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EVALUATION OF THE HEALTH ASPECTS OF CERTAIN COMPOUNDS FOUND IN IRRADIATED BEEF

AUGUST, 1977

LIFE SCIENCES RESEARCH OFFICE

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EVALUATION OF THE HEALTH ASPECTS OF CERTAIN COMPOUNDS FOUND IN IRRADIATED BEEF

August 1977

Prepared for

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
DEPARTMENT OF THE ARMY
WASHINGTON, D.C. 20314

under

Contract Number DAMD-17-76-C-6055

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LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
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Bethesda, Maryland 20014

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alkenes	food	irradiation

(over)

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

For more than 20 years, the U.S. Army has been investigating ionizing radiation as a possible preservation process for foods. It has developed a practical procedure for the preservation of beef; meat treated in this manner is now undergoing a multigeneration wholesomeness study in mice, rats and dogs. As an adjunct to this feeding study, the Army requested a thorough review of the possible toxicity to man of the volatile compounds detected in the irradiated beef. This is a report of that review. Sixty-five compounds have been

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Block 20-

identified in the irradiated beef. These include both saturated and unsaturated aliphatic compounds containing from 2 to 27 carbon atoms; certain of their alcohol, aldehyde and ketone derivatives; three aromatic hydrocarbons; and some sulfur, nitrogen and chlorine containing compounds. The concentrations of the individual compounds range from 1 to 700 μg per kg beef with a total concentration of 9.4 mg per kg.

The Committee reviewed the usual distribution of each compound in foods, water supplies and the atmosphere as well as its absorption, metabolic formation and disposition, acute and chronic toxicity and potential hazard for man. Virtually all of these compounds are ordinary constituents of human foods; many are approved additives or flavoring agents; and some are widely dispersed in our atmosphere and water supplies. The concentrations of the compounds reported in irradiated beef are within official guidelines, where such exist, while many others are below the amounts found in common foods or absorbed from other sources.

Tetrachloroethylene and benzene were scrutinized with especial care because of their possible carcinogenicity. The available evidence demonstrates that the tetrachloroethylene found in irradiated beef samples was not a radiolytic product, but was a contaminant probably arising from its use as a cleaning agent in the processing plant. Among different samples of beef, it was either not present, or its concentrations were no greater than those in nonirradiated beef. Irradiation with doses up to 120 kilograys (12.0 megarads) did not increase its concentration in the beef. The daily intake from air, water, and other foods is many times greater than that from irradiated beef. Benzene is suspected of being a possible human leukemogen although many experts dispute this claim. The small contribution from irradiated beef is not believed to constitute a significantly added risk.

On the basis of the available data, the Committee concluded that there were no grounds to suspect that the radiolytic compounds evaluated in this report would constitute any hazard to health to persons consuming reasonable quantities of beef irradiated in the described manner.

Block 19-

ketones
nitrogen-containing compounds
oxygen-containing compounds
sulfur-containing compounds
toxicity

AD_____

EVALUATION OF THE HEALTH ASPECTS OF CERTAIN COMPOUNDS FOUND IN IRRADIATED BEEF

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SUMMARY

For more than 20 years, the U.S. Army has been investigating ionizing radiation as a possible preservation process for foods. It has developed a practical procedure for the preservation of beef; meat treated in this manner is now undergoing a multigeneration wholesomeness study in mice, rats and dogs. As an adjunct to this feeding study, the Army requested a thorough review of the possible toxicity to man of the volatile compounds detected in the irradiated beef. This is a report of that review.

Sixty-five compounds have been identified in the irradiated beef. A number of nonvolatile compounds would not be detected by the analytical methods employed and were not considered in this study. Those identified include both saturated and unsaturated aliphatic compounds containing from 2 to 17 carbon atoms; certain of their alcohol, aldehyde and ketone derivatives; three aromatic hydrocarbons; and some sulfur-, nitrogen- and chlorine-containing compounds. The concentrations of the individual compounds range from 1 to 700 μg per kg beef with a total concentration of 9.4 mg per kg.

The Select Committee reviewed the usual distribution of each compound in foods, water supplies and the atmosphere as well as its absorption, metabolic formation and disposition, acute and chronic toxicity and potential hazards for man. Many of these compounds are found in human foods; some are approved additives or flavoring agents; and some are widely dispersed in our atmosphere and water supplies. The concentrations of the compounds reported in irradiated beef are within official guidelines, when such exist, while many others are below the amounts found in common foods or absorbed from other sources.

Tetrachloroethylene and benzene were scrutinized with especial care because of their possible carcinogenicity. The available evidence demonstrates that the tetrachloroethylene found in irradiated beef samples was not a radiolytic product, but was a contaminant probably arising from its use as a cleaning agent in the processing plant. Among different samples of beef, it was either absent, or its concentrations were no greater than those in nonirradiated beef. Irradiation with doses up to 120 kilograys* (12.0 megarads) did not increase its concentration in the beef. The daily intake from air, water and other foods is many times greater than that from

*In this report, absorbed dose is generally expressed in terms of the gray (Gy), as recommended by the International Organization for Standardization. Values expressed in terms of the rad are given in parenthesis. One rad = 10^{-2} Gy. In a few instances where graphs are reproduced from reports published before this convention was adopted, the older terminology is retained.

irradiated beef. Benzene is suspected of being a possible human leukemogen, although many experts dispute this claim. The small contribution to the general environmental burden of benzene from irradiated beef is not believed to constitute a significant added risk.

On the basis of the available data, the Committee concluded that there were no grounds to suspect that the radiolytic compounds evaluated in this report would constitute any hazard to health to persons consuming reasonable quantities of beef irradiated in the described manner.

FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the opinions of knowledgeable investigators who are actively engaged in work in specific areas of biology and medicine.

This technical report was prepared for the U. S. Army Medical Research and Development Command by the LSRO, FASEB, in accordance with provisions of contract number DAMD-17-76-C-6055. The report was written by the members of an ad hoc Select Committee on Health Aspects of Irradiated Beef with the assistance of the LSRO staff.

The Select Committee whose members are listed in Section IX accepts the responsibility for the contents of the report. Other scientists provided useful information to the Select Committee; however, the listing of their names does not imply that they endorse the study conclusions. Special appreciation is expressed to Dr. Walter M. Urbain, Special Consultant and to Dr. C. Jelleff Carr, Director Emeritus, LSRO, for their valuable assistance to the Select Committee in the preparation of this report.

The report was approved by the Select Committee, the Director of LSRO, and subsequently by the LSRO Advisory Committee composed of representatives of each constituent society of FASEB, under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the report was approved and transmitted to the U.S. Army Medical Research and Development Command by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of the individual members of its constituent societies.

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office

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I. INTRODUCTION

Although it has long been known that food can be subjected to irradiation and thus preserved for extended periods (1, 2, 5), only irradiated wheat and potatoes are currently accorded official sanction in the United States.

Radiation is defined by current statutes (21 CFR 179.21, formerly 121.3001) as a food additive (3), rather than as a process, so that rigorous standards of safety must be met before food processed by irradiation can be approved for human consumption by the Food and Drug Administration. In 1954, the Surgeon General's Office undertook an extensive program to meet these standards of wholesomeness* for irradiated foods, concentrating on products of special military significance, especially beef, chicken, pork and ham.

After many years of investigation, the Army was ready for a definitive study and called upon governmental and academic scientists to devise an experimental protocol that would determine unequivocally the safety or hazard of foods subjected to sterilizing doses of irradiation. Beef was chosen as the first food to be tested because of its wide consumption in the military and its popularity with the American public. Extensive discussions were held with officials of the FDA and with governmental and academic scientists. A task force was established under the aegis of the National Academy of Sciences - National Research Council to provide overall guidance. As a result, a comprehensive long-term experiment was designed that would evaluate the effect of feeding irradiated beef to several generations of mice, rats and dogs. The animals were to be subjected to a comprehensive toxicological study to uncover any acute or chronic harmful effects of this diet. On March 1, 1971, a contract was awarded to the Industrial Bio-Test Laboratories, Inc. of Northbrook, Illinois to conduct the prescribed study. At the suggestion of the Food and Drug Administration, the Army expanded its wholesomeness assay to include mutagenic and teratogenic effects as well as analysis of heavy metals, pesticide residues and organic volatile compounds.

The Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) was asked to undertake an evaluation of the possible toxicity of certain compounds found in irradiated beef. To accomplish this task, the staff of LSRO compiled relevant data on the distribution, metabolism and toxicology of

*With reference to irradiated foods, the term "wholesomeness" is generally used to encompass microbiological, nutritional and toxicological safety (4).

those compounds found in irradiated beef by the Army , LSRO also convened a committee of investigators in biochemistry, pharmacology, oncology, toxicology, food technology and nutrition to review the available data and to assess the health aspects of the compounds separately and in toto. The committee members are listed in Section IX (p. 111).

This report contains the findings and conclusions of this committee.

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*To facilitate referral, the references cited appear at the end of each section rather than in a single bibliography for the entire report. Consequently, some references appear in more than one section.

II. MATERIALS AND METHODS

The chemical analyses which form the basis of this report were performed on samples randomly selected from large batches of beef prepared for the animal-wholesomeness studies.

A. BEEF PREPARATION

Fresh beef was processed by a major packing house in a plant inspected by the U.S. Department of Agriculture (USDA), meeting all USDA requirements. Cleanup was done during the off-shift (usually at night). After cleanup, all equipment was sprayed with food-grade white oil and not rinsed before use, in accordance with the USDA approved procedures.

Cattle, approximately 500 kg live weight, were placed in restraining racks, stunned and hung on rails by their hind legs. They were slaughtered and dressed conventionally. The carcass was split to yield approximately 162 kg per side. These sides were washed with hot- and cold-water sprays and placed for 24 to 72 hours in a chilling room at -3° to 2°C. Refrigeration was of the ammonia type. The water in the plant was chlorinated to meet USDA requirements. The sides of beef were cut into front and hind quarters and transferred to the cutting table by a stainless steel conveyor. The quarters were then deboned, partially defatted and cut into large primal cuts.

For production lots 1, 2, 3 and 4, the meat from all portions of the carcass was cut by hand into 60- to 700-gram pieces. For production lots 5, 6 and 7, the meat was moved by the conveyor to a slicing machine which cut the meat into 2.5-cm strips. The hand-cut or machine-sliced meat was transferred to a meat tub holding 325 kg. When held in these tubs, the meat was always covered with a sheet of plastic. A sample of this meat, removed for chemical analysis, represents the "raw" beef shown in Table 1. The remainder was placed in a 650-kg capacity ribbon vacuum mixing machine and sodium chloride, sodium tripolyphosphate (TPP) and ice were added to the meat in the following proportions.

	Basic ratio kg	Typical batch kg
Deboned meat	100.000	650.000
NaCl	0.750	4.875
TPP	0.375	2.437
Ice	3.000	19.500

The mixer was evacuated to 200 to 250 torr and the meat was mixed with these additives for at least 20 minutes at 3° to 5°C. The mixture was then transferred to meat tubs, kept in coolers at -3° to 2°C for not more than 24 hours, and then loaded into a dumping machine which was a large, funnel-like piece of equipment used to fill the stuffing machine located on the lower floor. The stuffing machine filled casings to fit two types of containers: no. $6\frac{1}{2}$ casings for cans and no. 11 casings for pouches. On the clipping table the filled casings were cut into rolls containing 15 kg for cans and 11.5 kg for pouches.

Thirty of these rolls were placed on each of seven trees (210 rolls) and treated in stainless steel cookhouses which were normally employed as smokehouses. To produce the enzyme-inactivated beef, they were washed before use and smoke was not added. The total elapsed time from the mixer to cookhouse was at least 1, but not more than 24 hours. In the cookhouse, which was heated with hot steam coils, the meat was exposed to gradually increasing air temperatures:

First 3 hours	50° to 60° C
Next 6 hours	60° to 71° C
As required	71° to 90° C

To obtain the desired center temperature of 68° to 75° C and a yield of not more than 85 percent deboned weight of the raw beef, steam was injected to control the humidity of the cookhouse.

Next, the rolls were spray washed with cold water and the trees were transferred from the cookhouse to a cooler (-12° to 1°C) until the center temperature cooled to -3° to 5°C. This temperature must be reached within 12 hours. The rolls were kept in the cooler at -3° to -2°C for up to 8 days if their casings had not been removed. They were then placed in meat tubs and moved to the processing room for canning or pouching at 10°C. Four separate products were processed: frozen controls, thermally processed gamma irradiated and electron irradiated.

Frozen Controls

The rolls were placed on the cutting table, the casings were removed and the meat was cut into 1.3-kg pieces which were packed into spray-washed 404 x 700 cans, evacuated to 600 torr before sealing, packed 12 cans to a case and frozen at -40° to -18°C. The frozen samples were shipped to the feeding site and kept frozen at -20° to -18°C until used.

Thermally Sterilized

The rolls were placed on the cutting table, their casings were removed, the meat was cut into 0.37-kg pieces, and packed into 404 x 202 cans. The cans were evacuated to 600 torr, sealed, placed in retort baskets and thermally processed to F_o =5.8 (a minimum of 5.8 minutes at 121°C). The cans were labeled and packed 48 cans to the case. Samples were removed, incubated for 20 days at 35.6°C and were inspected for sterility by the USDA. The cases were then shipped to the feeding site and stored at 21° to 25°C until used.

Gamma Irradiated

The rolls were placed on the cutting table, their casings were removed and the meat was cut into 1.3-kg sections. These were packed into 404 x 700 cans which were then evacuated to 600 torr and sealed. Dosimetry labels were placed on the lids, the cans were packed 12 to the case, frozen at -40° to -18°C and shipped to the Natick Laboratories in the frozen state. Here they were stored at -45 to -35°C until irradiated with cobalt at an average dose of 56.0 kGy (5.6 megarads) at -30° ± 10°C, inspected and shipped to the feeding site where they were stored at 21° to 25°C until used.

Electron Irradiated

The rolls were taken from the cages, placed on the cutting table and the casings were removed. The meat was cut into 225-g slices and transferred to meat trays. The slices were packed into flexible pouches and vacuum sealed. Dosimeters were placed on the pouches, which were kept overnight at 1° to 2° C in meat tubs. The pouches were then inspected for vacuum integrity and packed eight pouches per box and eight boxes per case. They were frozen at -40° to -18°C and shipped to Natick, where they were stored at -45° to -25°C until irradiated with an average dose of 56 kGy (5.6 megarads) at -30°±10°C by a 10 MeV electron linear accelerator. After inspection, the irradiated pouches were shipped to the feeding site, where they were stored at 21° to 25° C until used.

B. ANALYTICAL (1, 2, 3)

All analyses were performed by the Analytical Chemistry Group, Food Sciences Laboratory, U.S. Army Natick Research and Development Command under the direction of Dr. C. Merritt, Jr.

Preparation of Meat Samples

"Uncooked" meat was removed from the container, cut into approximately one-inch cubes, ground through a quarter-inch plate, and mixed well.

"Cooked" samples were prepared to simulate the treatment accorded the beef prior to its consumption in the wholesomeness study. Meat cubes (300-400 g) plus their juices were placed in 2- to $2\frac{1}{2}$-inch thick layers in aluminum foil trays. The meat was covered and heated for 15 minutes in a convection type oven at 204° C. The covers were then removed while heating continued for 2 minutes more. The heated meat and juices were ground and mixed.

Collection of Total Condensate

The coarsely ground sample was weighed to the nearest gram and placed in a cylindrical vacuum bottle of approximately one liter capacity. The flask containing the sample was attached to a vacuum manifold, cooled to -196° C in a liquid nitrogen bath and the system evacuated to an absolute pressure of 1×10^{-3} torr. The volatile compounds were vacuum distilled at 30° ± 5°C. This temperature was maintained by periodically changing the water bath around the flask. The volatiles were collected in a smaller cylindrical sample bottle (approximately 400 ml) immersed in a liquid nitrogen bath. Both the vacuum manifold and the sample bottle had previously been evacuated to an absolute pressure of less than 1×10^{-3} torr. This distillation continued for 6 hours. The condensed distillate in the smaller cylindrical sample bottle represents the total condensate.

Fractionation of the Total Condensate

The total condensate (mostly water) was allowed to thaw. The flask was then immersed in a bath of dry ice and ethanol; in some instances acetone replaced the ethanol. When the sample had reached the temperature of dry ice mixture (about -80° C), the compounds volatile at this temperature were distilled from the total condensate and collected in a second sample bottle immersed in a liquid nitrogen bath. This thawing, freezing and distillation cycle was repeated five times, or until the absolute pressure in the bottle in the dry ice-ethanol bath was less than 5×10^{-3} torr. This process divided the total condensate into two fractions; the water fraction or the residue in the bottle immersed in the dry ice-ethanol bath; and the CO_2 fraction, the distillate collected at -196° C (liquid nitrogen bath).

Extraction and Concentration of the Water Fraction

The water fraction was extracted with three 10 ml portions of diethyl ether. The ether extract was vacuum distilled from a dry ice-ethanol bath. The distillate was condensed in a bottle immersed in a liquid nitrogen bath. The residue from this procedure is the concentrated water fraction.

Analysis of the Volatile Fractions

The fractions were subjected to gas chromatography. A support-coated open tubular (SCOT) column coated with 1, 2, 3 tris (2-cyanoethoxy) propane (TRIS), precooled to -100° C and programmed from -50° to 125° C at 5° C per minute was used for the CO_2 fraction. For the water fraction, a SCOT column coated with carbowax 20M was used, precooled to -50° C and programmed from 0°C to 200° C at 5° C per minute. The effluents flowed directly into the source of a time-of-flight mass spectrometer which allowed the acquisition of both qualitative and quantitative data.

Chloroform Extraction of Beef Residue After Collection of Total Condensate

This procedure has been used thus far only on four beef wholesomeness samples. All four of these samples were from a single procurement.

The beef residue was placed in a Waring blender with 250 ml of chloroform at room temperature. The mixture was blended for 5 minutes, filtered and the residue washed with 30 ml of chloroform. The combined filtrate and wash were placed in a separatory funnel, the residual water was removed and the chloroform was evaporated at 25° C in a rotary evaporator. The flask containing the residue was fitted with a cold finger and attached to a high-vacuum system.

The cold finger was filled with liquid nitrogen and the flask was evacuated. The residue was heated to 80° C with continual magnetic stirring. The distillate was collected on the cold finger for two hours and then washed into a test tube with several small aliquots of chloroform totaling 10-12 ml. The volume of chloroform was reduced to approximately 20 µl by evaporation under a gentle stream of nitrogen gas. The volume of the remainder was measured and a 0.2µl aliquot was analyzed by combined programmed temperature gas chromatography-mass spectrometry. The gas chromatographic

column was a SCOT column coated with carbowax 20M, precooled to 0°C and programmed from 0°C to 200°C at 5°C per minute with a helium carrier gas flow rate of 5 ml per minute.

The sensitivity of the analytical technique is approximately 1 ppb or 1 μg of compound detectable per kg of beef.

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III. RESULTS

Table 1 is a list of the 65 compounds and their concentrations detected in the cooked and uncooked samples of frozen, thermally sterilized and irradiated beef. The variously processed beef samples on which these analyses were made are aliquots of the batches used in the animal wholesomeness experiment. This use of identically treated beef permits a correlation between the chemical and the feeding studies. The amounts of the individual compounds range from 1 to approximately 700 µg per kg. The hydrocarbons are by far the most abundant, both in their number and their quantities; 70 percent of all the substances and which comprise almost 90 percent of the total weight, fall into this category. The saturated aliphatics (alkanes) predominate. Their content exceeds the combined total of alkenes and alkynes by 1.5 times and of the aromatic hydrocarbons by more than 60 times.

As might be expected, heat causes a significant loss of the volatile components, so the concentrations in the cooked samples are almost always lower than in the uncooked beef. Thus, ethane was found in the uncooked irradiated specimens, but none could be detected in the cooked samples. Methane, an even more volatile hydrocarbon, was absent from both the cooked and uncooked beef fractions, although theoretically, significant quantities should have been produced by irradiation.

The type of radiation, gamma rays by ^{60}Cobalt or high energy electrons by the linear accelerator, does not significantly affect the kind or amount of compounds produced. Consequently, no distinction has been made between these sources in considering the radiolytic products.

The presence of a compound in irradiated beef does not necessarily imply that it is a radiolytic product. With few exceptions, all of the compounds in Table 1 have been found in other foods, often in concentrations exceeding those in irradiated beef. As is evident from this table, many but not all of the compounds increase significantly after irradiation. Most of the aliphatic hydrocarbons are substantially more abundant in irradiated than in nonirradiated beef. However, the quantities of xylene and tetrachloroethylene are essentially the same whether or not the beef was irradiated, while acetonitrile, carbonyl sulfide, dimethyl disulfide, methanol and methyl heptane are present in greater amounts in the thermally sterilized than in the irradiated samples.

The kind and concentration of the resulting products will be markedly influenced by the fat content of the beef and by irradiation parameters such as dose, temperature and oxygen tension (see Section IV).

Table 1. Compounds identified in beef.

Compound	Cobalt irradiation[a]				Linac irradiation[a]				Thermally sterilized[a]				Frozen control[a]				Raw[a]	
	Cooked		Uncooked		Cooked		Uncooked		Cooked		Uncooked		Cooked		Uncooked			
	ppb	SD	ppb	SD	ppb	SD	ppb	SD	ppb	SD	ppb	SD	ppb	SD	ppb	SD	ppb	SD
Alkanes																		
Ethane	0	-	172	20.2	0	-	179	19.0	0	-	0	-	0	-	0	-	0	-
Propane	60	19.7	164	16.9	65	20.3	173	19.7	0	-	0	-	0	-	0	-	0	-
Butane	125	39.7	208	23.1	127	42.0	221	31.0	0	-	0	-	0	-	0	-	0	-
Pentane	170	54.1	205	21.4	180	52.8	203	49.7	4	1.9	8	2.1	2	0.7	1	0.7	1	-
Hexane	207	69.8	209	23.2	248	59.9	217	24.9	67	24.5	125	32.1	7	1.9	6	1.5	1	-
Heptane	281	96.1	417	43.6	298	88.1	438	50.4	62	16.6	102	25.9	8	2.2	10	1.7	1	1.1
Octane	284	91.2	348	37.4	302	84.9	367	42.2	0	-	47	8.3	0	-	0	-	0	-
Nonane	125[c]	-	266[c]	-	146[c]	-	-	-	0	-	0	-	0	-	0	-	0	-
Decane	175[c]	-	362	278-436[b]	184[c]	-	-	-	0	-	0[c]	-	0	-	0	-	1	0.4
Undecane	217[c]	-	176	108-267[b]	203[c]	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Dodecane	326[c]	-	207	98-317[b]	286[c]	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Tridecane	-	-	321	293-362[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Tetradecane	-	-	313	231-392[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Pentadecane	-	-	696	617-796[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Hexadecane	-	-	221	122-286[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Heptadecane	-	-	394	376-439[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Alkenes																		
Ethene	0	-	28	3.3	0	-	28	3.2	0	-	0	-	0	-	0	-	0	-
Butene	12	5.2	32	3.5	13	4.5	33	3.6	0	-	0	-	0	-	0	-	0	-
Pentene	2	1.0	36	4.5	2	0.9	38	3.6	3	0.8	4	4.4	3	1.0	0	-	0	-
Hexene	2	1.1	34	4.6	2	0.9	35	4.9	22	4.7	31	8.5	1	-	1	-	4	0.7
Heptene	46	14.1	111	13.5	45	15.7	116	14.5	12	5.7	31	8.0	2	6.2	1	-	0	-
Octene	22	8.4	95	11.7	20	7.4	97	10.1	0	-	0	-	0	-	0	-	0	-
Nonene	33[c]	-	59[c]	-	48[c]	-	61	9.1	0	-	0	-	0	-	0	-	0	-
Decene	101[c]	-	126	70-161[b]	116[c]	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Undecene	82[c]	-	78	54-103[b]	93[c]	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Dodecene	171[c]	-	156	113-229[b]	162[c]	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Tridecene	-	-	178	121-268[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Tetradecene	-	-	488	324-588[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Pentadecene	-	-	121	98-187[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Hexadecene	-	-	156	116-234[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Heptadecene	-	-	618	583-803[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Iso-alkanes																		
2-Methyl propane	27	8.1	45	6.9	28	9.2	47	7.4	0	-	9	1.4	0	-	0	-	0	-
2-Methyl butane	4	1.5	19	2.4	5	1.4	22	3.4	0	-	143	46.0	0	-	0	-	1	-
2-Methyl pentane	10	3.4	33	4.3	11	4.1	34	3.1	1	-	28	8.5	1	-	0	-	0	-
2-Methyl heptane	11	4.1	29	39.5	12	4.0	24	3.2	27	10.1	47	10.8	4	1.2	4	1.0	0	-

iso-alkenes																		
2-Methyl propene	2	0.8	37	4.6	2	0.9	39	5.3	0	-	4	1.0	0	-	0	-	0	-
Alkynes																		
Decyne	25[c]	-	23	18-27[b]	23[c]	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Undecyne	9[c]	-	4	0-6[b]	11[c]	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Dienes																		
Tetradecadiene	-	-	98	83-113[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Pentadecadiene	-	-	73	68-79[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Hexadecadiene	-	-	706	626-839[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Heptadecadiene	-	-	16	12-21[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Aromatic hydrocarbons																		
Benzene	15	5.1	18	2.5	14	5.0	19	2.1	2	-	0	-	3	-	0	-	0	-
Toluene	50	17.9	65	7.1	50	17.1	66	7.4	48	14.5	73	20.5	3	1.6	6	1.5	0	-
Xylene	4	1.9	1	0.2	3	1.2	1	0.5	7	1.4	7	4.7	1	-	1	-	0	-
Alcohols																		
Methanol	16	6.2	20	3.2	15	5.2	19	2.6	23	6.7	40	9.7	41	12.0	0	-	0	-
Ethanol	76	25.7	122	28.3	73	30.3	124	16.2	9	2.9	15	3.9	18	1.0	0	-	0	-
Ketones																		
Acetone	108	31.3	137	14.9	106	32.8	140	14.1	65	20.1	120	28.1	3	1.1	4	1.1	1	0.6
2-Butanone	71	24.3	88	10.0	72	24.7	90	9.3	5	1.6	10	2.5	5	3.3	0	-	0	-
Aldehydes																		
2-Methyl pentanal	11	3.2	10	1.8	10	3.3	11	1.9	0	-	0	-	0	-	0	-	0	-
Undecanal	-	-	76	52-89[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Dodecanal	-	-	63	53-78[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Tetradecanal	-	-	54	47-65[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Pentadecanal	-	-	46	41-52[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Hexadecanal	-	-	127	94-187[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Octadecanal	-	-	30	26-34[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Hexadecenal	-	-	33	27-41[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Octadecenal	-	-	398	371-432[b]	-	-	-	-	-	-	0[c]	-	-	-	0[c]	-	-	-
Sulfur compounds																		
Carbonyl sulfide	2	0.8	2	0.9	2	0.8	2	0.8	22	16.0	75	16.0	0	-	0	-	0	-
Hydrogen sulfide	2	0.8	2	0.6	1	0.6	1	1.7	1	1.4	5	1.3	0	-	0	-	0	-
Ethyl mercaptan	7	2.4	9	3.1	7	2.5	11	2.2	0	-	0	-	0	-	0	-	0	-
Dimethyl sulfide	4	1.3	5	1.5	4	1.4	6	1.4	0	-	0	-	0	-	0	-	0	-
Dimethyl disulfide	3	1.0	4	0.8	3	1.2	4	0.8	7	2.9	13	3.3	1	-	1	-	0	-
Choloro compounds																		
Tetrachloroethylene	9	3.5	8	1.5	9	3.1	8	1.8	9	3.2	11	2.8	10	3.0	12	1.8	4	0.8
Miscellaneous																		
Acetronitrile	3	1.0	1	-	3	0.9	1	-	21	12.7	57	14.2	6	9.1	3	0.7	0	-

[a] see text for treatment
[b] range of 4 determinations
[c] determined for only one sample

ppb = parts per billion
SD = standard deviation
0 = below detectable limits
- = not determined

NOTE: Unless otherwise indicated, figures represent averages of 10 samples for raw beef and approximately 50 samples for other treatments.

In this connection, it must be emphasized that the Committee considered only those compounds detected in the irradiated beef prepared and analysed according to the procedures described in the experimental section (pages 10 to 15). This approach permitted correlation of the Committee's study with the wholesomeness experiments in which the animals were fed beef composed and irradiated in the manner described. It is recognized that variations in the beef composition, the irradiation technique or the analytical procedures may modify these results.

During the course of this study, analytical techniques have been improved to allow determination of certain higher molecular weight compounds not originally reported in the quantitative analysis. This effort to improve the analytical methodology is a continuing project at the Natick laboratories. A recently developed, unpublished procedure utilizing dichloromethane appears to extract certain hydrocarbons more thoroughly than previous techniques. Very preliminary results on a single sample reveal significantly higher levels of pentadecane and heptadecene in the beef than those shown in this report. It is hoped that continued analytical refinements will ultimately ensure a comprehensive and accurate inventory of compounds in irradiated beef. However, at present, the Committee must confine its consideration to the best available data, recognizing both their qualitative and quantitative limitations.

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IV. VARIABLES AFFECTING RADIOLYTIC PRODUCTS

Irradiation produces volatile compounds in meat because the energy absorbed from the electron or gamma source is sufficient to ionize any atom with which it interacts. Interatomic bonds are broken, thus fragmenting the molecules and forming free radicals which can recombine to form new compounds. In most biological systems, water is the most abundant compound and the most obvious target of high energy irradiation. Transitory radiolytic products of water are formed; these react with other molecules or fragments to produce a number of compounds (2). Irradiation may also directly cleave bonds in organic molecules to produce free radicals. The recombination of these molecular fragments creates new compounds. Theoretically, irradiation of a complex matrix such as beef should produce a multitude of different fragments whose combination could result in numerous radiolytic products. In the frozen state, the number of new products would be reduced somewhat since the rigid structure would impede the reaction of molecular fragments. Many of these products would be nonvolatile and would have remained undetected by the analytical methods employed in this study. These compounds were not considered.

A. FAT CONTENT

Investigators have shown the major source of volatile compounds formed upon irradiation of beef to be the lipid fraction (5, 8, 9, 10). Protein is of secondary importance in their production and carbohydrates, vitamins, sterols and pigments make an even lesser contribution (8). This is graphically demonstrated by Figures 1 and 2. At a constant temperature and radiation dose, production of C_5 to C_8 alkanes was three to five times greater when high fat (35 percent) beef was irradiated than when lean specimens (5 percent) were similarly exposed. The concentration of acetaldehyde also increased with increasing fat content, albeit at a much lower rate. On the other hand, the amounts of benzene, toluene and dimethyl disulfide increased only slightly, if at all, after irradiation of the high fat compared with the lean beef samples. This is consistent with the contention that these latter compounds come from nonfat sources (8).

A major effort has been made to elucidate the mechanism of fat radiolysis. Merritt and Nawar and their respective co-workers have been the leading investigators in this area. Merritt et al. (10) were the first to point out that the chief products of fat irradiation are saturated and unsaturated aliphatic hydrocarbons. Subsequent studies confirmed and extended these observations (5, 9). Nawar's group (3, 11) using

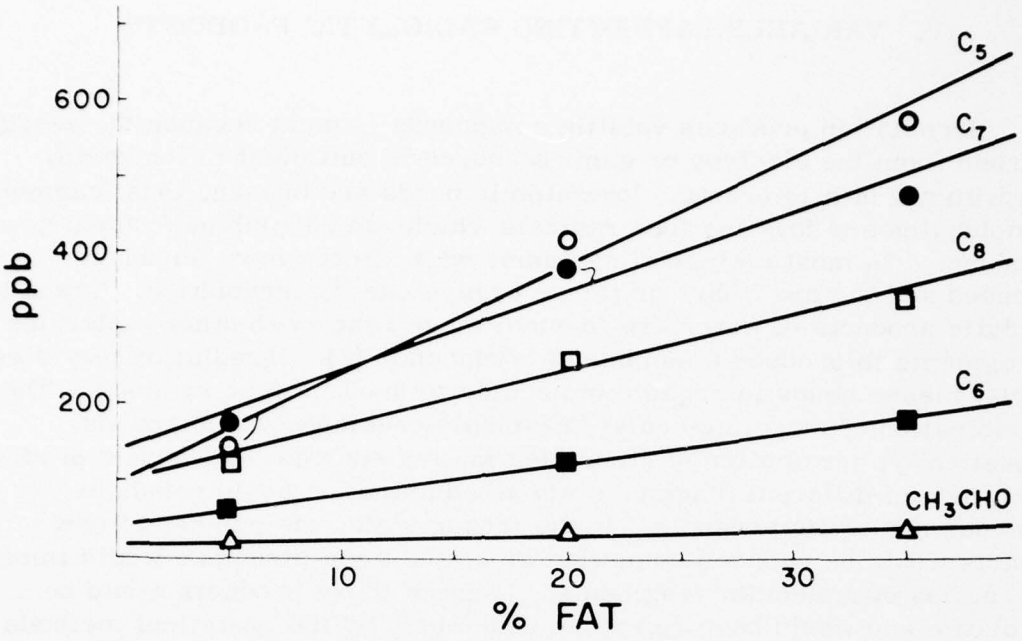


Figure 1. Effect of fat content on component concentrations. Irradiation temperature: -30°C, dose 45 kGy (4.5 megarads). From 7 with permission.

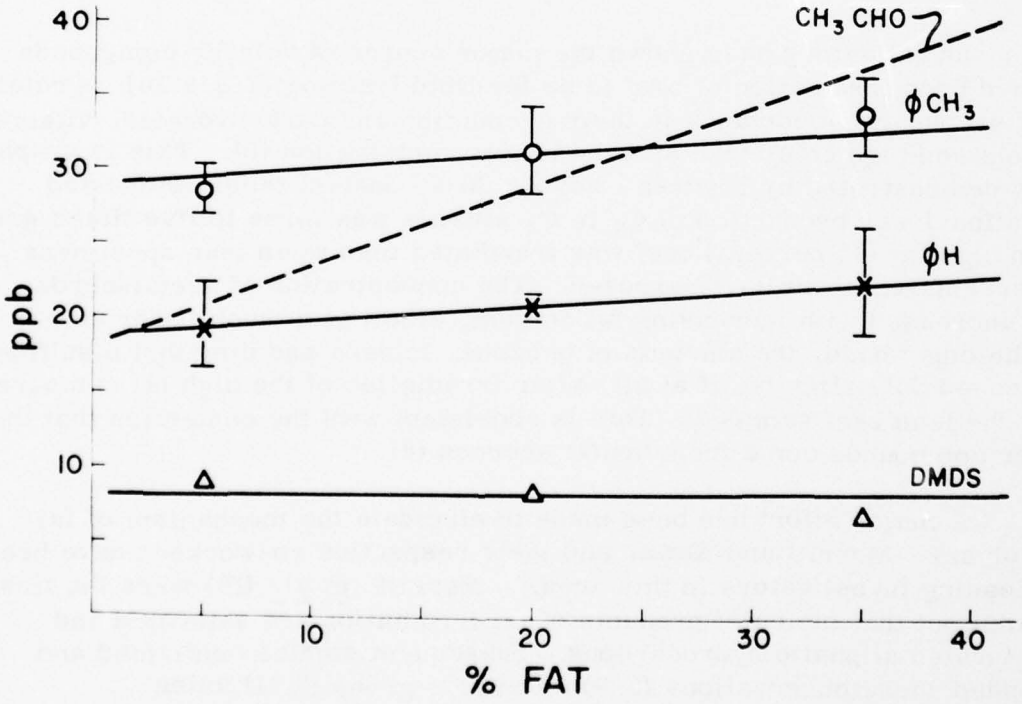
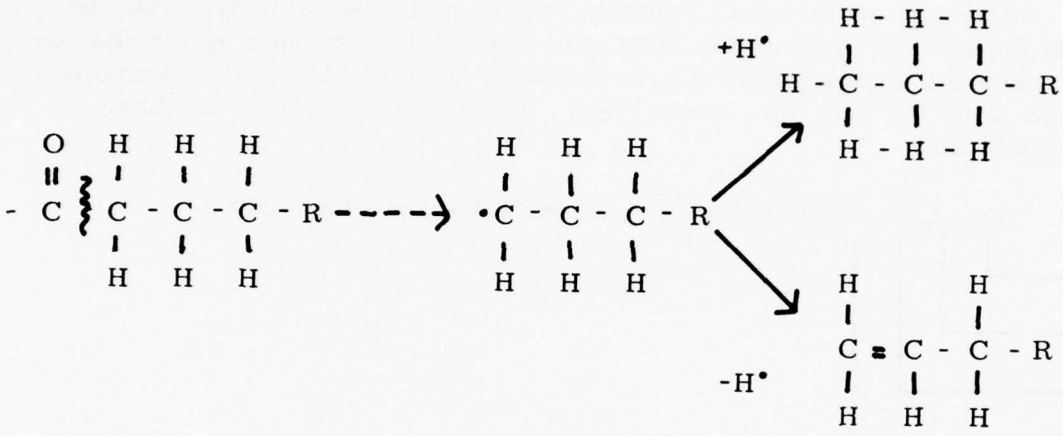
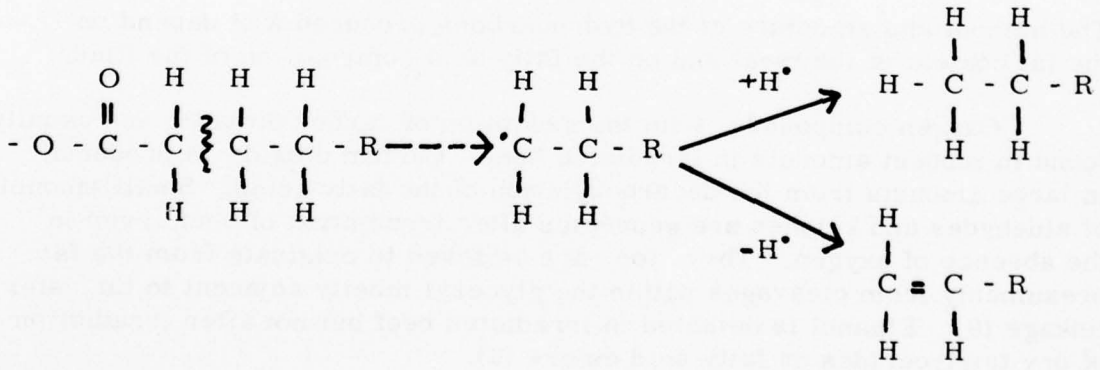


Figure 2. Effect of fat content on component concentrations. Irradiation temperature: 30°C, dose 45 kGy (4.5 megarads). From 7 with permission.

purified model systems, has been especially successful in defining the pathway of hydrocarbon formation. They have confirmed the production of homologous series of alkanes and alkenes when various fats are irradiated and have provided persuasive evidence of the mechanism involved. They have demonstrated that carbon bonds near the carbonyl group of the fatty acid or triglyceride are the most vulnerable to cleavage (3). If the carbon alpha to the carbonyl is ruptured, alkanes and alkenes are produced with one carbon less than the parent fatty acid:

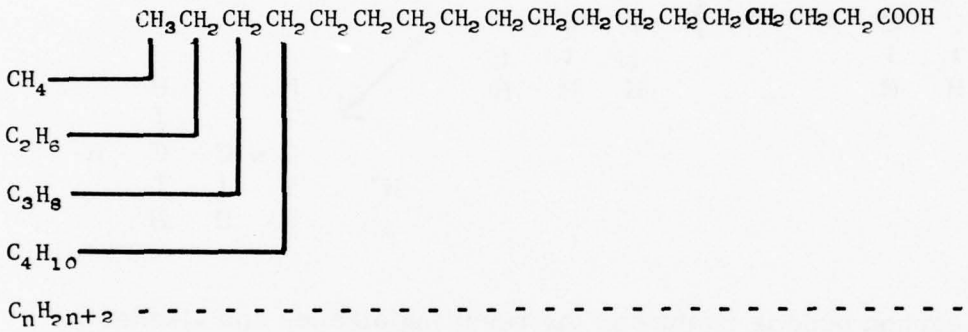


If the beta carbon bond is ruptured, the resulting alkanes and alkenes possess two carbons less than the original acid:



These C_{n-1} and C_{n-2} alkanes and alkenes are the most abundant products of lipid irradiation. If the original fatty acid is unsaturated, the production of an additional double bond by irradiation results in a diunsaturated hydrocarbon, or diene. Thus, oleic acid, the most common unsaturated compound in animal fat (C18:1), yields significant quantities of hexa- and heptadecadiene after irradiation. Similarly, irradiation will convert a diunsaturated fatty acid (linoleic) to a triene and a triply unsaturated member to a tetraene.

Although preferential scission occurs near the carbonyl grouping any of the carbon bonds in the fatty acid chain can be broken by irradiation to produce the entire gamut of hydrocarbons from C_1 to its C_{n-1} homologue. Stearic acid, for example, could yield all hydrocarbons from methane to heptadecane:



The amount and structure of the hydrocarbons produced will depend on the fat content of the meat and on the fatty acid composition of the lipid.

Oxygen compounds, with the exception of carbon dioxide, are usually found in modest amounts in irradiated beef. Carbon dioxide is produced in large amounts from the decarboxylation of the fatty acids. Small amounts of aldehydes and ketones are generated after irradiation of beef, even in the absence of oxygen. They, too, are believed to originate from the fat, presumably from cleavages within the glyceryl moeity adjacent to the ester linkage (9). Ethanol is detected in irradiated beef but not after irradiation of dry triglycerides or fatty acid esters (6).

The small amounts of sulfur and aromatic compounds resulting from radiolysis probably come from direct bond cleavage of amino acid moeities. Merritt et al. (8) could find no evidence for the rupture of peptide bonds. The main products from protein irradiation originated from the cleavage of side chains or end groups. The aromatic amino acids or those with sulfur groups seem the most radiosensitive. Benzene

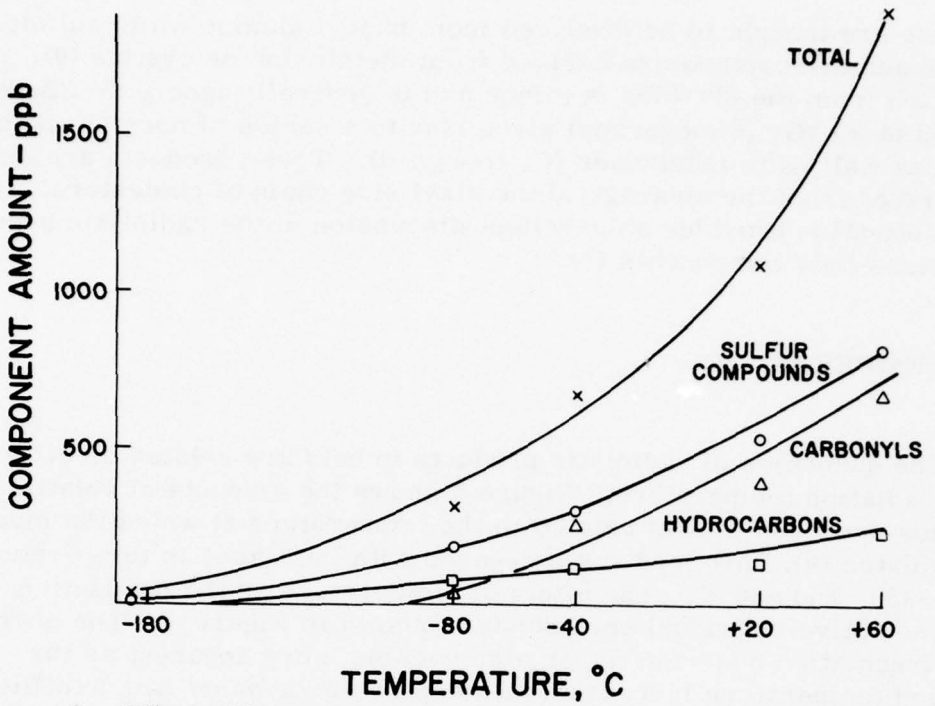


Figure 3. Effect of temperature on component concentration. Irradiation dose 45. kGy (4.5 megarads). From 9 with permission.

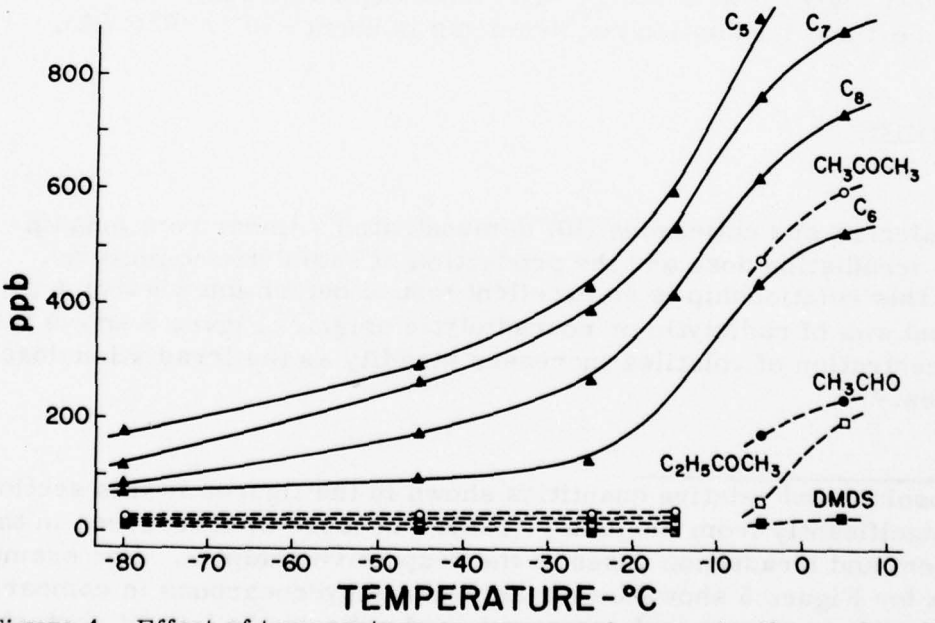


Figure 4. Effect of temperature on component concentrations. Irradiation 45. kGy (4.5 megarads). From 7 with permission.

and toluene are thought to be produced from phenyl alanine while sulfides, disulfides and mercaptans are derived from methionine or cystine (6). The contribution from the steroids is minor and is generally ignored. Cholesterol irradiated at 60 kGy (6 megarads) gives rise to a series of normal alkanes C_1 to C_7 as well as to isoalkanes (C_4 to C_8) (8). These products are most likely derived from the cleavage of the alkyl side chain of cholesterol. A recent publication provides an excellent discussion of the radiolytic products from various food components (4).

B. TEMPERATURE

The quantities of radiolytic products in beef are related directly to the irradiation temperature. Figure 3 shows the amounts of volatile compounds produced in beef related to the temperature at which the meat was irradiated (9). All products increased with increases in temperature between -185° and +60°C. The effect of temperature on the production of representative individual compounds is shown in Figure 4. The characteristic irradiation odor and flavor also became more apparent as the irradiation temperature increased. An expert flavor panel had no difficulty in identifying the beef irradiated at the higher temperatures (9).

Although volatiles are minimal at extremely low temperatures, economic considerations dictate that the irradiation be accomplished at a somewhat higher temperature. The most favorable compromise among quality, cost and irradiation requirements is about -30° ± 10°C (12).

C. DOSE

Merritt and colleagues (10) demonstrated a linear relationship between irradiation dose and the production of radiolytic compounds. In fact, this relationship is an excellent test to determine whether a given compound was of radiolytic or nonradiolytic origin. Figure 5 shows that the concentration of volatiles increases steadily as the irradiation dose increases.*

*The absolute and relative quantities shown in the figures in this section differ significantly from the data in Table 1 because of differences in the fat content and irradiation doses of the respective samples. For example, the data for Figure 5 shows lesser amounts of hydrocarbons in comparison with the sulfur and carbonyl compounds and reflects the low fat content (2 to 3 percent) of this sample. The values in Table 1 are from beef with a fat content of 10 to 12 percent.

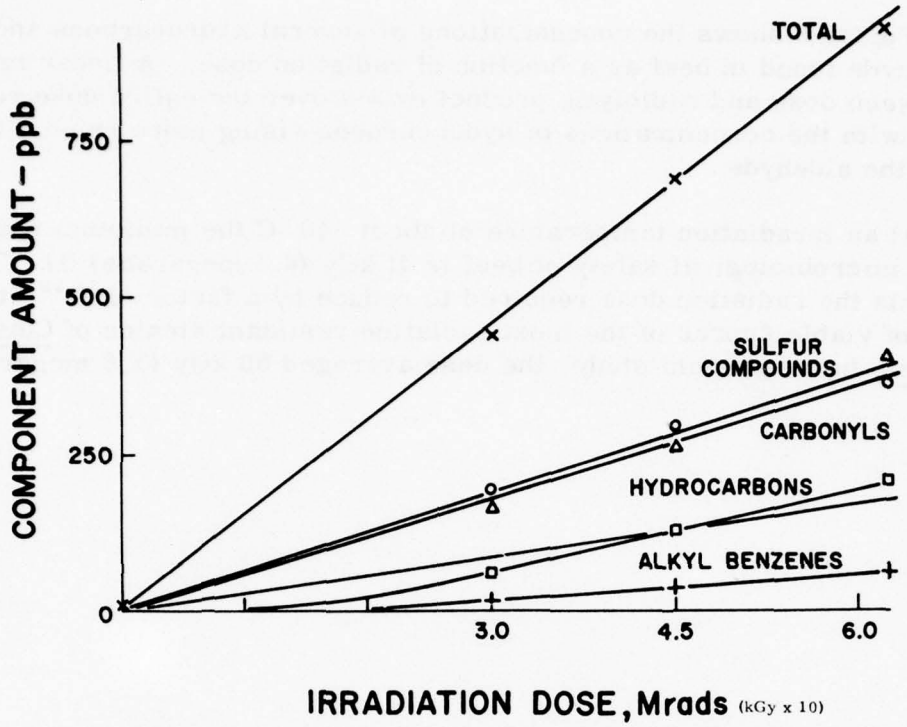


Figure 5. Effect of irradiation dose on component concentration. Irradiation temperature -40°C. From 9 with permission.

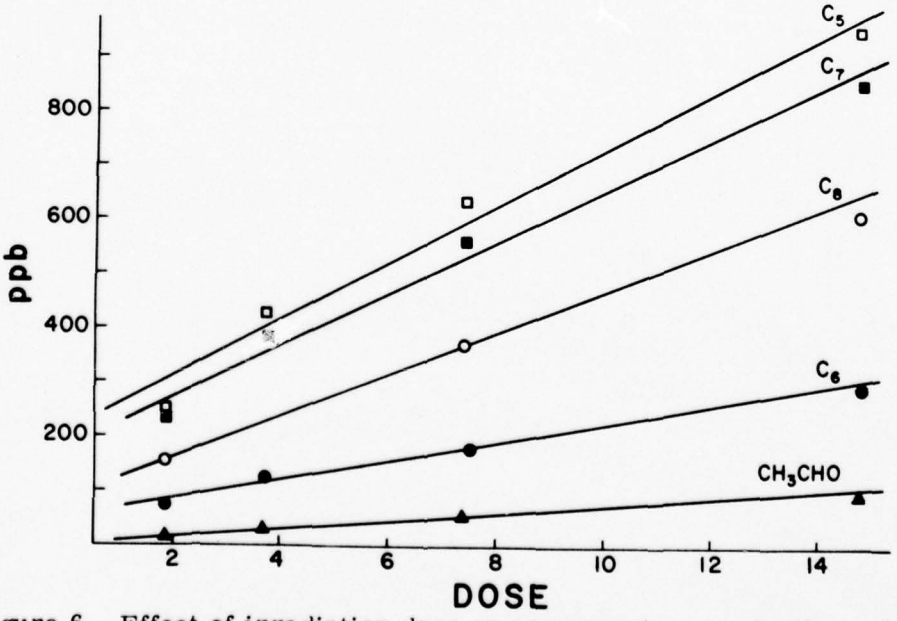


Figure 6. Effect of irradiation dose on component concentration. Irradiation at -30°C. From 7 with permission.
(To convert to kGy, multiply megarads by 10)

Figure 6 shows the concentrations of several hydrocarbons and of acetaldehyde found in beef as a function of radiation dose. A linear relationship between dose and radiolytic product exists over the entire dose range studied, with the concentrations of hydrocarbons rising more rapidly than those of the aldehyde.

At an irradiation temperature of about -40° C the minimum radiation dose for microbiological safety of beef is 41 kGy (4.1 megarads) (1). This represents the radiation dose required to reduce by a factor of 10^{12}, the number of viable spores of the most radiation-resistant strains of Clostridium botulinum. In the present study the dose averaged 56 kGy (5.6 megarads).

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V. EVALUATION OF HEALTH EFFECTS

Designating "safe" levels for the intake of specific substances is a perennial, elusive and perhaps impossible goal. The goal is an ever-moving target, constantly changing as new data become available and analytical techniques become more sensitive. Absolute safety can never be assured. Nevertheless, assessments of safety are often required even when data are not always adequate. In the face of this dilemma, prudence suggests that realistic guidelines be developed to gauge the potential benefit and hazard of products to which the public is exposed. Various expert commissions have attempted to formulate such guidelines for food additives, but no universally acceptable criteria have yet been established.

Perhaps the most widely employed concept is that of margin of safety, based on some fraction of the largest dose of a substance that can be given to animals or man without causing adverse effects. Often 1 percent of the minimum toxic dose, determined experimentally in animals is considered safe for man (2). This is an arbitrary measure, with the obvious dangers of extrapolating from animal to human exposures and of variations in individual sensitivity.

A more arbitrary guideline is the attempt by some bodies to specify an absolute quantity of a substance as "toxicologically inconsequential" or "toxicologically insignificant." Values of 1 to 10 parts per million in foodstuffs have been suggested by various groups. The Committee believes this "guideline" to be potentially dangerous for many substances, such as aflatoxin, plutonium, botulinum toxin, dioxin and others, are serious health hazards at even lower levels.

Other criteria of safety suggested by one or another working group include the long-term consumption of a substance without apparent hazard; its presence in foods; a chemical similarity with compounds of known low toxicity; its rapid metabolism to innocuous products or its occurrence as a natural constituent or metabolite of the human body. A frequently used criterion is that of "added burden." This is interpreted as the potential increment of hazard added to that received from other sources.

The Committee utilized all of these factors and other relevant evidence in its consideration of each compound in irradiated beef. No substance was arbitrarily dismissed because of "insignificant" or "inconsequential" concentrations. The Committee found, as have other groups concerned with similar questions, that the available experimental and clinical data were at times insufficient to permit an unequivocal decision.

In these cases, the Committee's judgment is based on the best available evidence.

The various types of evidence considered by the Committee are discussed in more detail below.

A. POSSIBLE ORIGIN

As stated in the introduction, the irradiation of food has been defined by the statutes of the Food and Drug Administration as an "additive" rather than as a "process" (21 CFR 179.21, formerly 121.3001) (4). Consequently, the regulations governing food additives must be applied to irradiated foods. Although most of the compounds under discussion increased significantly after irradiation, several showed no change from the frozen control levels and cannot be considered as radiolytic products. Other compounds increased more after thermal sterilization than after irradiation. The concentrations of these compounds in frozen control, thermally sterilized and irradiated beef are compared in Table 2.

Table 2. Concentration of selected compounds in irradiated and nonirradiated beef.

Compound	Cooked			Uncooked		
	Frozen control μg/kg	Thermally sterilized μg/kg	Irradiated μg/kg	Frozen control μg/kg	Thermally sterilized μg/kg	Irradiated μg/kg
Acetronitrile	6	21	3	3	57	1
Carbonyl sulfide	0	75	2	0	16	2
Dimethyl sulfide	0	7	3	0	13	4
Hexene	1	22	2	1	31	34
Hydrogen sulfide	0	1	2	0	5	2
Methanol	41	23	16	0	40	20
2-Methyl butane	0	<1	4	0	143	21
2-Methyl heptane	4	27	12	4	47	27
Toluene	3	48	50	6	73	66
Tetra-chloroethylene	10	9	8	12	11	8
Xylene	1	7	4	1	7	1

B. ACCEPTABLE DAILY INTAKE (ADI)

A working party commissioned by the Council of Europe and representing 17 countries reviewed the toxicology of a number of widely used flavoring substances and recommended acceptable levels of daily intake in those cases where it deemed the data to be adequate (1). Five of these compounds are among those detected in the irradiated beef. These compounds and their concentrations in beef are listed in Table 3, together with the Council of Europe's ADIs. For each of these compounds, the ADI is at least several hundred times the amount one would normally ingest from beef.

Table 3. Comparison of acceptable daily intakes and quantities from beef.*

Compound	ADI		Concentration in irradiated beef	Daily beef intake (DBI)	ADI/DBI
	concentration	mg	μg/kg	mg	(ratio)
Dimethyl sulfide	1.5 ppm	2.25	5	.0006	3750
Dodecanal	1.0 mg/kg	70.0	63	.0076	9210
Ethyl mercaptan	1 ppm	1.5	9	.0011	1364
Tetradecanal	3 ppm	4.5	54	.0065	692
Undecanal	5 ppm	7.5	76	.0091	824

*The following average values were assumed in this table: average weight - 70 kg; daily food consumption - 1.5 kg; daily beef consumption - 0.12 kg. According to the American Meat Institute, the per capita annual consumption in the U.S. was 43.3 kg of dressed beef in 1976.

C. DISTRIBUTION IN FOOD AND BEVERAGES

Virtually all the compounds detected in the irradiated beef are present in other foods. Some of the simpler compounds such as methanol, ethanol, butanone, etc. have been identified in almost every food or beverage in which they were sought. In many cases, the amounts in widely consumed foods are considerably greater than in the irradiated beef. Thus, cheese is richer in ketones, eggs in sulfur compounds, citrus fruits in aldehydes and apples in certain hydrocarbons. Although the presence of a compound in a common food does not assure its safety, it does provide a standard against which the amount in beef can be compared. In addition, these data help gauge the "added burden" of substances that might be ingested from irradiated beef. It seems unlikely that the irradiated meat would constitute a significant hazard if its consumption contributed only a very small fraction of the amount of a compound entering the body from other sources. Perhaps the most striking example of the relatively trivial

addition from beef to the overall intake is that of ethanol. A person consuming one kg of irradiated beef daily throughout a long lifetime would ingest only a small fraction of the ethanol contained in a single glass of wine.

D. AUTHORIZATION BY FOOD AND DRUG ADMINISTRATION

The Food and Drug Administration authorizes the use of certain solvents and additives in the preparation and preservation of various foods (3). In some cases, the compound is authorized by name; in other cases oils or solvents with well-defined boiling or melting point ranges are indicated. Thus, dodecanal is specifically authorized as a synthetic flavoring substance, methanol as an extractant and butane as an oxygen displacer. Implied rather than specific permission is given to certain alkanes found in irradiated beef since they fall within the authorized boiling range of "odorless, light, petroleum hydrocarbons," which is a permitted additive for a number of foods. Similarly, other aliphatic hydrocarbons in irradiated beef are present in mineral oil which is authorized for certain food usages. Table 4 summarizes the various regulations in which the compounds in irradiated beef are either explicitly or implicitly authorized in food. It should be noted that more than one-third of the compounds identified in irradiated beef are found in this list, including all of the straight-chain alkanes except pentane.

E. TOXICITY OF METABOLIC PRODUCTS

Any evaluation of the toxicity of a compound must include consideration of its metabolic transformations in the body. Unfortunately, these pathways are not always known so that the fate of some compounds must be inferred by analogy to related substances whose metabolism has been studied more thoroughly. Available evidence suggests that alkanes and the oxygenated compounds are converted through well-recognized metabolic pathways. The metabolism of alkenes, alkynes and certain other compounds is not as well known. The fate of the individual compounds will be considered in detail in the following sections of this report.

Table 4. Pertinent regulations of the Food and Drug Administration.

Authorized substance	Use	Residue limits	Compounds included	Regulations: Former reference	Regulations: Revised reference
				21 CFR	
Acetone	Extractant: spices	30 ppm	Acetone	121.1042	173.210
Butane	Additive "Generally recognized as safe"	None stated	Butane	121.101	182.1165
	Antioxidant	None stated		121.1060	-
Ethanol	Antimicrobial agent	2% by weight	Ethanol	121.104	182.1293
Hexane	Extractant:				
	Spices	25 ppm	Hexane	121.1045	173.270
	Hops	2.2%			
	Hop extract	25 ppm		121.1082	172.560
Isoparaffinic petroleum hydrocarbons	Cleaning vegetables	None stated	2-Methyl heptane*	121.1154	172.882
	Insecticide component	None stated			
	Fruit, vegetable coating	None stated			
	Vinegar, wine manufacturing	None stated			
	Defoaming agent	150 ppm (yeast)		121.1099	173.340
Light petroleum hydrocarbons	Fruit, vegetable coating	None stated	Nonane*	121.1182	172.884
	Vegetable cleaning	None stated	Decane*		
	Food processing	None stated	Undecane*		
	Vinegar, wine manufacturing	None stated	Dodecane*		
	Defoaming agent	150 ppm (yeast)	Tridecane* Tetradecane* Pentadecane* Hexadecane* Heptadecane*	121.1099	173.340
Methanol	Extractant:				
	Spices	50 ppm	Methanol	121.1044	173.250
	Hops	2.2%			
	Hop extract	100 ppm		121.1082	172.560
Mineral oil	Animal feed	0.06% of ration	Tetradecane* Pentadecane* Hexadecane* Heptadecane*	121.246	
	Release agent	0.02-0.6%		121.1146	172.878
	Binder	0.02-0.6%			
	Lubricant	0.02-0.6%			
	Defoaming agent	150 ppm (yeast)		121.1099	173.340
Petrolatum	Animal feed	0.06 % of ration	Heptadecane*	121.261	
	Coating	0.02-0.2%		121.1166	172.880
	Lubricant	0.02-0.2%		121.	
	Defoaming agent	150 ppm (yeast)		121.1099	173.340
Petroleum naphtha	Extractant	None stated	Heptane* Octane*	121.1203	172.250
Petroleum wax	Coating	None stated	Heptadecane*	121.1156	172.886
	Chewing gum base	None stated		121.1156	172.888
Propane	Additive - "Generally recognized as safe"	None stated	Propane	121.101	182.1655
	Antioxidant	None stated		121.1060	
Synthetic flavoring substances	Flavoring	None stated	2-Butanone Dodecanal Dimethyl sulfide Tetradecanal	121.1164	172.515

*Within range of specified melting or boiling points.

F. DATA FROM CONVENTIONAL TOXICITY STUDIES

Although considerable toxicity data are available for many of the compounds under consideration, they are of limited utility in the present study since routine tests generally utilize single exposures to large doses. This method enables investigators to establish the LD_{50}, LD_{100}, minimum lethal dose or least toxic dose of a compound. It provides a crude index which allows one to distinguish among highly, moderately, and mildly toxic compounds. It has little relevance, however, to the problem of irradiated beef where the concern is with possible toxicity induced by repeated ingestion of small doses of a compound. Long-term studies at modest dose levels would be more informative, but are rarely reported.

Another difficulty in utilizing the available toxicity data to evaluate the compounds in irradiated beef lies in the different routes of administration. A number of the volatile compounds in irradiated beef are widely used as industrial solvents and have been carefully studied for their safety in the factory or shop. However, the primary objective of these toxicological studies has been to establish permissible levels in the workroom atmosphere, and it is difficult to utilize such data in estimating the toxicity of a compound when ingested in food. In the first place, data on the retention of inhaled compounds are scarce and not always reliable. Then, too, a compound absorbed through the lungs may undergo a fate different from that it would experience after enteral absorption. This would be particularly true for compounds that are metabolized in the lung or the liver, so that one cannot interchange with any assurance toxicity data between the two routes.

G. CARCINOGENICITY, MUTAGENICITY AND TERATOGENICITY

In recent years, some compounds long believed to be innocuous, have been implicated in tumor production, genetic alteration or birth abnormalities. The traditional toxicological indices must therefore be increased to include these parameters. Regulations concerning the possible carcinogenicity of food additives are particularly stringent. Consequently, tetrachloroethylene (perchloroethylene), benzene and certain alkenes have been scrutinized with especial care despite their low concentrations in irradiated beef. Tetrachloroethylene in very high doses has recently been shown to cause liver tumors in mice (3). Benzene is a suspected leukemogen in man, while the alkenes in their metabolism produce epoxides, some of which may be carcinogenic. These will be discussed in the appropriate sections.

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VI. ANALYSIS OF INDIVIDUAL CHEMICAL CLASSES

A. HYDROCARBONS

1. Alkanes

Occurrence

The concentrations of alkanes in cooked and uncooked samples of frozen controls and of heat- and radiation-sterilized beef are listed in Table 5. The table also includes data on the occurrence of these compounds in air, water and food. These data strongly suggest that the straight-chain alkanes are products of radiolysis for the irradiated beef contains each in a higher concentration than in the thermally sterilized samples or in the frozen controls. On the other hand, the branched compounds, except for 2-methyl propane, are found in equal or greater amounts in the thermally sterilized meat.

It is also apparent from Table 5 that many of these alkanes are common in our environment. They are found in air and water and in many foods, including untreated or cooked meats, vegetables, fruits, nuts, and dairy products. Quantitative data are not generally available, but single studies on apples (24) and eggs (20, 21) reported concentrations of the higher alkanes comparable with or greater than those found in irradiated beef. There is also evidence that some of these compounds are produced during cooking and that their content in beef is higher after microwave than after conventional cooking (22).

The lower members of the alkane series are gaseous or highly volatile at normal temperatures. They are used widely in industry as fuels, lubricants, solvents and feed-stocks for numerous chemical processes. It is not surprising, therefore, that many of these members have been detected at significant levels in metropolitan atmospheres and water supplies.

Absorption and Metabolism

All of the volatile alkanes are absorbed through the pulmonary system and their retention closely parallels the fat content of the body and tissues. Rats exposed to high concentrations of hexane vapor (170 g/m^3) for 2 to 10 hours, revealed an average tissue concentration of approximately 4 mg per g of lipid (2). Equilibrium concentrations

Table 5. Presence of alkanes in various media.[2]

Compound	Irradiated beef μg/kg		Thermally sterilized beef μg/kg		Frozen beef control μg/kg		Atmosphere μg/m³	Water μg/l	Food μg/kg
	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked			
Ethane	0	176	0	0	0	0	Los Angeles - 120 Hawaii - 10		No quantitative data, detected in apples, chicken, strawberries, vinegar
Propane	63	169	0	0	0	0	Los Angeles - 90 Hawaii - 9		No quantitative data, detected in chicken
Butane	126	215	0	0	0	0	Los Angeles - 150		Detected in canned beef
Pentane	175	204	4	8	2	1	Los Angeles - 63 Simulated spacecraft - 53	U.S. (max) 1.0	Beef, canned - 160; rum, Jamaican - 50; also found in fruits, dairy products, vegetables
Hexane	228	213	67	125	7	6	Los Angeles - 40 Simulated spacecraft - 51		Beef, canned - 29; rum, Jamaican - 50; also found in approximately 15 other foods
Heptane	290	428	62	102	8	10	Zurich - 140 Simulated spacecraft - 120		Rum, Jamaican - 50; also found in approximately 20 foods
Octane	293	358	0	47	0	0	Zurich - 16 Simulated spacecraft - 58	Detected in U.S. drinking water	Eggs - 2800; beef, canned - 7.5; also found in approximately 20 foods
Nonane	136	266	0	0	0	0	Zurich - 9 Los Angeles - 26	U.S. drinking waters -0.03-10.0	Eggs - 1900; found in approximately 15 foods
Decane	180	362	±	0	±	0	Zurich - 10	U.S. (max) - 2.4	Apples - 420; also found in approximately 15 foods
Undecane	210	176	±	0	±	0	Zurich - 7	U.S. (max) - 0.02 Effluents from oil refineries, paper mills, etc.	Eggs - 1100; apples - 330; also found in approximately 20 foods
Dodecane	306	207	±	0	±	0	Zurich - 9	U.S. (max) - 0.4 Effluents from oil refineries, paper mills, etc.	Eggs - 4900; apples - 240; also found in approximately 20 foods

Tridecane	[b]	321	[b]	0	[b]	0		U.S. (max) - 0.3 Effluents from paper mills, oil refineries and textile mills	Eggs - 700; apples - 240; also found in fruits, nuts, meats, dairy products
Tetradecane	[b]	313	[b]	0	[b]	0		U.S. (max) - < 1.0 Effluents from textile mills	Eggs - 3600; apples - 380
Pentadecane	[b]	696	[b]	0	[b]	0		U.S. (max) - 0.5 Effluents from oil refineries, paper mills	Eggs - 22,200; apples - 380; also found in approximately 20 other foods
Hexadecane	[b]	221	[b]	0	[b]	0		Detected in U.S. drinking water Effluents from oil refineries, paper mills, nylon plants	Eggs - 1100; apples - 280; also found in meat, dairy products, vegetables
Heptadecane	[b]	394	[b]	0	[b]	0			Eggs - 1200; apples - 240; also found in meat, vegetables, dairy products, fruits
2-Methyl propane	28	46	0	0	0	0	Los Angeles - 28		Found in whiskey
2-Methyl butane	5	21	0	143	0	0	Los Angeles - 105		Found in apples
2-Methyl pentane	11	34	1	28	1	0			Rum, Jamaican - 10; also found in apples and cheese
2-Methyl heptane	12	27	27	47	4	4	Zurich - 16		Found in chicken

[a] Data on the distribution of the compounds listed in this and in subsequent tables have been compiled from numerous sources making it impractical to cite each reference. The following references have been especially valuable: 4, 8, 9, 25, and 32.
[b] Not determined.

in most tissues occurred in 4 to 5 hours. When exposure was terminated, the volatile hydrocarbons were rapidly eliminated unchanged in the expired air. This fact was demonstrated with human volunteers who breathed approximately 100 ppm of hexane for 4 hours. When returned to ambient air, the subjects rapidly eliminated the hexane and within 4 hours, its concentration in the expired air was less than 0.5 ppm (26).

Little is known about the absorption of alkanes from the gastrointestinal tract, but the degree of absorption seems to depend on the molecular dimensions of the hydrocarbon. Mineral oil, consisting largely of paraffins with 15 to 30 or more carbon atoms, is poorly absorbed (17). On ingestion, only 2 percent is absorbed and this presumably represents the shorter members of the series. Slight, but significant, absorption of hexadecane (3, 7) and heptadecane (31) occurred in rats fed small amounts daily. Longer chain paraffins were more poorly absorbed.

Because of their relative physiological and pharmacological inactivity, the metabolism of alkanes has not been studied as thoroughly as more reactive substances. Consequently, the precise metabolic disposition of many of these compounds is not known and can only be surmised from investigations on some of their homologues.

In common with a number of lipid-soluble organic compounds, the metabolism of alkanes and isoalkanes is catalyzed by microsomal mixed-function oxidases (MFO) (12). This ubiquitous, highly inducible enzyme system (5, 11) found in most tissues of all higher organisms, is generally associated with the metabolism of steroids and a wide variety of xenobiotics, including drugs and pesticides. Both low and high molecular weight alkanes such as butane (10), pentane (10), heptane (6), decane (15), and hexadecane (19) are oxidized to the corresponding alcohol by this system. Although all of the alkanes found in irradiated beef have not been studied, it seems reasonable to conclude that they are metabolized in the same way as their higher or lower homologues.

Among the simpler alkanes, the preferred site of attack is the tertiary CH bond, with the secondary bond the next most susceptible and the primary methyl group the most resistant to the hydroxylation reaction (10). Thus, n-butane on oxidation yields 2-butanol with only traces of the primary alcohol. Similarly, 2-pentanol is the major product of n-pentane hydroxylation. Significant amounts of 3-pentanol but only barely detectable amounts of the primary alcohol (1-pentanol) are also produced.

However, the higher alkanes are apparently oxidized preferentially at the terminal carbon atom. Heptane is oxidized by MFO to 1-heptanol (6)

and decane to 1-decanol, with equal facility by lung, kidney or liver microsomes (15).

The ultimate fate of the alcohols produced from the alkane hydroxylation is not known with certainty. Some are probably conjugated and excreted in the urine as glucuronides (18) while some may be metabolized further to the corresponding fatty acid (19, 23), a fate clearly demonstrated for hexadecane and octadecane. Hexadecane was converted by the MFO and other enzymes to cetyl alcohol and palmitic acid (19), the latter presumably by further oxidation of the alcohol. In contrast with their activity in decane oxidation, lung microsomes showed relatively low activity compared with liver microsomes in the oxidation of hexadecane, while the kidney microsomes were completely inactive. Alcohol dehydrogenase catalyzes the oxidation of lower aliphatic alcohols (30) to the corresponding aldehydes, which are then further oxidized to their acids.

Toxicity

The simplest alkane homologues -- methane, ethane and propane -- are generally considered to be innocuous when inhaled at concentrations below 1 percent by volume. Several thousand parts per million of these gases are necessary to produce any detectable physiological effect and even at these levels, the effect is a mild hypoxia resulting from the corresponding reduction of oxygen in the inspired air. No threshold limit values (TLV) have been established for their presence in workroom atmospheres.

TLVs for the intermediate alkane members range from 100 to 600 ppm (1) as shown in the following table:

Table 6. Threshold limit values for alkanes (1).

Compound	ppm	mg/m^3
Butane	600	1400
Pentane	600	1800
Hexane	100	360
Heptane	400	1600
Octane	300	1450
Nonane	200	1050

Propane and butane are used in processing and packaging of foods to remove and displace oxygen and have been approved as GRAS ("generally recognized as safe") by the Food and Drug Administration for this purpose (27). Petroleum hydrocarbon fractions are used as solvents to extract oils from various food preparations. These fractions are mixtures of hydrocarbons boiling within a given temperature range and may include hexane, methyl pentane, heptane, dimethyl butane, and other compounds. Their use, in accordance with good manufacturing practices, has been approved by the Joint FAO/WHO Expert Committee on Food Additives (17). Similarly, FDA regulations permit the use of hexane (21 CFR 173.270; 172.560, formerly 21 CFR 121.1045; 121.1082) 2-methyl heptane (21 CFR 172.882, formerly 21 CFR 121.1154) and heptane (21 CFR 172.250, formerly 21 CFR 121.1203) as solvents for foodstuffs under controlled conditions (27). Also mineral oil which contains high molecular weight hydrocarbons is employed for a variety of food and medicinal purposes (21 CFR 172.878, formerly 21 CFR 121.1146) (27,17).

Virtually no toxicological data are available for the individual higher molecular weight alkanes. Most of the available reports concern various industrial products consisting of complex hydrocarbon mixtures including some of the C_{10} to C_{17} alkanes. One of the few studies of these higher paraffin members given systemically is that of Jeppsson (16) who injected mice intravenously with emulsions containing various alkanes. The LD_{100} for pentadecane, hexadecane and heptadecane was approximately 10 g per kg for each of these alkanes. Hine and Zuidema (13) administered the following mixtures of paraffins and naphthenes intragastrically to rats at concentrations of 25 ml per kg without causing death in any of the animals: C_9 and C_{10}; C_{11} and C_{12} and C_{13} through C_{16}.

None of these alkanes has proved carcinogenic but some have enhanced ("promoted") the development of papillomas in mouse skin pretreated or "initiated" with subcarcinogenic doses of polycyclic aromatic hydrocarbons. Sicé (29) "initiated" the skin of female Swiss mice with 7,12 dimethylbenz (a)anthracene and subsequently applied a number of alkanes and alkanols. The skin tumor incidence/number of mice were as follows: hexane (0/30), octane (0/40), decane (2/30), dodecane (6/30), tetradecane (5/30), hexadecane (1/50) and octadecane (1/30). The latent period before tumor appearance promoted by dodecane and tetradecane was 20 to 60 weeks, compared with more than 50 weeks with decane and octadecane.

Similar conclusions were reached in an experiment with dodecane (28) and in a more recent series of experiments by Horton et al. (14) in which male C3H mice were treated repeatedly with 0.14 percent benzo(a) pyrene dissolved in certain alkanes or in decalin (decahydronaphthalene). Benzo(a) pyrene in decalin alone caused 33 percent malignant tumors while benzo(a) pyrene with dodecane, hexadecane, octadecane and eicosane produced

malignant skin tumors in all of the animals. These experiments suggest that chronic exposure to high concentrations of some alkanes can enhance the production of tumors "initiated" by polycyclic aromatic hydrocarbons, but gave no indication that the alkanes alone were carcinogenic.

Discussion

Each of the alkanes found in irradiated beef has occurred in other foods, sometimes more abundantly than in the irradiated beef. Their origin in many of these foods is unknown. A number of the alkanes are employed as solvents or in preparations approved for various purposes by the Food and Drug Administration and by official international bodies. The lower homologues are common industrial substances whose threshold limits in workroom atmospheres are several orders of magnitude greater than their concentrations in beef. A similar margin of safety exists for each of the compounds whose least toxic effect has been determined. Some of the metabolic products of these substances, where known, are either compounds normally found in the body or substances metabolized by known physiological processes to compounds believed to be nontoxic.

The Committee carefully reviewed the data demonstrating that several of the higher alkanes and alkanols may act as co-carcinogens or tumor-promoting agents in mice pretreated with polycyclic aromatic hydrocarbons. The possibility seems slight that alkanes in the quantities found in irradiated beef are co-carcinogenic. Not only were the alkanes applied in the presence of a large concentration of a known carcinogen, but their doses were huge in comparison with the amounts found in beef.

The available data on the alkanes suggest that the consumption of irradiated beef would not pose a significant increment of hazard to the amounts an individual would be unavoidably exposed.

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2. Alkenes and Alkynes

Although the most apparent effect of lipid irradiation is the production of n-alkanes with one or two less carbon atoms than the original fatty acids, significant amounts of unsaturated hydrocarbons are also produced. Simple alkenes are produced from saturated fatty acids while dienes are generated from the monounsaturated acids.

Occurrence

The alkenes and alkynes and their concentrations found in the variously treated beef samples are listed in Table 7 as are their concentrations in air, and in other foods.

As might be expected, almost the entire series of straight-chain alkenes, from C_2 to C_{17} has been identified in the irradiated beef. An exception is propene which was not detected. Heptadecene and hexadecadiene are the most abundant of the unsaturated hydrocarbons with 618 and 706 μg per kg beef, respectively. The former is presumably generated from stearic acid and the latter from oleic acid, among the most common fatty acid constituents in beef. Traces of decyne and undecyne have also been detected. The precursors of these highly unsaturated compounds are unknown.

Each of the mono-alkenes has been detected in certain foods, but there have been few attempts to provide quantitative data. A single report suggests that eggs are a particularly rich source of these compounds (13,14) and several of the higher homologues --- nonene, undecene, pentadecene, hexadecene and heptadecene --- appear to be present in concentrations considerably greater than any alkene in the irradiated beef. All alkenes from hexene through heptadecene have been found in cooked beef and most members of this series have also been detected in other meats and dairy products. The amounts are generally greater in beef when cooked by microwave than by conventional means (15), presumably because of the rapid rate of heating. Every member of the series C_2 to C_{17} has been found in coffee or in some fruit or vegetable (26). Some dienes and alkynes also appear in food, but no reference could be located to indicate the presence of pentadecadiene, hexadecadiene and undecyne in any food.

Ethene is exceeded only by methane as the major hydrocarbon emitted into the atomsphere from automotive exhausts (19). Lesser but significant amounts of other low molecular alkenes --- propane, methyl propene, butene and hexene --- are also present in exhaust fumes (18).

Table 7. Presence of unsaturated hydrocarbons in various media.[a]

Compound	Irradiated beef µg/kg Cooked	Irradiated beef µg/kg Uncooked	Thermally sterilized beef µg/kg Cooked	Thermally sterilized beef µg/kg Uncooked	Frozen beef control µg/kg Cooked	Frozen beef control µg/kg Uncooked	Atmosphere µg/m³	Food µg/kg
Alkenes								
Ethene	0	28	0	0	0	0	Washington - 800 Los Angeles - (ave) 120, (max) 820 Pasadena freeway -820; S. Pasadena - 410; Incinerator - 1400-5600	Found in apples, coffee, grapes, pears, peaches, mushrooms, tomatoes, strawberries
Butene	13	33	0	0	0	0	Los Angeles - 14	Found in coffee
Pentene	2	37	3	4	3	0		Found in coffee
Hexene	2	35	22	31	1	1		Found in coffee, currant, cooked beef
Heptene	46	114	12	31	2	1		Eggs - 900; also found in apples, cooked beef, milk
Octene	21	96	0	0	0	0		Eggs - 1300; also found in apples, cooked beef, cheese, chicken, coffee, milk, onions
Nonene	41	60	0	0	0	0		Eggs - 1800; also found in apples, cooked beef, cheese, chicken, coffee, milk, onions
Decene	114	126	[b]	0	[b]	0		Eggs - 300; also found in apples, cooked beef, cheese, coffee, milk, onions
Undecene	88	78	[b]	0	[b]	0		Eggs - 2600; also found in cooked beef, cheese, milk, onions
Dodecene	167	156	[b]	0	[b]	0		Found in cheese, eggs, milk, onions, cooked beef
Tridecene	[b]	178	[b]	0	[b]	0		Found in eggs, milk, onions, cooked beef
Tetradecene	[b]	488	[b]	0	[b]	0		Found in eggs, milk, onions, cooked beef
Pentadecene	[b]	121	[b]	0	[b]	0		Eggs - 2800; also found in cooked beef, onions, pork
Hexadecene	[b]	156	[b]	0	[b]	0		Eggs - 3900; also found in onions, pork, cooked beef
Heptadecene	[b]	618	[b]	0	[b]	0		Eggs - 137,900; also found in cooked beef, milk, onions
2-Methyl propene	2	38	0	4	0	0		Found in hops
Alkynes								
Decyne	24	23	[b]	0	[b]	0		Found in apples, potato chips
Undecyne	10	4	[b]	0	[b]	0		
Dienes								
Tetradecadiene	[b]	98	[b]	0	[b]	0		Found in approximately 15 foods; meat, fish, fruit, dairy products, beverages
Pentadecadiene	[b]	73	[b]	0	[b]	0		
Hexadecadiene	[b]	706	[b]	0	[b]	0		
Heptadecadiene	[b]	16	[b]	0	[b]	0		Eggs - 2000

[a] See table 5, footnote a.
[b] Not determined.

In freeways and in metropolitan areas, the concentration of ethene approximates 400 to 800 μg per cubic meter of air (1, 20) while other alkenes range from 5 to 10 μg per m^3 (21). Hydrocarbons with more than 12 carbon atoms are generally not present in the atmosphere in any significant amounts (16). Butene has been identified in drinking water supplies in the United States (6) but its concentration has not been reported.

The alkenes are employed in large quantities in the manufacture of various industrial products. The simpler members provide the raw material for plastics, those with 8 to 12 carbon atoms are common sources for plasticizers, and the longer members (C_{14} to C_{18}) are used in manufacturing alkyl sulfate detergents.

Metabolism

This class of compounds is oxidized in the body to epoxides by the microsomal mixed function oxidases (MFO). Although the epoxides exhibit a very short half-life and are extremely difficult to isolate, they have been demonstrated to be obligatory intermediates of alkene metabolism (12, 24, 25). They may be unstable and rearrange to unknown products or may be converted to their corresponding diols by epoxide hydratase (9). The diols may then be excreted unchanged, undergo further oxidation, or be conjugated with glucuronic acid (4, 5). Epoxides may react with glutathione with or without enzymatic mediation.

Boyland and Williams (3) reported the direct conjugation of aromatic epoxides by glutathione S-epoxide transferase found in rat liver, but this reaction has not been studied with the aliphatic epoxides. Both enzymatic and nonenzymatic conjugation would depend upon the stability of the aliphatic epoxide intermediate and upon the relative affinities of the glutathione transferase and epoxide hydratase for this epoxide.

The metabolic pathway of the dienes remains virtually unexplored. However a single study with butadiene suggests that this compound, too, is metabolized through the intermediate formation of epoxides. When butadiene was incubated with rat liver fractions, 3-butene 1, 2-diol and erythritol (1, 2, 3, 4-tetrahydroxybutane) were produced (8). These are the compounds one would expect if the oxidation of each double bond proceeded in the same way as those of the monounsaturated alkenes just discussed.

One can only speculate on the fate in the body of the two alkynes, decyne and undecyne, produced by beef irradiation. Phenylacetylene ($C_6H_5C{\equiv}CH$), one of the few related compounds that has been studied, was found to be relatively stable and only slowly metabolized to phenaceturic

acid. Williams (27) postulated an initial hydration of the triple bond to form the enol form of phenylacetaldehyde which was then oxidized to phenylacetic acid, the precursor of phenaceturic acid. He believed a similar pathway is taken by the fluoroalkynes to yield fluoroacetic acid. By analogy, the end products of decyne and undecyne metabolism would be decanoic and undecanoic acids respectively.

Toxicity

Toxicologists have largely ignored the volatile olefins, because they appear to act primarily as asphyxiants in high concentrations and exhibit no discernible harmful effects at low or moderate concentrations. All acute studies on the lower alkenes (C_1 to C_8) indicate that concentrations several orders of magnitude greater than those found in beef are necessary before any significant hazard is produced (2, 7, 11, 17). The Committee believed such studies to be irrelevant to its consideration of the much smaller quantities involved. Toxicity data for the higher alkenes are unavailable.

The demonstration that alkenes are converted metabolically to epoxides raises the possibility of carcinogen formation *in vivo*. Epoxidation of ethylenic bonds in vinyl chloride, polycyclic aromatic hydrocarbons and aflatoxin B, is thought by many to represent conversion of the pro-carcinogen to its reactive form. The question arises, therefore, whether alkene epoxides represent a carcinogenic hazard. Relevant data are currently confined to skin-painting experiments and have been summarized by Lawley (10). In a series of experiments with Swiss mice, Van Duuren *et al.* (23) found 1, 2-epoxybutane and 1, 2-epoxydodecane to be inactive but they considered 1, 2-epoxyhexadecane to be tumorigenic since it induced two papillomas and one squamous cell carcinoma in 41 mice surviving an average of 427 days. The Committee questioned the authors' conclusion that these data demonstrated the carcinogenicity of this compound. The possible presence of impurities in the large amounts of test substance used and the very few tumors induced in this experiment raise considerable doubt that 1, 2-epoxyhexadecane is truly a carcinogen.

Van Duuren (22) has pointed out that diepoxides are more apt to be carcinogenic than the monoepoxides. He speculates that this may result from the cross linking of DNA with a consequent alteration of its replication. The ability to effect such cross linkages would depend upon the interatomic distances between the epoxides. No information is available on the diepoxides that could theoretically be produced from the dienes detected in the irradiated beef.

Discussion

As is evident from this brief treatment of the alkenes and alkynes in irradiated beef, significant gaps exist in our knowledge of the metabolism and toxicity of many of these substances. The growing conviction that epoxides may be important in the carcinogenicity of certain chemicals emphasizes the importance of additional, systematic investigations on the metabolism and toxicology of the aliphatic unsaturated compounds.

The total alkenes and alkynes amount to 2.4 mg per kg of irradiated beef for an average daily consumption of approximately 0.3 mg. Each of the compounds under consideration has been identified in other foods, with the exception of penta- and hexadecadiene and undecyne. All of the alkenes from hexene through heptadecene (C_6 C_{17}) have been reported in conventionally cooked beef. As mentioned above, the only analogue of these compounds alleged to produce tumors is 1,2-epoxyhexadecane, a presumed epoxide of hexadecene. Hexadecene has been found in eggs, cooked beef, pork, onions and chicken broth as well as in irradiated beef.

Despite the widespread industrial use of many of these compounds in considerable quantities, no reports of acute or chronic toxicity could be found.

There is insufficient information to permit an unequivocal decision on the long-term effects of small quantities of alkenes. However, based on the available data, the Committee concludes that these compounds, consumed at levels found in irradiated beef, are not likely to represent a significant increment of hazard to that encountered by exposure from unavoidable sources.

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Table 8. Aromatic compounds in various media.*

Compound	Irradiated beef µg/kg Cooked	Irradiated beef µg/kg Uncooked	Thermally sterilized beef µg/kg Cooked	Thermally sterilized beef µg/kg Uncooked	Frozen beef control ug/kg Cooked	Frozen beef control ug/kg Uncooked	Atmosphere µg/m³	Water µg/l	Food µg/kg
Benzene	15	19	2	0	3	0	Los Angeles - (ave) 48 (max) 182 Toronto - (ave) 42 (max) 314 Zurich - 173 Gas stations (ave) 4000 (Max) 11,000 Near reclamation plant - 7360 Bulk loading facilities - 320 - 68,000 Simulated space-craft - 61	Highest reported in U.S. - 10.0 Canadian lake - 320 Miami - 0.1 Ottumwa - 0.1 Philadelphia - 0.2 Cincinnati - 0.3 Well water - Florida fire station - 300.	Beef, canned (headspace) - 2.0 ppb Rum, Jamaican - 120; Eggs - 2100; Haddock (stored Haddock (stored 14 days) - 200; also detected in approximately 20 other foods - no quantitative data available
Toluene	50	65	48	73	3	6	Delft -(ave) 11 (max) 76 Los Angeles - (ave) 120 (max) 720 Netherlands - tunnel (ave) 150 (max) 240 Toronto - (ave) 140 (max) 460 Nuclear sub-marine 738	Highest reported in U.S.-11.0 Canadian water-shed 375.0 Philadelphia - 0.7 Cincinnati - 0.1 Connecticut - 81-140 Effluents from textile mills	Beef, canned - 5.9; eggs 39,300; Haddock (stored 14 days)- 500; also detected but not analysed in approximately 30 foods
Xylene	4	1	7	7	1	1	Zurich - (ave) 31 (max) 91 Los Angeles (ave) 66 (max) 265 Simulated space-craft - 122	Highest reported in U.S.- 5.0 Effluents from oil refineries	No quantitative values; detected in approximately 20 foods

*See Table 5, footnote a.

3. Aromatic Hydrocarbons

Three aromatic hydrocarbons have been detected in irradiated beef: benzene, toluene and xylene (Table 8). Irradiation had little, if any effect upon the concentration of xylene, which is apparently not a radiolytic product. On the other hand, the amounts of benzene and toluene varied directly with the irradiation dose (Figure 7), presumably from the action of ionizing radiation upon the amino acid, phenyl alanine (21). Heating alone produced comparable amounts of toluene but not of benzene. Similarly, small amounts of benzene have also been produced after the irradiation of codfish (40).

All three of these compounds have extensive industrial uses and hundreds of millions of gallons are produced annually in the U. S. Of special significance is the presence of benzene in gasoline with the consequent ubiquitous contamination from automotive emissions. It has been estimated that over one billion pounds of benzene per year are emitted into the atmosphere. Significant quantities of benzene, as well as of toluene and xylene, have been detected in the air and water of virtually every metropolitan area in which they were sought.

All three of these compounds have been reported in numerous foods, including meat, vegetables, nuts, dairy products, and beverages (12). "Large" amounts of benzene have been reported in boiled beef (6) and in canned beef stew (6). Thus, conventional cooking, itself, will cause an increase of benzene, probably from amino acid precursors. Benzene and toluene (but not xylene) have also been detected in fruits, fish and eggs. In common with most of the compounds under consideration, quantitative data are scarce. Eggs appear especially rich in aromatics if a single report is typical, for their content of benzene and toluene is estimated to be more than a hundred times that in the irradiated beef (18, 19). Large amounts of both these compounds were also found in haddock kept under refrigeration for 14 days; as much as 200 μg per kg of benzene and 500 μg per kg of toluene (20).

Absorption and Metabolism

These compounds are usually rapidly absorbed through the lungs although significant absorption through the skin and gut is also possible. Human subjects exposed for 4 hours to 52 to 62 ppm benzene or to 98 to 130 ppm toluene had an apparent retention of 30 to 40 percent of the hydrocarbon inhaled during that period (28). No comparable data are available for xylene. After exposure ceased, elimination of the unchanged solvents through the lungs continued for many hours (29). When labeled benzene was given orally to rabbits, 43 percent was recovered unchanged in the expired air, 34.5 percent in the urine as phenolic conjugates and 0.5 percent in feces within 2 to 3 days (32). Similarly, benzene injected subcutaneously in mice was

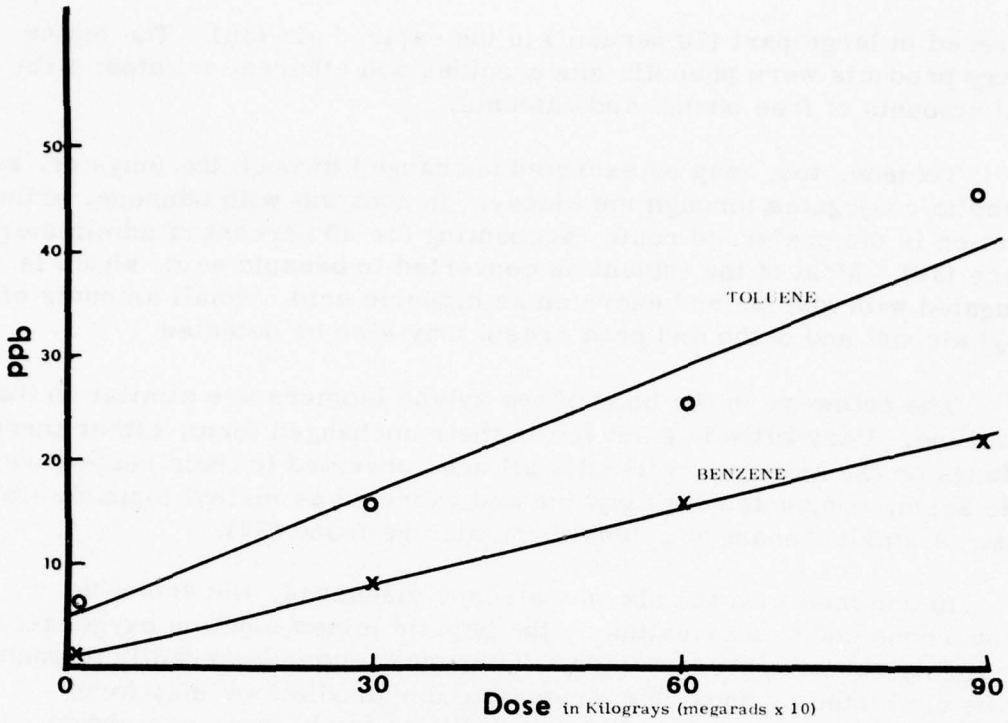


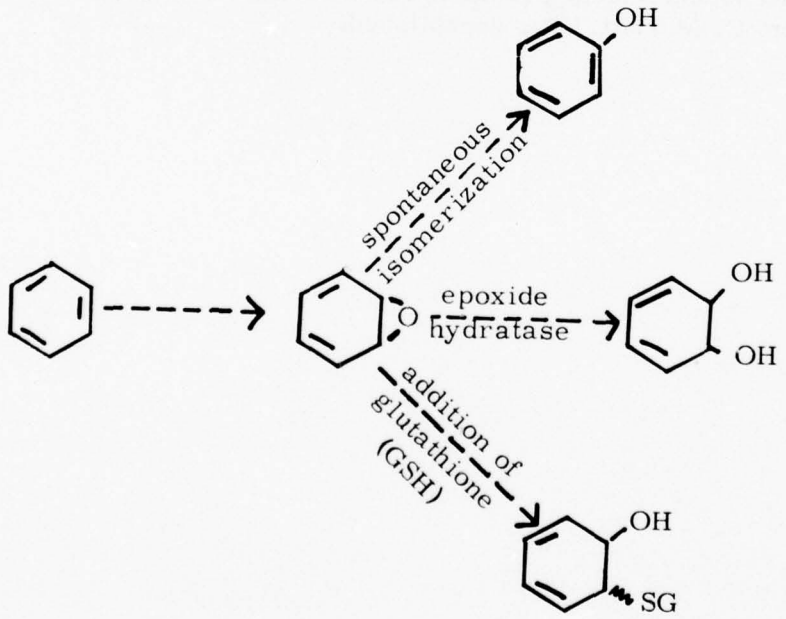
Figure 7. Benzene and toluene production as a function of irradiation dose. From C. Merritt, Jr., unpublished.

recovered in large part (70 percent) in the expired air (39). The major urinary products were phenolic glucuronides and ethereal sulfates, with small amounts of free phenol and catechol.

Toluene, too, may be excreted unchanged through the lungs or, as metabolic conjugates, through the kidney. In contrast with benzene, urinary excretion is the preferred route, accounting for 80 percent of administered toluene (30). Most of the toluene is converted to benzoic acid, which is then conjugated with glycine and excreted as hippuric acid. Small amounts of benzyl alcohol and ortho and para cresol may also be detected.

The pathways in the body of the xylene isomers are similar to that of benzene. Very little is excreted in their unchanged form, either through the lungs or the kidneys. Virtually all are converted to their respective toluic acids, conjugated with glycine and excreted as methyl hippuric (toluric) acids. A small amount of xylenols can also be found (37).

In common with the alkenes already discussed, the aromatic hydrocarbons undergo oxidation by the hepatic mixed function oxygenases to form highly reactive arene oxides. They may isomerize readily to phenols, may be converted by epoxide hydratase to dihydrodiols or may form glutathione conjugates (30). The possibilities for benzene are shown below:



Similar labile epoxides are intermediates in the metabolism of toluene and xylene.

Toxicity

Since several million workers in the United States are exposed to these aromatic hydrocarbons, they have been extensively investigated and are the subjects of recent reviews (24, 25, 27). The acute toxicities are summarized in Table 9.

Table 9. Acute toxicity of aromatic hydrocarbons.

Compound	Animal	Route	LD_{50} (mg/kg)	Reference
Benzene	Mouse	Intraperitoneal	468	26
	Rat	Inhalation	10,000 ppm/7 hr.	26
	Rat	Oral	930	7
	Rat (young)	Oral	3400	16
	Rat (old)	Oral	5600	16
Toluene	Mouse	Inhalation	5300 ppm	26
	Rat	Oral	5000	26
	Rat	Intraperitoneal	1640	26
Xylene	Rat	Oral	4300-5000	26

Benzene

Although deaths and severe central nervous disturbances have been reported from acute exposure to high concentrations of benzene in air (approximately 2.5 percent) (27), it is the chronic exposure to moderate levels of the compound that has aroused the greatest concern. Large amounts of the compound are clearly capable of depressing bone marrow activity. Exposure to benzene in a contaminated workroom atmosphere is well known to lead to blood dyscrasias, particularly to a reduction in the leukocyte count, and in some cases, to aplastic anemia.

Various types of leukemia have been reported to result from chronic benzene exposure and possibly to arise preferentially in those persons developing aplastic anemia. Blood dyscrasias and leukemias have also been noted among patients receiving certain drugs such as phenylbutazone, chloramphenicol and some sulfonamides. However, the evidence of an association of leukemias with blood dyscrasias in these patients and those exposed to benzene is equivocal and is based mainly on unconvincing case reports.

In an extensive epidemiological survey, Aksoy et al. (1) in 1974 reported a correlation between benzene exposure and leukemia. Among 28,500 shoemakers in Istanbul, 26 patients with acute or preleukemia were detected during a 7-year period for an annual incidence of 13 per 100,000, contrasted with 6 per 100,000 among the general population. The maximum benzene concentrations to which these workers were exposed were 210 to 650 ppm. The mean duration of exposure was 9.7 years. Thorpe (41), on the other hand, reporting the same year, failed to detect an increased incidence of leukemia among 38,000 petroleum workers who were at least potentially exposed to benzene. A recent report by the National Institute of Occupational Safety and Health (14) compared the deaths from leukemia among white, male employees exposed to benzene in a large rubber plant, with those in a control population during the same time period. Seven deaths among 748 men were recorded in the former group, or approximately five times the incidence among the controls. Largely on the basis of this report, the Occupational Safety and Health Administration on May 27, 1977 proposed a temporary emergency standard reducing the permissible workroom levels of benzene from 10 to 1 ppm (31). On June 8, 1977, the Environmental Protection Agency added benzene to its list of hazardous air pollutants (8). These studies have not eliminated the possibility that agents other than benzene may be responsible for leukemogenesis in the large study populations.

Stable or unstable chromosomal aberrations in man may be produced by high levels of benzene (several hundred ppm) (27). No correlation has been demonstrated between the persistence of these changes and the degree of benzene exposures.

Despite its status as a suspect leukemogen in man, attempts to induce leukemia in animals by benzene exposure have been unsuccessful (42). Benzene has frequently been used in skin painting experiments as a solvent without producing tumors. Because of this inability to induce leukemia in animals, Ward et al. (42) speculate that benzene may induce leukemia only in highly sensitive persons or by synergistic action with other environmental agents.

No developmental malformations were detected when pregnant mice were exposed continuously to doses ranging from 1 to 670 mg per m^3 of benzene vapor, but at the highest doses, the number of fetuses per litter was reduced (36).

The possibility that benzene may be a leukemogenic agent in man cannot be excluded on present evidence. After a recent critical review of the relevant data, the Committee on Toxicology (27) of the National Research Council concluded that benzene must be considered a suspect leukemogen but that more definitive data are required for an accurate assessment of its effects.

Toluene

Workmen's exposure to toluene is almost exclusively through the lungs or skin, so that very limited data are available on its oral toxicity. As shown in Table 9 the lethal dose by this route in the rat is 5000 mg per kg. In animals, the toxic effects are primarily on the central nervous system and range from light narcosis to prostration, depending upon the extent of exposure. No effects could be confirmed on the blood or blood-forming organs when toluene was administered either by inhalation or percutaneously, even at levels that produced marked central nervous effects.

In man, too, the acute effects are largely on the central nervous system (5). They are narcotic and result in muscular weakness, incoordination and mental confusion (24).

The TLV for workroom exposure is 100 ppm (375 mg per m^3). After chronic exposure to atmospheric concentrations of 100 to 1100 ppm for 2 weeks to 5 years, enlarged livers and macrocytosis were noted in about 20 percent of the subjects (11). Chronic exposure to approximately 200 ppm for 3 to 15 years produced no chromosomal changes in the lymphocytes (9). Various reports in the early literature report toxicity to blood and blood-forming organs by toluene. However, since industrial-grade toluene contains significant amounts of benzene, many of the reported effects may be attributable to this contaminant.

Xylene

The acute toxicity of xylene in animals approximates that of toluene (Table 9). Inhalation for 4 hours of mixed xylenes by rats and dogs in concentrations exceeding 500 ppm caused no apparent ill effects (4). Guinea pigs exposed to 300 ppm for 4 hours daily, 6 days per week for 2 months showed only slight liver and lung effects (38). Recent investigations with both dogs and rats revealed no gross or microscopic pathology nor any hematological disturbances, even with exposures as high as 805 ppm for 6 hours per day, 5 days per week for 13 weeks (4). Intraperitoneal injection of xylene into rats caused liver necrosis and diffuse nephritis (2). Liver and kidney damage has also been reported in man after inhalation of sufficient xylene to cause unconsciousness (23). However, the victims recovered fully and there is no evidence in the literature of irreversible damage to either kidney or liver (25).

Commercial xylene contains varying amounts of the ortho-, meta-, and para-isomers as well as other aromatic and aliphatic hydrocarbons, thiophene, pyridine and phenol. Consequently, many reports on the toxicity of xylene are unreliable since exposures were rarely to a pure preparation. Thus, even though early reports attributed a myelotoxic effect to xylene, it is now believed that xylene poses no threat to the blood and blood-forming organs. Its toxic effects are very similar to those of toluene and are reflected primarily in the form of headache, lassitude, fatigue and irritability, together with minor gastrointestinal symptoms (10). The TLV of xylene is 100 ppm or 435 mg per m^3.

Berenblum (3) painted the skin of white mice with xylene alone and with 3, 4 benzpyrene and xylene and concluded that xylene was neither carcinogenic nor co-carcinogenic. Pound (35) reported an increased incidence of skin tumors in mice pretreated with xylene and exposed to ultraviolet light, but he attributed this increase to the hyperplasia induced in the skin, rather than to carcinogenic properties of the xylene. Pre-treatment with croton oil or acetic acid caused similar increases in skin tumors.

Russian workers (17) investigated possible embryotoxic effects of xylene by exposing pregnant rats to para-xylene (115 ppm) continuously for 20 days. No teratogenic effects were noted.

Discussion

The presence of toluene and xylene in the irradiated beef would seem little cause for concern. Xylene does not appear to be a radiolytic

product, and the concentration of toluene in thermally sterilized beef is equal to or greater than that in the irradiated samples. Both are widely distributed in other foods, often in considerably greater amounts than in beef. The amount of benzene increases slightly upon irradiation, reaching a level of 15 to 19 μg per kg after exposure to 56 kGy (5.6 megarads). This would represent a daily intake from irradiated beef of approximately 2 μg.

Benzene has been detected in nonirradiated beef by numerous workers, but comparison with irradiated samples is made difficult by the lack of quantitative data. The only reported value is 2 ppb (6.4 μg per m^3) in the head space of canned beef (33). In semiquantitative studies the amount of benzene has been characterized as "large" in canned beef stew (6, 34), and boiled beef (6, 12). It has also been detected in roast beef (22) as well as in a score of other foods.

Unavoidable absorption from air and water supplies also contribute significantly to the daily intake of benzene. The average atmospheric concentration in metropolitan areas is about 45 μg per m^3 and may increase several fold during peak traffic periods. Body retention has been shown to be 25 to 30 percent (28, 13) of the total inhaled or about 100 μg daily. This is approximately 40 times the daily intake from irradiated beef. The recently imposed emergency measure for workroom atmospheres reduces the permissible levels from 10 to 1 ppm. Even at this sharply reduced limit, the average workman (after his 8 hour stint) would retain about 2.5 to 3.0 mg benzene or more than 1000 times that consumed in irradiated beef.

The Committee believed that a small addition of benzene from irradiated beef contributes only a trivial increment to the normal body burden and is unlikely to increase significantly whatever hazard exists from other sources.

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B. OXYGEN-CONTAINING COMPOUNDS

1. Alcohols

Methanol and ethanol, the simplest of the primary alcohols have been detected in both irradiated and nonirradiated beef (Table 10). The concentrations of methanol in the thermally sterilized samples are greater than those in the irradiated beef. Ethanol, on the other hand, increases significantly with irradiation.

Occurrence

Methanol and ethanol are produced in huge quantities for a variety of industrial, medicinal and household purposes. Methanol has been detected in water supplies of six of ten cities tested (3) and, as shown in Table 10, is a normal constituent of many meats, beverages, fruits, vegetables and dairy products. It is also found normally in the breath, blood and urine of man and is considerably elevated after ethanol consumption (7).

Ethanol can result from the natural or deliberate fermentation of numerous carbohydrate foods. Millions of gallons are consumed annually in numerous alcoholic beverages. Even those persons who conscientiously abstain from such beverages cannot avoid the ingestion of significant amounts of ethanol from other sources. As is apparent from Table 10, milligram quantities or more can easily be ingested daily from fruits, fruit juices, cheeses and various vegetables.

Metabolism

Because of its simple structure and high solubility, methanol is rapidly absorbed from the intestinal tract and distributed evenly in body water. Substantial amounts are eliminated through the lungs but its major metabolic pathway is through formaldehyde and formic acid to carbon dioxide and water. A small amount may escape conversion and be excreted unchanged in the urine. Small amounts of its glucuronide and of formic acid can also be found in the urine (11). Although it is generally assumed that formaldehyde is the initial oxidation product of methanol, it has not been detected in the blood and urine, presumably because of its rapid conversion to formic acid. Keeser (4) has found

Table 10. Distribution of methanol and ethanol.*

	Irradiated beef µg/kg		Thermally sterilized beef µg/kg		Frozen beef control µg/kg		Atmosphere µg/m³	Water µg/l	Food µg/kg
	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked			
Methanol	16	20	23	40	41	0	Near production plant - 18-33 Simulated spacecraft - 125 Incinerator - 500-4000	Detected in over half of city waters tested concentrations not reported	Passion fruit juice - 4000; Rum, Jamaican - 80; reported in over 50 foods; meats, beverages, fruit, dairy products, vegetables
Ethanol	75	123	9	15	18	0	Simulated spacecraft - 46	5.0 (highest reported in U.S. water)	Apple essence - 15,000; banana - 5,000; cheese (cheddar) - 620,000; cherry - 55,000; cucumber - 200-2000; grapefruit juice - 400,000 - 660,000; passion fruit juice - 100,000; pineapple - 60,500; raspberry - 10-100; strawberry - 1,510; vinegar - 1,650,000 (found in over 50 foods)

*See Table 5, footnote a.

it in the aqueous and vitreous humors of rabbits poisoned with methanol. Formaldehyde is a powerful inhibitor of retinal respiration (5) and the blindness often resulting from methanol consumption is believed attributable to this action.

Ethanol, too, is readily absorbed from the gastrointestinal tract. Some is excreted through the lungs and kidneys but most is metabolized to carbon dioxide and water. It is first oxidized in the liver to acetaldehyde, which is then converted to acetate or to its activated form, acetylcoenzyme A, which can enter the general metabolic pool of two carbon fragments.

Several enzyme systems are capable of oxidizing methanol and ethanol and their respective roles have not been entirely elucidated. Alcohol dehydrogenase is primarily responsible for the oxidation of ethanol and probably for that of methanol as well (2). Catalase (10) and a microsomal oxidizing system in the liver (7) may also participate under certain conditions or in certain animal species.

The subsequent oxidation of the acetaldehyde produced from ethanol is accomplished by acetaldehyde dehydrogenase with possible contributions from xanthine and aldehyde oxidases. Both of these enzymes rapidly metabolize acetaldehyde and other aliphatic aldehydes in vitro. However, only traces of xanthine oxidase are present in the human liver (9), so its significance in normal metabolism is questionable.

Toxicity

Although methanol or ethanol poisoning usually results from an overly generous consumption of alcoholic beverages, both compounds are sufficiently volatile to pose potential hazards to workmen exposed to their fumes. Consequently, threshold limit values have been established for workroom environments in the U.S. For ethanol, the TLV is 1000 ppm (1900 mg per m^3), and for methanol it is 200 ppm (260 mg per m^3) (1).

The relative toxicity of these alcohols by different routes in different animals can be judged from representative values taken from the Registry of Toxic Effects of Chemical Substances (8) (Table II).

Table 11. Toxicity of methanol and ethanol.

Substance	Animal	Route	LD_{50} mg/kg
Methanol	Mouse	Subcutaneous	9800
	Monkey	Inhalation	1000 (ppm)
Ethanol	Mouse	Subcutaneous	8285
	Rat	Intravenous	1440
		Intravenous	1973
	Rabbit	Oral	6300
	Guinea pig	Oral	5560

Discussion

The ethanol in a single beer or cocktail or glass of wine would equal that present in many tons of irradiated beef, so that the potential contribution of irradiated beef is trivial and can be disregarded as a health hazard. Virtually every category of foodstuffs -- fruits, vegetables, dairy products, juices, -- contains significant quantities of ethanol.

Methanol also is widely distributed among foodstuffs and consumed in significant amounts. It has been detected in the water supplies of six of ten American cities where such analyses were made. While data are not available on man's normal intake of methanol, it is believed to be far higher than the 3 to 4 μg that would be contributed daily from irradiated beef.

The Committee believes the amounts of these alcohols consumed from irradiated beef do not constitute a hazard to health.

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2. Aldehydes

Occurrence

A number of aldehydes were detected in the irradiated uncooked beef samples (Table 12). Apart from 2-methyl pentanal, all were relatively long chain compounds ranging from 11 to 18 carbon atoms. The total aldehyde content was approximately 0.8 mg per kg of beef. Almost half of this amount came from octadecenal and presumably arose from oleic acid, a major constituent of beef fat. The concentrations of the remaining aldehydes varied from 0.011 to 0.127 mg per kg of beef.

As is evident from Table 12, the free fatty aldehydes are widely distributed in nature. They have been identified in yeast (7), bacteria (5), fruit (9), vegetables (9) and in various mammalian tissues (6, 12, 14, 26). Some are natural flavoring substances and are present in relatively large amounts in certain foods, especially in citrus fruits. In fact, the longer chain aldehydes are the major flavor constitutents of most citrus oils (2). Thus, a kilogram of orange oil contains 140 to 4500 mg undecanal (17), 760 to 4600 mg dodecanal (2, 17) and 130 to 1240 mg tetradecanal (2). Hexadecanal has also been identified as one of the major aldehydes in orange oil, although its concentration was not reported (2).

Dodecanal and tetradecanal have been used as fragrances for approximately 50 years and undecanal since the 1940's. Approximately 20,000 pounds each of undecanal and dodecanal and 2000 pounds of tetradecanal are used annually in soaps, detergents, creams, lotions and perfumes.

The fatty aldehydes have also been identified as naturally occurring components in mammalian lipids, where they occur both in free and in bound forms. Gilbertson *et al.* (13) have isolated from the hearts of rat, dog and cow all the aldehydes found in irradiated beef except for 2-methyl pentanal. The total free aldehyde concentrations were approximately 20 to 40 mg per kg of fresh heart muscle. Hexadecanal and octadecanal were the principal aldehydes present, with the former accounting for roughly half of the total. Only trace amounts of undecanal and dodecanal were detected. More recently, Ferrell and Radloff (8) measured the free fatty aldehydes (C_8 to C_{18}) in normal and infarcted hearts. In the apparently normal hearts of two men, 46 and 72 years old respectively, they found 1.91 and 9.14 μ moles of aldehydes per 100 mg lipid (21). The principal free aldehydes detected were dodecanal, hexadecanal, heptadecanal, octadecanal and octadecenal. The infarcted hearts showed increased aldehyde concentrations, especially of tetradecanal. Free aldehydes have also been found in rat brain (26), human serum (12) and mouse liver(6).

Table 12. Distribution of aldehydes found in irradiated beef.*

Compound	Irradiated beef uncooked µg/kg	Other foods µg/kg
2-Methyl pentanal	11	Found in beef, chicken, coffee, crisp bread, garlic, meat, onion, peanut, tomato, vinegar
Undecanal	76	Bilberry - 50; oil, orange - 140,000-4,500,000; oil, roasted peanut - 150; also found in fruit, meat, dairy products
Dodecanal	63	Bilberry - 20; milk, dry - 5; beef, cooked - 100; egg - 7400; citrus oils - 760,000-4,600,000; tomato - 7710; oil, roasted peanut - 93; found in approximately 20 foods: dairy, fruit meat, beverages
Tetradecanal	54	Bilberry - 20; citrus oils - 130,000 - 1,240,000; oil, roasted peanut - 230; also found in grape, lemon
Pentadecanal	46	
Hexadecanal	127	Found in beef, bilberry, chicken, citrus fruits, cranberry, pork
Octadecanal	30	Found in beef, chicken, pork
Hexadecenal	33	Oil, roasted peanut - 63
Octadecenal	398	

*None of these aldehydes were found in the thermally sterilized, frozen control or raw beef samples.

Metabolism

It is generally stated that aldehydes are readily oxidized in the animal body to the corresponding acids and converted by beta oxidation to carbon dioxide and water (27). This oxidation is catalyzed by three enzyme systems: aldehyde dehydrogenase (20), aldehyde oxidase (22) and xanthine oxidase (23). Although this pathway is well documented, there is growing evidence that reduction of the aldehyde to its alcohol also occurs and may actually be the favored route, especially for xenobiotic aldehydes and ketones. Kessler and Ferrell (16) isolated an alcohol dehydrogenase from the supernatant fraction of mouse liver homogenate capable of reducing aldehydes ranging from acetaldehyde to octadecanal. Bachur (1) has recently compiled a list of carbonyl-reducing enzymes whose characteristics are remarkably similar. All these enzymes are found in the cytoplasm and are widely distributed in tissues. Bachur has termed this ubiquitous class of enzymes "cytoplasmic aldo-keto reductases."

Toxicity

Toxicity data on these aldehydes are disappointingly sparse. Oral rat LD_{50} values for 2-methyl pentanal (25), undecanal (24), dodecanal (3), and tetradecanal (18) are all greater than 5 g per kg body weight. Rats survived inhalation of 8000 ppm of 2-methyl pentanal for 4 hours with no deaths (25). After intraperitoneal injection of tetradecanal, hexadecanal and octadecanal into mice, the LD_{50}s were 2.2, 2.0 and 1.3 g per kg body weight respectively (11). Data on oral ingestion are available only for tetradecanal which was fed to mice at levels of 166 mg per kg for 130 days with no apparent toxic effect (11). No data have been found on the toxicity of hexadecenal and octadecenal.

Discussion

The long chain aldehydes are important flavor components of fruits and other foods. With the exception of pentadecanal and octadecenal, each of the compounds found in the irradiated beef has been reported in other foods and most of them have been detected in cardiac tissue. Those that have been tested have very low toxicities and what is known of their metabolism suggests that these aldehydes are readily converted to innocuous materials. Some of these compounds or their close relatives are utilized by the food industry to simulate the odor and taste of natural foods (15). The Food and Drug Administration (19) sanctions the use of dodecanal and tetradecanal for this purpose as well as several close relatives of undecanal; namely, undecalactone, undecenal, undecanone, undecyl alcohol and undecenyl

acetate (21 CFR 172.515, formerly 21 CFR 121.1164) (19). The Council of Europe (4) has approved undecanal itself as a flavoring adjuvant as well as dodecanal and tetradecanal. Similarly, these three aldehydes have been approved as food flavors by an expert committee for the British Ministry of Agriculture (10). The acceptable daily intakes are roughly 1000 to 10,000 times the amounts of the respective aldehydes from the irradiated beef.

The Committee does not believe the amounts of the various aldehydes found in irradiated beef constitute a significant increment of hazard to the consumer.

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3. Ketones

Occurrence

The two simplest ketones, acetone and 2-butanone (methyl ethyl ketone) were present in concentrations of 139 and 89 μg per kg respectively in the irradiated beef (Table 13). No other ketones were detected.

Both compounds are widely distributed in nature. Acetone has been detected in virtually every food examined. Its presence has been reported in over 70 foods including beverages, fruits, vegetables and meat. Amounts in excess of 1 mg per kg (1 ppm) have been reported for beer, butter, certain cheeses, milk, eggs, strawberries and other foods. It is found in significant amounts in diesel exhaust (25), in the effluents from wood burning (14) and solid waste incineration (27), in drinking water (8) and in ambient air (10) (Table 13). It normally is found in small amounts in the tissues and fluids of man and other animals. In severe diabetes, when fat is the predominant metabolic substrate, as much as 100 grams per day of acetone, beta hydroxybutyric and acetoacetic acids (ketone bodies) may be produced and excreted. Comparable amounts may also be produced during starvation.

2-Butanone is also a ubiquitous food constituent, found naturally in a variety of fruits, vegetables, meats and dairy products. It is also widely used as a flavoring constituent in certain beverages, ices, candy and baked goods.

Metabolism

Because of its extreme solubility, acetone is readily absorbed into the blood stream after inhalation. Whether inhaled or ingested, acetone is rapidly excreted through the lungs with the kidneys serving as secondary excretory organs (6, 7, 26).

Small amounts are oxidized to carbon dioxide or converted to formate or acetate. Price and Rittenberg (19) administered 1 to 7 mg per kg of labeled acetone to rats and found that about half was exhaled as carbon dioxide within 24 hours. The labeled atom which appeared in a number of compounds, including glycogen, urea and cholesterol, suggested that acetone was split to one or two carbon atoms and utilized in various metabolic cycles.

Recently, Leibman (13) demonstrated that acetone, in common with various other aliphatic and aromatic ketones, can be reduced by the broad

Table 13. Presence of ketones in various media.*

Compound	Irradiated beef µg/kg		Thermally sterilized beef µg/kg		Frozen beef control µg/kg		Atmosphere µg/m³	Water µg/l	Food µg/kg
	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked			
Acetone	107	139	65	120	3	4	4800 (24 hour average near production plant) Simulated spacecraft - 140 Fireplace - 5,000 - 10,000	1.0 (Seattle) 6.3-9.5 (Connecticut)	Beef, canned - 650; beer - 1400; butter (oil) - 42000, butter (sweet cream) - 130; carrot - 200; cheese (cheddar) - 8500; cheese (blue) - 510; cherry essence - 16000; eggs - 22300; grapefruit juice - 100; ham (cured) - 650; ham (uncured) - 500; milk (dry) 450-1260; milk (whole) 3000; pea - 350; oil (roasted peanut) - 3600; rum, Jamaican - 250; soybean, raw - 1600; strawberry - 1300; whiskey - 200; detected in over 70 foods of all types
2-Butanone	72	89	5	10	5	0	Simulated spacecraft - 590		Beef, canned - 14-110; butter - 160; cheese (cheddar) - 180; eggs - 9600; grapes - 10; lingonberry juice - 6; milk, dry - 15-75; peanut oil - 130; pear - 1000; rum - 30; detected in almost 60 foods

*See Table 5, footnote a.

category of liver and kidney enzymes which Bachur (3) has termed the cytoplasmic aldo-keto reductases. However, in contrast with other aldehydes and ketones, only a small fraction of absorbed acetone is reduced to alcohol and excreted as its glucuronide (26).

Little can be added concerning the metabolic pathways of 2-butanone, the other ketone detected in irradiated beef. Variations from acetone seem related to differences in physical properties of the two ketones. Since 2-butanone is somewhat less volatile than acetone, less is excreted unchanged through the lungs and more is subjected to enzymatic action (21). It appears that butanone can be reduced to a secondary alcohol by the liver and kidney cytoplasmic reductases described above (13). DiVincenzo et al. (5) also detected 3-hydroxy-2 butanone and 2, 3-butanediol after butanone administration indicating that some of the compound follows an oxidative pathway.

Toxicity

Both acetone and 2-butanone are generally considered to be relatively nontoxic. The Joint FAC/WHO Expert Committee on Food Additives (11) has approved the use of acetone as a solvent in accordance with "good manufacturing practices" and states that "many years of human industrial experience have shown no evidence of organ damage." The Food and Drug Administration (17) also permits the use of 2-butanone in foods as a synthetic flavoring substance (21 CFR 172.515, formerly 21 CFR 121.1164), as does the British Ministry of Agriculture (9) and the Council of Europe (4). The latter permission has been on a temporary basis since 1973, pending additional medium term toxicity studies on a sensitive species but no study of this type has been reported. McCann et al. (15) reported that acetone was nonmutagenic in Salmonella typhimurium.

For inhalation, the threshold limit values adopted by the American Conference of Governmental Industrial Hygienists for workroom atmosphere (2) are 1000 ppm (2400 mg per m^3) for acetone and 200 ppm (590 mg per m^3) for butanone. Oglesby et al. (18) stated that a study of thousands of mill workers exposed to acetone revealed at most, only minor irritation of eyes and nose at levels of 2500 to 3000 ppm.

2-Butanone is widely used as an industrial solvent and although workers frequently complain of its objectionable odor, it has been stated that exposure to 700 ppm in the air gave no evidence of permanent ill effects (7). Dermatoses are common among workers handling butanone and numbness of fingers and arms were reported by some exposed to 300 to 600 ppm (22). The question of possible neuropathy was reconsidered when

a recent outbreak occurred among workers in a color-print and plastic-coated fabric plant exposed to 2-hexanone, a close relative of butanone (1). Saida and co-workers (20) produced extensive peripheral nerve changes in rats exposed to hexanone but none upon continuous exposure to butanone at concentrations of 1125 ppm for up to 55 days. Similar results were obtained with cats (24). DiVincenzo et al. (5) attributed the toxicity of the hexanone to the enzymatic formation of 2,5-hexanedione, which produced a marked peripheral neuropathy in rats. However, when rats were exposed to the combined vapor at a ratio of one part hexanone to five parts of butanone (225:1125 ppm) a marked potentiation of the peripheral neurotoxicity was observed (20).

Lethal concentrations by different routes in different species are summarized in Table 14.

Table 14. Toxicity of ketones.

Compound	Animal	Route	LD_{50}	LD_{100}	Reference
Acetone	Mouse	Inhalation		46000 ppm	11
		Inhalation		3200 ppm	23
		Oral	10,700 mg/kg		23
		Oral	9,700 mg/kg		11
		Intravenous		4 ml/kg	11
		Intraperitoneal	1297 mg/kg		16
	Rabbit	Oral	5300 mg/kg		16
		Intragastric	5340 mg/kg		11
		Intravenous		6-8 ml/kg	11
		Percutaneous	>20 ml/kg		23
	Dog	Oral	8000 mg/kg		11
2-Butanone	Mouse	Intraperitoneal	616 mg/kg		16
	Rat	Oral	3400 mg/kg		16
		Oral	2730-5490 mg/kg		11
		Oral	6860 ml/kg		23
		Inhalation	8000 ppm/8 hr.		23
	Rabbit	Percutaneous	>10 ml/kg		23

Discussion

Acetone and butanone have been detected in scores of foodstuffs. In some commonly consumed foods, their concentrations exceed by a large margin the quantities found in irradiated beef. Both have been approved by official bodies, including the Food and Drug Administration for use as a food extractant or additive. Both are widely used in industry without evidence of chronic human toxicity. Their metabolic products pose no apparent hazard. For these reasons, the Committee believes that the amounts of acetone and butanone present in irradiated beef can be consumed without harm.

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C. SULFUR-CONTAINING COMPOUNDS

Occurrence

Five sulfur-containing compounds -- four sulfides and one thiol -- were detected in irradiated beef: carbonyl sulfide, dimethyl disulfide, dimethyl sulfide, ethane thiol and hydrogen sulfide (Table 15). All were found in both cooked and uncooked samples. The thermally sterilized samples contained considerably more carbonyl sulfide than did the irradiated specimens. No ethane thiol or dimethyl sulfide was detected in the nonirradiated beef, while the amounts of hydrogen sulfide and dimethyl disulfide were approximately the same in irradiated and thermally sterilized samples.

Sulfur compounds originate from many natural and anthropogenic sources. Significant amounts of hydrogen sulfide are constantly added to the atmosphere from volcanic and geothermal activity, from anaerobic bacterial action and from various industrial processes. Natural gas is rich in hydrogen sulfide and the processing techniques to remove this contaminant give rise to considerable amounts of carbonyl sulfide. Carbonyl sulfide is also generated when fossil fuels containing sulfur are burned. In man and other animals, hydrogen sulfide is liberated in the intestinal tract and is a readily detectable component of flatus (9) and fecal material. In addition to these sources, hydrogen sulfide, carbonyl sulfide and other sulfur containing compounds are also found in numerous foods, as shown in Table 15.

Sulfur-containing compounds are present in a wide variety of foodstuffs and are especially prominent in many vegetables, meats and roasted products such as coffee, cocoa and peanuts. Volatile sulfur compounds were detected in 21 of 23 varieties of vegetables investigated (7). The broadest distribution was noted for hydrogen sulfide, dimethyl sulfide and dimethyl disulfide, with a somewhat more limited occurrence of ethane thiol. Carbonyl sulfide was found in large amounts in horseradish and was readily detectable in other vegetables and meats.

In general, the concentration of these compounds increases significantly after heating. Dimethyl disulfide has been reported in almost all cooked vegetables (13). It represents, for example, over 25 percent of the total volatiles from fresh cabbage and almost 40 percent of those from cooked samples (10). It is also evident after heating beef, when it may reach levels of 100 μg per kg and contribute to the off-flavor of overcooked meat (19). Hydrogen sulfide is a major contributor to the flavor and off-flavor of cooked

Table 15. Distribution of sulfur compounds.*

Compound	Irradiated beef μg/kg		Thermally sterilized beef μg/kg		Frozen beef control μg/kg		Atmosphere μg/m³	Foods μg/kg
	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked		
Carbonyl sulfide	2	2	22	75	0	0	183 (Los Angeles)	Horse radish - 12000; found in chicken, cabbage, parsley
Dimethyl disulfide	3	4	7	13	1	1		Haddock (stored 14 days) - 150,000; cheddar cheese - 70,000; beef (cooked) - 100; eggs - 7400; also found in: onion, garlic, peas, cabbage, rutabaga, potato, beans, cauliflower, tomato, sprouts
Dimethyl sulfide	4	6	0	0	0	0		Haddock (stored 14 days) 200,000; cheddar cheese - 1000; Baltic herring - > 1000; found in: canned beef, onion, leek, garlic, pea, beans, cabbage, cauliflower, rutabaga, carrot, sprouts, potato, celery, parsnip, tomato
Ethane thiol	7	10	0	0	0	0	Simulated spacecraft - 35	Beef, canned - 170-290; carrot, canned - 50; fish protein concentrate - 37; potato, boiled - 100-200; also found in: onion, leek, peas, cauliflower, parsnip
Hydrogen sulfide	2	2	1	5	0	0	1-20 140-1400 (flatus)	Beef, broth - 6000-8000; beef, canned - 1100 - 2900; beer - 110; broccoli - 10,000; cabbage - 300,000 - 790,000; cauliflower - 20,000; cheese cheddar - 3-50,000; oryzae - 20; gouda - 48; trappist - 1900; chicken, boiled - 580-730; chicken, broth - 35; chicken, cooked - 180-1000; corn - 14-131; eggs, boiled - 178; fish protein concentrate - 850; grapefruit juice - 900; orange juice - 1600; potato, boiled - 150-525

*See Table 5, footnote a.

meats, fish, certain vegetables such as broccoli and cabbage, and especially of eggs.

The origin of these compounds has not been extensively investigated, but methionine is generally believed to be their major precursor (2, 3), especially for dimethyl sulfide and dimethyl disulfide. The Maillard reactions in heat-processed foods give rise to various alkyl thiols and carbonyl sulfide (13), while hydrogen sulfide can arise from various sulfur-containing amino acids, peptides and proteins and from thiamine. Cysteine, however, appears to be its chief precursor.

Metabolism

Surprisingly few studies have been conducted on the fate of the simple sulfur volatiles identified in the irradiated beef. By analogy with the body's treatment of known sulfur xenobiotics, oxidation would appear to be the preferred pathway of metabolism.

Hydrogen sulfide is very susceptible to oxidation. It is oxidized so readily that its reported levels in the atmosphere may reflect artifically low values resulting from oxidation during sampling and analysis (8). Although documentation could not be found, it seems likely that hydrogen sulfide is also oxidized in the body. In any event, the simplest organic sulfide -- dimethyl sulfide -- has been shown to undergo such oxidation, first to the sulfoxide (16) and then to the sulfone (6).

The disulfides, on the other hand, are first reduced to the corresponding mercaptans by a nonspecific nucleotide-dependent disulfide reductase (15). The resulting mercaptans maybe partially eliminated, unchanged in the expired air and urine, but the bulk is oxidized as described above and excreted in the urine as its sulfone (14) or as inorganic sulfate (17). Ethane thiol undergoes a similar transformation, being partially excreted unchanged in the breath and urine and partially as the sulfone and inorganic sulfate in the urine. Ethane thiol may also be methylated by S-methyl transferase to produce its S-methyl analogue (18). In addition, thiols undergo conjugation with glucuronic acid (17) although this has not yet been demonstrated with ethane thiol.

No reports could be found on the metabolism of carbonyl sulfide. Since it is readily hydrolyzed by water to hydrogen sulfide and carbon dioxide, its fate in the body would presumably be the same as these products.

Toxicity

The ingestion of volatile sulfur compounds is largely self-limiting because of their strong odor and taste. Small amounts may be

necessary to impart a desirable, characteristic flavor to the food, but excessive quantities discourage consumption by all except those with highly insensitive or idiosyncratic tastes. The quantities detected in the irradiated beef fall well within the range naturally present in many foods. The Council of Europe (4) has approved the addition of several of these compounds as flavoring adjuncts. The acceptable daily intake for dimethyl sulfide has been set by this group at 1.5 ppm of ingested food (about 1.5 to 2.5 mg per day) and for ethane thiol at 1 ppm (about 1.0 to 1.5 mg per day). No level has been set for hydrogen sulfide, presumably because of the self limiting aspect mentioned above. British authorities have recommended approval of hydrogen sulfide, dimethyl sulfide, dimethyl disulfide and ethane thiol as food flavoring adjuvants (5). The Food and Drug Administration imposes no limit in its approval of dimethyl sulfide (21 CFR 172.515, formerly 21 CFR 121.1164) as a synthetic flavoring substance that may be safely used in foods (12).

The threshold limit value for workroom atmospheres has been set at 0.5 ppm (1 mg per m^3) for ethane thiol and at 10 ppm (15 mg per m^3) for hydrogen sulfide (1). The acute toxicities of these compounds are summarized in Table 16, constructed from data collected by the National Institute for Occupational Safety and Health (11). Hydrogen sulfide in high concentrations acts directly upon the nervous system causing paralysis of the respiratory center and olfactory system. It also decreases the oxygen carrying capacity of hemoglobin by the formation of sulfhemoglobin. Little is known of the acute effects of the other sulfur-containing compounds.

Table 16. Toxicity of sulfur compounds.

Compound	Animal	Route	LD_{50}
Dimethyl sulfide	Mouse	Oral	3700 mg/kg
	Rat	Oral	535 mg/kg
Ethane thiol	Mouse	Inhalation	2700 mg/kg
	Rat	Intraperitoneal	450 mg/kg
		Inhalation	4420 ppm/4 hr
		Oral	682 mg/kg
Hydrogen sulfide	Mouse	Inhalation	683 ppm/1 hr
	Rat	Inhalation	713 ppm/1 hr

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Discussion

The amounts of the sulfur-containing compounds in irradiated beef are extremely small, in no case exceeding 10μg per kg. Three of the five compounds (carbonyl sulfide, dimethyl disulfide and hydrogen sulfide), were more abundant in the thermally sterilized than in the irradiated samples. Each (except carbonyl sulfide) has been detected in numerous foods and in every case where quantitative analysis has been performed the quantity in meat, fish, eggs, fruits and vegetables is far greater than that in irradiated beef. Most of those compounds have been approved by official bodies as flavoring adjuvants at levels several orders of magnitude greater than their concentrations in irradiated beef.

The Committee believes the concentrations of sulfur-containing compounds in irradiated beef are trivial and pose no hazard to the consumer.

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D. NITROGEN-CONTAINING COMPOUNDS

Occurrence

Acetonitrile was the only nitrogen containing organic volatile detected in the irradiated beef. It was present in both the cooked and uncooked irradiated samples (Table 17). The concentrations did not differ significantly from those in the frozen controls and were considerably less than those in the thermally sterilized beef. This suggests that acetonitrile is not a radiolytic product, a suggestion strengthened by the observation that nitriles can be produced from amino acids at conventional cooking temperatures.

No report could be found listing acetonitrile as a normal constituent of food. However, Vollmin and colleagues (8) have shown that nitriles are produced in abundance when amino acids are subjected to high temperatures. Acetonitrile was a major product when the following amino acids were heated to 700° C: glycine, alanine, proline, serine, cysteine, methionine, aspartic acid, ornithine and gamma amino butyric acid. Lien and Nawar (4) using milder treatments, detected the formation of acetonitrile when beta alanine was heated at 200° C <u>in vacuo.</u> The compound apparently resulted from successive decarboxylation and dehydrogenation as follows:

$$\underset{\displaystyle NH_2}{CH_2} - CH_2 - COOH \xrightarrow{\Delta} \underset{\displaystyle NH_2}{CH_2}-CH_3 + CO_2 \longrightarrow CH_3CH{=}NH + H_2 \longrightarrow CH_3C{\equiv}N + H_2$$

Lien and Nawar also demonstrated that triglycerides and amino acids interact readily to produce secondary amides which hydrolyze to form nitriles.

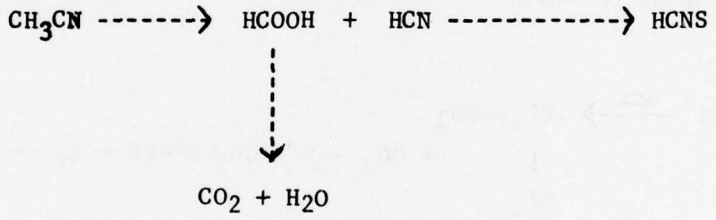
Acetonitrile has been identified in cigarette smoke (2) and in the urine of smokers (5). About one mg of acetonitrile is produced from the smoke of a single cigarette. The average urinary excretion among smokers was more than 100 μg per liter with heavy smokers excreting twice this amount. The highest value detected among nonsmokers was less than 10 μg per liter of urine.

Table 17. Acetonitrile in beef.

Irradiated		Thermally Sterilized		Frozen Control	
cooked	uncooked	cooked	uncooked	cooked	uncooked
μg/kg		μg/kg		μg/kg	
3	1	21	57	6	3

Metabolism

The alkyl cyanides (e.g., acetonitrile) are hydrolyzed to hydrocyanic acid and a corresponding acid with one less carbon atom. The cyanide is converted to thiocyanate which is excreted unchanged, while the acid is metabolized through normal metabolic pathways to carbon dioxide and water (9).



Toxicity

The acute toxicity of acetonitrile is summarized in Table 18. It was the least toxic of all alkyl nitriles tested in mice and rabbits (9). Pozzani et al. (6) subjected rats to acetonitrile vapor for seven hours daily for 90 days. No deaths were encountered even at levels of 655 ppm, but two monkeys exposed to this concentration died within 23 and 51 days respectively. One monkey inhaling 330 ppm survived the 91 day test period as did three dogs. No gross pathology was evident but histological examination revealed focal emphysema and diffuse proliferation of alveolar septa.

The TLV is 40 ppm (70 mg per m^3) (1). This value seems based primarily on the study by Pozzani et al. (6) on human subjects who inhaled the vapor at this concentration for 4 hours. Two of the three subjects reported no adverse effects. No cyanide could be detected in their blood nor was there any increase in the level of urinary thiocyanate. The third subject experienced a slight tightness and a sensation of coolness in the chest after the exposure. There was a slight increase in his urinary thiocyanate.

Table 18. Acute toxicity of acetonitrile.

Animal	Route	Minimum Lethal Dose	LD_{50}	Reference
Mouse	Intraperitoneal		250 mg/kg	6
Rat	Intragastric		1.7-8.5 g/kg	6
			3.8 g/kg	7
	Subcutaneous		5 ml/kg	3
	Intraperitoneal		0.95-5.62 g/kg	6
	Intravenous (portal)		0.71 ml/kg	6
	Intravenous (tail)		1.68 ml/kg	6
	Inhalation	8000 ppm/4 hr.	16000 ppm/4 hr	6
	Percutaneous		1.25 ml/kg	6
			5.0 ml/kg	1
	Inhalation		2800 ppm/4 hr	6
Guinea pig	Inhalation		5655 ppm/4 hr	6

Discussion

Acetonitrile is a nonradiolytic product. The minute amounts in irradiated beef are no greater than in frozen controls, and are considerably less than in thermally sterilized samples. Evidence also points to its rapid hydrolysis in the body. The Committee concludes that ingestion of acetonitrile in the amounts found in irradiated beef should have no harmful effects.

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E. HALOGEN-CONTAINING COMPOUNDS

Tetrachloroethylene

Occurrence

Tetrachloroethylene (perchloroethylene) was the only organochlorine compound detected in the irradiated beef in concentrations sufficient to permit quantitative analysis ($\gtrsim$ 1 ppb). Its concentration (8 to 11 µg per kg) (Table 1) was not significantly different from the amounts detected in the nonirradiated samples, nor did it increase with higher irradiation doses.

Because of its nonflammability and its excellent solvent ability, tetrachloroethylene is found in a number of consumer and industrial products. It is the leading dry cleaning solvent in the U.S.; it is used extensively to degrease metals; it serves as a solvent for silicones; it is an intermediate in the synthesis of fluorocarbons and at one time it was used extensively as a human and veterinary antihelminthic. Its production on a world-wide basis exceeds one million tons per year, with approximately one-third of this amount produced in the United States.

As is evident from Table 19, it has been detected above the North Atlantic and in the air of rural and metropolitan areas. It is present in the ocean waters, in rivers and in municipal water supplies; in aquatic organisms, fish, birds, mammals and man; in fruits, vegetables, beverages and dairy products. It was detected in eight of ten water utilities surveyed by the Environmental Protection Agency (4) as well as in other drinking water sources.

Metabolism

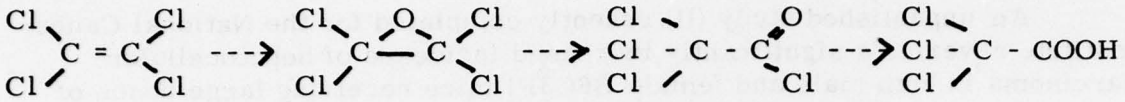
Tetrachloroethylene usually enters the body through the lungs although absorption is also possible through the skin or gastrointestinal tract. Regardless of the absorptive site, virtually all is excreted unchanged through the lungs. Daniel (3) administered ^{36}Cl-labeled tetrachloroethylene by stomach tube to rats and recovered 98 percent of the radioactivity in the expired air within 48 hours. After exposure to tetrachloroethylene, human subjects excrete most of the absorbed compound through the lungs within 24 hours. Following repeated exposures, a prolonged exponential decay of the compound in the subjects' breath was observed extending for 14 days or more (14). An epoxide, which has been recovered as an intermediate, is the first metabolic product. This

Table 19. Distribution of tetrachloroethylene.*

Irradiated beef µg/kg		Thermally sterilized beef µg/kg		Frozen beef control µg/kg		Raw beef µg/kg	Atmosphere µg/m³	Water µg/l	Food µg/kg	Miscellaneous µg/kg
Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked					
9	8	9	11	10	12	4	Sea stations - 0.005 Rural Northwest - 0.15 Western Ireland - 0.2 Los Angeles - .07-31 New Jersey - <0.1-72 Submarine - 814 Simulated spacecraft - 14	Atlantic - 0.005 Liverpool Bay 0.12 (ave) 2.6 (max) Municipal water Miami, Ottumwa, Philadelphia, Cincinnati, N.Y.C., Tucson, Lawrence, Grand Forks - 0.07 - 0.46 Connecticut - Trace -220 Italian wells (combined tri- and tetrachloroethylene) Suburban - 4-38 City - 38-450 Provincial - 39-348	Dairy products - milk - 0.3 Cheese (cheshire) - 2. Butter - 13 Meat - Beef steak - 0.9 Beef fat - 1.0 Pig liver - 5. Oils and fats - Margarine - 7. Olive oil - 7. Vegetable oil - 0.01 Castor oil - 3. Beverages Fruit juices (canned) - 2 Instant coffee - 3. Tea (packet) - 3. Fruits and vegetables Potatoes - 0.7 Apples - 2. Pear - 2. Tomato - 1.2 Bread - 1.	Marine algae - 13-22 Invertebrates - 0.05-15. Fish Fresh - <0.1-11 Liver - 1-41 Birds Muscle - 0.7 Liver - 1.5-3.2 Eggs - 1.3 - 39. Kidney - 6.4 Seal Blubber - 0.6-19 Liver - 0-3.2 Human tissue Fat - 0.1-29.2 Kidney - 0.5-6 Liver - <0.5-4.3 Brain - <0.5 Effluents from textile mills

*See Table 5, footnote a.

undergoes a slow rearrangement to trichloroacetyl chloride, which is then rapidly hydrolyzed to yield trichloroacetic acid as the major end product (16).



Only traces of trichloroethanol were excreted in the urine of mice, rats or man after exposure to tetrachloroethylene vapor (6).

Toxicity

Tetrachloroethylene is relatively nontoxic after acute exposure. This is evident from the LD_{50} values shown in Table 20, as well as by the TLV for workroom exposure (100 ppm or 535 mg per m^3) (1). Men exposed to 1000 ppm showed slight inebriation in 45 minutes but no narcosis after 95 minutes. At levels of 2000 ppm, light narcosis was produced within a few minutes (2). Stewart et al. (14) exposed human volunteers to 100 ppm for 7 hours daily for 5 consecutive days. Over half complained of mild eye, nose or throat irritation developing within the first few hours and then usually subsiding. About one-quarter reported mild headaches. Neurological, pulmonary and laboratory tests were normal. However, Kylin et al. (9) reported significant fatty degeneration of the liver in mice chronically exposed to atmospheres of 200 ppm for 4 hours daily, 6 days weekly for periods of 1 to 8 weeks.

Table 20. Acute toxicity of tetrachloroethylene.

Animal	Route	LD_{50}	Reference
Mouse	Oral	5.2 ml/kg	15
	Intraperitoneal	4.6 g/kg	7
	Intraperitoneal	5.7 g/kg	5
Rat	Inhalation	>3000 ppm (8 hr)	12
	Inhalation	>9000 ppm (3 hr)	2
Dog	Intraperitoneal	3.4 g/kg	8

Pregnant mice and rats were exposed to 300 ppm tetrachloroethylene for 7 hours daily on days 6 to 15 of gestation (15). Cesarean sections were performed on gestation days 21 in rats and 18 in mice. The tetrachloroethylene exposure to the pregnant mice and rats caused no significant maternal embryonal or fetal toxicity nor any teratogenicity.

An unpublished study (11) recently completed for the National Cancer Institute revealed a significantly increased incidence of hepatocellular carcinoma in both male and female B6C3F1 mice receiving large doses of tetrachloroethylene. The compound dissolved in corn oil was administered by gavage five days per week for 78 weeks followed by an observation period of 12 weeks. The time-weighted-average dosages for male mice were 1072 and 536 mg per kg per day; and for female mice were 772 and 386 mg per kg per day.

Both treated and control mice displayed various neoplastic and other lesions. However, primary malignant tumors of the liver were significantly higher in the mice receiving tetrachloroethylene (Table 21).

Table 21. Incidence of hepatocellular carcinoma in mice receiving tetrachloroethylene.

Treatment	Males	Females
Controls	7/79	0/20
Low dose	32/49 ($P < 0.001$)	19/48 ($P < 0.001$)
High dose	27/48 ($P < 0.001$)	19/48 ($P < 0.001$)

The time to the first observed tumor was 27 weeks for male mice and 41 weeks for the females.

Male and female rats were also exposed to two dose levels of tetrachloroethylene administered as described above: 941 and 471 mg per kg per day for male rats and 962 and 481 mg per kg per day for female rats. There was no observable carcinogenic effect of the compound in rats but the results were considered inconclusive because of the poor survival of the animals. Half of the high dose males and females died within 44 and 66 weeks, respectively. Lesions indicative of pneumonia were observed in most control and dosed aminals alike at necropsy. A high incidence of the tetrachloroethylene-treated rats displayed toxic nephropathy.

Discussion

It has been convincingly demonstrated that the small amounts of tetrachloroethylene detected in irradiated beef were of extraneous rather than radiolytic origin. Thus, there is no significant difference in concentration between the irradiated and nonirradiated beef. In some samples of beef supplied by the same processing firm, no tetrachloroethylene could be detected. In the numerous publications on irradiated beef and other meats, the presence of tetrachloroethylene has never been reported. Similarly, Merritt and co-workers (10), with many years' experience with beef irradiation, state they have rarely detected chlorinated hydrocarbons, and in these rare cases, the amount detected was independent of the radiation dosage. To verify the nonradiolytic origin of the tetrachloroethylene in beef, two separate samples of beef were exposed to increasing doses of gamma irradiation. The levels in the nonirradiated samples were zero and 6 μg per kg respectively. After exposure of each sample to 30, 60, 90 and 120 kGy (3, 6, 9, 12 megarads) the tetrachloroethylene concentrations were unchanged, remaining at 0 in the one sample and 6 μg per kg in the other.

Nevertheless, the recent indictment of tetrachloroethylene as a carcinogen --- even at levels a million times greater than its intake from beef --- intensified a search for the source of contamination. It was discovered that tetrachloroethylene was used as a cleaning solvent in the meat packing plant and stored near the beef processing area. To trap the compound, samples of lard, free from tetrachloroethylene, were placed at various sites. In one of the beef-processing areas the lard was found to contain 91 ppb of tetrachloroethylene, indicating a significant atmospheric contamination at this site.

The Committee concludes that the small amount of tetrachloroethylene detected in each type of beef sample represents a contaminant from prior stages of preparation, unrelated to the irradiation process. The exercise of reasonable care during all processing stages should eliminate this compound from beef.

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VII. GENERAL DISCUSSION

As pointed out earlier, this report reviews only those compounds which were detected in beef irradiated and analysed by the techniques employed at the Natick Laboratories and described in the experimental section (see pages 10 - 15). Major modifications of these irradiation, isolation or analytical procedures could change the nature or amount of the compounds identified. In attempting to assess the possible health hazards of these compounds, the Committee was often frustrated by the paucity of information on their toxicity. Such gaps in our knowledge have been pointed out in the individual sections and additional studies in these areas are highly desirable.

By conventional toxicological standards, the concentration of each compound was low, as was the total of all the compounds. For those compounds where such data are available, the least toxic doses are several orders of magnitude greater than the contribution from irradiated beef. There seems no chance that the volatile compounds in the irradiated meat could cause an acute intoxication following its consumption.

Evaluation of possible chronic toxicity is a more difficult and uncertain task. Virtually every compound under consideration has been found in significant amounts in commonly consumed natural and processed foods. Very few of these products have been subjected to the long-term animal studies or to the rigorous epidemiological surveys that would detect subtle or slowly developing pathology or carcinogenesis. The Committee gave this problem particular attention and examined closely all data related to chronic toxicity or carcinogenicity.

Several alkanes and alkenes and one aromatic hydrocarbon (benzene) produced by beef irradiation have been implicated as carcinogens or co-carcinogens under certain conditions.

Several higher alkanes promoted tumor production when painted on mouse skin pretreated with carcinogenic doses of polycyclic aromatic hydrocarbons. These results were considered to have little relevance to the effect of alkanes in irradiated beef. Not only were the routes of administration quite different, but the doses required in these experiments were huge compared with the amounts consumed in beef.

Relatively little is known of the fate and action in the body of the various alkenes found in irradiated beef. It is now generally accepted that epoxides are obligatory intermediates in their metabolism and epoxides are viewed by many investigators as potential carcinogens. This view is

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supported by the findings that such potent carcinogens as benzo(a) pyrene, aflatoxin and vinyl chloride seem to owe their carcinogenicity to their conversion to epoxide intermediates. On the other hand, many epoxides or compounds having epoxide metabolic intermediates are considered to be noncarcinogenic. Of all the epoxide metabolic intermediates likely to be formed from the alkenes in irradiated beef, very few have been tested for tumorigenicity. Of these, only epoxyhexadecane has caused increased tumor production and these results are equivocal. It appears that there may be structural features which cause some epoxides to be carcinogenic while others are not. It is not possible at this time to designate the specific structural or electron distribution characteristics that impart carcinogenicity to an epoxide intermediate. Additional studies are desirable, but available evidence does not implicate the alkenes in irradiated beef as carcinogens when ingested in the amounts present in these samples.

Animal studies have failed to demonstrate a leukemogenic action of benzene and more definitive studies are necessary. The amount of benzene ingested from irradiated beef is less than 2 μg per day, an extremely small fraction of that absorbed from such unavoidable sources as the atmosphere, municipal water supplies and numerous foods. The amount consumed from the irradiated beef is believed to add an insignificant increment to the usual body burden.

The Committee considered, too, the possibility that interactions in the body among the various volatile compounds in irradiated beef might cause toxicity. The possibility of additive or synergistic effects cannot be excluded. There are, however, no known or suspected dangerous interactions among these compounds and the lack of data renders unprofitable any further speculation along these lines at the present time.

VIII. CONCLUSION

The Committee has examined the available evidence on the possible health effects of the various volatile compounds identified in beef prepared by low-temperature irradiation preservation. In its opinion, the data do not demonstrate or suggest that the volatile compounds present any significant increment of hazard to the public from the normal consumption of beef processed in this way.

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