Deciphering potential health impacts of radioactive medical waste: *in silico* analysis of iodine-131 and technetium-99m's biological interactions

Katarina Baralić[1], Predrag Božović[2], Biljana Radović[1], Marijana Ćurčić[1], Biljana Antonijević[1], Danijela Đukić Ćosić[1]

[1]Department of Toxicology "Akademik Danilo Soldatović", University of Belgrade - Faculty of Pharmacy, Vojvode Stepe 450, 11221, Belgrade, Serbia
[2]University of Belgrade, Vinča Institute of Nuclear Sciences, Department of Radiation and Environmental Protection, Belgrade, Serbia

Corresponding author:
Katarina Baralić
telephone: +381 11 3951 248
fax: +381 11 3972-840
email: katarinab@pharmacy.bg.ac.rs

Abstract

Purpose: The escalating use of radioactive materials in medical applications has generated a significant increase in radioactive medical waste, posing substantial risks to healthcare workers, patients, and general public. Iodine-131 and technetium-99m, crucial radionuclides with widespread applications in nuclear energy, medical treatments, and diagnostics, have emerged as focal points of interest. However, understanding the combined impact of these radionuclides on human health, particularly in mixtures, is lacking, while adequate attention to identifying and validating biomarkers for early detection is crucial for assessing individual susceptibility and enabling timely interventions to mitigate potential health risks. Hence, the current study aims to explore how these radioisotopes, individually and in combination, induce hazardous effects by applying toxicogenomic data mining.

Methods: Comparative Toxicogenomics Database (CTD), Search Tool for Interactions of Chemicals (STITCH), GeneCards database (https://www.genecards.org), GeneMANIA server (https://genemania.org), and ToppGene suite (https://toppgene.cchmc.org) were used for *in silico* analysis.

Results: Iodine-131 interacted with 13 genes related to thyroid function, glucose transport, and cellular processes, while technetium-99m interacted with 10 genes associated with cellular transport, endocrine functions, and cellular responses. The combined interactions revealed a diverse range of molecular functions, biological processes, and pathways, emphasizing the interplay between thyroid functions and cellular transport mechanisms. The overlapping SLC5A5 gene suggested a shared mechanism between the two radioisotopes.

Conclusion: The coexistence of iodine-131 and technetium-99m in a mixture may result in complex and dynamic interactions within biological systems, underscoring the necessity for a comprehensive understanding of their combined effects on health, particularly in scenarios involving simultaneous exposure.

Keywords: toxicogenomics, bioinformatics, radioactive contamination, hazard assessment, *in silico* analysis

1. Introduction

Recent advancements in medical use of radioactive materials have increased the amount of radioactive medical waste (excess liquids from treatments, contaminated glassware, packaging, and fluids from patients exposed to radioactive substances), with diagnostic procedures, treatments, and research being primary contributors [1,2]. Radioactive medical waste poses risks to healthcare workers, patients, visitors, support staff, and general public due to potential exposure from mishandling, transportation incidents, or inadequate disposal practices [3]. Among the myriad of radionuclides used in medicine, iodine-131 and technetium-99m are of particular interest due to their widespread applications. Iodine-131 is a vital radioisotope used in nuclear energy and medical treatments, while technetium-99m is the most frequently used medical radioisotope, employed in tens of millions of diagnostic procedures annually [4]. Iodine-131, generally safe, has potential side effects categorized as early (gastrointestinal symptoms, radiation thyroiditis, etc.) and late (secondary cancers, pulmonary fibrosis, etc.). Once inside the body, I-131 will be absorbed by the thyroid gland exposing it to radiation and potentially increasing the risk for thyroid cancer or other thyroid-related problems [5]. Likewise, the potential accumulation of Technetium-99 in the thyroid gland and gastrointestinal tract, coupled with the elevated risk of adverse health effects from radiation exposure, mirrors the general susceptibility associated with exposure to radioactive substances, heightening the likelihood of developing cancer [6]. Delving into the toxicity mechanisms of iodine-131, technetium-99m, and their combination is indispensable for guaranteeing the safe and efficient utilization of these isotopes in medical contexts, as well as for promoting appropriate waste management practices. Furthermore, the current available information on the toxicity of the mixture of these two radioactive substances is lacking, necessitating further comprehensive studies and research to better understand the potential combined effects and associated risks. In Serbia, the examination of radiation effects on animals is prohibited due to ethical considerations and concerns for animal welfare, as stated in "The Law on Animal Welfare" (published in "Official gazette of the Republic of Serbia", br. 41/2009). However, there is a possibility of exploring the impacts of radiation exposure through alternative means. One such approach involves *in silico* testing, utilizing computational methods and data analysis techniques. Using online resources that aggregate data on the correlation between various stressors and alterations in gene expression, with potential implications for the development of various diseases, allows for data mining, analysis, and discussion of the observed connections [7]. Toxicogenomics data mining cover the extraction and analysis of data concerning the impact of chemicals on genes and patterns of gene expression [8], but can also be applied on different stressors, including radiation. Exploring databases such as the Comparative Toxicogenomics Database (CTD) allows the identification of genes affected by radiation exposure. The insights derived from databases such as CTD and toxicogenomics data mining facilitate the identification of potential biomarkers, signaling pathways, and molecular mechanisms, thereby contributing to a more comprehensive understanding of the biological processes underlying radiation exposure [9,10].

In light of these factors, the present toxicogenomic data mining study seeks to: (i) investigate the mechanisms by which iodine-131 and technetium-99m elicit their hazardous effects, with a particular focus on the potential combined impact of these radioisotopes originating from radioactive waste; and (ii) demonstrate the effectiveness of the suggested methodology in efficiently elucidating the effects and sources of damage arising from radiation exposure.

2. Material and methods

In our exploration of chemical-gene interactions related to iodine-131 and technetium-99m, we utilized two databases: Comparative Toxicogenomics Database (CTD) available at https://ctdbase.org and Search Tool for Interactions of Chemicals (STITCH) accessible at http://stitch.embl.de. Despite providing valuable insights into iodine-131 – gene interactions, CTD did not offer data on technetium-99m. To bridge this gap, we applied STITCH database to extract the data on gene interactions involving both iodine-131 and technetium-99m. We later assessed all the extracted genes, taking into account the potential for simultaneous exposure to both radioactive substances. To explore the functions of the identified genes, we employed two resources: GeneCards database (https://www.genecards.org) and PANTHER knowledgebase (https://www.pantherdb.org). Subsequently, we systematically categorized the genes according to their respective functions. After this step, we delved into the interactions among the identified genes using the GeneMANIA server, a valuable resource accessible at https://genemania.org. Furthermore, we utilized gProfiler (https://biit.cs.ut.ee/gprofiler/gost) for functional profiling, applying its algorithms to explore the impact of these radioactive chemicals on gene functions and associated gene ontology terms, and ToppGene suite (https://toppgene.cchmc.org) to gain more

information about the specific molecular functions, biological processes, and pathways associated with the identified genes. All data presented in this research was obtained in February 2024. Detailed steps of the applied *in silico* analysis are presented in the Fig. 1.

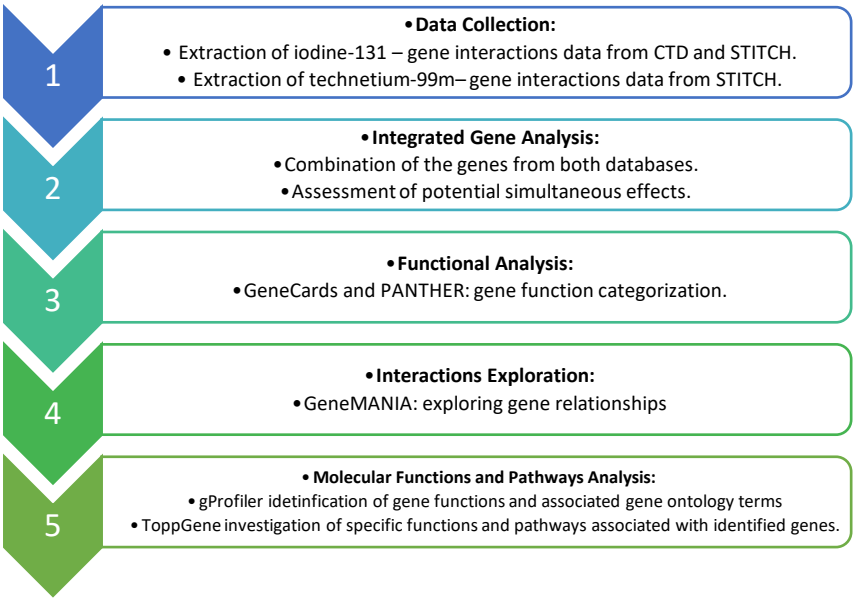


Figure 1. Detailed steps of the applied *in silico* analysis: iodine-131 and technetium-99m's biological interactions.

3. Results and discussion

Our data mining results have indicated that iodine-131 exhibited interactions with 13 genes: PAFAH1B1, ATXN1, PCNA, SOX2, TG, TSHR, SLC5A5, SLC2A1, TPO, PAX8, HUG1, SLC2A14, and KLK8. These genes were primarily linked to thyroid function (TG, TSHR, SLC5A5, TPO, PAX8), glucose transport (SLC2A1, SLC2A14, SLC5A5), and cellular processes (PCNA, PAFAH1B1, SOX2, ATXN1, HUG1, KLK8). Meanwhile, technetium-99m interacted with 10 genes: SLC5A5, CHGA, RFX6, FUT4, LPIN2, ALB, SST, CHEK2, MMP1, and SLC6A3, mostly associated with cellular transport (sodium/iodide cotransporter (SLC5A5), sodium-dependent dopamine transporter (SLC6A3)), endocrine functions (CHGA, SST), and diverse cellular responses (RFX6, FUT4, LPIN2, ALB, CHEK2, MMP1).

The PANTHER tool analysis further elucidated the functional roles of these genes (Fig. 2). Proteins associated with iodine-131 exhibited diverse functions, including antioxidant activity (TPO), binding (SOX2, PAX8), catalytic activity (SOX2), molecular function regulation (PCNA), molecular transducer activity (TSHR), transportation regulation (SOX2, PAX8), and transporter activity (SLC5A5, facilitated glucose transporters (SLC2A1, SLC2A14)). Similarly, proteins related to Technetium-99m are implicated in binding (RFX6), catalytic activity (FUT4, MMP1, LPIN2, CHEK2), transcriptional regulation (RFX6, LPIN2), and transporter activity (SLC6A3, SLC5A5).

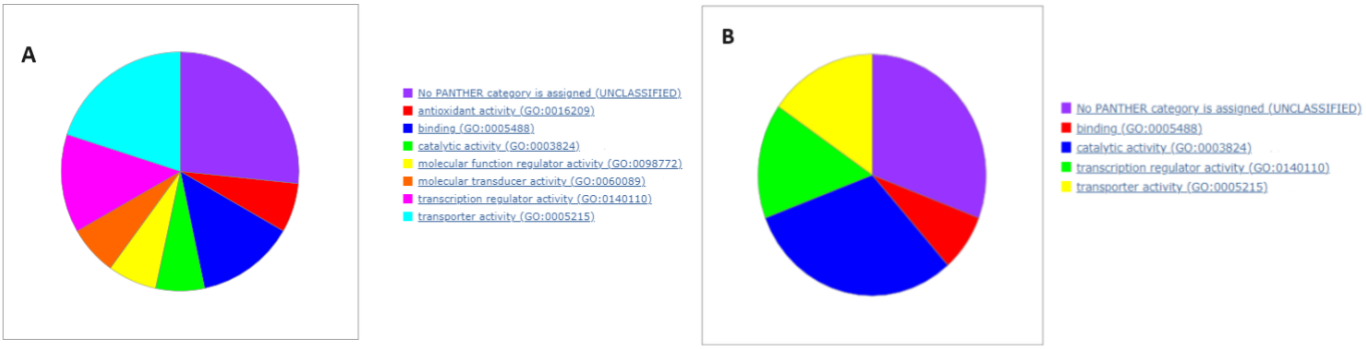
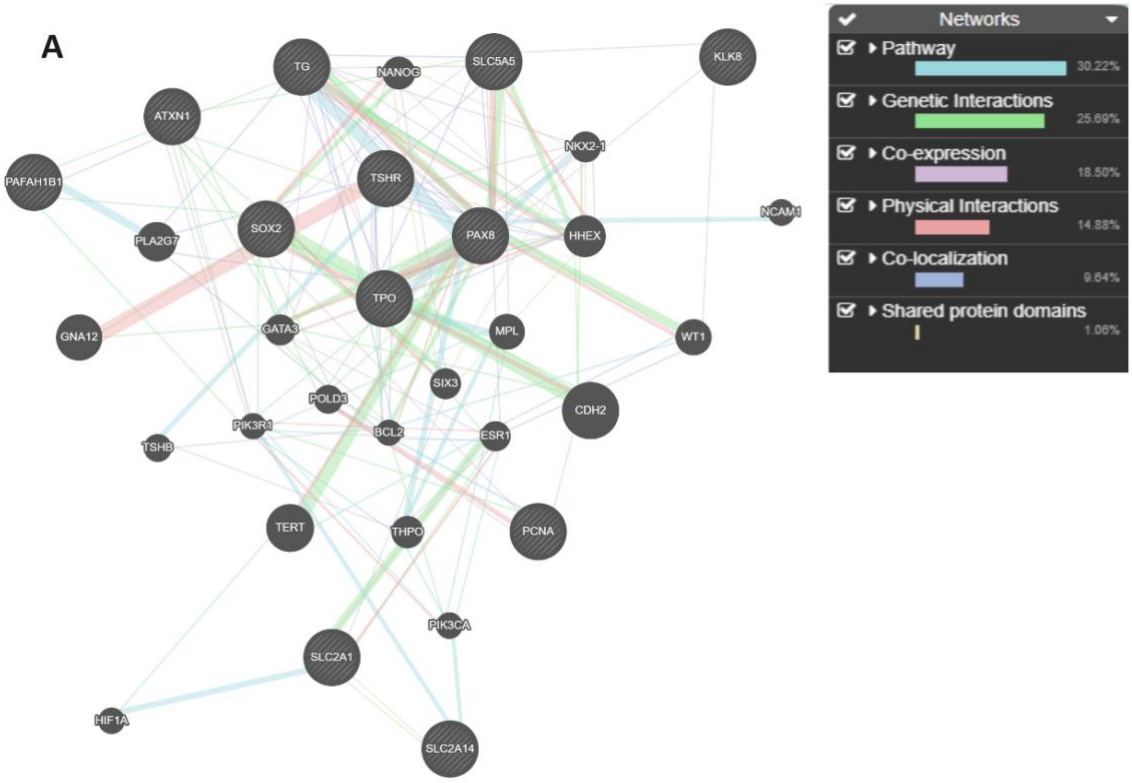


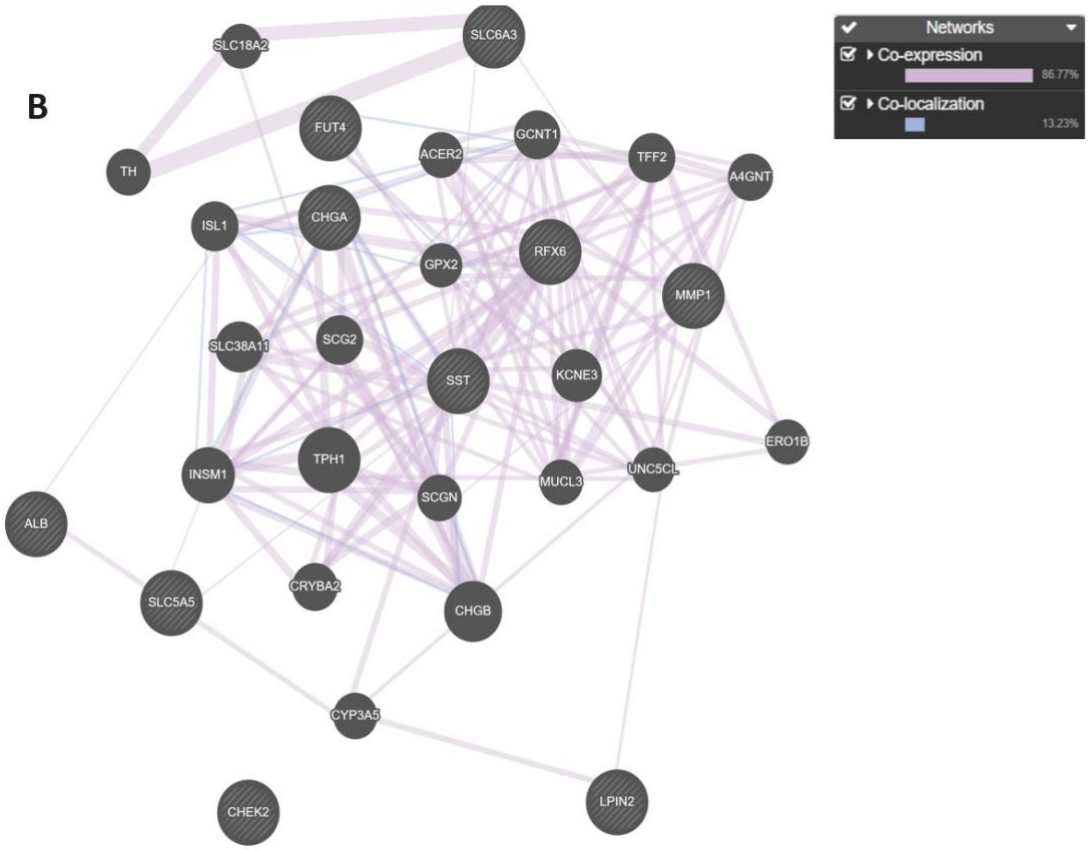
Figure 2. Functional roles of genes associated with: A. Iodine-131 – antioxidant activity: TPO; binding: SOX2, PAX8; catalytic activity: SOX2; Molecular function regulator activity: PCNA; Molecular tranducer activity:

TSHR; transportion regulator activity: SOX2, PAX8; transporter activity: SLC5A5, SLC2A1, SLC2A14. B. Technetium-99m – Binding: RFX6; Catalytic activity: FUT4, MMP1, LPIN2, CHEK 2; Transcription regulator activity: RFX6, LPIN2; Transporter activity: SLC6A3, SLC5A5. PANTHER (https://www.pantherdb.org).

Further, we investigated the interactions among the identified genes related to iodine-131 and technetium-99m, both individually and combined (Fig. 3). Among the genes linked to iodine-131, significant proportions were observed in shared pathways (30.22%), genetic interactions (25.69%), co-expression (17.80%), physical interactions (14.88%), and co-localization (9.64%). In contrast, technetium-99m genes predominantly displayed co-expression (86.77%) and co-localization (13.23%). In the combined gene set co-expression (37.17%), genetic interactions (27.55%), shared pathways (14.17%), co-localization (10.50%), and physical interactions (10.17%) were noted.

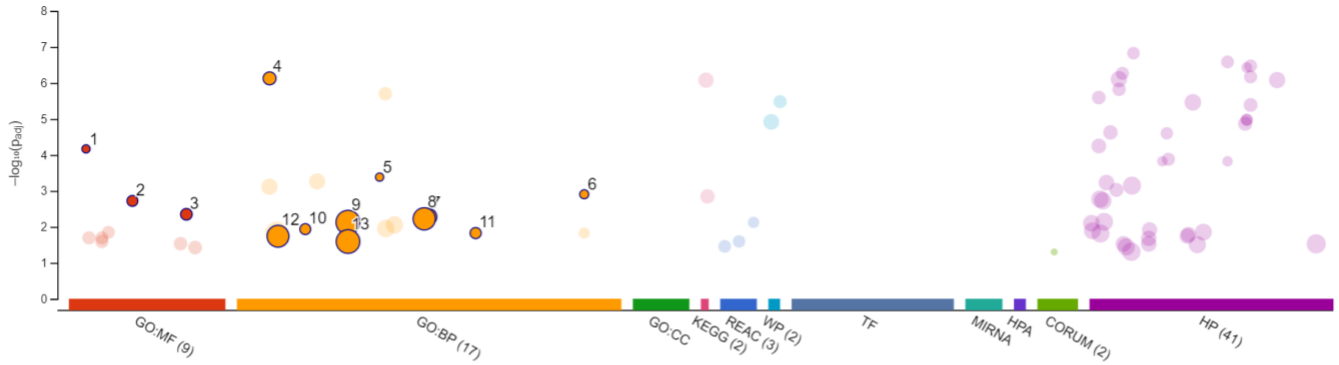
Figure 3. Network characteristics of: A. Iodine-131 and B. Technetium-99m related genes (https://genemania.org). **Co-expression:** Genes with similar expression patterns across conditions. **Physical Interaction:** Gene products that physically interact in protein-protein studies; **Genetic Interaction:** Genes functionally associated based on perturbation effects; **Shared Protein Domains:** Genes with the same protein domain; **Co-localization:** Genes expressed in the same tissue or proteins found in the same location; **Pathway:** Genes participating in the same reaction within a pathway; **Predicted:** Functional relationships between genes predicted through various methods, including orthology mapping and data source integration.


A
TG
NANOG
SLC5A5
KLK8
ATXN1
NKX2-1
PAFAH1B1
TSHR
NCAM1
PLA2G7
SOX2
PAX8
HHEX
TPO
GNA12
GATA3
MPL
WT1
SIX3
POLD3
CDH2
PIK3R1
BCL2
ESR1
TSHB
TERT
THPO
PCNA
PIK3CA
SLC2A1
HIF1A
SLC2A14
Networks
Pathway
30.22%
Genetic Interactions
25.69%
Co-expression
18.50%
Physical Interactions
14.88%
Co-localization
9.64%
Shared protein domains
1.06%


B
SLC6A3
SLC18A2
FUT4
GCNT1
ACER2
TFF2
A4GNT
TH
ISL1
CHGA
RFX6
GPX2
MMP1
SLC38A11
SCG2
SST
KCNE3
ERO1B
TPH1
INSM1
MUCL3
UNC5CL
SCGN
ALB
CRYBA2
SLC5A5
CHGB
CYP3A5
LPIN2
CHEK2
Networks
Co-expression
86.77%
Co-localization
13.23%

Both gProfiler and ToppGene were employed to comprehensively investigate the impact of these radioelements on gene functions and associated gene ontology terms. In Figure 4, functional profiling for Iodine-131 is presented, while the ToppGene results, including key gene ontology terms, pathways, and associated diseases, are summarized in Table 1. For technetium-99m, functional profiling analysis is depicted in Figure 5, while Table 2 provides information on molecular functions, cellular components, and associated diseases. In Table 1 and 2 terms are presented in descending order of statistical significance, with a significance threshold of $p < 0.05$ as the cut-off criterion. The Supplementary Table 1 contains gene enrichment results elucidating the functional implications and pathways associated with the mutual gene, SLCA5, concerning its involvement with both Iodine-131 and Technetium-99m. The use of both gProfiler and ToppGene enabled a thorough examination of the obtained genes, considering that each tool employs distinct algorithms and databases, offering complementary perspectives on functional enrichment. It is important to note that the extraction process for technetium-99m did not yield information on biological functions and pathways in either gProfiler or ToppGene.

Figure 4. Functional profiling of the genes connected to Iodine-131 (gProfiler; https://biit.cs.ut.ee/gprofiler/gost)

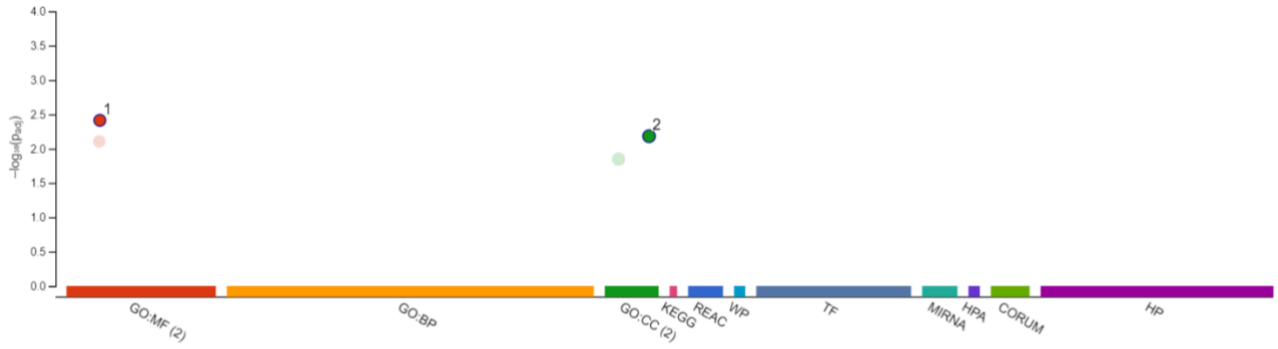


ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:MF	GO:0004996	thyroid-stimulating hormone receptor activity	6.809×10^{-5}
2	GO:MF	GO:0033300	dehydroascorbic acid transmembrane transporter ...	1.903×10^{-3}
3	GO:MF	GO:0055056	D-glucose transmembrane transporter activity	4.479×10^{-3}
4	GO:BP	GO:0006590	thyroid hormone generation	7.457×10^{-7}
5	GO:BP	GO:0038194	thyroid-stimulating hormone signaling pathway	4.153×10^{-4}
6	GO:BP	GO:1904588	cellular response to glycoprotein	1.246×10^{-3}
7	GO:BP	GO:0048839	inner ear development	5.264×10^{-3}
8	GO:BP	GO:0048513	animal organ development	5.995×10^{-3}
9	GO:BP	GO:0032501	multicellular organismal process	7.477×10^{-3}
10	GO:BP	GO:0015705	iodide transport	1.161×10^{-2}
11	GO:BP	GO:0070837	dehydroascorbic acid transport	1.492×10^{-2}
12	GO:BP	GO:0007399	nervous system development	1.823×10^{-2}
13	GO:BP	GO:0032502	developmental process	2.562×10^{-2}

Table 1. In-depth gene ontology terms (molecular functions, biological processes and cellular components), pathways, and associated diseases extracted for Iodine-131 based on identified genes (TopGenne's ToppFun function - https://toppgene.cchmc.org/enrichment.jsp).

	ID	Name	pValue	Input genes	Genes in annotation
Molecular functions	GO:0004996	thyroid-stimulating hormone receptor activity	9.985E-7	2	3
	GO:0033300	dehydroascorbic acid transmembrane transporter activity	9.304E-6	2	8
	GO:0055056	D-glucose transmembrane transporter activity	2.587E-5	2	13
	GO:0016500	protein-hormone receptor activity	8.364E-5	2	23
	GO:0005355	glucose transmembrane transporter activity	1.073E-4	2	26
	GO:0015149	hexose transmembrane transporter activity	1.073E-4	2	26
	GO:0015145	monosaccharide transmembrane transporter activity	1.435E-4	2	30
	GO:0051119	sugar transmembrane transporter activity	1.635E-4	2	32
	GO:0090482	vitamin transmembrane transporter activity	2.828E-4	2	42
	GO:0015144	carbohydrate transmembrane transporter activity	3.545E-4	2	47
	GO:0140906	halogenase activity	6.030E-4	1	1
	GO:0008507	sodium:iodide symporter activity	6.030E-4	1	1

	GO:0140905	haloperoxidase activity	6.030E-4	1	1
	GO:0004447	iodide peroxidase activity	6.030E-4	1	1
	GO:0033222	xylose binding	1.205E-3	1	2
Biological processes	GO:0006590	thyroid hormone generation	8.215E-10	4	25
	GO:0042403	thyroid hormone metabolic process	5.318E-9	4	39
	GO:0038194	thyroid-stimulating hormone signaling pathway	3.097E-7	2	2
	GO:1904588	cellular response to glycoprotein	9.285E-7	2	3
	GO:0018958	phenol-containing compound metabolic process	1.046E-6	4	143
	GO:0006575	cellular modified amino acid metabolic process	1.443E-6	4	155
	GO:0015705	iodide transport	1.112E-5	2	9
	GO:0070837	dehydroascorbic acid transport	1.112E-5	2	9
	GO:0009725	response to hormone	1.514E-5	6	1092
	GO:1904587	response to glycoprotein	1.698E-5	2	11
	GO:0060119	inner ear receptor cell development	2.078E-5	3	96
	GO:0042445	hormone metabolic process	2.131E-5	4	306
	GO:0051960	regulation of nervous system development	2.272E-5	5	667
	GO:0008344	adult locomotory behavior	3.477E-5	3	114
	GO:0060113	inner ear receptor cell differentiation	4.053E-5	3	120
Cellular components	GO:0043626	PCNA complex	5.741E-4	1	1
	GO:0044796	DNA polymerase processivity factor complex	5.741E-4	1	1
	GO:0016323	basolateral plasma membrane	7.174E-4	3	322
	GO:0009925	basal plasma membrane	9.520E-4	3	355
	GO:0070557	PCNA-p21 complex	1.147E-3	1	2
	GO:0045178	basal part of cell	1.168E-3	3	381
	GO:0016234	inclusion body	1.363E-3	2	97
	GO:0008247	1-alkyl-2-acetylglycerophosphocholine esterase complex	1.720E-3	1	3
	GO:0005901	caveola	1.748E-3	2	110
	GO:0045121	membrane raft	1.932E-3	3	454
	GO:0098857	membrane microdomain	1.944E-3	3	455
	GO:0043073	germ cell nucleus	2.109E-3	2	121
	GO:0044853	plasma membrane raft	3.092E-3	2	147
	GO:0090724	central region of growth cone	3.438E-3	1	6
	GO:0097180	serine protease inhibitor complex	3.438E-3	1	6
Pathways	M39353	thyroxine thyroid hormone production	4.046E-9	4	25
	M39791	thyroid hormones production and peripheral downstream signaling effects	9.345E-9	5	93
	M47626	medicus reference tsh tg signaling pathway	1.793E-6	3	29
	M13103	autoimmune thyroid disease	1.072E-5	3	52
	MM14769	vitamin C ascorbate metabolism	2.469E-5	2	9
	MM14833	thyroxine biosynthesis	3.085E-5	2	10
	M27120	thyroxine biosynthesis	3.085E-5	2	10
	MM1526	vitamin C pathway	4.520E-5	2	12
	M47627	medicus reference tsh duox2 tg signaling pathway	4.520E-5	2	12
	MM14829	metabolism of amine derived hormones	1.045E-4	2	18
	M27118	metabolism of amine derived hormones	1.045E-4	2	18
	MM14718	cellular hexose transport	1.167E-4	2	19
	M27058	cellular hexose transport	1.432E-4	2	21
	M39454	Nrf2 pathway	2.125E-4	3	141
	MM15928	glycolysis and gluconeogenesis	8.570E-4	2	51
Diseases	C0010308	congenital hypothyroidism	1.122E-11	4	13
	DOID:0050328	congenital hypothyroidism (implicated via orthology)	1.570E-11	4	14
	DOID:0050328	congenital hypothyroidism (is implicated in)	2.060E-10	3	4
	cv:C0010308	congenital hypothyroidism	1.029E-9	3	6
	C1848805	thyroid dyshormonogenesis 1	1.029E-9	3	6
	C0018021	goiter	1.801E-9	3	7
	C1563716	thyroid dysgenesis	4.554E-7	2	3
	C0151516	thyroid hypoplasia	4.554E-7	2	3
	C1578691	myxedema, congenital	9.106E-7	2	4
	C0920350	autoimmune thyroiditis	9.106E-7	2	4
	C0342200	endemic cretinism	9.106E-7	2	4
	C0750940	tremor, rubral	1.816E-5	2	16
	C0750937	ataxia, appendicular	1.816E-5	2	16
	C0427190	ataxia, truncal	1.816E-5	2	16
	C0278161	ataxia, motor	1.816E-5	2	1



ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:MF	GO:0015373	monoatomic anion:sodium symporter activity	3.868×10^{-3}
2	GO:CC	GO:0098992	neuronal dense core vesicle	6.578×10^{-3}

Figure 5. Functional profiling of the genes connected to technetium-99m (gProfiler; https://biit.cs.ut.ee/gprofiler/gost)

Table 2. In-depth gene ontology terms (molecular functions and cellular components) and associated diseases extracted for Tehnetium-99m based on identified genes (TopGenne's ToppFun function - https://toppgene.cchmc.org/enrichment.jsp).

	ID	Name	pValue	Input genes	Genes in annotation
Molecular functions	GO:0015373	monoatomic anion:sodium symporter activity	3.458E-5	2	18
	GO:0015296	monoatomic anion:monoatomic cation symporter activity	7.330E-5	2	26
	GO:0008507	sodium:iodide symporter activity	5.024E-4	1	1
	GO:0015370	solute:sodium symporter activity	7.559E-4	2	83
	GO:1903981	enterobactin binding	1.004E-3	1	2
	GO:0005334	norepinephrine:sodium symporter activity	1.506E-3	1	3
	GO:0015111	iodide transmembrane transporter activity	1.506E-3	1	3
	GO:0015294	solute:monoatomic cation symporter activity	1.675E-3	2	124
	GO:0005330	dopamine:sodium symporter activity	2.007E-3	1	4
	GO:0008509	monoatomic anion transmembrane transporter activity	2.312E-3	2	146
	GO:0015103	inorganic anion transmembrane transporter activity	2.700E-3	2	158
	GO:0015293	symporter activity	2.768E-3	2	160
	GO:0017083	4-galactosyl-N-acetylglucosaminide 3-alpha-L-fucosyltransferase activity	3.010E-3	1	6
	GO:0015081	sodium ion transmembrane transporter activity	3.117E-3	2	170
	GO:0035240	dopamine binding	3.511E-3	1	7
Cellular components	GO:0098992	neuronal dense core vesicle	1.214E-4	2	35
	GO:0031045	dense core granule	3.014E-4	2	55
Diseases	DOID:10763	hypertension (biomarker via orthology)	5.193E-5	3	227
	DOID:783	end stage renal disease (is implicated in)	8.020E-5	2	40
	DOID:783	end stage renal disease (is marker for)	1.063E-4	2	46
	C0030305	Pancreatitis	1.753E-4	2	59
	DOID:0080600	COVID-19 (is marker for)	2.000E-4	2	63
	C0086692	benign neoplasm	2.330E-4	2	68
	C0027651	neoplasms	2.330E-4	2	68
	DOID:11054	urinary bladder cancer (is implicated in)	2.835E-4	2	75
	C0006142	malignant neoplasm of breast	3.083E-4	4	1074
	DOID:6000	congestive heart failure (is marker for)	3.389E-4	2	82
	cv:C5700336	classic dopamine transporter deficiency syndrome	3.393E-4	1	1
	613135	parkinsonism-dystonia 1, infantile-onset	3.393E-4	1	1
	C0035457	rhinitis, allergic, perennial	3.393E-4	1	1
	DOID:1614	male breast cancer (is implicated in)	3.393E-4	1	1
	C0014867	esophageal varices	3.393E-4	1	1

3. Discussion

Data mining conducted in this study uncovered interactions involving 13 genes for iodine-131 and 10 genes for technetium-99m. Iodine-131 predominantly targeted genes related to essential thyroid functions, glucose transport, and diverse cellular processes. In contrast, technetium-99m primarily interacted with genes associated with cellular transport, endocrine functions, and various cellular responses. PANTHER tool analysis provided deeper insights into the functional roles of these genes, revealing diverse activities for iodine-131-associated proteins, including antioxidant, binding, catalytic, and transporter functions. Similarly, proteins related to technetium-99m were implicated in binding, catalytic activity, transcriptional regulation, and transport functions. The genes associated with iodine-131 display a wide array of interaction patterns, including shared pathways and genetic interactions. In contrast, technetium-99m genes predominantly exhibit co-expression, indicating a more focused set of interactions. This underscores the crucial role of iodine in thyroid function and its significance in thyroid-related diseases [11]. The functional profiling results for genes connected to Iodine-131 revealed molecular activities and biological processes associated with thyroid function and development, aligning with expectations based on the literature data. For example, the study which investigated the impact of varying doses of iodine-131 (64 - 277 µCi) administration on thyroid function in rats, revealed significant and dose-independent prolonged hypothyroidism, as evidenced by altered hormone levels, reduced thyroid gland volume and weight, and severe histopathological abnormalities including necrotic follicles and inflammatory reactions [12]. Another animal study examined age-related susceptibility to thyroid cancer, assessing the carcinogenic potential of 131I in rats at different ages. Findings revealed significant tissue damage at 1 week of age, varied apoptosis and proliferation changes with age, and the development of papillary carcinomas in rats irradiated at 1 week, suggesting that even low dose rates could induce thyroid carcinomas with a short latency [13]. Human studies also acknowledged Iodine-131 connection with thyroid-related effects. The research explored the lasting effects of iodine-131 treatment for thyrotoxicosis, revealing that patients initially euthyroid but later displaying an elevated plasma thyroid-stimulating hormone faced an increased likelihood (2-5% per year) of developing overt hypothyroidism [14]. Concerns regarding the cancer-causing impact of radioiodine exposure on the thyroid gland arise from its distinct susceptibility to radiation, indications of detectable rises in thyroid cancer at doses as minimal as 0.1 Sv, and the observation that the radiation dose to the thyroid, stemming from ingested or inhaled radioiodine, surpasses that to other organs by 500-1000 times [15]. The identification of GO terms such as "thyroid-stimulating hormone receptor activity," "thyroid hormone generation," and "iodide transport" in our study after the functional profiling also underscores the direct involvement of these genes in thyroid physiology. The significance scores, represented by p-values, indicate a high level of statistical confidence in these associations. Furthermore, the results suggest a broader impact on cellular processes, including transmembrane transporter activities like "dehydroascorbic acid transmembrane transporter" and "D-glucose transmembrane transporter." The enrichment of terms related to inner ear development, animal organ development, and nervous system development implies potential links between Iodine-131 exposure and these aspects of embryonic and organismal development. On the other hand, the functional profiling results for genes associated with technetium-99m revealed only 2 important GO terms, suggesting a specific impact on cellular transport mechanisms and neuronal vesicle dynamics. The identification of GO terms such as "monoatomic anion:sodium symporter activity" indicates a potential role of these genes in the transport of ions, specifically sodium, which could have implications for cellular homeostasis. Additionally, the association with "neuronal dense core vesicle" suggests a connection to neurotransmitter release and neuronal communication [16]. While the neuronal dense core vesicle term has a higher p-value, suggesting a lower level of confidence, its presence hints at a potential link between technetium-99m and processes related to neuronal vesicle function.

Following the comprehensive ToppGene enrichment, iodine-131-associated genes displayed a complex interaction profile, emphasizing the thyroid's central role. The analysis highlighted functions and pathways linked to thyroid-stimulating hormone receptor activity, thyroid hormone generation, and associated metabolic processes, thereby validating the earlier functional profiling findings. Furthermore, iodine-131 exhibited molecular functions, such as thyroid-stimulating hormone receptor activity, transmembrane transporters for dehydroascorbic acid and D-glucose, and protein-hormone receptor engagement, while influencing crucial biological processes like thyroid hormone generation, metabolic activities, thyroid-stimulating hormone signaling, and cellular responses to glycoproteins. Linked pathways of iodine-131 included thyroxine hormone production, downstream signaling effects, autoimmune thyroid disease, and ascorbate metabolism. The cellular components associated with iodine-131 exposure encompass a diverse array, including the PCNA complex, DNA polymerase processivity factor complex, various plasma membrane regions such as the basolateral and basal parts, as well as specific complexes like PCNA-p21. Notably, inclusion bodies and membrane structures

like caveolae, membrane rafts, and microdomains are implicated. Additionally, the involvement of the germ cell nucleus and central region of growth cone signifies potential impacts on reproductive and neurological processes [16]. These findings highlight the interactions of Iodine-131 with cellular structures involved in DNA repair, membrane organization, and developmental processes.

In contrast, molecular functions associated with technetium-99m included various symporter activities, such as monoatomic anion:sodium, monoatomic anion:monoatomic cation, sodium:iodide, and solute:sodium. Cellular components associated with genes connected to technetium-99m exposure include neuronal dense core vesicles and dense core granules. The contrast between the two exposures lies in their focus: iodine-131 appears to influence a broader range of cellular processes, including DNA maintenance and cellular membrane organization, whereas technetium-99m is specifically linked to components involved in neuronal vesicle function. These distinctions highlight the unique cellular effects of each isotope, with iodine-131 potentially influencing a broader range of cellular functions, while technetium-99m specifically targets processes within neuronal cells. Neither biological processes, nor pathways related to technetium-99m could be extracted. This suggests a knowledge gap in understanding the biological effects and pathways affected by technetium-99m, prompting the need for further research in this area.

While both isotopes share potential implications for the central nervous system, Iodine-131's focus on thyroid-related disorders contrasts with Technetium-99m's broader and multi-organ impact, highlighting the diverse health risks associated with these radioactive elements. As suggested earlier, Iodine-131 was found connected to congenital hypothyroidism, where insufficient thyroid hormone production from birth leads to developmental issues [17]. Additionally, it was associated with abnormalities in thyroid hormone production, as seen in thyroid dyshormonogenesis 1, and suggested to cause goiter, an enlargement of the thyroid gland, in response to disrupted hormone production. Furthermore, iodine-131 exposure has been suggested to contribute to abnormal thyroid development, thyroid dysgenesis, and potential underdevelopment, indicated by thyroid hypoplasia. Autoimmune thyroiditis, an inflammatory thyroid condition, was found connected to iodine-131 exposure. Beyond thyroid-related disorders, neurological symptoms such as tremors and ataxia, suggest potential impacts on the central nervous system.

On the other hand, the compilation of cardiovascular, renal, digestive, respiratory, neoplastic, and neurological disorders associated with technetium-99m exposure raises notable concerns regarding its potential toxicity across various organ systems. The observed links to congenital hypertension, and congestive heart failure imply potential cardiovascular toxicity, impacting both blood pressure regulation and cardiac function. The connections to pancreatitis and esophageal varices underscore potential toxicity affecting the pancreas and liver, respectively. Additionally, the associations with perennial allergic rhinitis, benign neoplasms, urinary bladder cancer, and breast cancer raise concerns about potential respiratory, allergic, and cellular toxicity in the respiratory and urinary systems, as well as breast tissue. The link to neurological disorders, including classic dopamine transporter deficiency syndrome and parkinsonism-dystonia 1, infantile-onset, suggests potential neurological toxicity, particularly in movement-related functions.

These distinctions indicate that, when present together, iodine-131 may contribute to a wider spectrum of cellular functions, while technetium-99m may exert more specific effects within neuronal cells. However, the shared involvement of the SLC5A5 gene in the genetic interactions of iodine-131 and technetium-99m underscores a common pathway related to iodine transport mechanisms, emphasizing the interconnectedness of their biological effects. The SLC5A5 gene functions as a sodium-coupled antiporter of neutral amino acids, playing a crucial role in the bidirectional exchange of L-glutamine and other neutral amino acids, contributing to cellular metabolism, differentiation, and various physiological processes, while also serving as a receptor for endogenous viruses in microbial infections [18,19]. Mutations in this gene can lead to a malfunction in the synthesis of thyroid hormones [20], while mutations that deactivate SLC5A5 might result in a deficiency in iodide transport, causing congenital dyshormonogenic hypothyroidism in humans, which may occasionally coincide with the development of goiter [21]. As seen in the supplementary material, this gene is associated with sodium:iodide symporter activity, iodide transmembrane transporter activity, and various symporter activities involving ions and monoatomic anions. The biological processes linked to SLCA5 involve cellular responses to thyroid-stimulating hormone, glycoproteins, forskolin, and gonadotropin stimuli, as well as iodide transport, thyroid hormone generation, and processes related to vascular transport. The pathways involving SLCA5 include organic anion transporters, thyroxine biosynthesis, metabolism of amine-derived hormones, and others associated with transmembrane transport and metabolism. The gene is implicated in several diseases, including thyroid dyshormonogenesis, congenital hypothyroidism, goiter, cholangiocarcinoma, thyroid neoplasms, kidney neoplasms, gliomas, ovarian neoplasms, melanoma, liver carcinoma, breast carcinoma, and prostatic neoplasms.

The extensive role of SLC5A5 in thyroid function highlights its importance and indicates possible connections to different cancers and metabolic issues.

While the methodology applied in this research has been valuable in uncovering the mechanisms behind the health effects of iodine-131 and technetium-99m, individual and combined, it is essential to acknowledge certain limitations associated with this approach. Firstly, the reliance on online sources like the CTD database and STITCH introduces concerns about the reliability and completeness of the interactions within these sources, potentially impacting the findings. Secondly, in toxicogenomics *in silico* investigations related to the health effects of iodine-131 and technetium-99m, the data hinges on statistical associations among stressor-gene-disease relationships. As a result, critical factors such as the dose-response relationship, exposure route, exposure duration, and individual sensitivity of exposed subjects cannot be fully taken into account. Thirdly, it is noteworthy that no genes were identified in CTD for technetium-99m, and some gene ontology terms could not be extracted, suggesting limitations in available knowledge and the need for further laboratory investigation. These limitations stress the need for cautious interpretation of findings and highlight the ongoing necessity for experimental validation.

4. Conclusion

Increased medical use of radioactive materials has heightened radioactive waste levels, posing risks to healthcare workers, patients, and the public, underscoring the need for urgent efforts to manage and mitigate these hazards for public health and environmental protection. The current study reveals distinct molecular interactions of iodine-131 and technetium-99m with genes, highlighting iodine's focus on thyroid functions and technetium's emphasis on transporter activities. However, when considering the combined interactions, a broader spectrum emerges, highlighting the complex interplay between thyroid functions and cellular transport mechanisms. Notably, the shared SLC5A5 gene suggests overlapping thyroid-related mechanism of both radioactive substances, and could potentially serve as a biomarker of the biological effects induced by exposure to iodine-131 and technetium-99m mixture. In summary, the presence of iodine-131 and technetium-99m in a mixture could result in complex interactions within biological systems, while understanding these interactions is crucial for assessing their collective health impacts, especially in simultaneous exposure.

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