deemed feasible, nevertheless [90,91]. Among several synthetic fibers, aromatic polyamide (Nomex), polyester (Dacron), and polyamide (Nylon), in decreasing order of stability, are progressively more susceptible to radiation damage [25]. Axially aligned surface scratches were observed in nylon-6,12 fibers exposed to 15 kGy gamma, increasing with dose [92–94]. Infrared spectra showed no new species, but peaks became sharper with increasing dose. The authors attributed both morphological and spectral changes to increased fiber crystallinity.

#### 2.5. Potential effects of radiation on evidence: paper

The Smithsonian Institution strongly discourages mailing of vulnerable museum specimens, citing multiple adverse irradiation effects on living specimens and a wide variety of natural and synthetic materials, including cellulosic materials such as paper, which begins to show damage above 2 kGy [22]. Various studies have reported onset of significant effects on physico-chemical properties of paper for gamma exposures occurring between 7 and 15 kGy [78,80,81,95–100]. Recent recommendations for

decontamination of documents without ill effects are in the range of 4–7 kGy [101,102]. Using a dose of 18 kGy on papers of various compositions, Flores [103] found evidence of macromolecular chain degradation and decreased tear resistance, being more pronounced for papers with higher wood pulp content. Measurements of copy paper following gamma irradiation of 25 and 50 kGy showed decreasing reflectance, primarily in the blue region of the visible spectrum [99]. Ultraviolet fluorescence of paper was significantly altered after e-beam irradiation as used to sterilize the U.S. Mail, but inks were unaffected [104].

#### 2.6. Potential effects of radiation on evidence: electronic materials

Electronic devices are particularly susceptible to radiation effects. Irradiation is damaging to electronic devices, causing displacement of lattice atoms and ionization, both of which are highly detrimental to semiconductor performance [105–107]. Engineering guidelines call for serious hardening of integrated circuits that will be exposed to 0.1 kGy [108]. Onset of failure is also strongly dependent on dose rate [105]. In a recent study, data recovery from CMOS memory was compromised at 0.25 kGy gamma, becoming unrecoverable by 1.5 kGy [109]. Another study noted the onset of damage at 0.9 kGy and 1.1 kGy for EEPROM and EPROM memory, respectively [110]. Indeed, assessment of magnetic damage has been proposed for forensic dosimetry [111].

#### 3. Materials and methods

#### 3.1. Preparation of Samples for Irradiation

Blood stains, hairs, and latent fingerprints were supplied by the FBI Laboratory for exposure to four different radiation sources at SRNL: alpha, beta, gamma, and neutron. Use of human samples was approved by the FBI Institutional Review Board.

For nuclear DNA testing, blood samples were collected into EDTA tubes from three volunteers (two males and one female). One blood drop was applied onto white cotton sheeting material and allowed to air dry (three stains per donor for each dose). For mitochondrial DNA testing, forcibly removed hair samples were collected from three volunteers. Five hairs from each individual were adhered on multiple index cards with transparent tape. One such card was prepared for each dose for all four radiation types and others as controls. No visible tissue was present on hair roots of selected hairs when observed under a stereomicroscope.

Groomed sebaceous and eccrine touch fingerprints [112] were deposited by a single individual on approximately  $1.5 \text{ cm} \times 5 \text{ cm}$  aluminum and photocopy paper substrates. Aluminum was chosen above other metallic materials to minimize neutron activation. Thumb or index finger fingerprints were made by triplicate serial impressions. Each latent fingerprint was divided, with half destined for irradiation at SRNL and the other half retained as a

comparison control. These controls experienced the same shipping and processing, but were not irradiated. Additional positive and negative fingerprint controls were also prepared, not to be shipped, but processed.

The number of samples tested addresses several considerations: to provide reasonable confidence in the results by using replicate treatments and controls; to minimize the cost of irradiation and subsequent sample processing; and to confront the severe limitation imposed by the size and number of samples that can be placed equidistant around a radioactive source so that all samples would receive the same dose. Triplicate measurements are commonly accepted in experimentation and quality control, particularly for their value in revealing outliers [113]. Thus, a treatment set consisted of five hairs or three blood stains from each of three individuals (not all of the hairs were ultimately sequenced). For fingerprints, a treatment set consisted of triplicate sebaceous and eccrine samples on each of the two substrates.

All treatment sets (index cards with attached hairs, blood stains on fabric, and fingerprints on aluminum and paper substrates) were prepared in sufficient number for subsequent irradiation at each dose for all four radiation types. For each type and level of irradiation, 15 hairs, 9 bloodstains, and 12 fingerprints were exposed. All samples were placed into individual zippered plastic bags, both to protect sample integrity and to identify for each one the radiation treatment intended and received. The bagging is typical for post-event containment.

Similar blood, hair, and fingerprint samples from each donor were prepared to remain at the FBI Laboratory; others were prepared for shipping to and from SRNL, but not to be irradiated, i.e., travel controls.

#### 3.2. Irradiation of samples

SRNL conducted the irradiation treatments on the materials using the following radiation sources: neutron, gamma, alpha, and beta. No sample received more than one radiation treatment. Dose levels were selected based on preliminary testing involving latent print stability in radiation fields (unpublished) and for evaluation of various evidence containment systems [114].

The source material for alpha irradiation was an aliquot of plutonium (<sup>239</sup>Pu and <sup>240</sup>Pu) that was affixed via flame mounting to a stainless steel planchet (Fig. 1a). Estimated alpha activity of the source plate was  $5 \times 10^7$  disintegrations per minute. Samples were irradiated in groups of three for increasing lengths of time to achieve doses of 0.12, 12, and 1200 kGy across the plane of the planchet, with each exposed directly to the attenuated source and at a distance of <0.1 mm. Hair samples had to be removed from tape and placed on aluminum foil during exposure because the tape, if left intact, would shield the alpha radiation.

The source material for beta irradiation was a sealed strontium-90/yttrium-90 ( $^{90}$ Sr/ $^{90}$ Y) source with a dose rate of 0.015 Gy h<sup>-1</sup>



Fig. 1. Irradiation sources: (a) alpha planchet; (b) beta <sup>90</sup>Sr/<sup>90</sup>Y; (c) dry <sup>60</sup>Co irradiator; (d) SRNL neutron activation analysis facility.

(Fig. 1b). Samples were exposed to doses 0.5, 1, 5, and 10 Gy. Total exposure for each sample was calculated based on duration of exposure.

A dry <sup>60</sup>Co Irradiator (J.L. Shepherd Model 484, San Fernando, CA) allowed for simultaneous *in situ* gamma irradiation of all sample materials without risk of contamination (Fig. 1c). The samples were housed in a 25 cm  $\times$  25 cm radiation chamber for the entire irradiation period. Dose rate of the irradiation was estimated to be 4.7 kGy h<sup>-1</sup>. Samples were exposed for periods of time to achieve six doses ranging from 0.5 to 9000 kGy.

Source material for neutron exposure was provided by the SRNL neutron activation analysis facility, which houses six doubly-encapsulated californium-252 (<sup>252</sup>Cf) pods totaling 20 mg of source material (Fig. 1d). Pods were submerged at a depth of 4 m in concentric tanks of deionized and heavy water, which moderates the thermal neutron flux. Samples were encapsulated inside high density polyethylene cylindrical containers (approximately 6 cm  $\times$  2 cm) to prevent contamination during exposure, resulting in some attenuation. Estimated thermal neutron flux during exposure was  $1.7 \times 10^7$  neutrons cm<sup>-2</sup> s<sup>-1</sup>. The exposures were completed in four time steps of increasing dose. Neutron fluence at each of the four increments ranged from  $1 \times 10^{10}$  to  $1 \times 10^{13}$  neutrons cm<sup>-2</sup>, with sample exposures ranging from 8 min to 15 days, corresponding to doses of 1.7, 17, 170, and 1700 Gy. Blood stains did not receive the highest neutron dose.

#### 3.3. Post-irradiation analysis

After irradiation, SRNL held the samples until declared safe and then returned them to the FBI Laboratory for latent fingerprint development and DNA processing—mitochondrial for hairs and nuclear for blood stains. Other than allowing time for any nuclear activation to decay, no radiological decontamination measures were used, although these may be indicated in many operational scenarios [115–118]. Following treatment, SRNL returned the samples to the FBI using containment bags furnished by the FBI.

Post-irradiation visual inspection of all specimens was conducted to note any gross physical property changes that may correlate with, or act as a precursor to, the deterioration of the functionality of sample materials. In addition, cotton fibers and paper (i.e. paper index cards and photocopy paper), as media on which biological samples were placed, also provided evidentiary materials for post-irradiation evaluation.

#### 3.4. Nuclear DNA processing and assessment

All processing followed procedures used in the FBI Laboratory for DNA case work [119]. Specimens approximately  $\sim 5 \text{ mm} \times 5 \text{ mm}$ were cut from each swatch. The samples were extracted with the Qiagen EZ1 Advanced XL BioRobot (Qiagen, Valencia, CA), eluted in 50 µl TE-4 buffer (Qiagen), then dried down via vacuum centrifugation and reconstituted in 15 µl TE-4 buffer. Quantification of total human and male DNA was assayed using the Quantifiler DUO kit (Life Technologies, Carlsbad, CA). Total human DNA of 1 ng was targeted in an AMPFISTR Identifiler Plus amplification at 27 cycles (Life Technologies). Samples were then injected on a 3130xl Genetic Analyzer and genotyped using GeneMapper ID software (both, Life Technologies). Typing was conducted for the 13 CODIS core loci [120], plus D2S1338, D19S433, and amelogenin (AMEL).

Changes attributable either to transportation conditions or to irradiation were assessed by comparing DNA quantity and DNA profiles of the travel controls and the irradiated samples to those obtained for the control samples retained at the FBI Laboratory.

#### 3.5. Mitochondrial DNA processing and assessment

Prior to subsequent processing for DNA, a microscopic inspection was performed of hairs. Inspection would also suggest radiologically-induced changes in base material properties that may potentially compromise their suitability for evidence examination. Three hair samples collected from one individual that were exposed to the highest radiation levels within each radiation treatment were extracted, hypervariable regions 1 and 2 were amplified using amplicon sizes of 275 bp, then sequenced following FBI DNA Casework Unit procedures in use at the time of analysis [121]. A single HL60 positive control sample (ATCC, Manassas, VA) and a single negative DNA control sample also were sequenced per radiation treatment. Individual 2 cm hair fragments were washed with xylene, water, and 5% Terg-a-zyme (Alconox, White Plains, NY), mechanically ground, then digested with proteinase K (AMRESCO, Solon, OH). DNA was extracted with phenol/chloroform/isoamyl alcohol (PCIA 25:24:1; Sigma, St. Louis, MO) and sequentially concentrated (Microcon 100; Merck KGaA, Darmstadt, DE). Mitochondrial DNA hypervariable region 1 (two primer sets, HV1A and HV2B) and hypervariable region 2 (two primer sets, HV2A and HV2B) were amplified using the primers shown in Table 3. By convention, "L" indicates the light strand and "H" the heavy strand, followed by the number of that base [122].

PCR amplifications were quantified using the 2100 Bioanalyzer and DNA 1000 Series II LabChip kit (both, Agilent Technologies, Germantown, MD). When extraction or amplification/quantification were not successful within an exposure treatment, analysis of additional hairs was attempted. If DNA from hairs exposed to the highest treatment level could not be amplified, hairs exposed to the next-highest level of radiation were attempted. Following quantification [123], samples were cycle-sequenced (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems, Foster City, CA) and then sequenced on a 3130xl Genetic Analyzer (Life Technologies). Data were analyzed using Sequencing Analysis v5.2 (Life Technologies) and Sequencher v4.7 software (GeneCodes, Tallinn, EE).

Sequences obtained from hairs exposed to each type of radiation were compared to those obtained from travel control hairs as well as to control hairs from the same individual that were not shipped.

#### 3.6. Latent fingerprint processing and assessment

All specimens were paired with their non-irradiated halves, both of which received all subsequent processing and photographic documentation. Assessment of potential value for comparison, as would be done with casework samples, was conducted before each processing step. The sequence of processing steps and formularies of each process were those used in casework [124] and specimens were assessed for quality of friction ridge detail following each step. Latent fingerprints were assessed relative to their value, were they to be used for subsequent comparison. This bears some similarity to the assessment of "suitability" used in the study by Neumann et al. [125] during the Analysis phase of the ACE-V protocol. In the present study, suitability does not necessarily imply adequacy for identification.

Latent fingerprints on aluminum substrates were processed by cyanoacrylate fuming and examined using visual and reflective ultra violet (RUVIS, 254 nm) light sources. They were then processed using cyanoacrylate fluorescent dye RAM (Rhodamine 6G/Ardrox/MBD) and examined at 365 nm, 450 nm, and 532 nm.

In accord with standard practice, gamma-irradiated latent fingerprints on paper substrates were processed first with DFO (1,8-diazafluorene-9-one) then by ninhydrin [126,127]. Samples exposed to alpha, beta, and neutron radiation were designated for

processing with only ninhydrin to mimic certain operational situations. When processing unknown hazardous materials, DFO processing is often omitted due to logistic and safety concerns. Development conditions for DFO (100 °C, dry heat) [128,129] could lead to contamination or detonation. Ninhydrin processing occurs at a lower temperature (85 °C, high humidity), and even will develop at room temperature, though at a slower rate [126]. Lastly, all specimens on paper were processed using physical developer.

The effects of radiation were assessed in two ways. Each latent fingerprint was judged, relative to its control, whether it showed any degradation, or became of no value for comparison, after irradiation. Quality of each irradiated sample was assessed by certified latent fingerprint examiners as degraded (D), improved (I), or unchanged (U). Degradation was assessed when the control half had recognizable development which was superior to the test half. The fraction of specimens at each dose that suffered degradation was tabulated. Potential usefulness of each irradiated sample for comparison was judged as of value (V), of debatable (i.e., inconclusive) value (DV), or not identifiable, i.e., no value (NV). Even if both halves may lack sufficient detail for identification (NV), one half can still show degradation. If the control was of no value, the results for the exposed sample were deemed

#### Table 2

Proportion of samples exhibiting disruptive change after irradiation.

inconclusive. Irradiated samples judged to be NV were tabulated, but not if the control was also NV.

#### 3.7. Microscopy and assessment of hairs and fibers

Fibers were removed from the perimeter of the cotton fabric holding the irradiated blood samples, distant from the stain itself. Irradiated hairs were removed from the index cards to which they were taped. Hairs and fibers were mounted between glass slides and cover slips using Permount mounting medium (Thermo Fisher Scientific, Waltham, MA). Any changes in microscopic appearance relative to the controls were noted. Fibers were also examined by fluorescence microscopy using four excitation wavelengths: UV (330–380 nm), violet (380–420 nm), blue (450–490 nm), and green (510–560 nm). The color and intensity of fluorescent emission were recorded and compared to the controls.

#### 4. Results and discussion

The proportions of samples that exhibited disruptive change after various types and level of irradiation are summarized in Table 2.

	nuDNA		mtDN	4			Ecci	rine			Sebad	eous			Cottor	n Fibers		Hairs	
no. per dose	9		5				3	3			3	3				9		15	
	type	extr	amp	type		рар	era	me	tal	pap	era	me	tal		micros	fluoresc	>CD	>CF	degr
						degr	NV	degr	NV	degr	NV	degr	NV						
Alpha, Gy					_		I.												
1.2E+02	0	0	0	0		1.0	inc	1.0	1.0	1.0	inc	1.0	0.7		0	0	0.1	0	0
1.2E+04	0	0	0	0		0 <sup>b</sup>	inc <sup>b</sup>	0.3	0.3	1.0	inc	0.7	0.3		0	0	0	0	0
1.2E+06	0	0	0	0		0 <sup>b</sup>	inc <sup>b</sup>	1.0	0.7	0 <sup>b</sup>	inc <sup>b</sup>	1.0	0		0	0	0.1	0	0
Beta, Gy					_									_					
0.5	0	0	0	0		1.0	inc	1.0	1.0	0.5 <sup>c</sup>	incc	1.0	0.3		0	0	0.3	0.4	0
1	0	0	0	0		1.0	inc	1.0	1.0	1.0 <sup>b</sup>	inc <sup>b</sup>	1.0	0		0	0	0.2	0.1	0
5	0	0	0	0	_	n/a	n/a	1.0	0.7	1.0 <sup>c</sup>	Incc	1.0	0.3		0	0	0.3	0.3	0.1
10	0	0	0	0		1.0 <sup>b</sup>	inc <sup>b</sup>	1.0	1.0	0 <sup>b</sup>	Inc <sup>b</sup>	1.0	0.3		0	0	0.1	0.1	0.1
Gamma, Gy					_														
5.0E+02	0	n/a	n/a	n/a		0.3	0	1.0	0	1.0 <sup>c</sup>	0.5¢	1.0	0.3		0	0	0.2	0	0
1.0E+03	0	n/a	n/a	n/a		0.3	0	1.0	0	0 <sup>b</sup>	0 <sup>b</sup>	0.7	0.3		0	0	0.3	0	0
9.0E+03	0	n/a	n/a	n/a		0.7	0.3	1.0	0.7	0 <sup>b</sup>	0 <sup>b</sup>	1.0	0.7		0	0	0.3	0.1	0.2
9.0E+04	0	0	0	0		1.0 <sup>b</sup>	1.0 <sup>b</sup>	1.0	1.0	1.0 <sup>c</sup>	1.0 <sup>c</sup>	1.0	0.3		0	> B,G	0.3	0.5	0.2
9.0E+05	partial	0.8	0.8	0.8		1.0 <sup>b</sup>	1.0 <sup>b</sup>	1.0	1.0	1.0 <sup>b</sup>	1.0 <sup>b</sup>	1.0	0.7		break	> B,G	0.1	0	1.0
9.0E+06	1.0	1.0	1.0	1.0		n/a	n/a	1.0	1.0	n/a	n/a	1.0	0		break/	no UV;	n/a	n/a	1.0
															yellow	<v< td=""><td></td><td></td><td></td></v<>			
Neutron, Gy					_						_								
1.7	0	0	0	0		1.0	inc	1.0	0.3	0.5¢	incc	1.0	0		0	0	0.1	0.3	0
17	0	0	0	0		0.3	inc	1.0	0	0 <sup>b</sup>	inc <sup>b</sup>	0	0		0	0	0.1	0.3	0
170	0	0	0	0		0.5¢	inc	1.0	1.0	0 <sup>c</sup>	Incc	0.7	0		0	0	0.3	0.2	0
1700	n/a	0	0	0		0 <sup>b</sup>	inc <sup>b</sup>	1.0	0.3	0 <sup>b</sup>	inc <sup>b</sup>	1.0	0.7		n/a	n/a	0.1	0.5	0.1

<sup>a</sup>All specimens on paper substrates processed by ninhydrin and physical developer; gamma-exposed specimens processed with DFO additionally. <sup>b</sup>Based on 1 specimens at given dose.

<sup>c</sup>Based on 2 specimen at given dose.

degr = degradation; NV = no value (relative to control); inc = inconclusive (control was NV); n/a = not available or not processed.

symbols >, < indicate an increase or decrease in a characteristic.

B, G, V, UV = blue, green, violet, or UV excitation for fluorescence; CD = cortical disruption; CF = cortical fusi.

Color code reflects proportion of samples exhibiting change after irradiation: green (none), yellow (<0.5), red ( $\geq0.5$ ).

#### 4.1. Nuclear DNA analysis

All samples exposed to alpha, beta, and neutron irradiation showed no decrease in amplifiable DNA and yielded correct, full STR profiles (Table 2) and the correct sex typing results (AMEL), although no samples were exposed to neutron irradiation at 1700 Gy. Samples irradiated with gamma at or below 90 kGy produced full STR as well as the correct AMEL profiles. There was no decrease in the DNA yields or in the quality of the STR profiles (data not shown).

For the samples irradiated at 900 kGy gamma, the amount of DNA recovered, based on the Quantifiler assay, was very low—the concentration was reduced by a factor of 10–100 in comparison to the samples exposed to lower doses. In addition, though the 900 kGy gamma samples all yielded the correct sex typing results, only partial STR profiles were obtained. Generally speaking, the longer amplicons tended to be more susceptible to degradation. Ten loci (D21S11, D7S820, CSF1PO, D13S317, D16S539, D2S1338, vWA, TPOX, D18S51, and FGA) failed in every sample to yield PCR products that met SWGDAM match interpretation thresholds [130]. The remaining loci (D8S1179, D3S1358, TH01, D19S433, and D5S818) could be typed for some samples but not for others. The smallest locus, D3S1358, was successfully typed for all but one of the samples.

These results show much greater resistance to DNA damage than that reported by Abbondante [59]. She first noted DNA degradation at 1 kGy gamma. Partial STR profiles resulted after a dose of 66 kGy alpha radiation and after 100 kGy gamma. No DNA results were obtained after 500 kGy. A possible explanation for these widely-differing results is that Abondante used a Chelex extraction from blood, which utilizes a chelating resin that produces single stranded DNA, while the present study uses a silica-based DNA extraction method. One major disadvantage of Chelex is that it is not efficient in the removal of inhibitors, which will cause a reduction in the efficiency of the PCR. In addition, the presence of Chelex resin particles that can be carried over, even after a removal step, may sometimes inhibit the PCR process. Finally, DNA extraction methodology using Chelex requires heating to 100°C, which can/will degrade DNA. If the sample is already somewhat degraded, as it is with the higher level doses, then it will be further degraded leading to a decrease in PCR amplification product (especially of the larger amplicons).

The blood DNA in the present study was extracted on an EZ1 robot that uses a silica-based DNA extraction method (magnetic silica beads). The cells are lysed using Proteinase K and a lysis buffer under high salt (chaotropic) concentrations, the DNA then binds with the silica beads. Further washing and spinning removes unwanted contaminants and inhibitors while the DNA is still adsorbed to the beads. The adsorbed DNA can be finally eluted by rehydration with aqueous low salt solutions. The eluted DNA is double stranded. Silica methods have been shown to produce a higher quality DNA with efficient removal of contaminants and inhibitors, while also working well on DNA recovery from degraded samples [131–135]. The samples irradiated at 9000 kGy gamma resulted in no STR/AMEL profiles for any sample and the quantification showed no DNA present. Alternative techniques might possibly produce usable profiles, e.g., miniSTRs

[136–138], single nucleotide polymorphisms [137,139,140], or massively parallel sequencing [141–143].

#### 4.2. Mitochondrial DNA analysis

DNA extraction was successfully performed on all hairs exposed to each radiation treatment, with the exception of those subjected to 9000 kGy gamma treatment, which were physically degraded (brittle and fragmented) and not suitable for extraction (Table 2).

DNA amplification was successful for all hairs exposed to each radiation treatment that were suitable for extraction, with the exception of the 900 kGy gamma radiation treatment. Only one of five hairs exposed to 900 kGy gamma contained amplifiable DNA, which may have been due to biological variability or unrealized experimental error. All extracted hairs exposed to the 90 kGy gamma radiation level were successfully amplified and sequenced. Therefore, hair samples exposed to  $\leq 9$  kGy gamma radiation were not analyzed.

Mitochondrial DNA sequences were obtained for all hairs that produced quantifiable DNA amplification products. All sequences from exposed hairs were identical to the sequences of non-exposed hairs from the same individual (both travel controls and previously sequenced hairs). In addition, the sequence quality (e.g., background level, peak heights, peak resolution) of non-exposed hairs and exposed hairs was comparable, and independent of radiation type. As was the case with nuclear DNA, mitochondrial DNA showed greater resistance to degradation than was reported by Abbondante [59].

#### 4.3. Latent fingerprints

Nearly all irradiated latent fingerprints were fainter, smudged, smeared, and degraded relative to the controls (Table 2). The columns labeled "degr" denote the fraction of specimens at each dose that suffered degradation. Thus, a value of 1.0 in Table 2 indicates degradation of every sample. Results for latent fingerprints on a paper substrate are not fully informative for alpha, beta, and neutron irradiation. This is because ninhydrin processing of these samples (without using DFO before), followed by physical developer, usually failed to develop usable ridge detail on control or irradiated samples. This nonsuccess was likely due to use of natural fingerprints from a single person, as control samples produced immediately prior to development developed normally. Despite the lack of usable ridges, in many cases we were still able to judge whether degradation occurred; the reduced number of specimens on which such conclusions were based is footnoted in Table 2. Processing with DFO was more successful, particularly for eccrine prints.

Results of experiments designed to study the effect of some treatment or a process that involves natural latent fingerprint quality are inevitably convolved with other variables, including distortions due to pressure and movement, usable area, residue composition (matrix), as well as image capture conditions. Despite some degradation due to irradiation, a latent print may still be of value, illustrated by several instances in Table 2 where the proportion judged of no value (NV) is less than the proportion of prints at that dose that showed degradation. If a control was NV,

#### Table 3

Primers for the mitochondrial DNA hypervariable regions.

HV1 primers		HV2 primers	HV2 primers						
A1 (L 15997)	5'-CAC CAT TAG CAC CCA AAG CT-3'	C1 (L 048)	5'-CTC ACG GGA GCT CTC CAT GC-3'						
B2 (H 16237)	5'-GGC TTT GGA GTT GCA GTT GAT-3'	D2 (H 409)	5'-GGG GTT TGG TGG AAA TTT TTT G-3'						
A2 (L 16159)	5'-TAC TTG ACC ACC TGT AGT AC-3'	C2 (L 177)	5'-TTA TTT ATC GCA CCT ACG TTC AAT-3'						
B1 (H 16391)	5'-GAG GAT GGT GGT CAA GGG AC-3'	D1 (H 409)	5'-CTG TTA AAA GTG CAT ACC GCC-3'						

the mated irradiated specimen was excluded from the tabulation, and the reduced number of specimens on which a conclusion was based is indicated by footnote in Table 2.

For both eccrine and sebaceous fingerprints, and on both substrates, degradation occurred at even the lowest doses (Figs. 2 and 3). However at various intermediate or higher doses, the proportion of samples exhibiting damage was lower, or even none at all (Table 2). Despite evidence degradation by radiation, fingerprints often retained sufficient information to be of potential value for comparison.

Largely due to the fact that both DFO and ninhydrin were used only with the paper substrates irradiated by gamma, only those samples were successfully developed (Fig. 3). Since 7 of 20 controls were NV for gamma exposures of paper, those results must be interpreted cautiously (Table 2). Embrittlement and fragility of the paper substrate precluded processing of the 9000 kGy samples.

Our results for exposure of fingerprints to four radiation types are consistent to one another but they contrast sharply with those previously reported for fingerprints, and indeed with what was observed for other evidentiary materials examined in the present study. We observed degradative effects at doses as low as 0.0005 kGy beta, 0.002 kGy neutron, 0.12 kGy alpha, and 0.5 kGy gamma. These values are 3–5 orders of magnitude lower than reported damage thresholds of 56 kGy beta [77], 250 kGy alpha [91], and 100 kGy gamma [74]. One study reported no damage on non-porous substrates even after 1000 kGy [74]. It is important to note, however, that observation of degradation may not necessarily compromise the value of a fingerprint for comparison, even at the highest doses used.

One factor that may have promoted increased radiolytic degradation in our study was humidity during radiation exposure. The mean relative humidity of the radiological laboratory during the summer months is usually about 70% and humidities as high as 100% did occur. It is well known that radiolysis of water occurs. The radiolysis of water molecules produces hydroxyl radicals and other

highly reactive species [144–148]. The lipids, proteins, and salts that make up latent print residues [149] are likely to be degraded by these water radiolysis products. The same circumstances would apply to DNA in the absence of a host repair mechanism. Additional studies would be needed to better assess the influence of humidity during irradiation but this is one plausible explanation for our results.

Another factor to consider in the apparent disparity in fingerprint damage due to ionizing radiation exposure is the definition of "degradation" applied to exposed prints when compared to reference prints. Few of the previous studies clearly defined what constituted degradation due to exposure, and some of those determinations seem to have been made by personnel other than latent fingerprint examiners. One study implies an increase in quality of the fingerprint based on a relative increase in minutiae count [76], but minutiae could possibly be created by degradation of ridges. In Evans et al. [91], evaluation focused on the ability to develop fingerprints either prior to or after deposition of fingerprints and exposures to alpha radiation, rendering results at least somewhat less comparable to this study. Other studies note the effects of a variety of developers on irradiated fingerprints [57,74,77]. In each of these cases, a variety of image quality resulted, but was focused on usability of the print. In the present work, latent fingerprint examiners compared the irradiated and control samples, focusing on both an absolute determination of degradation, irrespective of usability, as well as value determinations. Finally, we note, in consideration of the apparent disparity, that we and nearly all authors of similar studies note the dependence on environmental conditions (e.g., water content of prints and relative humidity during deposition, irradiation, and pre-development evaluation) as well as the individual variations among donors of eccrine and sebaceous fingerprints. All these factors, and no doubt others, contribute to the differences in results.



**Fig. 2.** Examples of radiation effects on latent fingerprints deposited on an aluminum substrate. All latent fingerprints were processed by cyanoacrylate fuming with reflected ultraviolet imaging (RUVIS). Unexposed controls are on the right. Value for potential comparison for each half is indicated in square brackets as V (of value), DV (debatable value), or NV (no value). (a) Sebaceous, 0.5 Gy beta [NV,V]; (b) sebaceous, 1 Gy beta [DV,V]; (c) eccrine, 5 Gy beta [NV,V]; (d) eccrine, 0.5 kGy gamma [V,V]; (e) eccrine, 1 kGy gamma [V,V]; (f) sebaceous, 0.5 kGy gamma [V,V]; (g) sebaceous, 1 kGy gamma [NV,V]; (h) sebaceous, 900 kGy gamma [V,V]; (i) sebaceous, 9000 kGy gamma [DV,V]; (j) sebaceous, 2 Gy neutron [NV,V].



Fig. 3. Examples of gamma radiation damage to latent fingerprints on a paper substrate. Unexposed controls are on the right. All latent fingerprints were processed with DFO and ninhydrin. Value for potential comparison for each half is indicated in square brackets as V (of value), DV (debatable value), or NV (no value). (a) Sebaceous, 1 kGy [V,V]; (b) sebaceous, 900 kGy [NV,V]; (c) eccrine, 9 kGy [NV,DV]; (d) eccrine, 900 kGy [NV,V].

#### 4.4. Visual and microscopic analysis of hair, paper, and fiber

All irradiated hairs were examined microscopically and subsequently compared to the control hair samples using high magnification comparison microscopy. The microscopic examination and comparison of hair evidence involves comparing all microscopic characteristics present in the cuticle, cortex, and medulla in the corresponding regions of the hairs in the question and known hair samples. Microscopic characteristics such as cortical fusi and cortical disruption are among these human hair characteristics examined and compared [150]. Increased numbers of cortical fusi were observed for all beta and neutron doses, and at 9 and 90 kGy gamma (Table 2 and Fig. 4d). The prevalence of cortical fusi was unchanged in the alpha-irradiated hairs. For all four types of radiation, one or more hairs exhibited cortical disruption (Fig. 4c). This was never observed in more than onethird of the hairs, however, and there was no dose dependence. If a questioned hair recovered from a crime scene exhibited these observed increased number of cortical fusi and/or cortical disruptions, which were not exhibited in the known hair sample collected from the subject, it is possible the known hair sample would be excluded as a possible source of the questioned hair. Cortical disruptions and cortical fusi are microscopic characteristics observed in non-irradiated hairs as well, and therefore, the hair examiner may not be aware the questioned hair had been irradiated.

Localized distention and/or departure from axial linearity commenced at 5 Gy beta, 9 kGy gamma, and 1.7 kGy neutrons



Fig. 4. Examples of radiation damage to hairs from a single individual: (a) non-irradiated control; (b) cortical fusi, 100 Gy alpha; (c) cortical disruption, 5 Gy beta; (d) localized cortical distention, departure from linearity, and cortical fusi, 9 kGy gamma.

(Fig. 4d), but was not observed after alpha irradiation. It is important to note that it is not possible to definitively ascribe this particular observation to radiation effects, as it could arise from potential mechanical damage from using microtweezers in removing hairs from the index cards. Hairs exposed to 900 kGy gamma were broken into small pieces 1 mm or smaller. Some form of damage (breakage, local distention, and/or bending) was ubiquitous after 900 kGy gamma and 1.7 kGy neutrons. Hairs exposed to 9000 kGy were damaged to such an extent that they could not be removed from the index cards for microscopy.

The paper index cards onto which the hairs were affixed showed slight discoloration at 0.9 kGy gamma, becoming brown at 9 kGy, and blackened at 90 kGy (Fig. 5). At 90 kGy, both the paper and the plastic enclosure bag were disintegrating.

The photocopy paper substrate and its plastic enclosure bag appeared unaffected by gamma doses up to 9 kGy, but both showed brittleness and minor discoloration after 90 kGy (Fig. 6). After 9000 kGy exposure, both materials disintegrated into tiny fragments that precluded fingerprint processing.

The observed onset of visible darkening is a factor of ten higher than reported by Kubat et al. [95] but consistent with the results of Bouchard et al. [21].

Cotton fabric showed slight discoloration at 90 kGy gamma dose, darkening at each successive dose (Fig. 7). After 9000 kGy, the fabric background was significantly darkened, while the blood stain lightened in color. At that dose, the plastic enclosure bag was very brittle and yellowed, becoming more so in the months following radiation exposure. The samples and fabric exposed to alpha, beta, and neutron radiation showed no visible or microscopic changes.

The only microscopic change in irradiated white cotton fabric was fiber transverse cleavage beginning after 900 kGy and yellowing after 9000 kGy gamma (Table 2). Fluorescent intensity from blue and green excitation began to increase at 90 kGy gamma and was strongly increased at 9000 kGy. This observation differs from the results of Colella et al. [90], who reported no spectral changes for white cotton (unlike their results for colored fibers) even for doses of 1000 kGy. Fluorescence in the green increased from pale red to red for 90 kGy, becoming much brighter red for



Fig. 6. Embrittlement of copier paper and degradation of polyethylene bag (90 kGy gamma).

9000 kGy samples. After 9000 kGy gamma, the blue fluorescence from UV excitation that was observed in lower dose samples was quenched. A possible explanation is radiation-induced damage of polyaromatic compounds in optical brighteners. Fluorescence due to violet excitation changed from blue, which had been observed at lower energies, to white. Fluorescent intensity from blue and green excitation returned to control levels.

#### 5. Conclusions

This study showed that successful development of nuclear and mitochondrial DNA profiles is possible—up to a point—following exposure to varying levels of gamma, neutron, beta, and alpha radiation exposure. Complete nuclear and mitochondrial DNA profiles were obtained up to a gamma dose of 90 kGy. Only partial profiles were obtained at 900 kGy gamma. An exposure of 9000 kGy gamma destroyed all DNA. In general, irradiation negatively affected the quality of latent fingerprints. All irradiation



Fig. 5. Darkening of index cards used to mount hairs. Hairs were exposed to (a) 0.5; (b) 0.9; (c) 9; (d) 90 kGy gamma.



Fig. 7. Blood stains on cotton fabric after exposure to gamma irradiation at 9 kGy (left) and 90 kGy (right).

types were destructive for most fingerprint samples at all doses, appearing fainter, smudged, and smeared, although many samples retained sufficient detail to be of potential value for comparison. Although results are limited, DFO was much more effective than ninhydrin for developing latent prints on paper, particularly for eccrine prints. On metal substrates, both eccrine and sebaceous prints often retained value for potential comparison after neutron exposure. Although variable from one hair to another, physical changes were observed for all types and levels of irradiation. Negligible microscopic changes were observed in papers and fibers, except above 90 kGy gamma. Fluorescence of fibers began to change above that dose. Paper, fibers, and plastic became extremely brittle leading to breakage after a gamma dose of 900 kGy.

In this study, consistent with previously reported work, the effects of radiation on subsequent forensic examination of materials of evidentiary interest show considerable variability. It is difficult, therefore, to make specific recommendations about a threshold dose where forensic processing, and exposing personnel to a hazardous environment to collect the evidence, would assuredly be pointless. Only for electronic devices is there uniform agreement on radiation dose levels that cause unrecoverable damage (and that dose is extremely low), but is very dependent upon the manufacturing specifications and materials used to produce electronic equipment. For DNA, fingerprints, hairs, and fibers the reported doses where degradation is so severe as to negate the value of the evidence varies by a factor of ten or much more. Although the mechanisms are entirely different, this situation is reminiscent of the similarly wide variance in radiation doses that cause negative effects in living systems.

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