

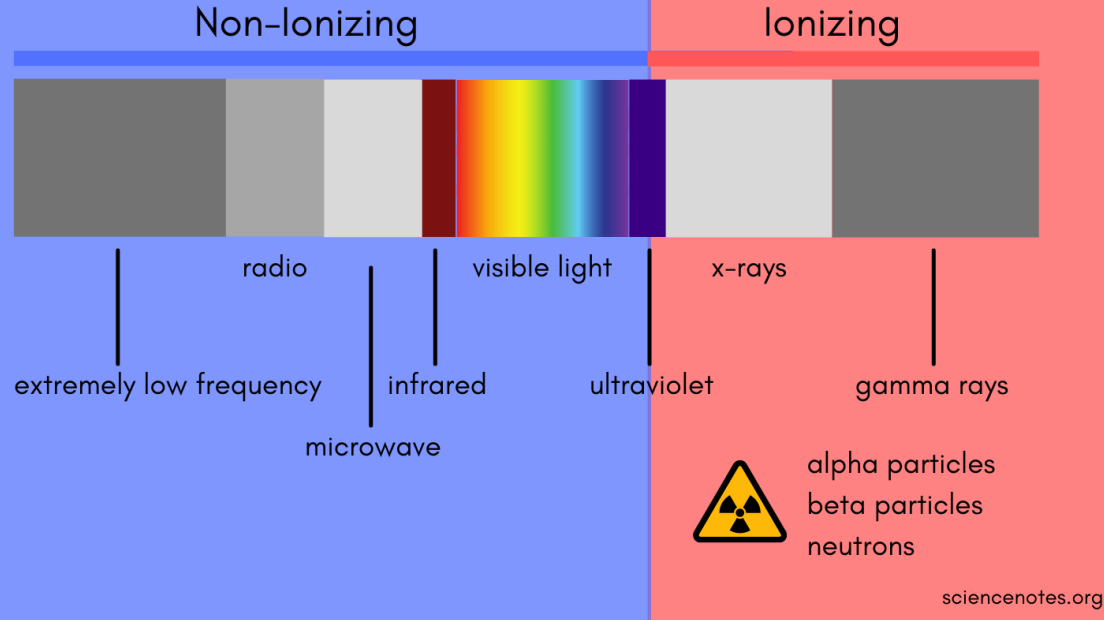
Can ionizing radiation cause isotopic fractionation of organic compounds?

Work in progress to constrain the integrity of
irradiated organic samples and reference materials

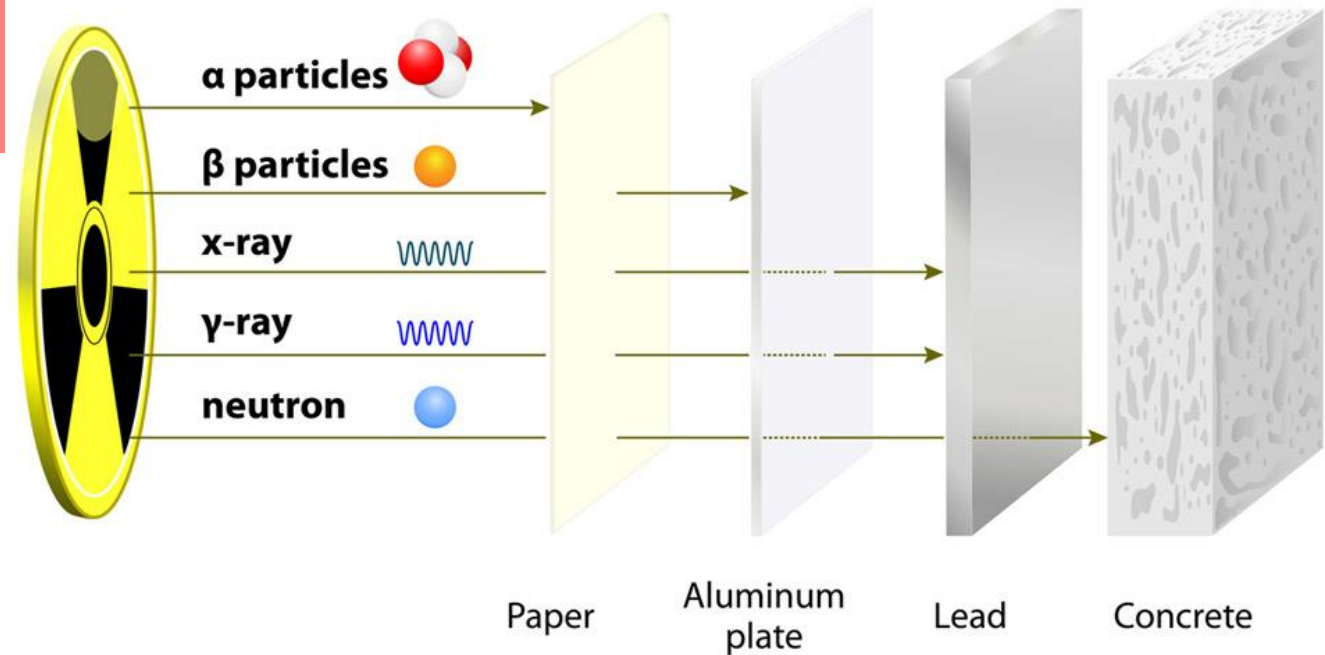
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Non-Ionizing and Ionizing Radiation



Types of Radiation



ABC of radiation, David N. Ruzic,
"Illinois EnergyProf":

<https://www.youtube.com/watch?v=qjkTzk8NAxM>

Food irradiation, David N. Ruzic:

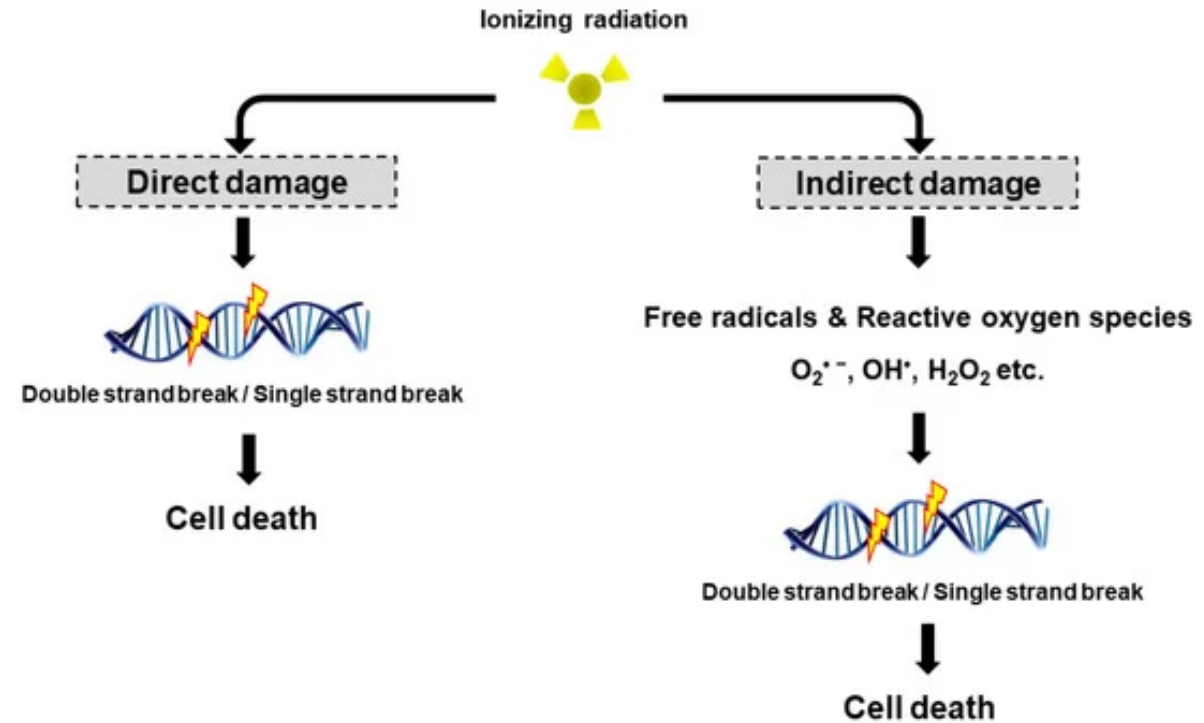
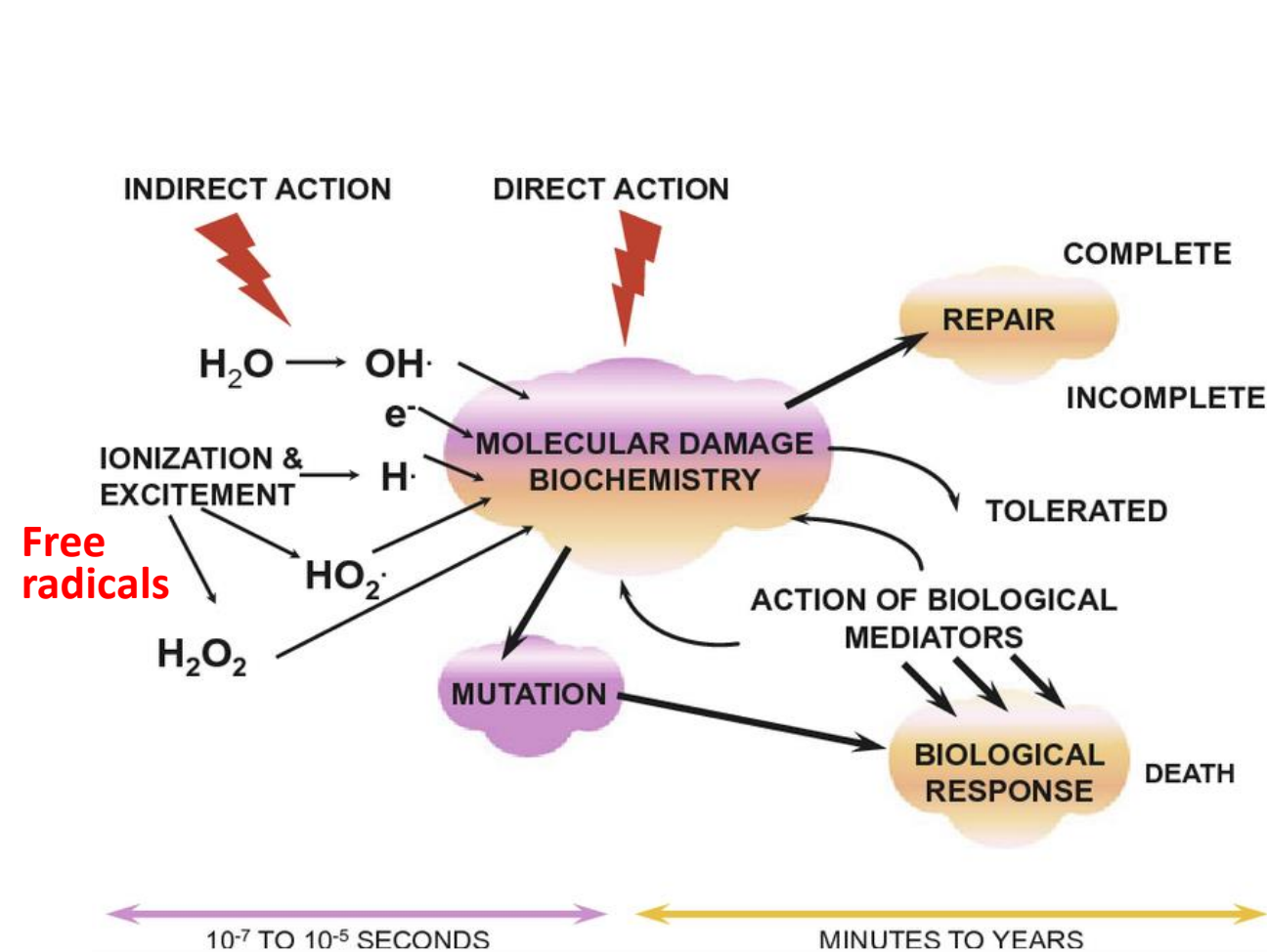
<https://www.youtube.com/watch?v=BRMG5SsbYkY>

How much [radiation] is too much? David N. Ruzic:

<https://www.youtube.com/watch?v=niFizj29h5c>

Source: www.nuclearsicherheit.de

Biochemical Effects of Ionizing Radiation



From: <https://www.semanticscholar.org> (after G. Avery)

From: *Int. J. Mol. Sci.* **2017**, 18(12), 2749;
<https://doi.org/10.3390/ijms18122749>

Sterilization by ionizing radiation is a common procedure and is even mandated in some countries when importing organic materials and scientific samples.



- Food biosecurity
- Agriculture
- Horticulture
- Radiobiology
- Radiotherapy dosimetry
- Polymer modification
- Vaccine development
- Research sample irradiation
- Medical product dose verifications
- Sterilisation of medical products and devices, including donated human tissue

Comparison of identical types of glass vials containing USGS86 peanut oil stable isotope reference material prior to γ irradiation (left), and after mandatory sterilization (right) in Australia *via* a dose of 30 kGy γ irradiation from a ^{60}Co source (from [Schimmelmann et al., 2020](#)).

Sterilization of food, pharmaceuticals, equipment etc. *via* γ irradiation

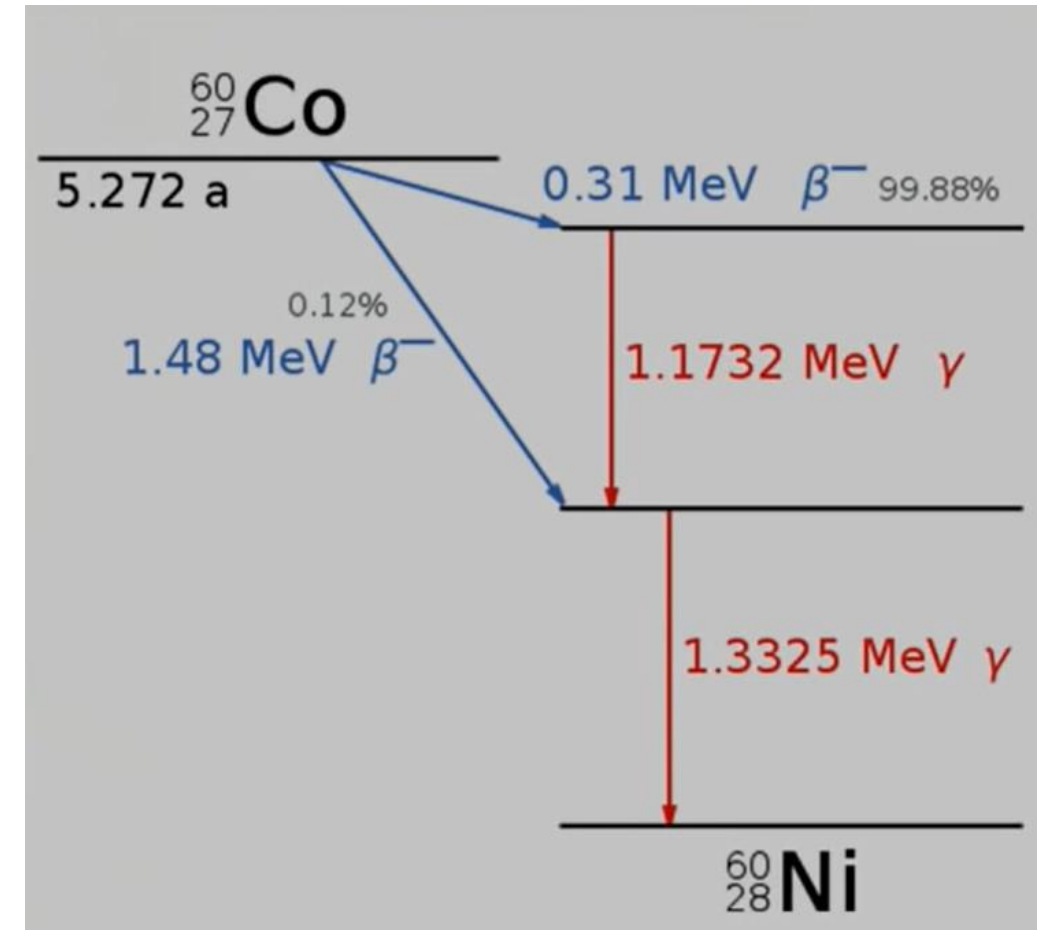
During Soviet times in the USSR, all army food rations were routinely irradiated.

NASA irradiates food supplies for space travel.

Radura symbol used for irradiated food in USA
(<https://commons.wikimedia.org/wiki/File:Radura-Symbol.svg>).



Typical radiation source:



Hypotheses to be tested:

Ionizing radiation for commercial sterilization of organic samples is **unable to generate carbon and nitrogen isotope fractionation that is measurable in bulk samples** *via* elemental analyzer (EA) interfaces.

Compound-specific isotopic measurements are far more sensitive and may be able to detect isotope fractionation, especially after higher dose rates.

Compound-specific **hydrogen** stable isotope ratios in organics are expected to be **even more susceptible** to fractionation than other isotope ratios, *via* exchange with water hydrogen.

The potential of ionizing radiation towards **isotopic fractionation in organics increases in the order $\gamma < \beta < \alpha$** .

Strong ionizing radiation can **dealkylate, aromatize, and cross-link** organic molecules, and thus cause chemical and isotopic changes.

Units of Radiation Dose

Système International (SI) units have been adopted by the *International X-ray and Radium Protection Committee* (ICRP). Ambient absorbed doses of radiation are reported in **gray per hour (Gy/h)** or **sievert per hour (Sv/h)**. Doses both in Gy and Sv are referring to joule (J) per kilogram ($1 \text{ J} = 1 \text{ W/sec}$).

When ionizing radiation interacts with the human body, it gives its energy to the body tissues. The **absorbed dose is the amount of energy absorbed per unit weight of the organ or tissue** and is expressed in units of gray (Gy). One gray dose is equivalent to one joule radiation energy absorbed per kilogram of organ or tissue weight.

Equal doses of all types of ionizing radiation are not equally harmful to human tissue. Alpha particles produce greater harm than do beta particles, gamma rays and X-rays for a given absorbed dose, so 1 Gy of alpha radiation is more harmful than 1 Gy of beta radiation. **To account for the way in which different types of radiation cause harm in tissue or an organ, radiation dose is expressed as equivalent dose in units of sievert (Sv)**. The dose in Sv is equal to the total external and internal "absorbed doses" multiplied by a "radiation weighting factor" and is important when measuring occupational exposures.

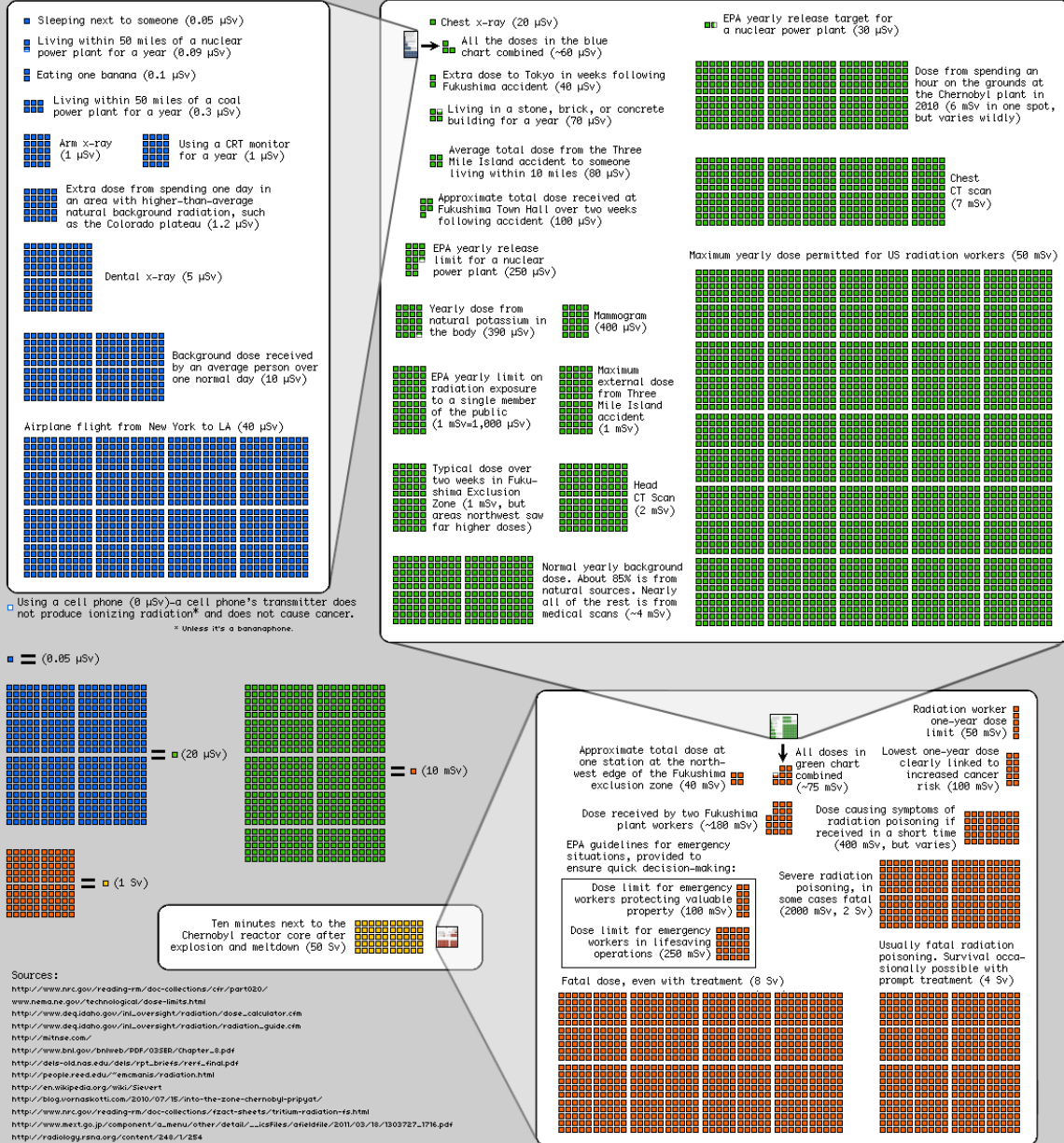
See more detailed information here: https://www.ccohs.ca/oshanswers/phys_agents/ionizing.html#section-4-hdr

Foods permitted to be irradiated under U.S. Food and Drug Administration’s (FDA) regulations (21 CFR 179.26; <https://packagingguruji.com/fda-guidelines-packaging-foods/>)

Food	Purpose	Dose
Fresh, non-heated processed pork	Control of <i>Trichinella spiralis</i>	0.3 kGy min. to 1 kGy max.
Fresh foods	Growth and maturation inhibition	1 kGy max.
Foods	Arthropod disinfection	1 kGy max.
Dry or dehydrated Enzyme preparations	Microbial disinfection	10 kGy max.
Dry or dehydrated spices/seasonings	Microbial disinfection	30 kGy max.
Fresh or frozen, uncooked poultry products	Pathogen control	3 kGy max.
Frozen packaged meats (solely NASA)	Sterilization	44 kGy min.
Refrigerated, uncooked meat products	Pathogen control	4.5 kGy max.
Frozen uncooked meat products	Pathogen control	7 kGy max.
Fresh shell eggs	Control of <i>Salmonella</i>	3.0 kGy max.
Seeds for sprouting	Control of microbial pathogens	8.0 kGy max.
Fresh or frozen molluscan shellfish ¹	Control of <i>Vibrio</i> species and other foodborne pathogens	5.5 kGy max.

Radiation Dose Chart

This is a chart of the ionizing radiation dose a person can absorb from various sources. The unit for absorbed dose is "sievert" (Sv), and measures the effect a dose of radiation will have on the cells of the body. One sievert (all at once) will make you sick, and too many more will kill you, but we safely absorb small amounts of natural radiation daily. Note: The same number of sieverts absorbed in a shorter time will generally cause more damage, but your cumulative long-term dose plays a big role in things like cancer risk.



How do radiation doses from sterilization *via* γ irradiation compare to sources and doses of other radiation in our environment? For simplicity, we assume equivalency of Gy and Sv.

From: <https://xkcd.com/radiation/>

■ Sleeping next to someone (0.05 μSv)

■ Living within 50 miles of a nuclear power plant for a year (0.09 μSv)

■ Eating one banana (0.1 μSv)

■ Living within 50 miles of a coal power plant for a year (0.3 μSv)

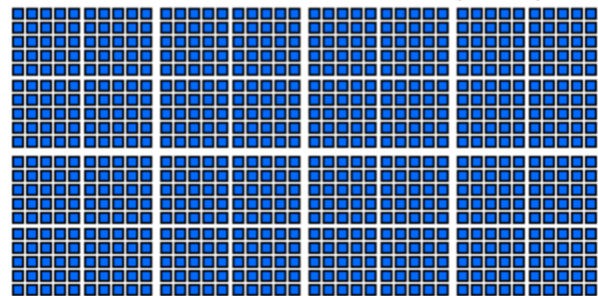
■ Arm x-ray (1 μSv) ■ Using a CRT monitor for a year (1 μSv)

■ Extra dose from spending one day in an area with higher-than-average natural background radiation, such as the Colorado plateau (1.2 μSv)

■ Dental x-ray (5 μSv)

■ Background dose received by an average person over one normal day (10 μSv)

■ Airplane flight from New York to LA (40 μSv)



■ Chest x-ray (20 μSv)

■ All the doses in the blue chart combined (~60 μSv)

■ Extra dose to Tokyo in weeks following Fukushima accident (40 μSv)

■ Living in a stone, brick, or concrete building for a year (70 μSv)

■ Average total dose from the Three Mile Island accident to someone living within 10 miles (80 μSv)

■ Approximate total dose received at Fukushima Town Hall over two weeks following accident (100 μSv)

■ EPA yearly release limit for a nuclear power plant (250 μSv)

■ Yearly dose from natural potassium in the body (390 μSv)

■ Mammogram (400 μSv)

■ EPA yearly limit on radiation exposure to a single member of the public (1 mSv=1,000 μSv)

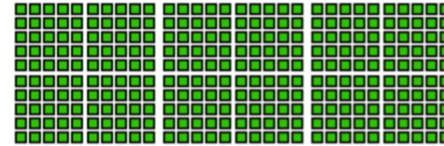
■ Maximum external dose from Three Mile Island accident (1 mSv)

■ Typical dose over two weeks in Fukushima Exclusion Zone (1 mSv, but areas northwest saw far higher doses)

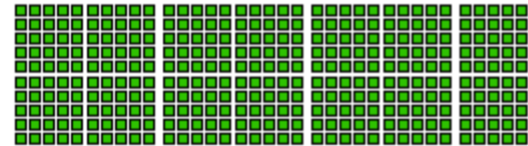
■ Head CT Scan (2 mSv)

■ Normal yearly background dose. About 85% is from natural sources. Nearly all of the rest is from medical scans (~4 mSv)

■ EPA yearly release target for a nuclear power plant (30 μSv)

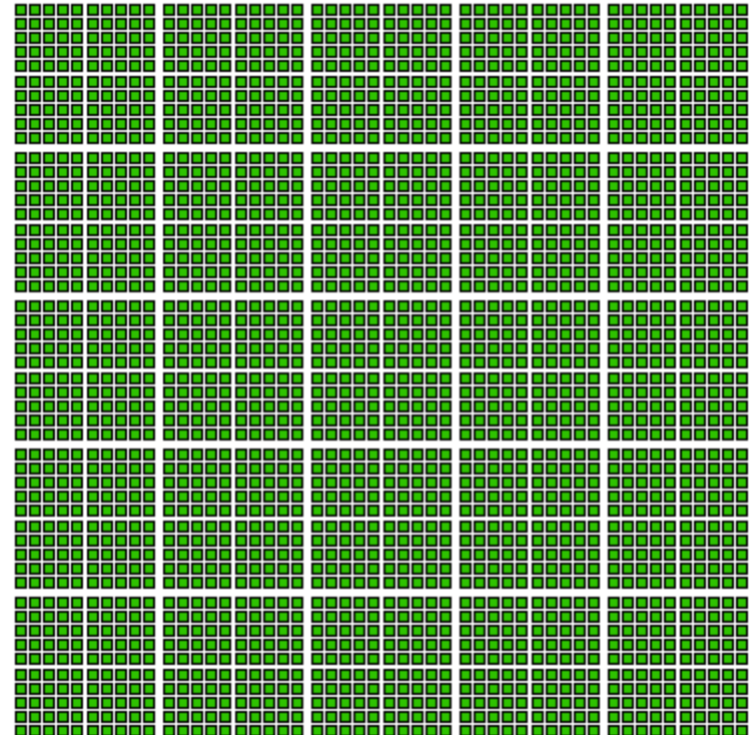


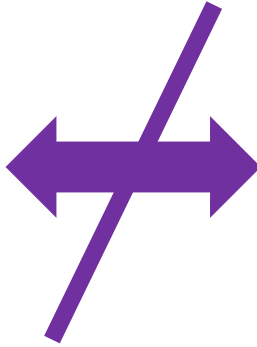
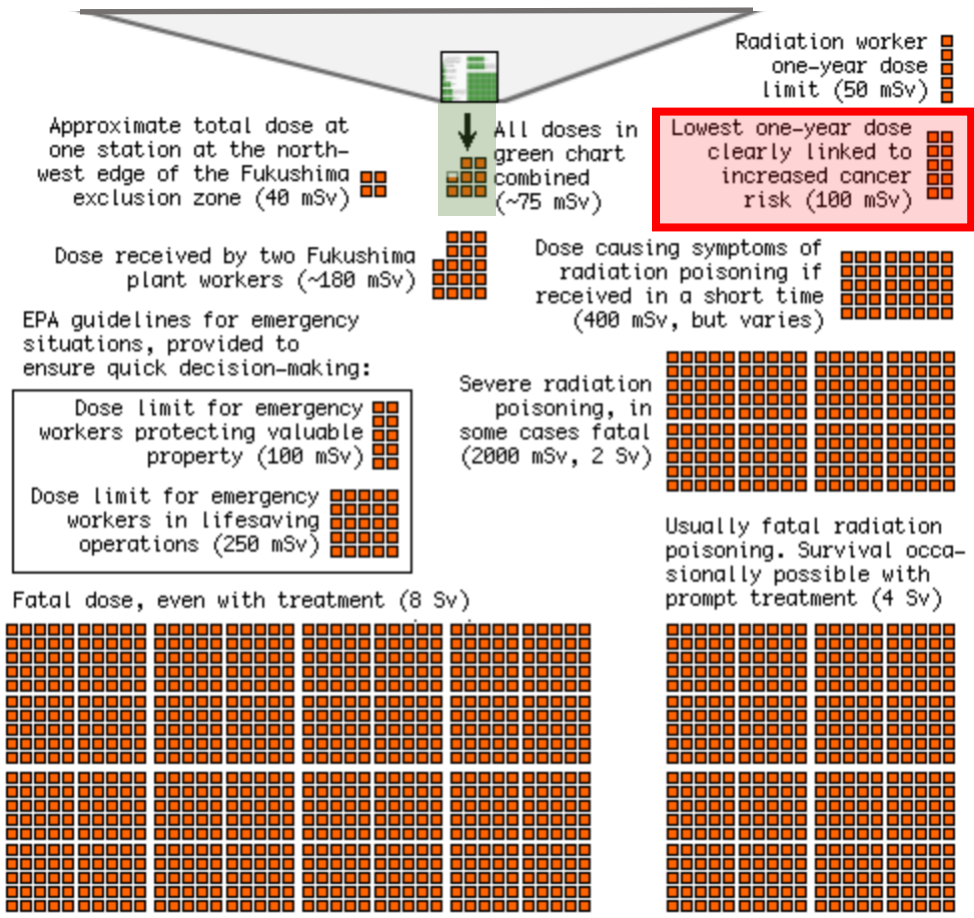
Dose from spending an hour on the grounds at the Chernobyl plant in 2010 (6 mSv in one spot, but varies wildly)



Chest CT scan (7 mSv)

Maximum yearly dose permitted for US radiation workers (50 mSv)





Food	Dose
Fresh, non-heated processed pork	0.3 kGy min. to 1 kGy max.
Fresh foods	1 kGy max.
Foods	1 kGy max.
Dry or dehydrated Enzyme preparations	10 kGy max.
Dry or dehydrated spices/seasonings	30 kGy max.
Fresh or frozen, uncooked poultry products	3 kGy max.
Frozen packaged meats (solely NASA)	44 kGy min.
Refrigerated, uncooked meat products	4.5 kGy max.
Frozen uncooked meat products	7 kGy max.
Fresh shell eggs	3.0 kGy max.
Seeds for sprouting	8.0 kGy max.
Fresh or frozen molluscan shellfish ¹	5.5 kGy max.

Our human experience of ionizing radiation cannot compare with the much higher dose rate of ionizing radiation used in commercial sterilization.

Evidence for and against isotope fractionation by ionizing radiation

Dahl et al. ([1988b](#)) suggested that $^{13}\text{C}/^{12}\text{C}$ ratios of kerogens (i.e., the insoluble fraction of sedimentary organic matter) in middle Cambrian – Lower Ordovician uranium-rich Alum Shale were proportional to the natural log of the ~100 ppm uranium concentration after intense α radiation and fractionation by a radiation dose of $10^8 - 10^9$ Gy over ~500 million years.

However, Lewan and Burchardt ([1989](#), and refs. therein) noted that significant biochemical/isotopic heterogeneity of total organic carbon in sedimentary sequences over time casts doubt on the hypothesis that $^{13}\text{C}/^{12}\text{C}$ ratios of bulk organic carbon can exhibit the effects of radiolysis. In fact, the $^{13}\text{C}/^{12}\text{C}$ ratio of kerogen is quite insensitive to isotopic post-depositional shifts, and completely blind to isotopic fractionations *between* individual molecules. It would require a significant radiolytic loss of an isotopically distinct component (like CH_4) to render the remaining solid organic matter isotopically fractionated relative to the starting material prior to radiolysis. However, Yang et al. ([2018](#), [2020](#)) described significant dealkylation, aromatization, and cross-linking in Alum Shale organic matter owing to radiolysis, which may have been accompanied by some isotope fractionation.

The radiolytic loss of a methyl group can yield methane if a hydrogen atom is either abstracted from organic matter or becomes available in the form of a radiolytically generated hydrogen atom from water ([Garrett et al., 2005](#)). The possible radiolytic origin of some hydrocarbons in natural gas plays has been proposed in recent publications (e.g., [Silva et al., 2019](#); [Naumenko-Dèzes et al., 2022](#); [Wang et al., 2022](#); [Boreham et al., 2022](#)).

As part of a ring-test for the development of ten food matrix stable isotope RMs USGS82 to USGS91 ([Schimmelmann et al., 2020](#)), a participating lab in Australia received a package with RM aliquots in glass vials after **mandatory sterilization by Australian Biosecurity and Irradiation with a “low dose” of 30 kGy γ irradiation** from a ^{60}Co source ([ANSTO, 2021](#)). The irradiation had turned the formerly clear glass of the vials to brown. Leftover subsamples of irradiated RMs were returned to the USA to evaluate their isotopic integrity. Direct **bulk carbon and oxygen isotopic comparisons** at the USGS laboratory in Reston, Virginia between original and γ irradiated RMs **showed no indication that γ irradiation had introduced isotopic fractionation larger than the analytical precision of analyses**. Exceptions were the oxygen isotopic compositions of USGS82 (honey from Vietnam), USGS89 (porcine collagen) and USGS91 (rice flour) that contained relatively large amounts of water and expressed enrichment in ^{18}O by up to 0.8 ‰ after subsamples had returned from Australia to the USA. Instead of γ irradiation, this oxygen-isotopic fractionation was attributed to the evaporative and desorptive loss of water from food matrix samples during repeated subsampling and return transport of small aliquots in spacious glass vials ([Schimmelmann et al., 2020](#)).

Gorman et al. ([2021](#)) used elemental analyzer (EA) based measurements of the **bulk carbon and nitrogen isotope compositions of 50 kGy γ irradiated marine faunal tissue, leaves, and soils** to show that bulk isotope results from irradiated and respective control samples were **statistically indistinguishable**. This result is expected for a closed-system pool of solid organic matter in the absence of a significant loss of volatile compounds containing carbon and nitrogen. The isotopic results allow no statement about possible chemical structural and inter- or intramolecular isotopic changes.

We consider the previous results inconclusive for the following reasons.

- (i) The measurement of **bulk isotope ratios via EA is insensitive** to isotopic and biochemical changes at the organic compound-specific level.
- (ii) No hydrogen isotope comparisons were performed with original and non-irradiated materials. In the presence of high activation energies, **hydrogen isotope ratios in organics are more sensitive than those of carbon and nitrogen** because **organic hydrogen can potentially exchange with hydrogen from water**.
- (iii) The possible effect of **α -irradiation exposure was not evaluated**.

Free radical reactions facilitate the exchange between organic hydrogen and hydrogen in water

Organic-rich Messel Shale was powdered, pre-extracted with an organic solvent, dried, mixed with heavy water ($^2\text{H}_2\text{O}$), and heated to 330 °C for 3 days in a sealed container. Ca. 8 weight % of organic matter were converted to hydrocarbons that excluded olefins (i.e., hydrocarbons with C=C double bonds)(Hoering, 1984).

A mechanism for the production of polydeuterated hydrocarbons is advanced, based on a model wherein the **molecular fossils that are chemically bonded into kerogen matrix are released as free radicals.** During the subsequent chain reactions, migration of olefinic bonds in intermediates and attack by **deuterium free radicals result in multiple deuteration with retention of the carbon skeleton** of the molecular fossil.

Presumably, one step that can lead to multiple deuterium uptake is the exchange of a hydrogen radical with deuterium oxide

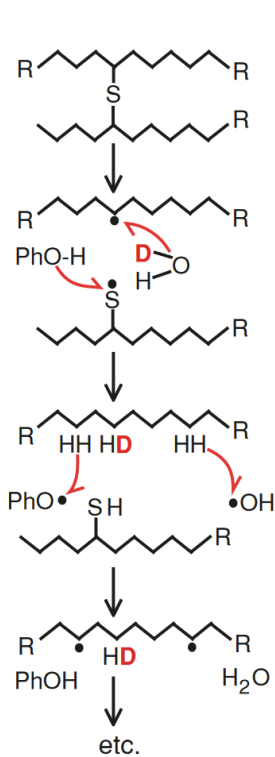


The resulting deuterium atom is then available for organic deuteration.

Hoering TC (1984) Thermal reactions of kerogen with added water, heavy water and pure organic substances. *Organic Geochemistry* 5: 267–78.

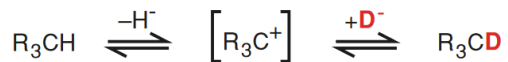
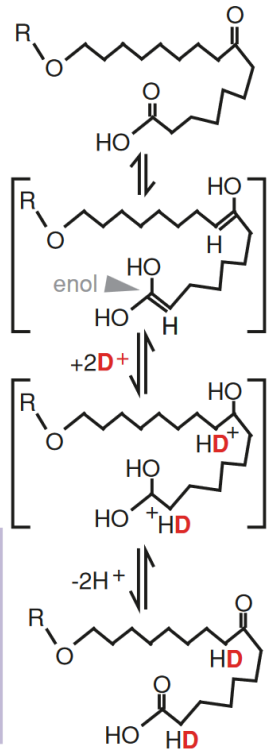
[https://doi.org/10.1016/0146-6380\(84\)90014-7](https://doi.org/10.1016/0146-6380(84)90014-7)

Mechanisms of hydrogen isotopic exchange (examples refer to hypothetical kerogen molecule shown to the left)

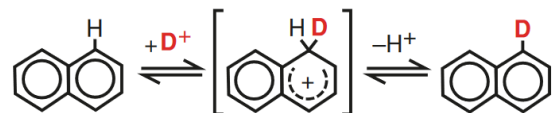


1. Cleavage of bonds initiates a chain of radical reactions with participation of water, sulfides, or organic molecules (phenol PhOH is used on the left), resulting in irreversible H-exchange.

2. Ionic exchange of organic H in α -position to carboxyl and carbonyl groups via enolization.

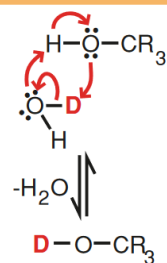


3. Ionic exchange of tertiary C-bound H.



4. Ionic exchange of aromatic hydrogen.

5. Ionic exchange of N, O, S-bound H.



Radicals and ionic reaction pathways facilitate the exchange of organic carbon-bound hydrogen and hydrogen in water.

Schimmelmann et al., 2006, *Annu. Rev. Earth Planet. Sci.* **34**: 501-533.

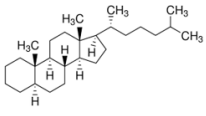
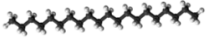
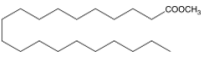
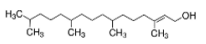
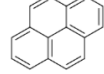
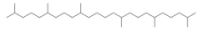

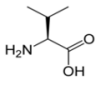
<https://doi.org/10.1146/annurev.earth.34.031405.125011>

Experimental approach:

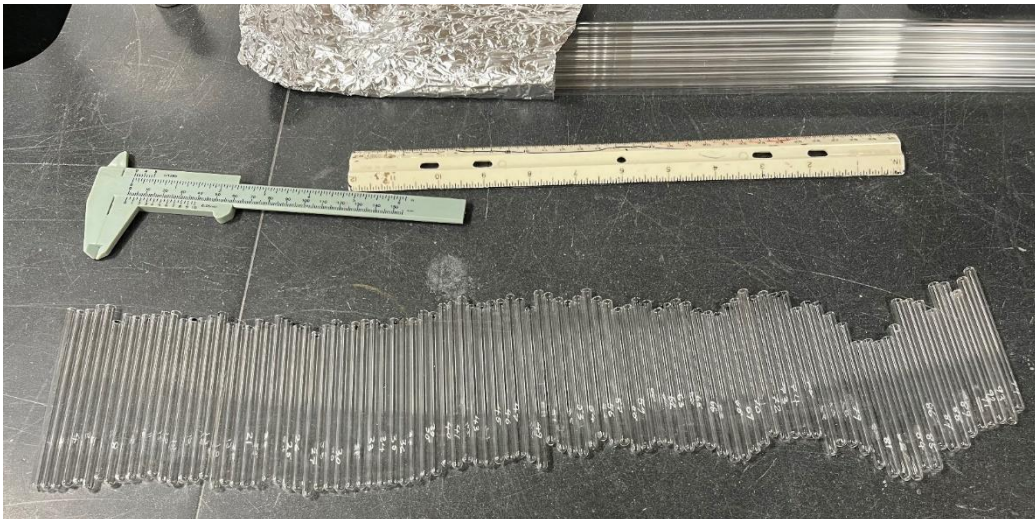
221 laboratory experiments (plus blanks) expose individual, chemically diverse and pure model organic compounds to **α , β , or γ irradiation over months to three years** in sealed glass tubes, followed by chemical-compositional and **compound-specific isotopic analyses**. By using added water with a high abundance of deuterium (^2H), we sensitively monitor any radiologically-induced H-transfer into the pool of stable, originally ^2H -depleted carbon-bound organic hydrogen.

The outcomes of this study will be an improved organic-molecular understanding of which functional groups and carbon skeletal configurations lend themselves to chemical and stable isotopic changes during exposure to different kinds of natural radiation, and the relevant doses at which such changes become measurable.

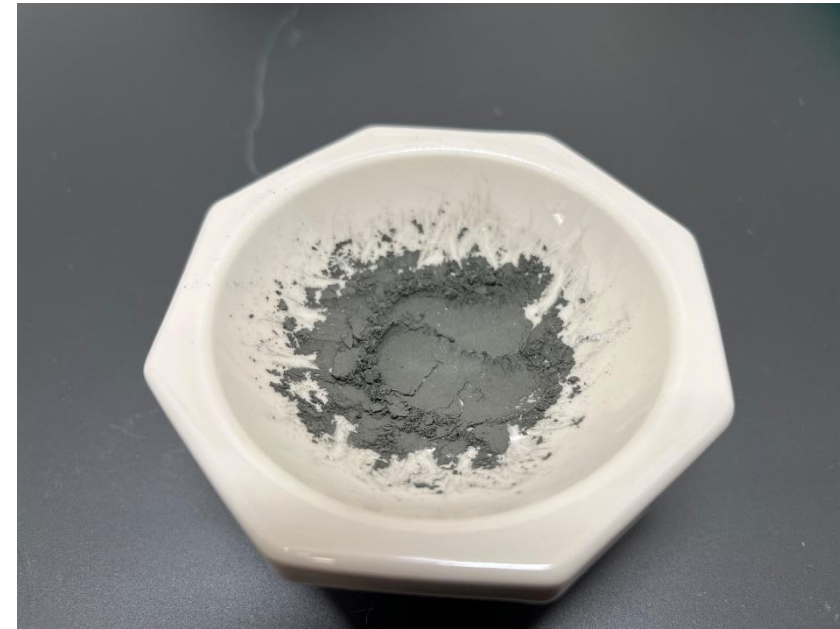
Table 1. Preliminary selection of available pure organic compounds and the rationale for their use in radiation exposure experiments.

Chemical name	Chemical structure	Rationale for use	Available characterization	Source
5 α -Cholestane C ₂₇ H ₄₈		Representative triterpenoid biomarker, <u>naphthenic</u> component of petroleum	C and H isotope ratios to be determined	Sigma-Aldrich #C8003
<i>n</i> -Icosane C ₂₀ H ₄₂		<u>Similar to</u> leaf wax biomarkers, aliphatic (unbranched) component of petroleum	C and H isotope ratios determined	Sigma-Aldrich #44818
<i>n</i> -Icosanoic acid methyl ester C ₂₁ H ₄₂ O ₂		<u>Similar to</u> common fatty acids	C and H isotope ratios determined in ring-test	USGS70 (Schimmelmann et al., 2016)
Phytol C ₂₀ H ₄₀ O		Side chain of chlorophyll, important biomarker for isotopic records	C and H isotope ratios to be determined	Sigma-Aldrich #W502200
Pyrene C ₁₆ H ₁₀		Representative polyaromatic, aromatic component of petroleum	C and H isotope ratios to be determined	Sigma-Aldrich #48570
Squalane C ₃₀ H ₆₂		Common biomarker, aliphatic (branched) component of petroleum	C and H isotope ratios determined	Sigma-Aldrich #234311
Squalene C ₃₀ H ₅₀		Isoprenoid lipid with multiple <u>unsaturations</u>	C and H isotope ratios to be determined	Sigma-Aldrich #442785
L-Valine C ₅ H ₁₁ NO ₂		Common amino acid, to study amine & carboxyl chemistry	C, H, and N isotope ratios determined in ring-test	USGS73 (Schimmelmann et al., 2016)

Sample preparation for α irradiation with uranium oxide



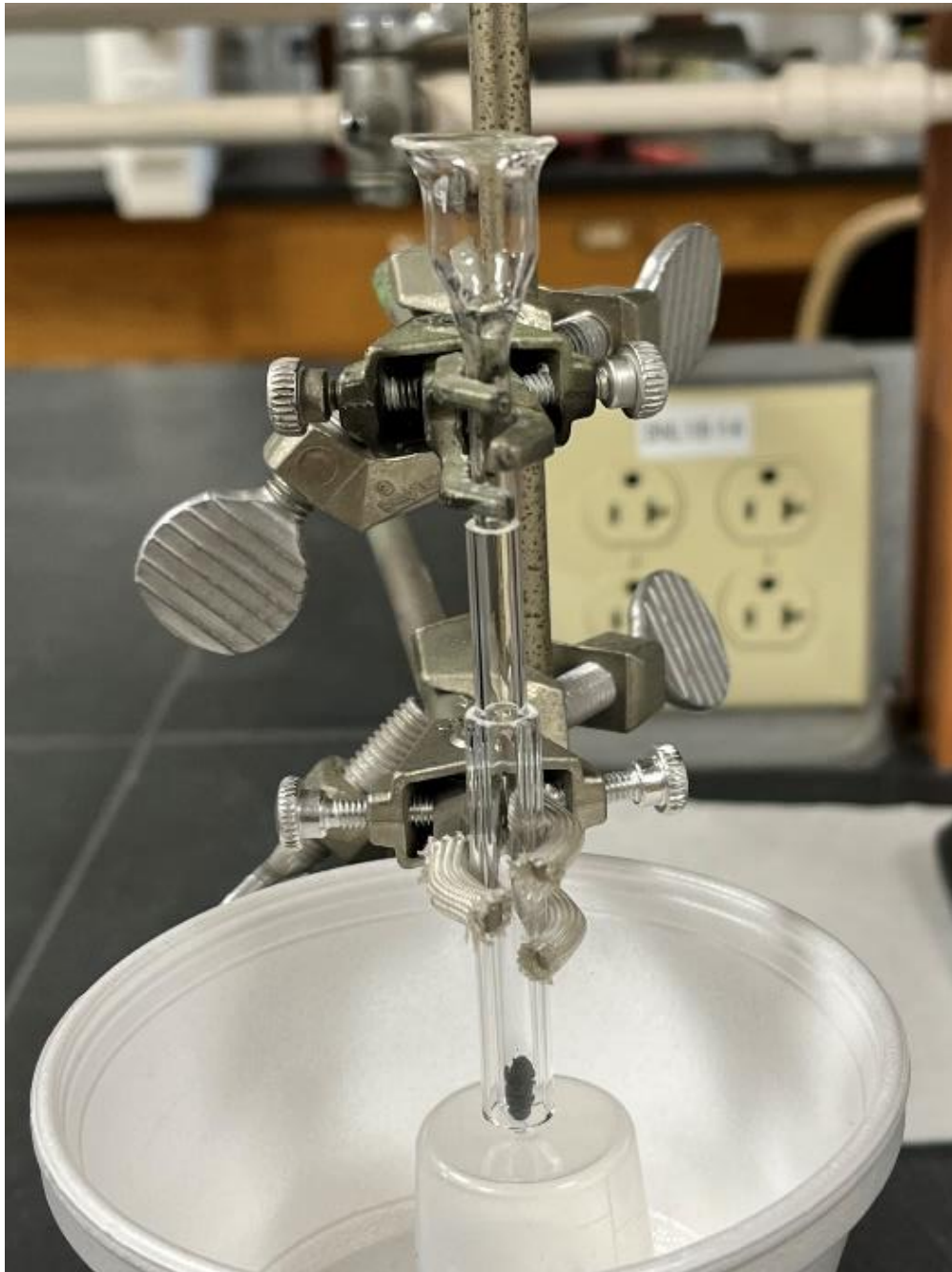
Annealed and engraved sample tubes



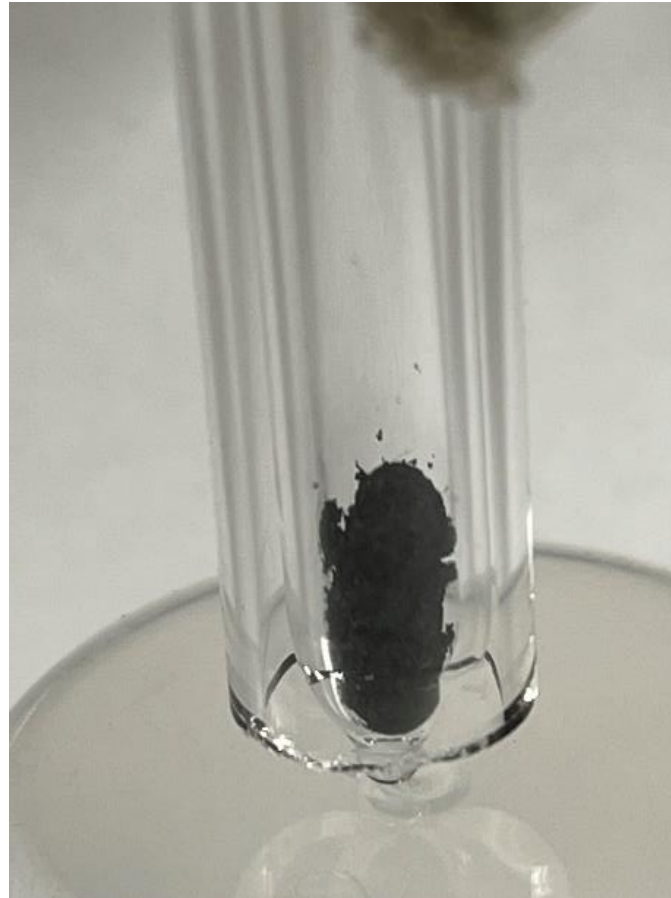
Black uranium oxide mixed with organic substrate



Filling apparatus behind plexiglass shield



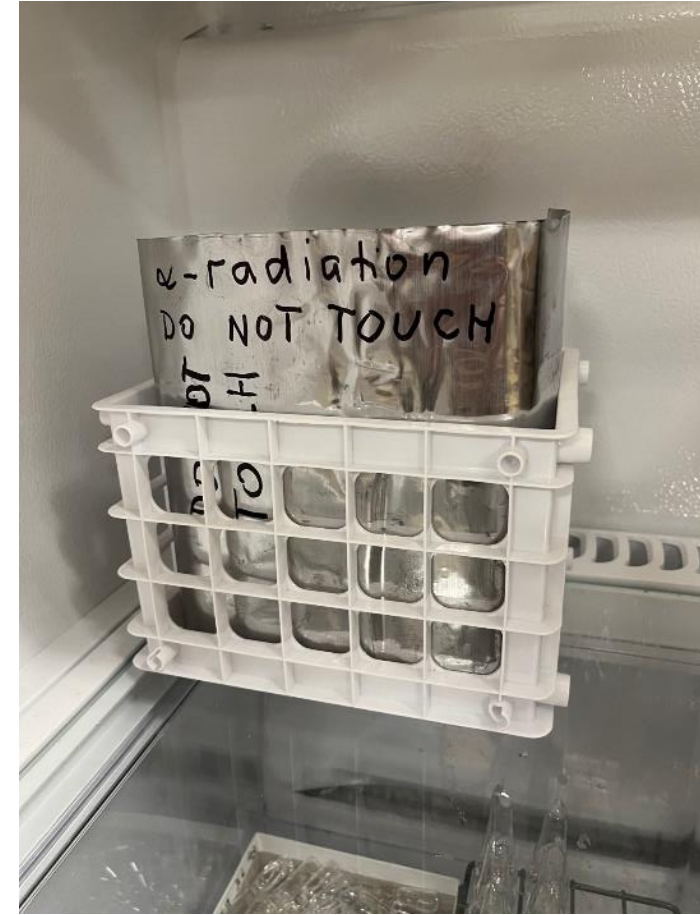
Organic samples for irradiation without uranium oxide are mixed with acid-washed and pre-annealed, highly porous diatomaceous earth (SiO_2) →



Water is added optionally to glass tubes after the filling with solids.

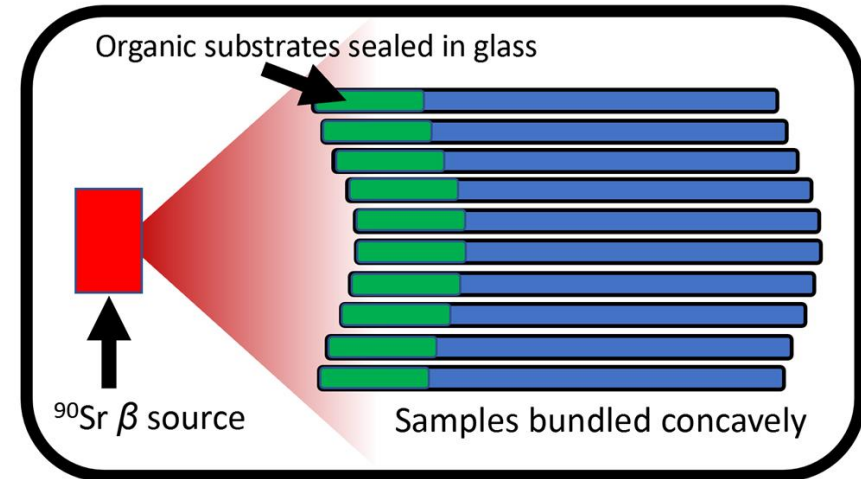
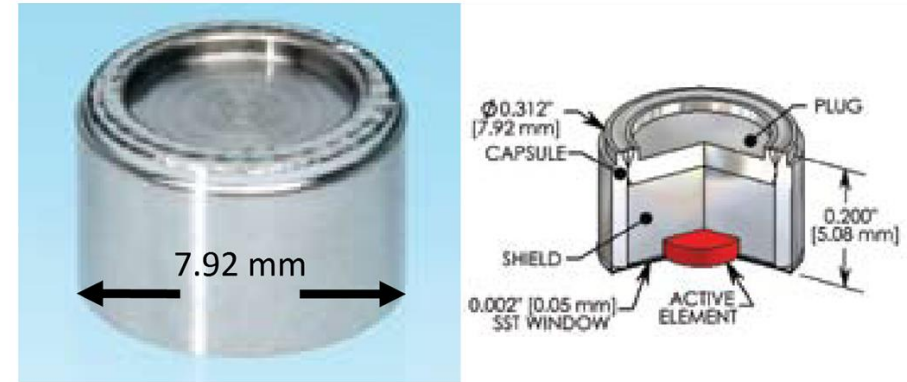
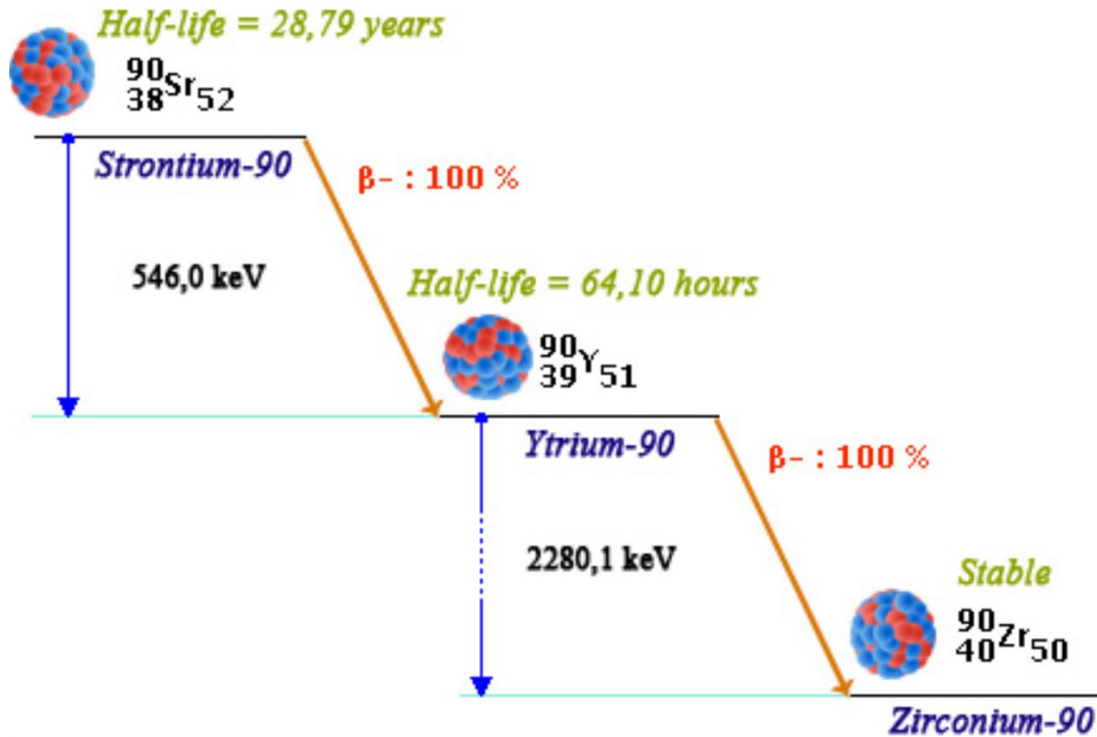


Flame-sealing of filled glass tube after freezing of lower end in liquid nitrogen and evacuation in connection with a vacuum line.



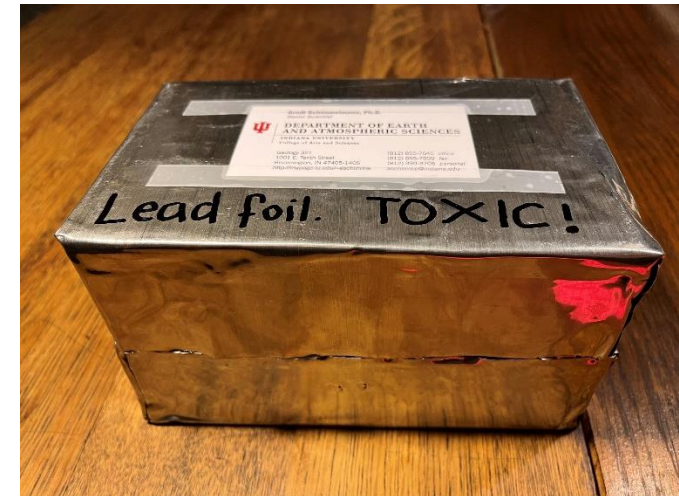
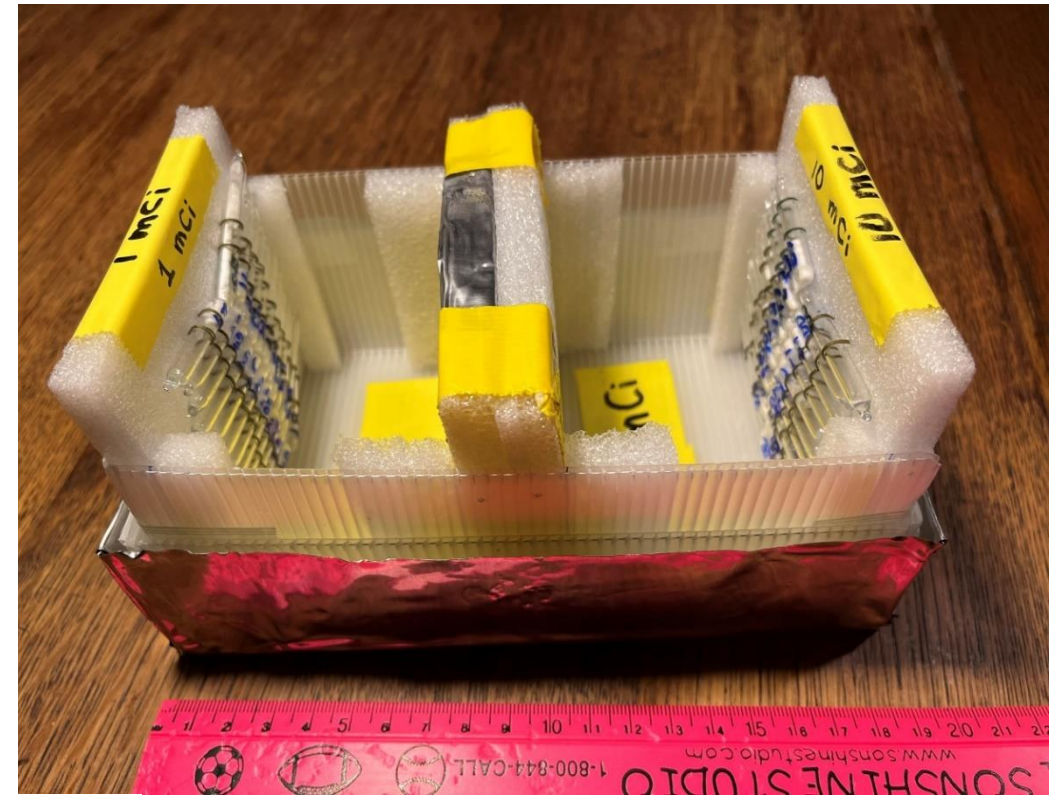
α irradiation experiments in 6-mm o.d. Pyrex[®] tubes placed in a refrigerator behind lead foil

β irradiation with ^{90}Sr



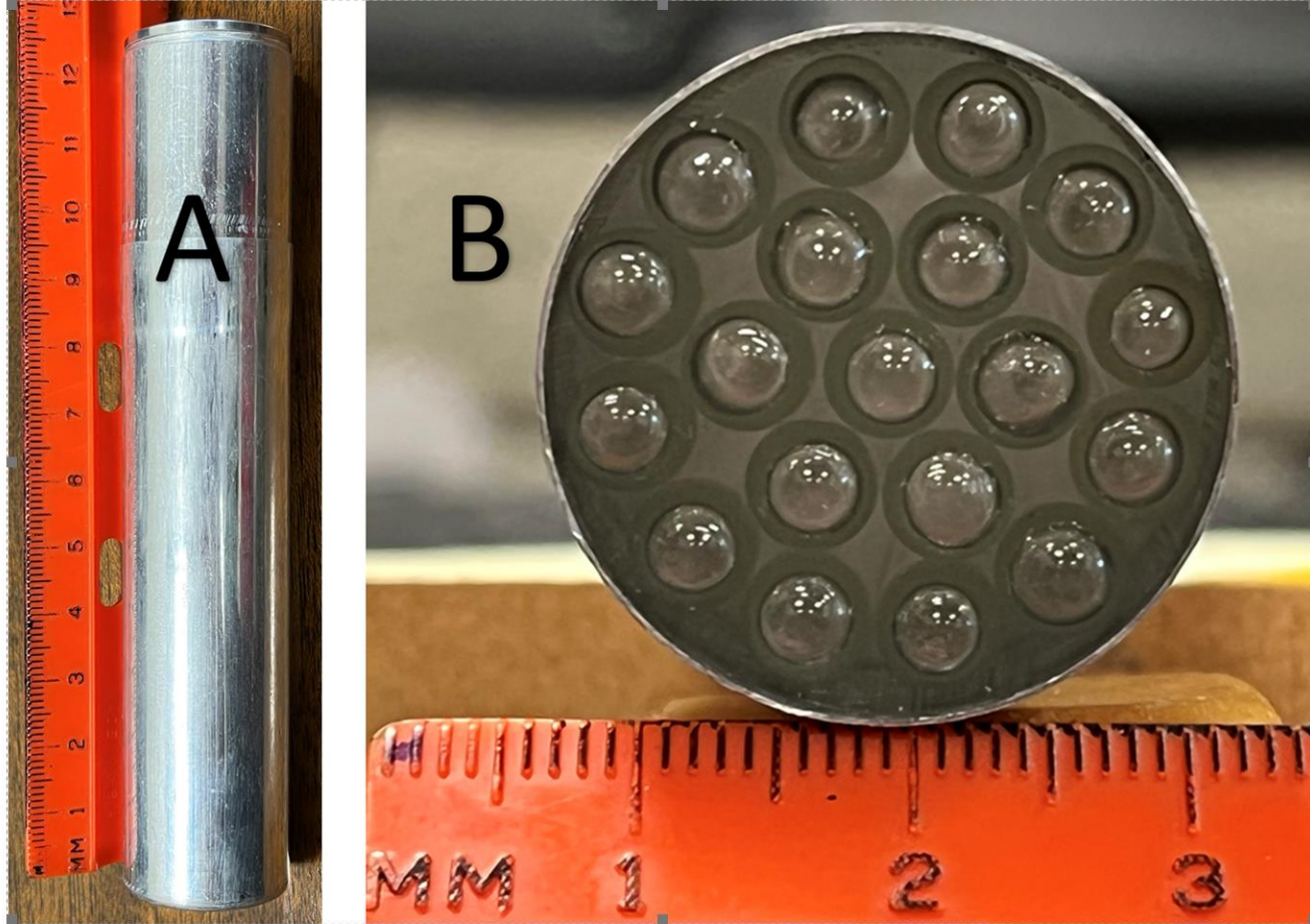
(Left) β cascade decay of ^{90}Sr to ^{90}Zr is being used for β irradiation in sealed glass tubes (from *Brookhaven National Laboratory*, Nudat 2). (Right) Sealed disks containing up to 370 MBq [10 mCi] of ^{90}Sr are used for β irradiation of organics in sealed glass tubes (from Eckert & Ziegler, 2021). (1 becquerel (Bq) = 1 event of radiation emission or disintegration per second).

β irradiation with ^{90}Sr



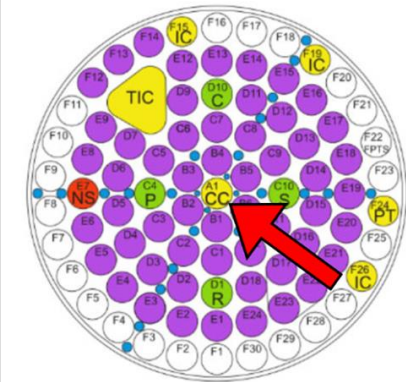
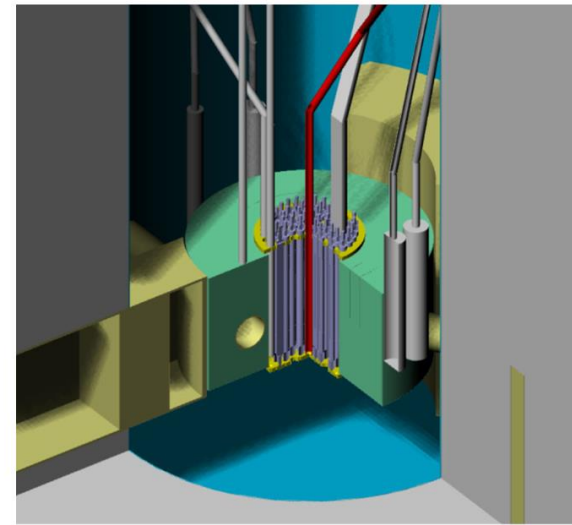
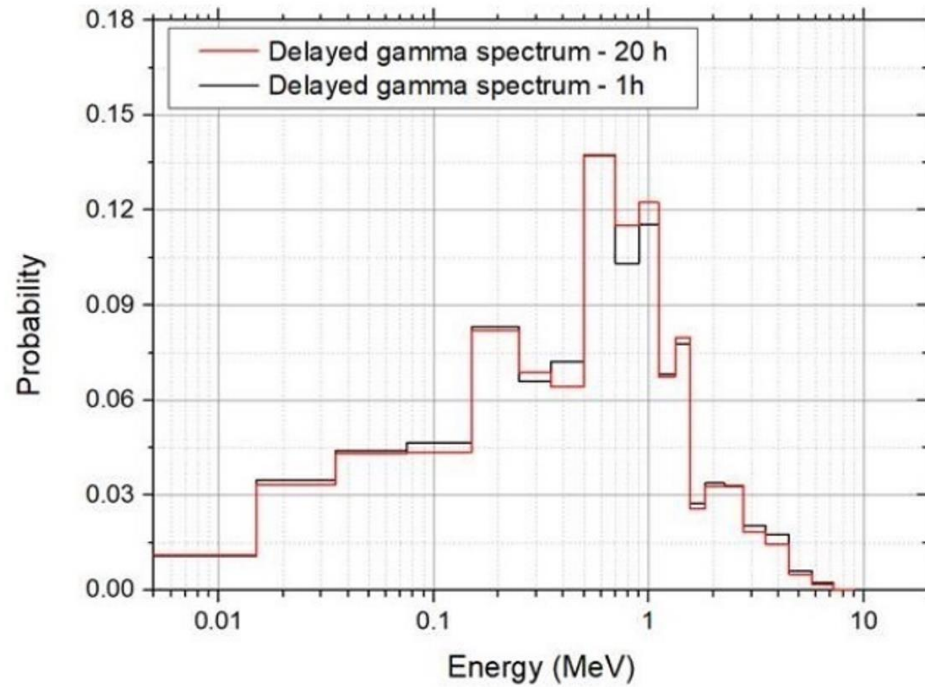
β irradiation setup where steel disks containing up to 370 MBq [10 mCi] of ^{90}Sr are positioned in the center; (4) sealed Pyrex[®] glass tubes are sewn onto foam in distal positions. Lead foil separates the two sets of experiments using 37 and 370 MBq ^{90}Sr sources. The entire setup is wrapped in lead foil and housed in a refrigerator.

γ irradiation with delayed γ rays



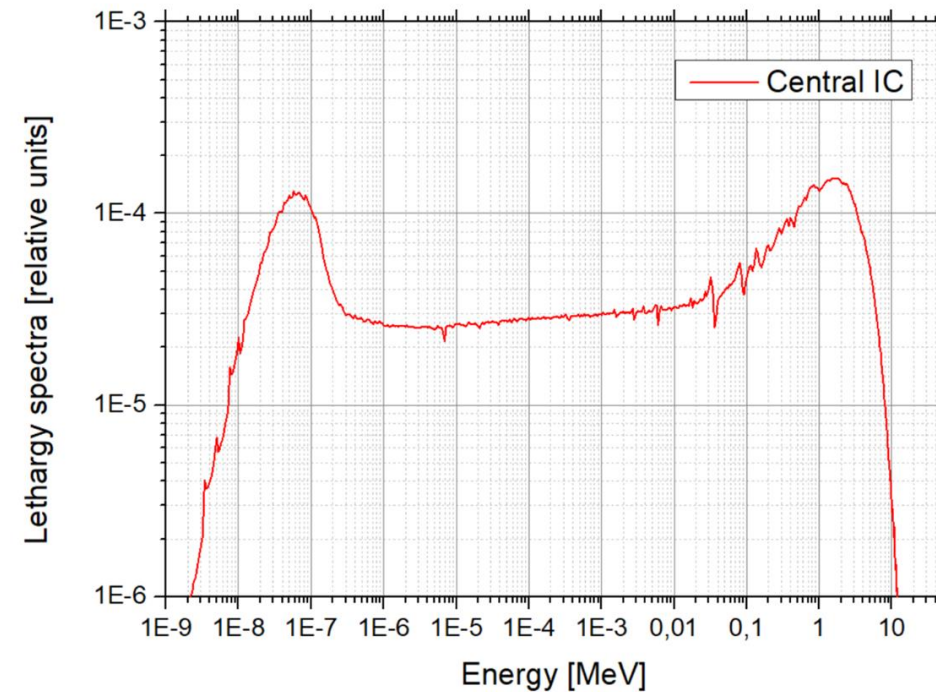
γ irradiation experiments in 5-mm o.d. Pyrex[®] tubes before and after irradiation.

(A) Aluminum tubes are being used at the Jožef Stefan Institute to hold samples for γ irradiation. **(B)** With the tube's inner diameter of ~ 2.4 cm, we can fit two bundles of 5-mm o.d. sealed glass tubes with a length of ~ 4.2 cm, each bundle containing up to 19 samples. Two aluminum tubes can be stacked in the reactor's central channel for simultaneous γ irradiation of up to 76 samples.



- Fuel element
- Irradiation channel
- Control rod
- Neutron source
- Measurement position

(Above) Delayed γ ray energy spectrum in the TRIGA nuclear reactor. (Top right) Position of central channel for γ irradiation. (Bottom right) γ energy distribution across central channel. Received γ doses are measured individually for each exposure session and will be added cumulatively over time (from [Institut "Jožef Stefan", 2021](https://ric.ijs.si/)).

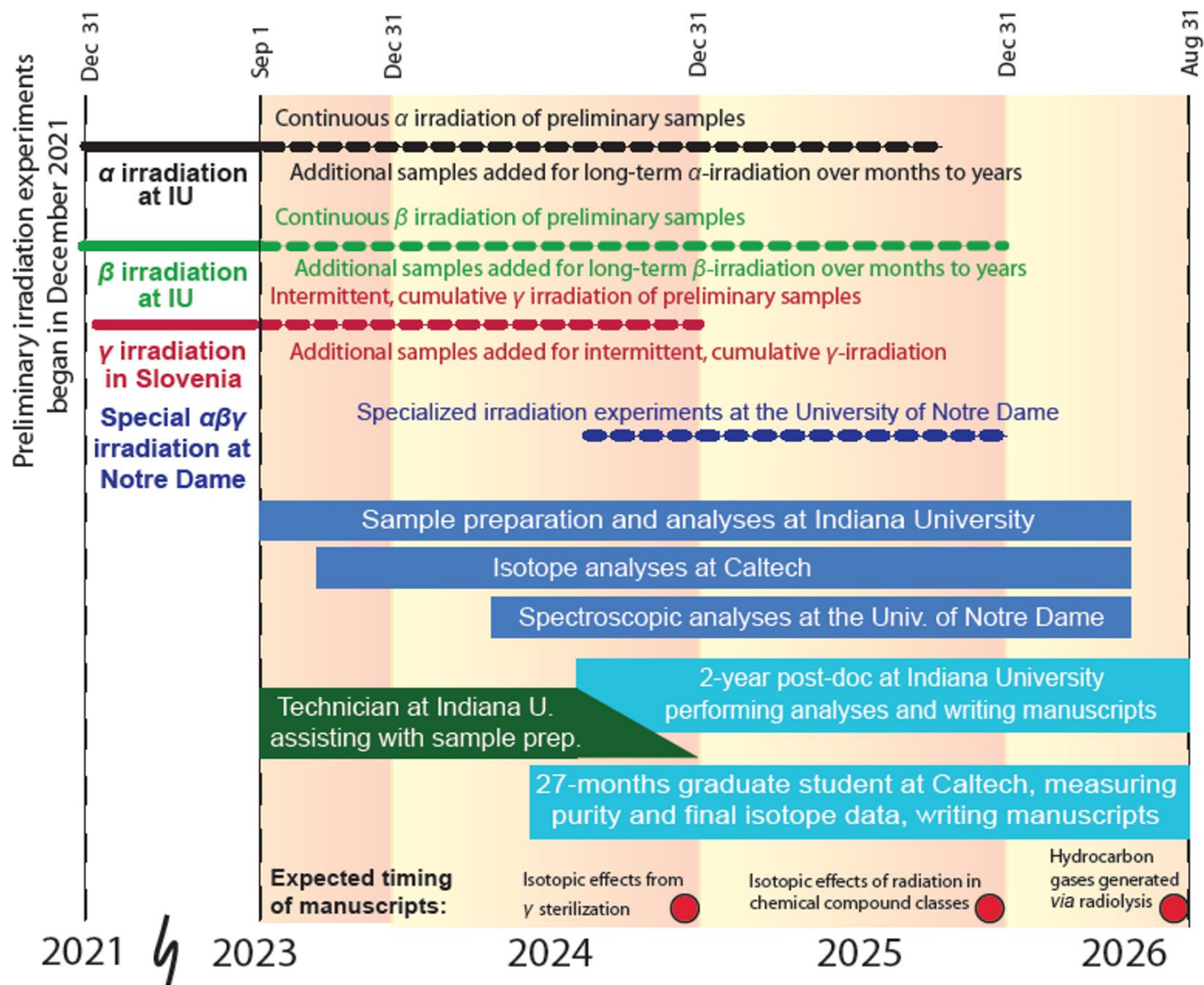


Current State of Affairs as of April 2023

A collaborative research proposal has been submitted to the *U.S. Department of Energy, BASIC Energy Sciences*. If funded, a 3-year project will begin with measurements in September 2023.

Many separate α and β irradiation experiments have been running continuously and in parallel for more than 15 months at Indiana University in refrigerators since December 2021.

γ irradiation of numerous samples has been performed in the TRIGA reactor at the Jožef Stefan Institute in Slovenia, both at routine 25 kGy sterilization conditions, and at more than 1 MGy as an extreme test to explore hydrocarbon gas generation and associated isotope fractionation.



THANK YOU FOR YOUR ATTENTION



Feel free to email questions to me: arndtschimme@gmail.com

More than 221 experiments are running in parallel, including experiments with and without waters of different isotopic composition, with and without an additional free-radical initiator diethyl disulfide, and blanks.

Time or Dose				² H ₂ O (wt. % in water)			Control Experiments			
Dose	<i>α</i> (mos)	<i>β</i> (days)	<i>γ</i> (kGy)	100	1	0.01 ¹	no water	no sub ²	with DEDS ³	Blank
Low ⁴ :	6	30	25	X ⁵	X		X	X		X
Med:	12	60	100	X	X	X	X	X	X	X
High:	24	90	500	X	X	X				X

¹ Natural abundance of ²H in water, i.e. deionized meteoric water.

² sub = solid substrate, uranium oxide (*α* irradiation) or diatomaceous earth (*β*, *γ* irradiation).

³ DEDS = diethyl disulfide, a free-radical initiator.

⁴ Low-dose experiments conducted prior to start of project to provide preliminary estimates of time, amount, etc.

⁵ Each 'X' indicates a condition to be tested, and – with the exception of blanks – represents 24 discrete sample tubes (8 compounds x 3 radiation sources).