EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Non-clinical data for regulatory decision-making on the efficacy of medical countermeasures

Non-clinical development of countermeasures for radio-nuclear threats

Dr Yannick SAINTIGNY, PhD

The dose: a question of physic… and biology

Radioactivity (Becquerel, Bq)

Physical Activity of a Radioactive Source

Quantifies the rate of nuclear decay.
Reflects how many atoms disintegrate per second.
Unit: 1 Bq = 1 disintegration/second.

Absorbed Dose (Gray, Gy)

Energy Deposited per Unit Mass

Measures the energy imparted by ionizing radiation.
Represents the physical quantity of radiation energy absorbed.
Unit: 1 Gy = 1 joule/kilogram.

Effective Dose (Sievert, Sv)

Biologically Weighted Dose for Risk Assessment

Adjusts absorbed dose to reflect biological impact.
Incorporates radiation type and tissue sensitivity.
Unit: Sv = Gy × radiation weighting × tissue weighting.

Dr Yannick Saintigny, PhD
CEA – France
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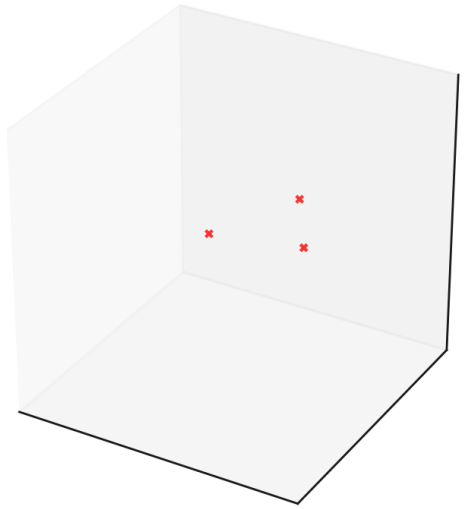
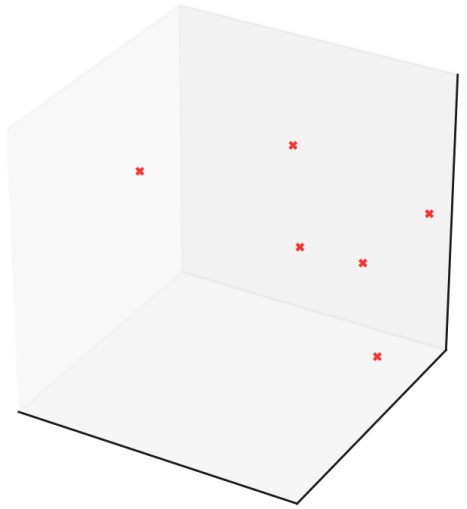
Ionization tracks in 1 μm³ of water

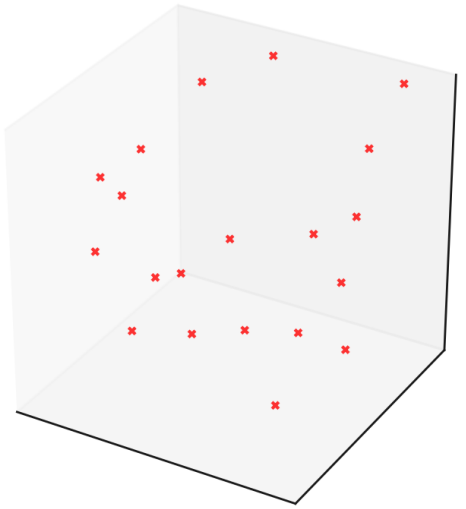
Gamma Rays (X-rays)
LET ≈ 0.3 keV/μm



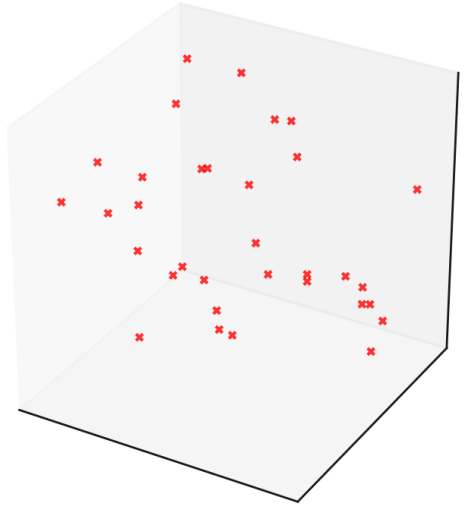
Beta Rays
LET ≈ 0.5 keV/μm



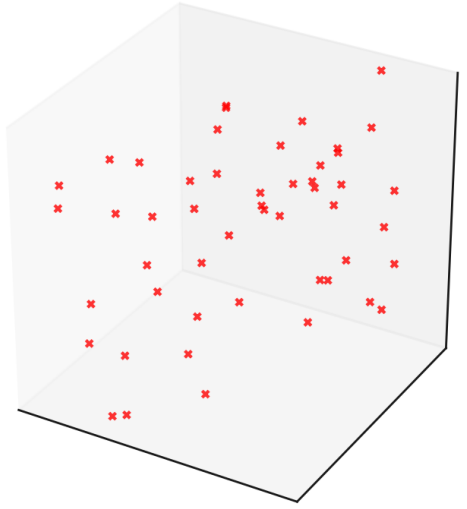
Protons
LET ≈ 2.0 keV/μm



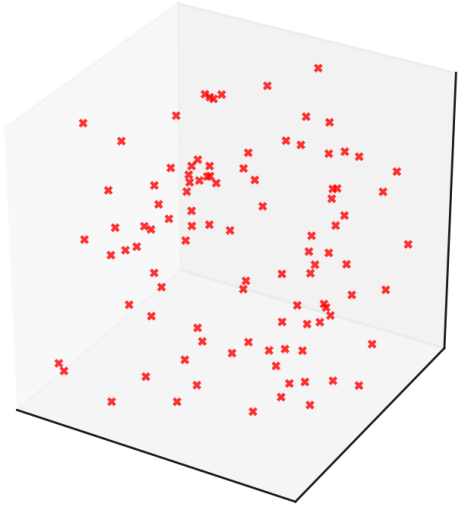
Neutrons (via recoil protons)
LET ≈ 10.0 keV/μm



Alpha Particles
LET ≈ 20.0 keV/μm



56Fe Ions
LET ≈ 100.0 keV/μm



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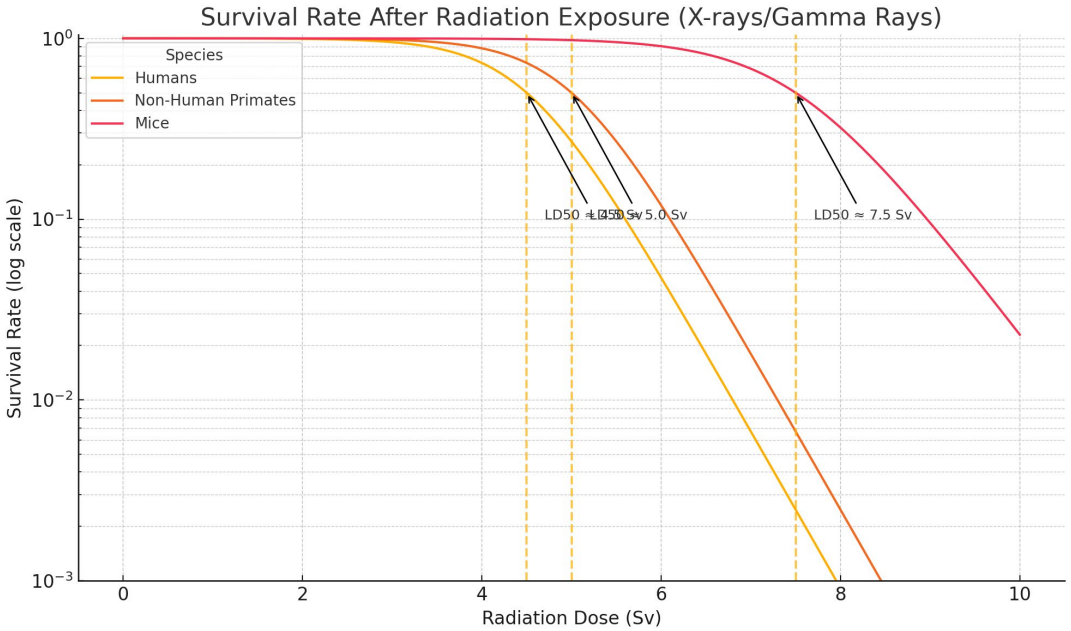
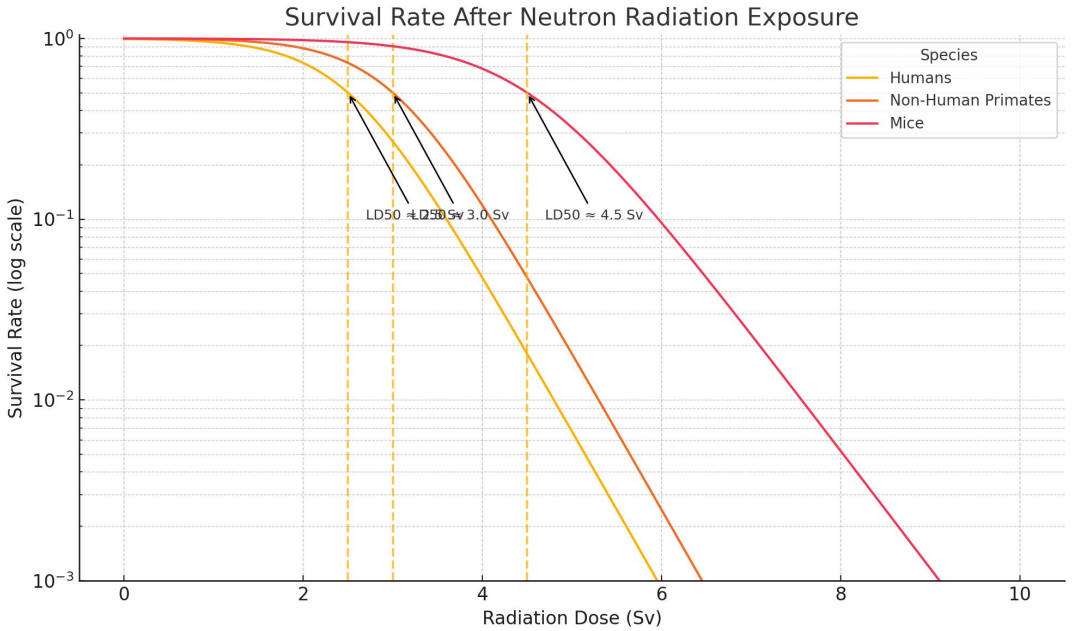
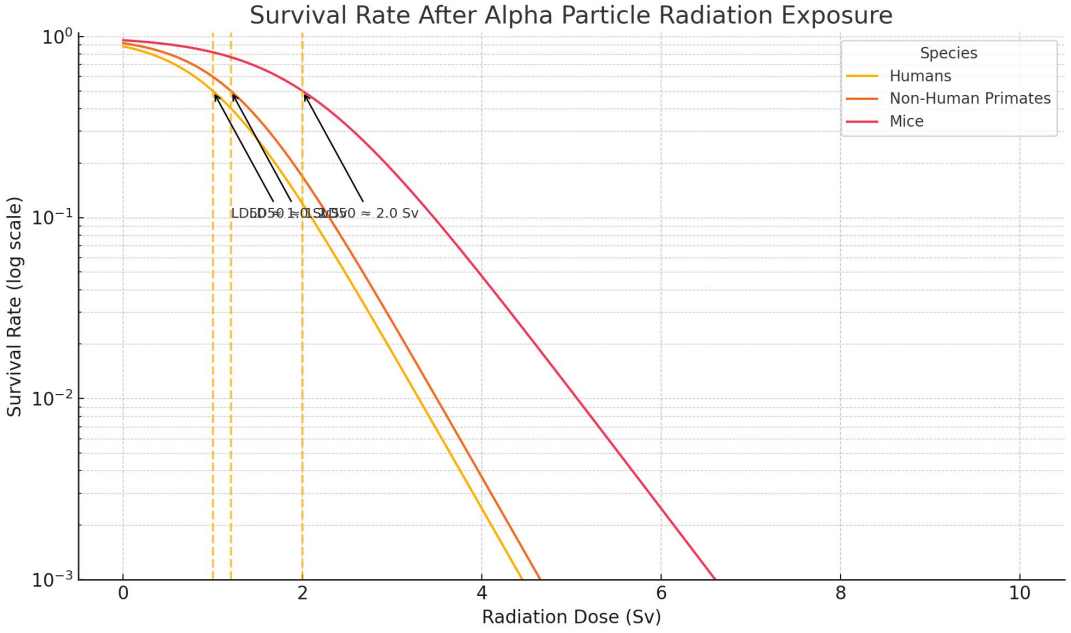
Impact on survival on humans or animals

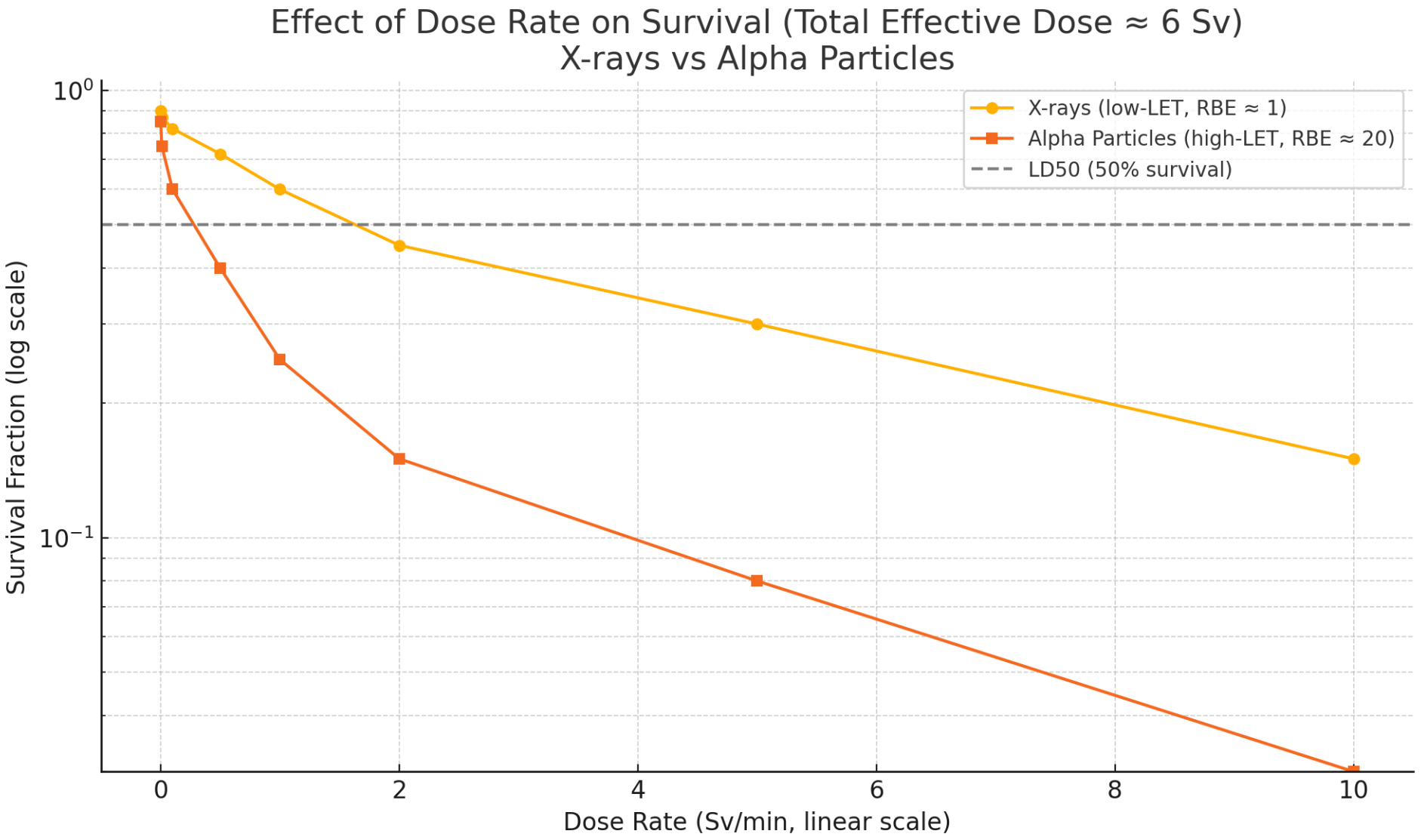






LD50 (Sieverts)	Human	NHP	Mice
X-rays	4,5	5,0	7,5
Neutron	2,5	3,0	4,5
Alpha	1,0	1,2	2,0

Dose rate impact on survival



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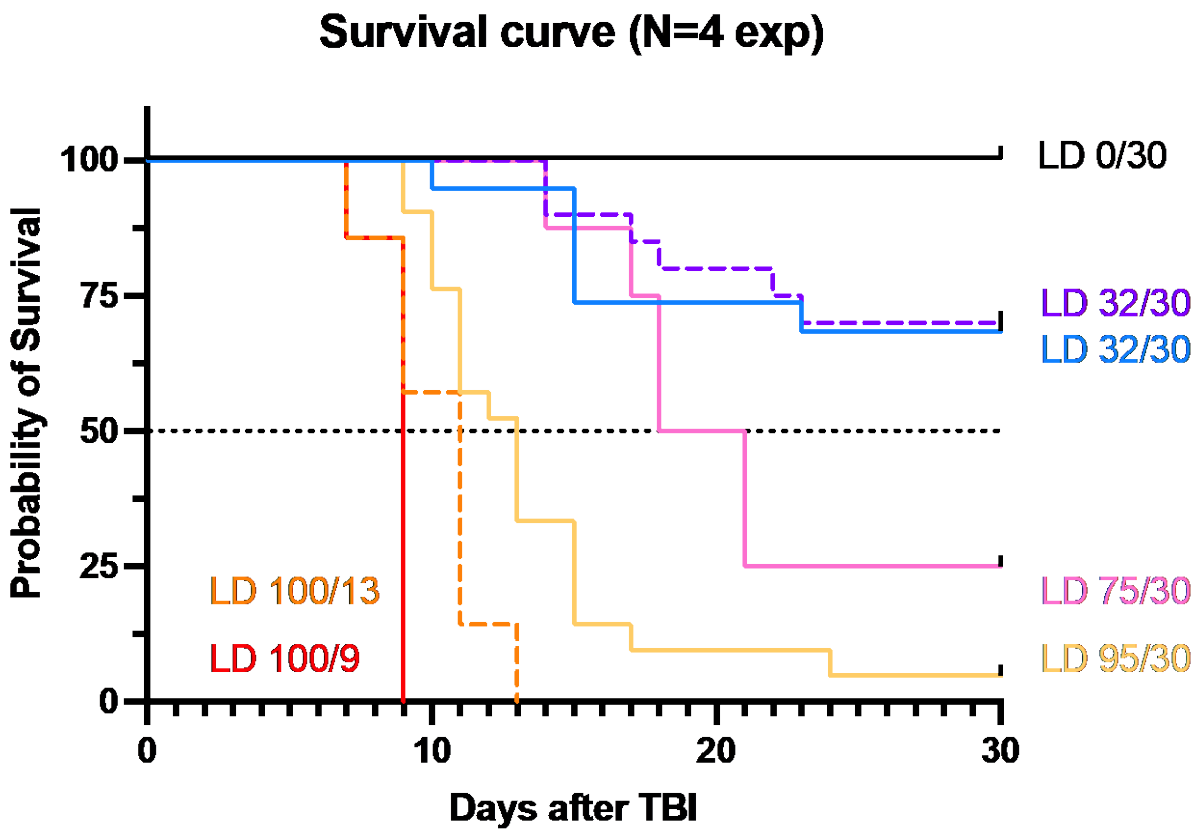
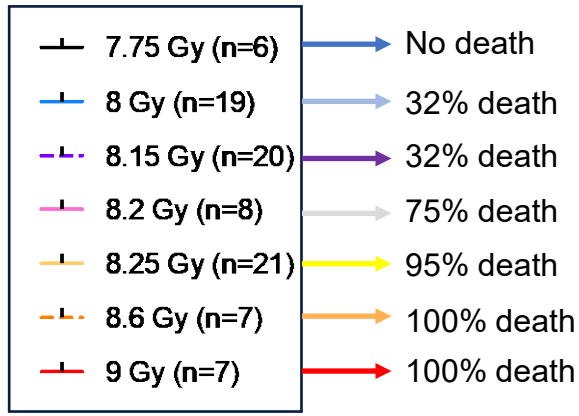
Mouse models of H-ARS

(Hematopoietic-Acute Radio-induced Syndrome)

Total Body Irradiation (TBI) - γ-rays (^{137}Cesium) - 0.95 Sv/min - GSR-D1® irradiator





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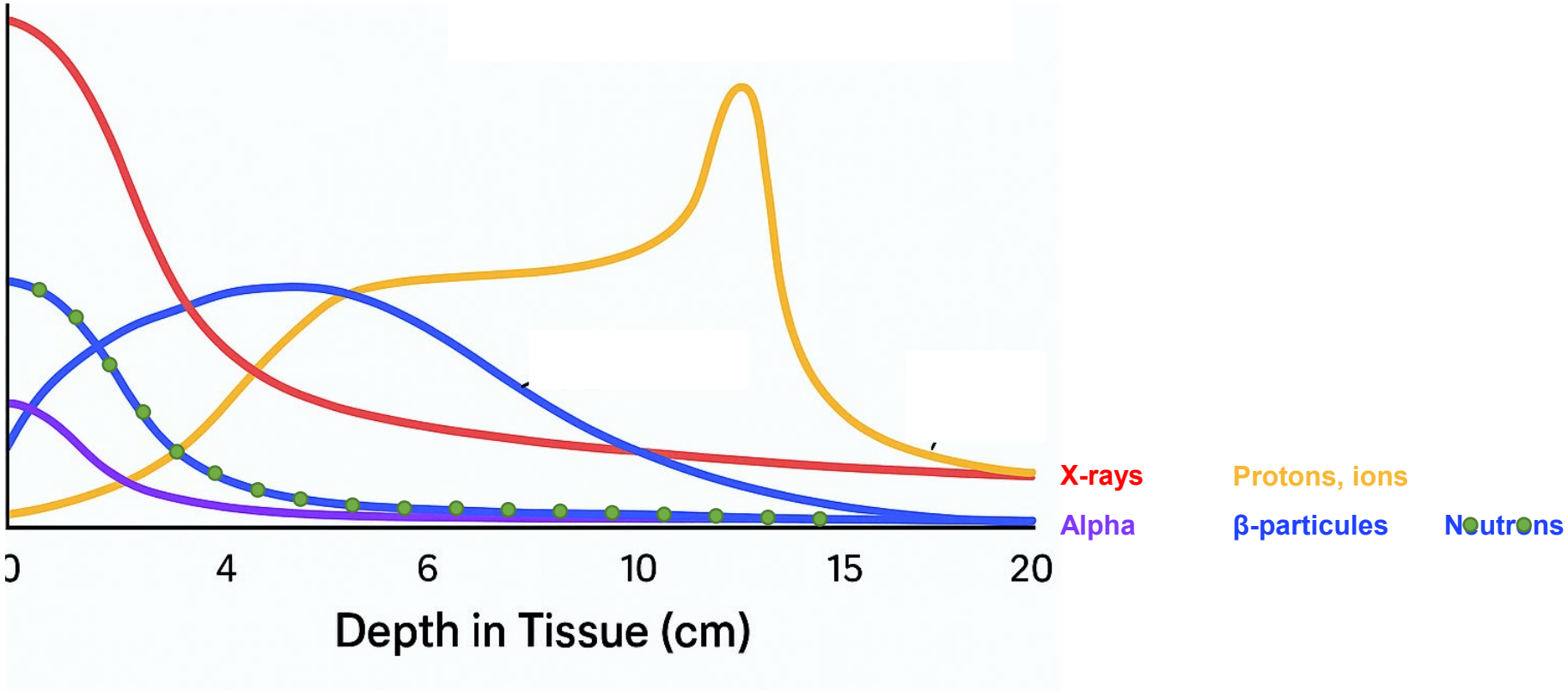
Dose distribution vs depth



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Key dosimetry parameters

A. Radiation quality

B. Energy (acceleration) of the particle

B. Dose

C. Dose rate

The validation of radiological dosimetry and dosimetric interpolation between multiple irradiation devices is therefore a crucial point.

Dr Yannick Saintigny, PhD
CEA – France
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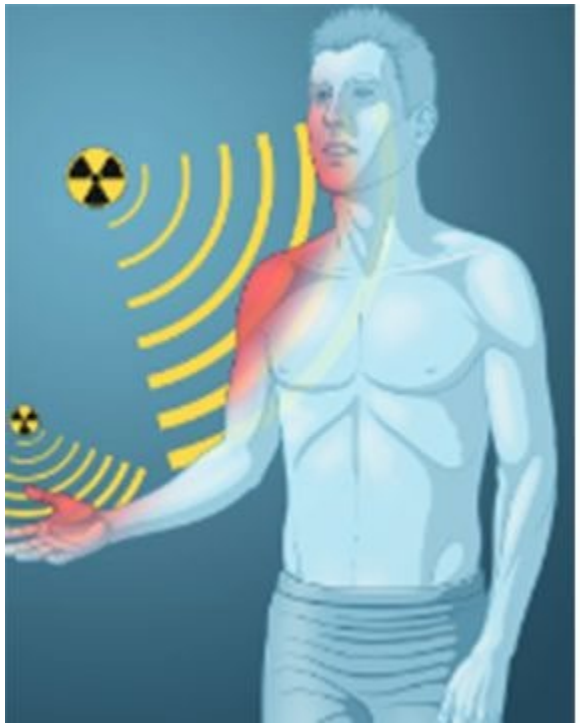
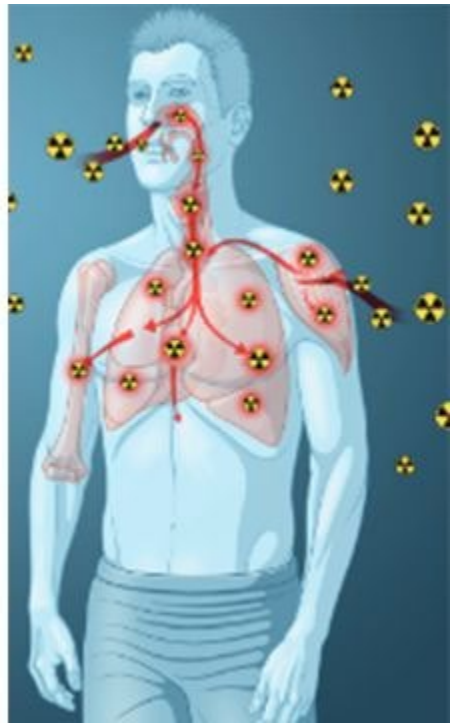
Irradiation vs Contamination:

Irradiation



Contamination



External

Internal

Radiation toxicity

Radiation and Chemical toxicity

Dr Yannick Saintigny, PhD
CEA – France
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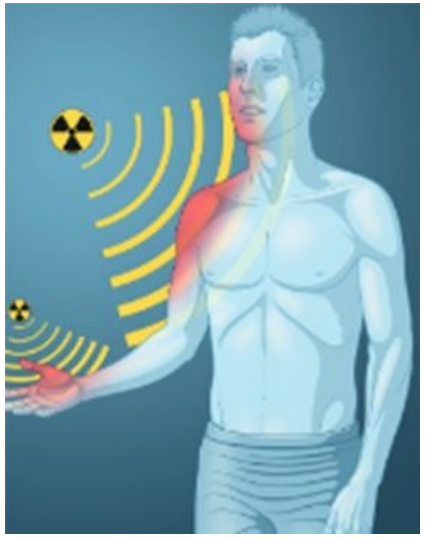
Irradiation in a CBRN threat context

Nuclear or improvised weapons:

- Detonation of a nuclear weapon.
- Detonation of an improvised nuclear device.
- Destruction of a facility in which radioactive material is used or stored in sealed or unsealed form.
- Irradiation of first responders with protections against directcontamination

Radiations of health concern:

- ɣ-rays (high energy electromagnetic radiation)
- Neutrons (direct primary high-speed neutrons and secondary netrons from activation of surrounding materials)
- β-particles (high-energy electrons emitted)



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CEA – France
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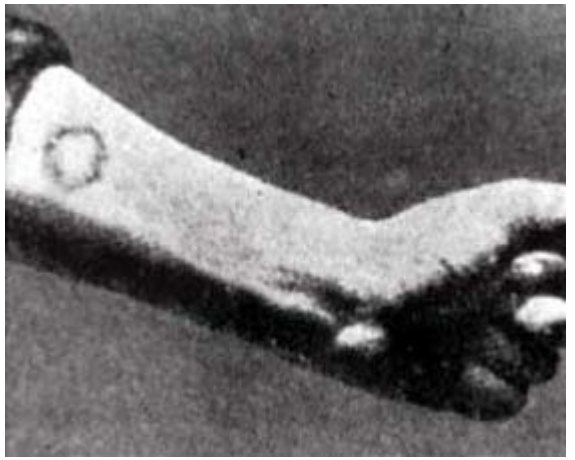
Acute Radiation Syndrome (ARS)

➢ Conditions

- **High enough dose**
- **Penetrating radiation**
- **Whole body irradiation**
- **Acute exposure**

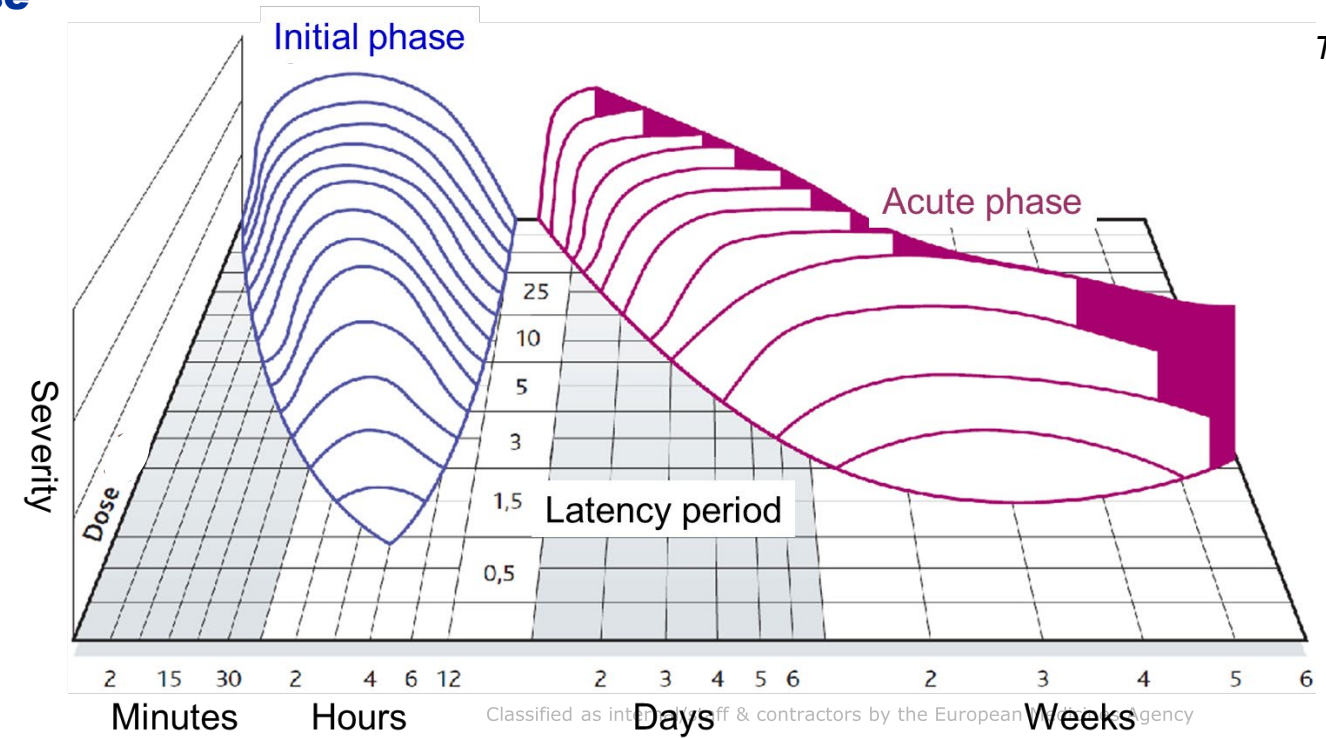
➢ Stages

- **Prodromal Reaction**
- **Latent Period**
- **Acute Phase**

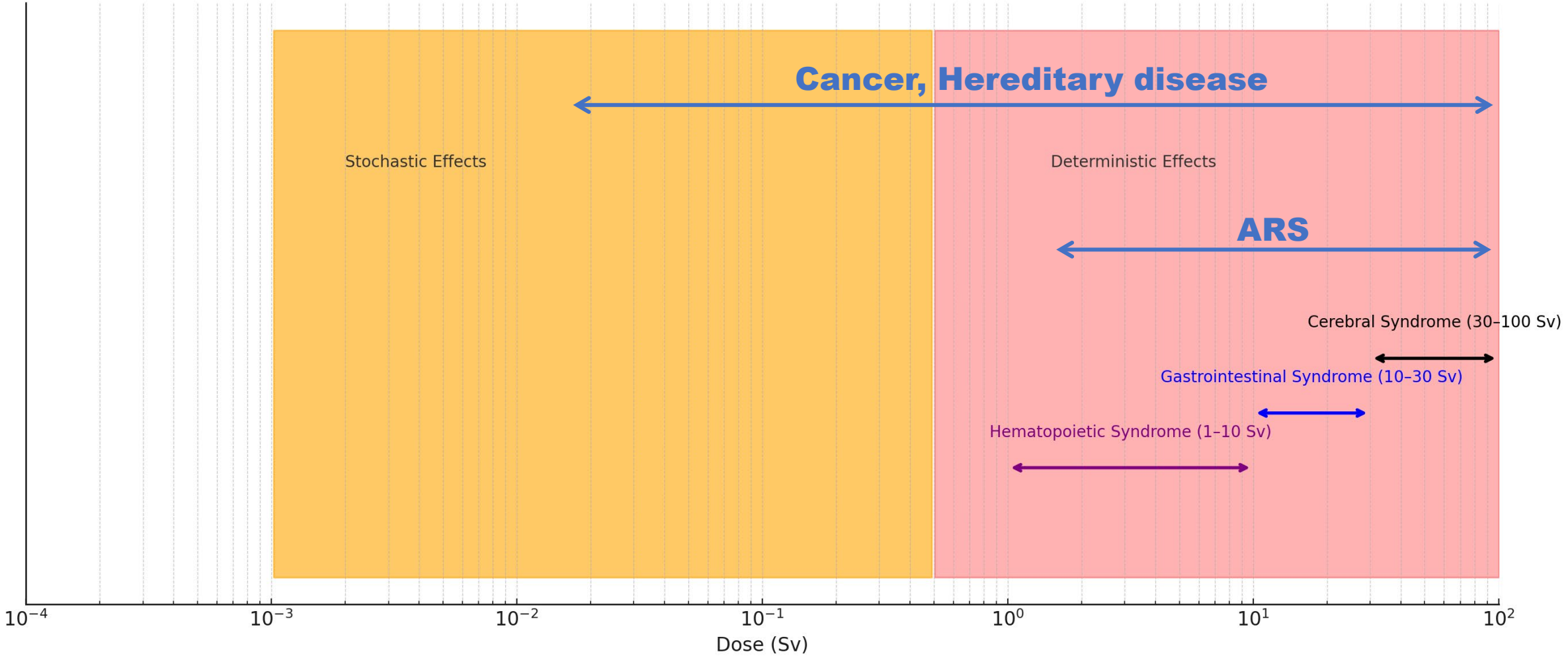


Skin burn by radium salts (10h exposition)

Test performed by Pierre Curie on his own arm in 1901.



Stochastic vs Deterministic effects



Onset and severity are affected by dose rate and LET

Dr Yannick Saintigny, PhD
CEA – France
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Preclinical Species Overview

- **Mouse**:
  - Relevance for mechanistic
  - Partial Body Irradiation (PBI) models can be performed
  - Only small-animal model compatible with human therapies (ATMPs, biologics)
  - Allow to develop sequential treatment strategies
  - Recreates nuclear-type injuries seen in tactical or reactor incidents
  - Rapid, reproducible, cost-efficient for early decision-making under CBRN constraints

- **Rat**:
  - Relevance for combined injury
  - Allow to develop sequential treatment strategies
  - Relevant for contamination (inhalation) models as lungs are more representative of human lungs than those of mice (lobular organization)
  - Rapid, reproducible, cost-efficient for early decision-making under CBRN constraints

Dr Yannick Saintigny, PhD
CEA – France
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Preclinical Species Overview

- **Minipig**:
  - High relevance for combined injury (infection, burn or trauma)
  - High relevance for skin contamination
  - Closest physiological match to humans: blood volume, organ radiosensitivity, repair kinetics
  - Replicates dose-dependent nuclear injuries
  - Replicates human conditions of transfusion, antibiotics treatments and monitoring
  - Genomic biodosimetry studies demonstrated that minipigs do not reproduce the modulation of gene expression profiles observed in humans
  - Easily available

- **NHP**:
  - Translational gold standard for ARS
  - Genomic biodosimetry studies demonstrated that NHP reproduce the modulation of gene expression profiles observed in humans
  - High degree of physiological, immunological, and genetic similarity to humans
  - Broad range of validated reagents and endpoints that are directly translatable to the clinical setting
  - Extremely limited access, costly

Endpoints Overview - Irradiation

Primary endpoint:

- animal survival (30/60/90 days) including sex differences → first proof of efficacy
- weight loss and recovery

Secondary endpoints:

- pancytopenia (lymphopenia is **not** sufficient) and recovery → predict infection and bleeding
- bone marrow cellularity loss and regeneration (Ki-67, CFU-GEMM, %$CD45^+$, $CD33^+$, $CD19^+$) → durable hematopoiesis
- GI integrity (crypt count, plasma citrulline, barrier integrity) → predicts survival beyond hematologic recovery
- Organ function panels (renal, hepatic, cardiac) → quantify systemic recovery / Multiple Organs Failure risk
- profiling of pro-inflammatory cytokine/chemokine response
- cytogenetics (micronuclei, dicentrics)
- immune response profiling and macrophages & T-cell infiltration
- Extracellular vesicles production (EVs) and cfDNA
- neuroinflammation
- behavioral recovery and cognitive performances → operational readiness indicators after exposure

Each parameter mirrors a human clinical outcome — transforming animal data into regulatory-ready proof of benefit

Mouse model of H-ARS (TBI dose > 8 Gy)

MOUSE SURVIVAL

WEIGHT LOSS

LEUCOPENIA

NEUTRO-THROMBOPENIA

ANEMIA

Contamination in a CBRN threat context

Nuclear or improvised weapons:

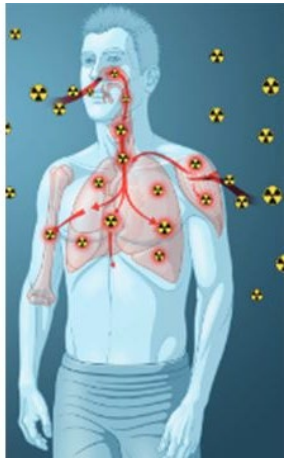
- Detonation of a nuclear weapon.
- Dissemination of RN by a radiological dispersion device (« dirty bomb »).
- Detonation of an improvised nuclear device.
- Destruction of a facility in which radioactive material is used or stored in sealed or unsealed form.

Isotopes and material of health concern:

- Alpha-emitting RN of the actinide series: uranium (U), plutonium (Pu), americium (^{241}Am), neptunium (^{237}Np) and curium (Cm).
- Fission products generated: iodine-131 (^{131}I), caesium137 (^{137}Cs), strontium (^{90}Sr), tritium (^{3}H).
- Activation products such as cobalt-60 (^{60}Co).
- ^{60}Co and ^{137}Cs sources used for gamma irradiation in industrial or medical setting can also be diverted.

Expected routes of exposure:

- Surface contamination of intact skin => external exposure (risk of secondary internal exposure through inhalation)
- Surface contamination of damaged skin => external/internal exposure
- Wound contamination => partially external/internal exposure
- Inhalation (+ unavoidable ingestion) => internal exposure



Dr Yannick Saintigny, PhD
CEA – France
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Endpoints Overview - Contamination

Primary endpoint:

- animal survival (30/60/90 days) including sex differences → first proof of efficacy
- radionuclide phagocytosis, fixation and release by target organs
- biodistribution and decorporation kinetics → Internal (inhalation, ingestion, wound uptake) and/or External (cutaneous)
- weight loss and recovery

Secondary endpoints:

- inflammatory markers secretion (local and systemic) as THP-1 macrophages if inhalation
- skin necrosis, erythema
- Blood coagulation
- Bone repair
- pancytopenia (lymphopenia is **not** sufficient) and recovery → predict infection and bleeding
- bone marrow cellularity loss and regeneration (Ki-67, CFU-GEMM, %$CD45^+$, $CD33^+$, $CD19^+$) → durable hematopoiesis
- GI integrity (crypt count, plasma citrulline, barrier integrity) → predicts survival after ingestion
- Organ function panels (renal, hepatic, cardiac) → quantify systemic recovery / Multiple Organs Failure risk
- cytogenetics (micronuclei, dicentrics)
- immune response profiling and macrophages & T-cell infiltration
- behavioral recovery and cognitive performances → operational readiness indicators after exposure

Dr Yannick Saintigny, PhD
CEA – France

Combined Injury Syndromes

Different exposition models for multiple « real » scenarii :

- Irradiation + trauma and/or burn → battlefield realism
- Internal / external contamination + trauma and/or hemorrhage → dirty-bomb scenario

Hemorrhage:

- Rat is an acceptable model to screen and for mechanistic studies
- Minipig is the physiologically relevant large animal model for detailed studies

Pulmonary injury (dust obstruction):

- Mouse is an acceptable model to screen and for mechanistic studies
- Rat for detailed studies on MCM sprays

Burn injury:

- Burn is a systemic disease: only animal models allow integrated response
- Cell therapy proposed for cutaneous radiation syndrome and combined injuries (autologous, syngenic, xenogeneic)
- Rat is an acceptable model to screen and for mechanistic studies
- Minipig skin closer to human than rodents (vascularization) but non flexible for mechanistic studies

Trauma injury:

- Combined injuries by trauma exacerbate lethality and impaired healing (Multiple Organs Failure) by accelerating systemic inflammation and impairing tissue regeneration.
- Rat is an acceptable model to screen and for mechanistic studies
- Minipig is the physiologically relevant large animal model for detailed studies of MOF progression

Dr Yannick Saintigny, PhD
CEA – France
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How to improve the European ecosystem ?

- Research should move forward to GI-ARS, as H-ARS is already relatively well-covered
- Proposals should involve –omic, RNAscope platforms to build a translatable efficacy signature
- Extracellular vesicules are anticipated to be a key issue
- Supportive care to animal models needs to be standardized (antibiotics, fluids, transfusions, analgesia)
- NSG mice + AACHEN minipigs could be considered as Europe's dual non-clinical architecture for NR MCM countermeasures
- Harmonisation of regulatory procedures:
  - EMA « under exceptionnal circonstances »
  - FDA « Animal Rule »
  - USA/JAPAN/EU/UK models and endpoint harmonisation
  - GLP-compliance in pivotal studies
  - Shared databases, biobanks, SOPs
  - NATO STANAG 2541: Medical CBRN guidance
  - IAEA and UNSCEAR guidance

Contributors

Yannick Saintigny
Laurent Gauthier
Stéphanie Moreno
Olivier Gremy

Michel Drouet
Juliette Peltzer
Sabine François
Alexandra Bourgois

Alain Chapel
Marc Benderitter

Alan H. Tkaczyk

Elizabeth A. Ainsbury

Aleš Tichý

Katalin Lumniczky

Ingars Reinholds
Indriķis Muižnieks

Matthias Port

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