Gamma Radiation Effects on Cigarettes*

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Cigarette smoke is a highly complex mixture of substances, some of which are known to be irritating or harmful to living tissue (1). Numerous attempts, such as smoke filtration, tobacco blending, alterations of curing and processing methods, etc., have had different degrees of success in alleviating or modifying these smoke effects.

One of these attempts, which has not been fully investigated, has been the application of gamma irradiation [Co-60] to cigarettes prior to smoking (2, 3). Cigarette smoke condensate [CSC] from irradiated and non-irradiated cigarettes was subjected to vacuum distillation. The most volatile CSC fraction of the irradiated cigarettes possessed a different infrared spectrum than the standard CSC fraction, and the amount of tar (the pot residue) in the smoke of the irradiated tobacco was about 20% less than the amount for standard smoke. Also, about 50% fewer free radicals were detected in the smoke of irradiated tobacco. A free-radical mechanism has been proposed as one of the major pyrolytic synthetic pathways for the production of polynuclear aromatic hydrocarbons [PAH] (4). The possibility of decreased formation of PAH as a result of fewer free radicals and other suggested changes in smoke chemistry prompted a more detailed study of the smoke of irradiated cigarettes. If any beneficial changes in smoke chemistry would occur, then gamma irradiation of manufactured cigarettes would be one way of decreasing some of the deleterious constituents of smoke. Accordingly, smoke condensates from University of Kentucky 1R1 reference cigarettes, irradiated at 30 and 60 Mrad dose levels, were compared with the CSC from the non-irradiated 1R1 cigarettes. The yields of total particulate matter [TPM], nicotine, tar, several major PAH, and selected CSC fractions are reported.

MATERIALS AND METHODS

Irradiation of 1R1 Cigarettes

The University of Kentucky 1R1 reference cigarettes were irradiated in the carton in a Cobalt 60 source at the Oak Ridge National Laboratory at 30 and

60 Mrad ($\pm 20\%$) levels. The cartons were frequently shifted to achieve uniform dose.

Total Particulate Matter [TPM]

TPM data were determined by the Tobacco Research Council procedure (5). Pretreatment and smoking conditions were: 48-hour conditioning at 60% relative humidity, puff of 2-second duration, one puff per min, 35 ml draw, and 23 mm butt length. Five cigarettes were smoked onto each Cambridge filter pad.

Gas Chromatographic [GC] Analysis of Nicotine

Nicotine was determined by GC analysis of the TPM solutions on a 10 ft $\times$ 1/8 in. stainless steel column, packed with 10% Carbowax 20 M - 2% KOH and operated isothermally at 190° C, with a 35 ml/min helium flow rate. Freshly distilled 7-methyl-quinoline was used as an internal standard. The response factor for nicotine was determined by placing 20 ml of nicotine stock solution (0.5 mg/ml in 2-propanol), 1 ml of 7-methyl-quinoline stock solution (0.3 mg/ml in 2-propanol), and one and one-half Cambridge filter pads in a vial. The resulting samples were mixed on a shaker for 1 hour. GC peak areas were obtained by using an electronic integrator and the response factor (K) was calculated to be 1.17, from the following equation:

$$K = \frac{\text{Area nicotine} / \text{mg nicotine}}{\text{Area 7-methyl-quinoline} / \text{mg 7-methyl-quinoline}}$$

The nicotine in the TPM samples was determined as follows: 1.0 ml of the 7-methyl-quinoline stock solution was added to the TPM samples which were analyzed by GC, after mixing on a shaker for 1 hour. Retention times for nicotine and 7-methyl-quinoline, measured from the point of injection, were 7.9 and 13.9 min, respectively.

Collection of Cigarette Smoke Condensate [CSC]

The smoke from 450 cigarettes, obtained under the same smoking conditions used for TPM, was collected in two dry-ice traps. The CSC was dissolved with acetone, and the solvent removed at 70 mmHg (30° C) on a rotary evaporator. The residue was dried in a desiccator over Drierite** to constant weight.

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** Reference to a company or product name does not imply approval or recommendation by the USDA.

Solvent Partitioning of Cigarette Smoke Condensate [CSC]

The fractionation procedure was based upon a large-scale CSC fractionation method used for biological testing (6). The dry CSC was dissolved in 350 ml each of 1.0 N NaOH and diethyl ether. The resulting organic and aqueous phases were cross extracted (6 times) with 50 ml portions of base and ether. The combined ether fraction was extracted with 2.0 N HCl (2×150 ml) and the aqueous and organic fractions were cross extracted (6 times with 50 ml portions of ether and acid). The organic fraction was dried over anhydrous Na₂SO₄ and filtered, and the solvent removed to yield the CSC neutral fraction, F15.

The HCl extract was adjusted to pH 11.3 with NaOH pellets, cooled in ice-water, and extracted with diethyl ether (2×150 ml and 6×50 ml). The ether extract was dried and concentrated to yield the basic fraction, F5.

The NaOH extract was adjusted to pH 6.1 with 2.0 N H₂SO₄ and extracted with ether (2×150 ml and 6×50 ml). Treatment of the organic phase, as above, resulted in the weak acid fraction, F8. The aqueous phase was further acidified to pH 2.0 and extracted with ether (4×150 ml). The ether solution was dried and concentrated to give the strong acid fraction, F10. All CSC fractions were brought to constant weight over Drierite.

Chromatographic Separation of CSC Neutrals

The CSC neutral fraction F15 was chromatographed on 100 g of silicic acid, according to the method of Swain et al. (7). The following fractions were eluted from the column: 800 ml of petroleum ether [fraction PE], 800 ml of 25% benzene in petroleum ether (v/v) [fraction BP], 1800 ml of benzene [fraction B], 1400 ml of ether [fraction E], and 600 ml of methanol [fraction M]. All eluates were filtered, the solvent removed on a rotary evaporator, and the residues brought to constant weight.

PAH Analysis

The PAH of fraction BP were separated from other material by gel filtration, according to a recently developed technique (13). Briefly, gel filtration [GF] was performed on Bio-Beads SX-12, packed in three Chromatronix LC columns (1.25 cm × 109.2 cm), connected in series. Samples, dissolved in benzene, were placed on the column with a 1.0 ml injection loop. Benzene was pumped by a Chromatronix pump at a flow rate of 120 ml/h and 8 ml fractions were collected. The PAH-containing fractions, GF fractions 33–40, were combined and reduced in volume to about 100 µl. GC analysis was performed on 10 ft × 1/8 in. stainless steel columns of 5% Dexsil 300 GC on Chromosorb W/AW. The helium flow rate was 50 ml/min, and after 5 min at 100° C, the oven was programmed to 325° C at 2°/min. Individual PAH were identified

by GC retention data, UV spectra, and mass spectral data. Methodology will be detailed in the near future (14).

RESULTS AND DISCUSSION

Previous work (2, 3) on gamma irradiated cigarettes had indicated some changes in the composition of volatile smoke compounds. Similar irradiation of various plant materials decreased ascorbic acid and sulfhydryl content, increased evolution of ethylene and respiration changes in enzyme activities, and changed the molecular configuration of proteins, the carbohydrate degradation, the amino acid concentration, and the general texture (8 – 12). Such information might allow predictions as to the type of reactions which could take place in selected irradiated plant material. However, in a system as complex as tobacco, it is difficult to predict the products resulting from irradiation of the tobacco. The different smoke constituents arising from altered leaf precursors would be combined with the usual smoke constituents. Consequently, it was hoped that fractionation of the CSC into acidic, basic, and neutral constituents, as well as subfractionation of the CSC neutrals would indicate gross smoke composition changes due to irradiation.

Figure 1. Gas chromatogram of a 1R1-TPM solution for nicotine determination (retention times: nicotine – 7.9 min, 7-methyl-quinoline – 13.9 min).

Table 1. Comparison of smoke analyses of non-irradiated and irradiated 1R1 cigarettes.

	Yields (mg/cig.)		
	1R1	30 Mrad 1R1	60 Mrad 1R1
Total particulate matter (TPM)	47.0	44.8	41.6
Nicotine	2.86	2.71	2.45
Tar	44.2	42.1	39.2

The comparison of the effect of radiation on TPM, nicotine, and tar levels in non-irradiated and irradiated cigarettes is given in Table 1. As previously reported, tar levels decreased in smoke of irradiated tobacco (2, 3). Nicotine was conveniently determined by gas chromatography of TPM solution samples, using 7-methylquinoline as the internal standard. It was noted that the chromatographic column gave sharp peaks, without tailing (Fig. 1). Calculations showed a decrease for nicotine with increasing radiation doses. However, at the 60 Mrad level, an 11% decrease in tar or TPM and a 14% decrease in nicotine were not considered to be highly significant changes.

Since some of the effects observed with increased dose levels might be due to differences in moisture contents of the irradiated tobacco, water levels in the cigarettes were determined (oven-drying: 95° C, 3 hours). This, however, revealed that all cigarettes contained identical 11% water levels.

The decrease in nicotine suggested that the yields of other classes of compounds might also be altered by irradiation. Accordingly, CSC was fractionated into acidic, basic, and neutral constituents (Table 2). A decrease in the basic fraction F 5 was noted at the 60 Mrad level, but the other yields were essentially unchanged. The biologically active weak acids (F8) decreased at the 30 Mrad level but increased at the 60 Mrad level. The ether-soluble CSC neutrals (F15) were then separated by column chromatography on silicic acid into five fractions (Table 3), according to methods used to produce bioassay fractions (7). Fractions BP and M, which have shown biological activity, did not differ appreciably in yields as a result of the irradiation. Fraction BP contains the PAH of the smoke and, thus, the decreased yield of free radicals observed by previous investigators (2, 3) might have led to a decreased yield of this fraction with increasing radiation

Table 2. Effect of irradiation on the yields of cigarette smoke condensate (CSC) fractions.

Fraction	% by weight of dry CSC		
	1R1	30 Mrad 1R1	60 Mrad 1R1
F 5 (bases)	9.1	9.2	7.2
F 8 (weak acids)	8.6	6.3	9.0
F 10 (strong acids)	4.1	5.0	4.5
F 15 (neutrals)	31.0	28.7	34.3
Total ether-solubles	55.8	49.2	55.0

Table 3. Effect of irradiation on the yields of chromatographic fractions of cigarette smoke condensate (CSC) neutrals.

Fractions	% by weight of F 15 ^a			
	1R1	30 Mrad 1R1	60 Mrad 1R1	Commercial cigarettes ^b
PE	11.9	10.3	10.9	11.9 ± .7
BP	8.9	9.1	10.5	10.6 ± 1.0
B	22.3	26.0	23.7	25.3 ± 2.6
E	46.7	45.2	44.7	41.2 ± 3.0
M	10.2	9.3	10.3	10.2 ± 3.0
Total	104.0	96.0	94.0	99.2 ± 4.0

^aBased on amount of material placed on column.

^bData obtained in this laboratory from ten chromatographic fractionations of F 15 neutrals obtained from kg quantities of cigarette smoke condensate from commercial cigarettes.

doses. However, this effect was not observed. The variations in yields observed for the F 15 subfractions of 1R1 condensate were similar to variations obtained in the fractionation of large quantities of condensate from commercial cigarettes.

The final and most important point on the effects of radiation on smoke PAH formation was examined. The recently developed gel filtration — gas chromatography technique for concentrating and analyzing smoke PAH was applied to the BP fractions from standard and irradiated cigarettes (14). The resulting gas chromatograms of the concentrated PAH-containing gel fractions indicated that the fingerprint pattern for the PAH were identical. That is, the same PAH were produced in the same proportion in the smoke of irradiated and standard cigarettes. A typical chromatogram is shown in Fig. 2. Quantitative calculations of percent composition of selected major PAH showed that irradiation of the cured, processed cigarette tobacco had not produced any measurable changes in the PAH formation (Table 4).

It was concluded that gamma irradiation of the reference cigarettes did not result in any major yield changes in smoke fractions of biological significance or in PAH composition. Decreased yields may have occurred in water-soluble smoke constituents that were not extracted into the ethyl ether solvent. Another factor may be responsible for lack of drastic changes in irradiated cigarettes, namely, moisture contents. Publi-

Table 4. Contents of major polycyclic aromatic hydrocarbons (PAH) in fraction BP.

	% Composition ^a		
	1R1	30 Mrad 1R1	60 Mrad 1R1
Fluoranthene	10.2	9.7	10.1
Pyrene	20.0	22.5	20.1
Chrysene	5.5	6.6	5.7
BaP/BeP	3.9	5.0	3.7

^aBased on total gas chromatography peak areas, assuming unitary response.

Figure 2. Gas chromatogram of refined smoke PAH fraction of non-irradiated 1R1 cigarettes.

(1: fluoranthene, 2: pyrene, 3: chrysene, 4: benzo(e)pyrene/benzo(a)pyrene)

shed reports have indicated that changes were greatest when plants were irradiated in their high-moisture, green state. Since it would not be practicable to irradiate green tobacco plants, irradiation of freshly harvested or partially cured tobacco leaf might produce more significant changes in smoke composition.

SUMMARY

Smoke from gamma irradiated 1R1 reference cigarettes was compared to smoke from the non-irradiated cigarettes. Total particulate matter, nicotine, and tar levels decreased with increasing radiation dose. Nicotine was determined by gas chromatography, using 7-methylquinoline as an internal standard. Yields of solvent partition fractions of the smoke condensate and of chromatographic fractions of the condensate neutrals did not indicate significant changes in smoke composition resulting from gamma irradiation of the precursor cigarette tobacco. The composition of refined polycyclic aromatic hydrocarbon (PAH) fractions of smoke from irradiated cigarettes appeared to be identical to that of standard cigarettes. The expected decrease in PAH did not occur. It was concluded that gamma irradiation of cigarettes had no major effects on smoke composition.

ZUSAMMENFASSUNG

Der Rauch von 1R1-Versuchscigaretten, die mit Gammastrahlen bestrahlt worden waren, wurde mit dem Rauch nichtbestrahlter Cigaretten verglichen. Gesamtpartikelphase, Nikotin und Kondensat nahmen mit ansteigender Strahlendosis ab. Nikotin wurde durch Gaschromatographie unter Benutzung von 7-Methylchinolin als innerem Standard bestimmt. Die

Ausbeuten der Fraktionen der Lösungsmittelverteilung des Rauchkondensates und der Chromatographiefraktionen der Neutralanteile des Kondensates zeigten keine signifikanten Veränderungen der Rauchzusammensetzung durch Bestrahlung des zugrundeliegenden Cigarettentabaks. Die Fraktionen der gereinigten polycyclischen aromatischen Kohlenwasserstoffe (PAH) des Rauches von bestrahlten Cigaretten erwiesen sich in ihrer Zusammensetzung als identisch mit den entsprechenden Fraktionen des Rauches der Standardcigaretten. Die erwartete Abnahme des Gehaltes an Kohlenwasserstoffen trat nicht auf. Aus den Untersuchungen wird geschlossen, daß die Bestrahlung der Cigaretten mit Gammastrahlen keine größere Wirkung auf die Zusammensetzung des Rauches ausübt.

RESUME

On a comparé la fumée de cigarettes de référence 1R1 irradiées aux rayons gamma à celle de cigarettes non irradiées. La matière particulaire totale ainsi que les teneurs en nicotine et en condensat décroissent quand on augmente la dose d'irradiation. La nicotine a été déterminée par chromatographie en phase gazeuse, avec la 7-méthyl-quinoline comme étalon interne. Le rendement des fractions de condensat de fumée par partition de solvant, et des fractions chromatographiques de la phase neutre du condensat, n'indique pas de changement significatif dans la composition de la fumée après irradiation gamma du tabac. La composition des fractions HPA (hydrocarbures polycycliques aromatiques) raffinés est identique pour la fumée des cigarettes irradiées ou non. On n'a pas trouvé la baisse prévue en HPA. En conclusion, l'irradiation gamma des cigarettes n'a pas d'effet majeur sur la composition de la fumée.

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