10.64837/GSA.13.5.7

Global and Stochastic Analysis
Vol. 13 No. 5 (2026)
Received: 03rd July 2026 Revised: 07th September 2026 Accepted: 19th September 2026

MODELING OF CHOLESTEROL METABOLISM WITH STEADY-STATE ANALYSIS UNDER UNCERTAINTY USING FUZZY STOCHASTIC PETRI NETS

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Abstract. Cholesterol metabolism involves complex biochemical processes with significant inter-individual variability that cannot be fully captured by deterministic or standard stochastic models. This paper presents a Fuzzy Stochastic Petri Net (FSPN) framework to incorporate parameter uncertainty into metabolic modeling. Transition rates are represented using triangular fuzzy numbers, and uncertainty is analyzed using an α-cut-based continuous-time Markov chain approach. The analysis is performed in two phases, where crisp transition rates provide a baseline and fuzzy parameters enable uncertainty quantification. Structural constraints ensure bounded system behaviour and support steady-state analysis. Results reveal substantial variability across metabolic pools, with the LDL pool exhibiting 55.85% uncertainty. Sensitivity analysis identifies key pathways governing cholesterol accumulation. The proposed framework enables uncertainty-aware analysis of metabolic systems, providing more informative insights than conventional deterministic approaches.

1. Introduction

Cholesterol metabolism involves dietary intake, hepatic synthesis, transport through lipoproteins, and cellular uptake, making mathematical models useful for understanding system behaviour and predicting metabolic outcomes [1]. These processes are governed by discrete biochemical events and exhibit significant inter-individual variability due to genetic, dietary, and lifestyle factors, resulting in substantial differences in LDL cholesterol levels across individuals [4, 5]. This heterogeneity underscores the need for uncertainty-aware modeling approaches. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are key carriers in cholesterol transport.

Ordinary differential equation (ODE) models have provided valuable insight into metabolic regulation [2, 3], but they treat system dynamics as continuous and rely on fixed parameter values. In contrast, many biochemical processes such as receptor binding, vesicle transport, and lipoprotein interactions occur as discrete stochastic events. Stochastic Petri nets (SPNs) offer a natural framework for modeling such discrete-event systems with probabilistic timing [6, 7], and have been applied to biological pathways, including cholesterol-related processes [9].

2000 *Mathematics Subject Classification.* 60J27; 03E72; 68Q85.
Key words and phrases. Fuzzy Stochastic Petri Nets, uncertainty quantification, Cholesterol metabolism, Metabolic modeling, α-cut analysis.

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However, most existing SPN-based models assume precisely known transition rates and therefore do not explicitly address parameter uncertainty.

By combining SPNs with fuzzy set theory, parameter imprecision can be incorporated directly into discrete-event models [10, 16, 17]. Furthermore, fuzzy stochastic and hybrid Petri net approaches have been successfully applied to biological systems with uncertain kinetic parameters [14, 18]. Nevertheless, their application to cholesterol metabolism with structured biological constraints and steady-state uncertainty analysis remains limited. Existing studies have demonstrated the use of fuzzy stochastic and hybrid Petri nets for modeling biochemical reaction networks under parameter uncertainty, particularly focusing on fuzzy kinetic representations and hybrid dynamics [11, 18]. This work extends existing FSPN models by incorporating biologically structured constraints and steady-state uncertainty analysis specific to cholesterol metabolism.

In this study, a fuzzy stochastic Petri net (FSPN) framework is developed to model cholesterol metabolism under uncertain parameter conditions. Transition rates are represented by triangular fuzzy numbers, and uncertainty analysis is performed using an α-cut-based continuous-time Markov chain analysis. Biologically motivated structural constraints, including mass conservation and a recycling mechanism, are incorporated to ensure bounded system behaviour and enable steady-state analysis. The numerical results quantify how biological uncertainty influences cholesterol distribution across dietary, hepatic, LDL, HDL, tissue, and excretion pools.

1.1. Novelty and Contribution. While fuzzy stochastic and hybrid Petri net models have been previously applied to biological systems with uncertain kinetic parameters [16, 17, 14, 18], most existing approaches focus on dynamic simulation or hybrid continuous–discrete representations. In contrast, the present study develops a fuzzy stochastic Petri net framework tailored to cholesterol metabolism, incorporating biologically motivated constraints such as mass conservation and system-level recycling.

Unlike previous applications, this work emphasizes steady-state uncertainty quantification using α-cut-based continuous-time Markov chain analysis, producing interval-valued probabilistic interpretations of metabolic behaviour. This combination of biological constraints and steady-state probabilistic analysis distinguishes the proposed approach from existing fuzzy Petri net models.

The remainder of the paper is organized as follows. Section 2 presents the mathematical formulation of the FSPN model. Section 3 describes its application to cholesterol metabolism. Section 4 outlines the analysis methodology. Section 5 presents theoretical observations. Section 6 reports numerical simulations and discussion, and Section 7 concludes the study and indicates possible directions for future work.

2. Mathematical Formulation of the Problem

2.1. Stochastic Petri Nets with Fuzzy Parameters. The formulation is based on established fuzzy stochastic Petri net models for biological systems [12,

13, 17, 18], and is adapted here for steady-state metabolic analysis. A fuzzy stochastic Petri net(FSPN) is defined as

$$\mathrm{SPN}_{\mathrm{fuzzy}} = (P, T, A, \tilde{\Lambda}, M_0, F)$$

where P is the set of places, T is the set of transitions, and A is the set of input and output arcs. $\tilde{\Lambda}$ denotes the set of fuzzy transition rates, M_0 is the initial marking, and F is a mapping that assigns each transition a triangular fuzzy number. This formulation follows the fuzzy stochastic Petri net framework introduced in [16] and further developed in [17], while extending it to include biologically motivated constraints and steady-state uncertainty analysis.

Fuzzy parameters are included only in the transition rates, while the discrete token dynamics of the Petri net remain unchanged. Each fuzzy transition rate $\tilde{\lambda}_i$ is represented as a triangular fuzzy number (a_i, m_i, b_i), whose membership function is defined as follows:

$$\mu_{\tilde{\lambda}_i}(x) = \begin{cases} 0, & x \le a_i, \\ \dfrac{x - a_i}{m_i - a_i}, & a_i \le x \le m_i, \\ \dfrac{b_i - x}{b_i - m_i}, & m_i \le x \le b_i, \\ 0, & x \ge b_i \end{cases} \tag{2.1}$$

Triangular fuzzy numbers are employed due to their computational simplicity and suitability for α-cut-based uncertainty analysis. The triangular fuzzy number representation used for transition rates is illustrated in Fig. 1.

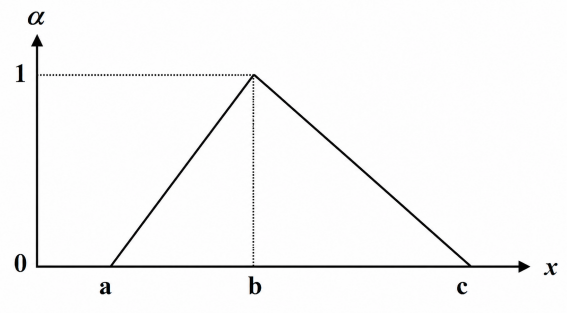


FIGURE 1. Triangular fuzzy number (a, m, b) and corresponding α-cuts.

Throughout the paper, a tilde ($\tilde{\cdot}$) denotes fuzzy quantities, while quantities without tildes denote deterministic values. In particular, $\tilde{\lambda}$ represents a fuzzy transition rate, and α-cuts are used to obtain interval-valued representations of these fuzzy parameters.

2.2. Continuous-Time Markov Chain Representation. For a live and bounded SPN, the underlying stochastic behaviour can be associated with a continuous-time

Markov chain (CTMC) [7]. The entries of the infinitesimal generator matrix Q are given by

$$q_{ij} = \begin{cases} \sum_{t_k \in T_{ij}} \lambda_k, & i \neq j, \\ -\sum_{k \neq i} q_{ik}, & i = j \end{cases} \tag{2.2}$$

where T_{ij} is the set of transitions that move the system directly from state i to state j. When transition rates are fuzzy, each triangular fuzzy transition rate is converted into an interval at a specified α-level. The resulting α-cut intervals define the admissible transition-rate ranges. These ranges are propagated numerically using an α-cut Monte Carlo procedure: transition-rate realizations are sampled within the corresponding α-cut intervals, and a CTMC generator is constructed for each realization. The stationary distribution of each resulting CTMC is then calculated numerically, allowing the lower and upper steady-state probability bounds to be obtained from the sampled stationary probabilities.

2.3. Biological and Parameter Modeling Approach. The use of fuzzy transition rates allows uncertainty in biological parameters to be incorporated directly into the stochastic dynamics of the Petri net. This formulation enables systematic propagation of parameter imprecision through the associated CTMC, providing interval-valued steady-state probabilities that reflect biological variability rather than single deterministic estimates. This approach is consistent with prior fuzzy Petri net studies on biochemical systems [16, 14].

In adapting the SPN framework to cholesterol metabolism, two main biological considerations are taken into account. First, tokens circulating through the network represent the movement of cholesterol among different pools, so the structure of the net is designed to prevent unbounded accumulation. Second, a recycling transition is introduced to conceptually return cholesterol to the system, thereby avoiding absorption into a terminal excretion state and enabling steady-state analysis. Rather than imposing explicit physiological concentration limits or detailed biochemical feedback mechanisms, uncertainty in the underlying biological processes is captured through fuzzy ranges on transition rates. Transition-rate parameters were collected from published experimental and clinical studies describing cholesterol absorption, synthesis, transport, and clearance. Due to inter-individual variability, each parameter is represented as a triangular fuzzy number, with the most likely value m_i corresponding to the mean or typical reported value, while a_i and b_i define realistic bounds of variation. Triangular fuzzy numbers provide a simple yet computationally efficient representation for the subsequent α-cut analysis.

Figure 2 illustrates representative fuzzy membership functions with an example α-cut, and Table 1 summarizes the biological data sources and primary sources of uncertainty considered in parameter estimation.

Unlike existing fuzzy or hybrid Petri net models that primarily emphasize dynamic simulation, the present formulation is structured to support steady-state probabilistic analysis under uncertainty.

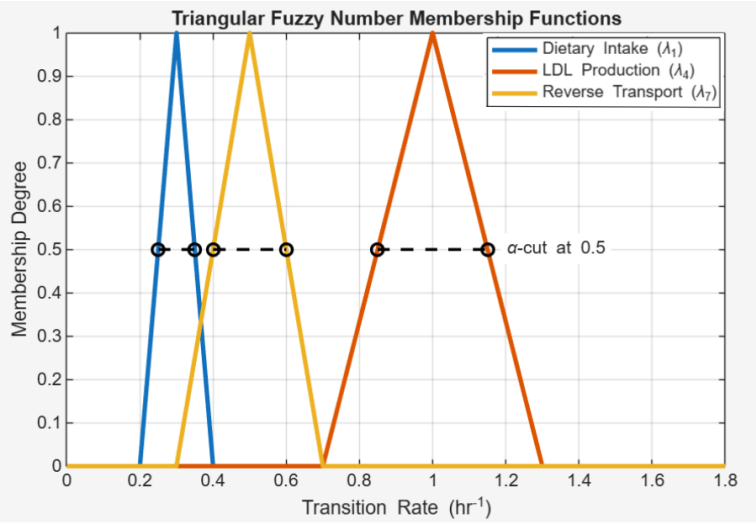


FIGURE 2. Triangular fuzzy membership functions for selected transition rates with an α-cut illustration.

TABLE 1. Biological data sources and sources of uncertainty for fuzzy parameter estimation.

Parameter type	Representative data sources	Primary uncertainty source
Dietary absorption	Clinical feeding studies, population surveys	Inter-individual variability
Hepatic synthesis	Enzyme activity and tracer studies	Measurement variability
LDL clearance	Receptor and transport studies	Genetic differences
HDL dynamics	Efflux and epidemiological studies	Methodological variation

3. Cholesterol Metabolism Model

3.1. Biological Foundation and Pathway Description. Cholesterol metabolism is a regulated physiological process involving intestinal absorption, hepatic synthesis, circulation through lipoproteins, uptake by peripheral tissues, and systemic elimination. Disturbances in this system are strongly associated with cardiovascular risk, and mathematical modeling provides a useful framework for analyzing pathway behaviour and evaluating intervention strategies [15, 5].

To capture both discrete biochemical events and uncertainty in biological parameters, the pathway is modeled using a fuzzy stochastic Petri net (FSPN), which enables the integration of stochastic dynamics with parameter imprecision, as established in fuzzy stochastic Petri net models for biological systems [16, 14]. The pathway considered includes dietary cholesterol intake, intestinal absorption, hepatic processing, transport via low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles, delivery to peripheral tissues, and reverse cholesterol transport back to the liver. A portion of cholesterol is subsequently eliminated through bile and fecal excretion [15].

In the FSPN representation, places correspond to cholesterol pools, and transitions represent biochemical transport or conversion processes. The resulting model consists of seven places and nine transitions capturing the principal routes of cholesterol flow within the system.

3.2. Structural Considerations and Recycling Transition. When biliary excretion is modeled as a terminal process, tokens ultimately accumulate in the excretion place and the dynamics terminate. In such cases, long-term probability distributions cannot be examined because the underlying process reaches an absorbing state. To enable steady-state behaviour to be analyzed, an auxiliary recycling transition t_9 is introduced from the excretion pool back to the dietary source ($P_7 \to P_1$). This transition is introduced as a modeling closure to prevent the excretion pool P_7 from becoming absorbing and to permit stationary-state analysis of the stochastic network. It should not be interpreted as a literal physiological return of excreted cholesterol to the dietary pool.

Under this closed-network representation, the steady-state probability associated with each place represents the long-run fraction of modeled stochastic occupancy, or equivalently the relative residence-time fraction, associated with that metabolic pool. Accordingly, the stationary probabilities provide a normalized description of the relative distribution of the modeled cholesterol token across the pathway under the assumed transition rates. They should not be interpreted directly as absolute physiological cholesterol concentrations, measured pool masses, or net excretion rates in the corresponding open physiological system. Instead, the closed recycling formulation provides a mathematical reference framework for assessing the relative redistribution of metabolic occupancy and the effect of transition-rate uncertainty, while the actual physiological excretion process remains an open-system process.

This modeling step is therefore introduced for analytical convenience and should be interpreted as a structural construct for steady-state analysis rather than as a detailed physiological re-circulation mechanism. The overall structure of the fuzzy stochastic Petri net model and its components is shown in Fig. 3 and in Table 2.

3.3. Fuzzy Parameter Specification. Transition rates in cholesterol metabolism vary across individuals due to genetic factors, dietary habits, and measurement uncertainty [5]. To reflect this variability, each transition rate is modeled as a triangular fuzzy number

$$\tilde{\lambda} = (a, m, b)$$

where a and b denote lower and upper bounds, and m represents the most likely value. Triangular fuzzy numbers provide a simple and computationally efficient way to propagate uncertainty through the FSPN using α-cut analysis. Table 3 summarizes the fuzzy transition rates used in the model (hr^{-1}).

The selected places and transitions capture the principal mechanisms of cholesterol transport and regulation, including dietary intake, hepatic processing, LDL-mediated tissue delivery, HDL-mediated reverse transport, and excretion. Although simplified, this structure reflects the essential mechanisms governing cholesterol redistribution and allows systematic analysis of how parameter uncertainty influences long-term metabolic behaviour.

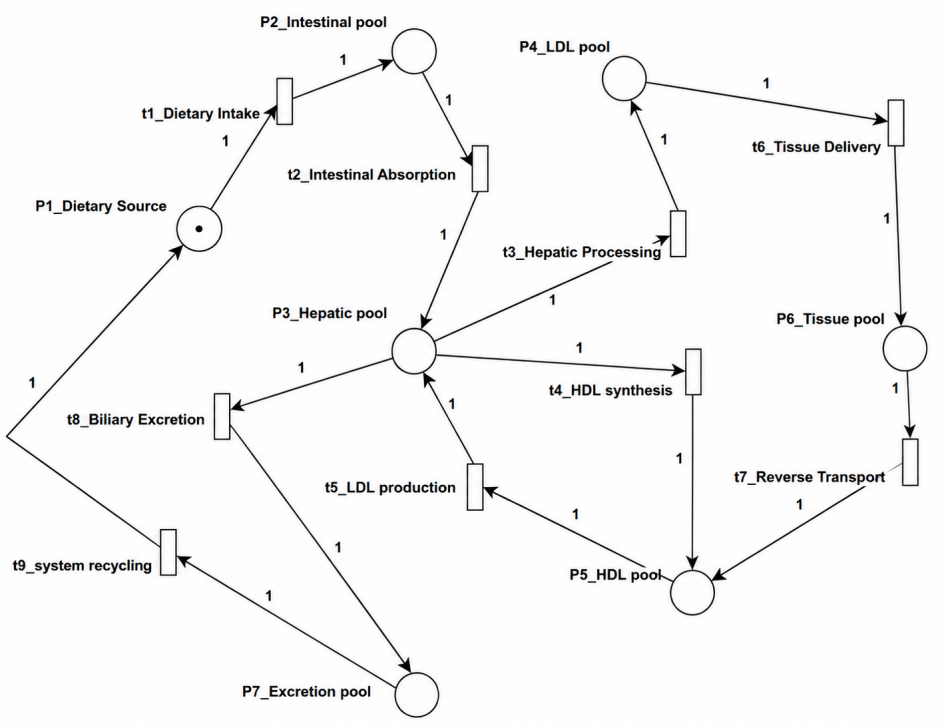


FIGURE 3. Fuzzy stochastic Petri net model of cholesterol metabolism.

4. Analysis Methodology

4.1. Uncertainty Analysis via α-Cuts. The analysis is carried out in two phases. Initially, the model is evaluated using the most likely (crisp) transition rates to obtain a baseline steady-state distribution. Subsequently, fuzzy transition rates are introduced, and uncertainty is propagated through the system using α-cut analysis, resulting in interval-valued steady-state probabilities. The crisp solution serves as a reference point for assessing the impact of parameter uncertainty, and the resulting interval estimates form the basis for the numerical results presented in the next section.

Although the parameters are fuzzy, the Petri net structure remains a discrete-event model, and all reachability computations are performed over a finite state space. Using the α-cut formulation defined earlier, the fuzzy transition rates are converted into interval-valued parameters, and steady-state probabilities are evaluated at each α-level, consistent with established fuzzy stochastic modeling approaches [16, 14]. For each sampled transition-rate realization within the corresponding α-cut intervals, a CTMC generator $Q(\lambda)$ is constructed and the associated stationary probability vector is calculated numerically. The collection of

TABLE 2. FSPN Components for Cholesterol Metabolism

Places (Metabolic Pools)

Component	Notation	Biological Meaning
Dietary Source	P_1	Cholesterol entering from diet
Intestinal Pool	P_2	Cholesterol during intestinal absorption
Hepatic Pool	P_3	Liver storage, synthesis, and processing
LDL Pool	P_4	Circulating LDL cholesterol
HDL Pool	P_5	Circulating HDL cholesterol
Tissue Pool	P_6	Peripheral tissue storage and demand
Excretion Pool	P_7	Cholesterol routed toward elimination

Transitions (Metabolic Processes)

Process	Transition	Modeled Flux
Dietary Intake	t_1	Dietary source $\to$ Intestinal pool ($P_1 \to P_2$)
Intestinal Absorption	t_2	Intestinal pool $\to$ Hepatic pool ($P_2 \to P_3$)
Hepatic Processing	t_3	Hepatic pool $\to$ LDL pool ($P_3 \to P_4$)
HDL Synthesis	t_4	Hepatic pool $\to$ HDL pool ($P_3 \to P_5$)
LDL Production	t_5	HDL pool $\to$ Hepatic pool ($P_5 \to P_3$)
Tissue Delivery	t_6	LDL pool $\to$ Tissue pool ($P_4 \to P_6$)
Reverse Transport	t_7	Tissue pool $\to$ HDL pool ($P_6 \to P_5$)
Biliary Excretion	t_8	Hepatic pool $\to$ Excretion pool ($P_3 \to P_7$)
System Recycling	t_9	Excretion pool $\to$ Dietary source ($P_7 \to P_1$)

TABLE 3. Fuzzy Transition Rates (Triangular Fuzzy Numbers $\tilde{\lambda}_i$)

ID	Transition	Flux	L_i	C_i	R_i
t_1	Dietary Intake	$P_1 \to P_2$	0.2	0.3	0.4
t_2	Intestinal Absorption	$P_2 \to P_3$	0.4	0.5	0.6
t_3	Hepatic Processing	$P_3 \to P_4$	0.6	0.8	1.0
t_4	HDL Synthesis	$P_3 \to P_5$	0.4	0.6	0.8
t_5	LDL Production	$P_5 \to P_3$	0.7	1.0	1.3
t_6	Tissue Delivery	$P_4 \to P_6$	0.5	0.7	0.9
t_7	Reverse Transport	$P_6 \to P_5$	0.3	0.5	0.7
t_8	Biliary Excretion	$P_3 \to P_7$	0.1	0.2	0.3
t_9	System Recycling	$P_7 \to P_1$	0.8	1.0	1.2

sampled stationary probabilities is then used to determine the lower and upper probability bounds at each α-level.

The collection of these results across all α-levels yields a fuzzy steady-state distribution that characterizes the range of long-term system behaviour under uncertainty in the transition rates. All stationary linear systems were solved numerically in MATLAB, and the resulting fuzzy probability intervals provide uncertainty bounds on the steady-state behaviour rather than single-point estimates.

4.2. Computation of Steady-State Probabilities. For the continuous-time Markov chain with infinitesimal generator $Q(\lambda)$ defined in (2.2), the steady-state

probability vector $\pi = (\pi_1, \pi_2, \ldots, \pi_N)$ satisfies the linear system

$$\pi Q = 0, \qquad \sum_{i=1}^{N} \pi_i = 1,$$

where N is the number of reachable states. The steady-state solution is obtained by solving a system of linear equations derived from the generator matrix, consistent with standard CTMC formulations. For the crisp parameter case ($\alpha = 1$), this system was solved numerically.

For fuzzy parameters, the α-cut intervals were propagated numerically using a Monte Carlo approach. For each α-cut, admissible transition-rate realizations were sampled within their corresponding intervals. For each realization, the corresponding CTMC stationary distribution was calculated by solving

$$\pi Q(\lambda) = 0, \qquad \sum_{i=1}^{N} \pi_i = 1.$$

The minimum and maximum stationary probabilities among the Monte Carlo realizations were used as numerical estimates of the lower and upper probability bounds at each α-level. Thus, the reported fuzzy probability intervals were obtained by α-cut Monte Carlo uncertainty propagation rather than by direct interval arithmetic on the stationary equations.

5. Theoretical Analysis and Model Properties

5.1. Structural Validation and System Liveness. Before computing steady-state probabilities, the structural integrity of the FSPN model was examined using the Petri Net Integrated Platform Environment (PIPE) tool. Table 4 summarizes the main structural results.

TABLE 4. Summary of Structural Validation Results (PIPE Analysis)

Property	**PIPE Result**	**Interpretation**
P-Invariant	$\sum M(P_i) = 1$	Mass is conserved across places; model is bounded
T-Invariant	Positive cycles present	Model admits cyclic behaviour (tokens continue circulating)
State-space	Bounded, safe, no deadlocks	Finite feasible state space; no trapping states

The reachability graph in Fig. 4 confirms that the system has a finite, deadlock-free state space. The existence of P-invariants ensures that the total number of tokens remains constant, thereby preventing unbounded accumulation. The inclusion of the recycling transition (t_9) eliminates absorbing states and ensures that all tokens eventually re-enter the system. These structural properties support the feasibility of steady-state analysis. Although a formal proof of ergodicity is not explicitly provided, the bounded, cyclic, and deadlock-free structure strongly indicates ergodic behaviour. These findings confirm that the model is bounded, safe,

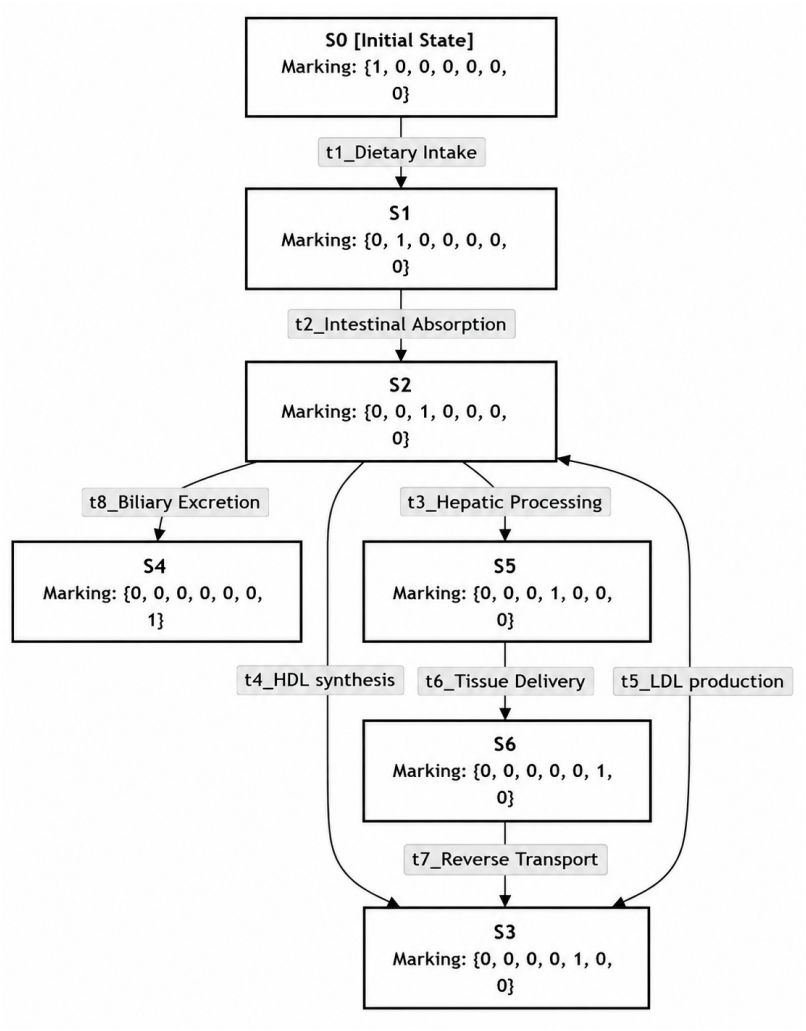


FIGURE 4. Reachability graph confirming bounded and deadlock-free behaviour of the FSPN model.

and structurally consistent. The existence of P-invariants ensures conservation of tokens across metabolic pools, while T-invariants indicate cyclic behaviour within the system. Together, these properties validate the correctness and stability of the proposed model.

5.2. Feasibility and Bounded Behaviour. Since the model preserves the total number of tokens, all steady-state probabilities satisfy

$$\sum_i \pi_i = 1,$$

and remain finite and non-negative for all feasible parameter values. This confirms that the model behaves in a physically meaningful way and does not predict unrealistic concentrations.

5.3. Existence and Uniqueness of Fuzzy Steady-State Solutions.

Theorem 5.1. *(Existence and Uniqueness) For each α-cut $\tilde{\Lambda}(\alpha) = [\Lambda^L(\alpha), \Lambda^R(\alpha)]$, the induced continuous-time Markov chain with generator $Q(\lambda)$, for any $\lambda \in \tilde{\Lambda}(\alpha)$, is finite, irreducible, and possesses a unique stationary distribution $\pi(\lambda)$.*

Proof. The initial marking M_0 contains one token, and the 1-token P-invariant established in Section 5.1 preserves the total number of tokens in the Petri net. Hence, every reachable marking contains exactly one token, and the net is 1-bounded. Since the model contains seven places, the set of reachable markings is finite. Consequently, the state space $\mathcal{S}$ of the underlying CTMC is finite. The inclusion of the recycling transition t_9 prevents the excretion state from becoming absorbing, and the validated cyclic structure ensures that the reachable CTMC states communicate under the modeled transitions. Thus, for each admissible transition-rate vector $\lambda \in \tilde{\Lambda}(\alpha)$, the induced CTMC is finite and irreducible. By the standard existence and uniqueness result for finite irreducible CTMCs [8], a unique stationary distribution $\pi(\lambda)$ exists. This holds for every admissible $\lambda \in \tilde{\Lambda}(\alpha)$ at each α-level. □

5.4. Sensitivity Analysis Methodology. Sensitivity analysis was performed to identify which transition rates exert the strongest influence on LDL cholesterol accumulation. Normalized sensitivity indices were computed via finite-difference approximations:

$$S_{\lambda_i} = \frac{\partial \pi_4}{\partial \lambda_i} \cdot \frac{\lambda_i}{\pi_4},$$

where π_4 denotes the steady-state probability of the LDL pool (P_4), and transition rates λ_i (hr^{-1}) represent fractional flow rates of cholesterol between metabolic pools. This sensitivity index quantifies the relative change in LDL pool occupancy resulting from a proportional change in the transition rate λ_i. The sensitivities are computed using a finite-difference approximation:

$$S_{\lambda_i} \approx \frac{\pi_4(\lambda_i + \Delta\lambda_i) - \pi_4(\lambda_i)}{\Delta\lambda_i} \cdot \frac{\lambda_i}{\pi_4(\lambda_i)},$$

with $\Delta\lambda_i = 0.01\lambda_i$ (1% perturbation). The computational cost increases with the number of α-levels and the size of the reachable state space. For the present model, the state space remains small and computationally tractable. However, scaling to larger biological systems may require state-space reduction techniques or approximate numerical methods in future work.

6. Results and Discussion

6.1. Numerical Implementation and Scenario Analysis. Numerical simulations were carried out in MATLAB, with reachability analysis performed in PIPE and fuzzy steady-state probabilities computed using the α-cut methodology described earlier. Three illustrative scenarios were examined:

Scenario 1: Baseline operation — All parameters at nominal fuzzy ranges (Table 3), producing balanced uncertainty-aware steady-state distributions representative of a typical metabolic state.

Scenario 2: Elevated LDL regime — Increasing LDL production (λ_3) by 20% and weakening reverse transport (λ_7) by 15% to simulate risk-prone metabolic configurations.

Scenario 3: Improved HDL support — Enhancing HDL synthesis (λ_4) by 25% to demonstrate a protective metabolic shift.

Detailed results for the baseline scenario (Scenario 1) are presented below. Scenarios 2 and 3 confirm that parameter adjustments produce biologically consistent shifts in cholesterol distribution, with Scenario 2 increasing LDL occupancy by 18.3% and Scenario 3 increasing HDL occupancy by 14.7% relative to baseline. These percentage changes were computed relative to the baseline Scenario 1 values.

6.2. Crisp Steady-State Results. Table 5 reports the crisp steady-state probabilities ($\alpha = 1$) computed using the most likely parameter values. These serve as a reference baseline against which the fuzzy uncertainty intervals can be compared.

TABLE 5. Stage 1: Crisp Steady-State Probabilities (π_C) at $\alpha = 1$

Pool ID	**Metabolic Pool Name**	$\pi_{\mathbf{C}}$
P1	Dietary Source	0.1114
P2	Intestinal Pool	0.0706
P3	Hepatic Pool	0.1557
P4	LDL Pool (Critical)	0.1744
P5	HDL Pool (Optimal)	0.2176
P6	Tissue Pool	0.2351
P7	Excretion Pool	0.0352
Sum		**1.0000**

All computed probability vectors satisfy $\sum_{i=1}^{7} \pi_i = 1.0000 \pm 0.0001$ (numerical tolerance). The crisp results show that cholesterol is distributed across all pools, with the tissue pool (P_6) having the highest steady-state probability (23.51%), followed by the HDL pool (P_5, 21.76%) and LDL pool (P_4, 17.44%). These results serve as a baseline for evaluating the impact of parameter uncertainty in the fuzzy analysis.

6.3. Fuzzy Steady-State Results with Uncertainty Intervals. Table 6 presents the interval-valued fuzzy steady-state probabilities obtained through α-cut analysis, while Figure 5 visualizes the uncertainty ranges for each metabolic pool.

Relative uncertainty for each pool is calculated as:

$$\text{Rel. Uncertainty} = \frac{\pi^R - \pi^L}{\pi^C} \times 100$$

where π^L, π^C, π^R denote lower, center, and upper probability bounds.

TABLE 6. Fuzzy Steady-State Probabilities Across All Metabolic Pools

State	Pool	π^L	π^C	π^R	Width	Rel. Uncertainty
P1	Dietary	0.0508	0.1114	0.1490	0.0982	88.15%
P2	Intestinal	0.0300	0.0706	0.0807	0.0507	71.81%
P3	Hepatic	0.1248	0.1557	0.2008	0.0760	48.81%
P4	LDL (Critical)	0.1350	0.1744	0.2324	0.0974	55.85%
P5	HDL (Optimal)	0.1651	0.2176	0.2902	0.1251	57.50%
P6	Tissue	0.2044	0.2351	0.3669	0.1625	69.12%
P7	Excretion	0.0149	0.0352	0.0397	0.0248	70.45%

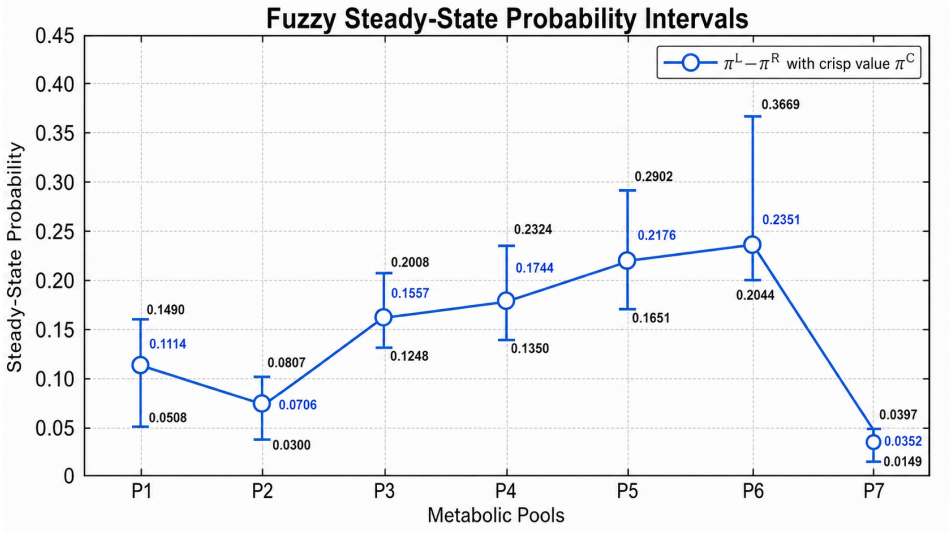


FIGURE 5. Fuzzy steady-state probability intervals for all metabolic pools. The central markers represent crisp steady-state probabilities ($\alpha = 1$), while the vertical bars denote the lower and upper bounds obtained through α-cut analysis.

The fuzzy results reveal substantial uncertainty across all pools, with relative uncertainties ranging from 48.81% (hepatic pool) to 88.15% (dietary source). The LDL pool shows a relative uncertainty of 55.85%, indicating significant variability in LDL cholesterol levels due to parameter imprecision. This highlights the sensitivity of LDL dynamics to parameter uncertainty. These findings further support the importance of incorporating uncertainty-aware modeling in analyzing complex metabolic systems. The steady-state results obtained using the analytical formulation are consistent with numerical simulation results generated using PIPE, confirming the validity of the proposed modeling approach.

6.4. Sensitivity Analysis Results. Numerical sensitivity analysis identified the LDL production pathway (t_3) and reverse transport (t_7) as the most influential parameters. For nominal (crisp) parameter values, the computed sensitivity indices are:

$$S_{\lambda_3} \approx 1.2 \quad \text{and} \quad S_{\lambda_7} \approx -0.9,$$

quantitatively confirming that LDL production (λ_3) and reverse transport (λ_7) exert dominant control over LDL accumulation. A positive S_{λ_3} indicates that increasing LDL production rate increases LDL pool occupancy, while a negative S_{λ_7} shows that enhancing reverse transport reduces LDL accumulation. Figure 6 illustrates how variations in λ_3 and λ_7 influence the LDL steady-state probability.

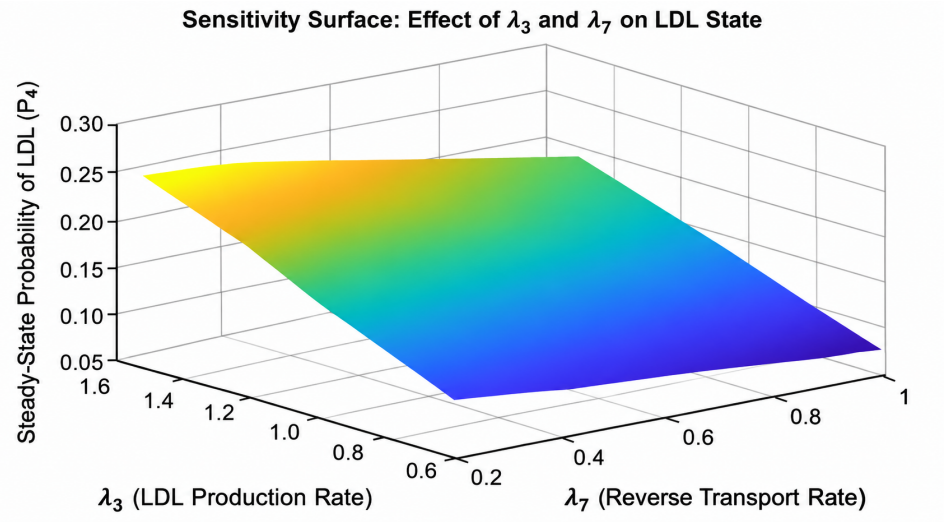


FIGURE 6. Sensitivity surface showing the effect of LDL production rate (λ_3) and reverse transport rate (λ_7) on the steady-state probability of the LDL pool (P_4). Increasing λ_3 increases LDL accumulation, whereas increasing λ_7 reduces LDL occupancy, consistent with biological expectations.

6.5. Clinical Relevance and Model Validation. The relative uncertainty of 55.85% obtained for the LDL pool (P_4) is consistent with clinical observations of substantial inter-individual variability in LDL cholesterol levels, as reported in population studies [5, 4]. This agreement indicates that the fuzzy probability intervals reflect genuine metabolic heterogeneity rather than modeling bias.

Unlike deterministic models that yield single-point estimates, the proposed framework provides uncertainty intervals that better represent biological variability in human populations. This makes the model particularly suitable for personalized metabolic assessment, where parameter uncertainty must be explicitly

quantified. The framework allows uncertainty-aware assessment of metabolic behaviour by producing probability intervals instead of single deterministic outputs. This supports decision-making under uncertainty, such as evaluating pathway risks or assessing intervention effects.

6.6. Comparative Analysis with Existing Approaches. The fuzzy SPN framework addresses several limitations of traditional biological modeling approaches. Table 7 summarizes the conceptual differences.

TABLE 7. Comparison of Modeling Approaches for Biological Systems

Model Type	**Uncertainty Handling**	**Dynamics Representation**	**Mathematical Basis**	**Limitation**
ODE Models	Point parameter values	Continuous flows	Differential equations	Cannot capture discrete molecular events
Crisp SPN [7]	No parameter uncertainty	Discrete stochastic behaviour	Markov chains	Assumes precise transition rates
Fuzzy Logic	Linguistic uncertainty	Rule-based	Fuzzy set theory	No temporal dynamics
Monte Carlo	Random variability	Sampling-based	Probability theory	High computational effort
Fuzzy SPN (This study)	**Epistemic + stochastic uncertainty**	**Discrete stochastic with fuzzy intervals**	**Fuzzy Markov processes**	**Computational cost increases with system size**

Epistemic uncertainty refers to uncertainty arising from limited biological knowledge and measurement variability. This comparison shows that the proposed model integrates discrete stochastic behaviour with explicit uncertainty quantification, providing probability intervals that capture parameter imprecision. This integration of fuzzy uncertainty with stochastic Petri net dynamics for metabolic pathway analysis represents a methodological contribution beyond conventional deterministic or purely stochastic models.

6.7. Limitations. This study assumes triangular fuzzy numbers for all transition rates, which may not capture asymmetric or multi-modal uncertainty distributions. The recycling transition t_9 is a structural simplification introduced to enable steady-state analysis by preventing the excretion pool from becoming absorbing. It does not represent a literal physiological re circulation mechanism. Accordingly, the resulting stationary probabilities should be interpreted as normalized relative pool occupancies or residence-time fractions within the closed analytical representation, rather than as direct measures of absolute cholesterol concentrations, pool masses, or net excretion rates in the corresponding open physiological system. Computational cost may increase for larger biological networks, although the current cholesterol metabolism model remains tractable. Future work could

explore more complex fuzzy number representations, open-system formulations, and scalability improvements.

7. Conclusion and Future Work

This study develops a fuzzy stochastic Petri net framework for modeling cholesterol metabolism by integrating discrete-event dynamics with fuzzy parameter uncertainty. Structural validation confirms that the model is bounded, cyclic, and free of deadlocks, ensuring the feasibility of steady-state analysis. The α-cut approach produces interval-valued steady-state probabilities that capture the effects of biological parameter imprecision on metabolic behaviour.

The results demonstrate how uncertainty in transition rates influences the distribution of cholesterol across LDL, HDL, tissue, and excretion pools. In particular, the uncertainty range observed for the LDL pool aligns with clinically reported inter-individual variability, highlighting the relevance of uncertainty-aware modeling for metabolic assessment. These findings indicate that the proposed framework provides a more informative representation of metabolic dynamics compared to conventional deterministic approaches. Future work will focus on incorporating real patient data, extending the framework to larger biological networks, investigating non-Markovian transitions, and refining fuzzy parameter estimation methods to improve physiological realism.

Acknowledgment. The authors would like to express their sincere gratitude to the editors and anonymous reviewers for their valuable comments and constructive feedback, which significantly contributed to the improvement of this paper.

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