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Pesticide Residue Induced Hepatotoxicity: Determination for Animal Studies Based on Fuzzy Logic

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Abstract: Exposure to an excessive number of environmental stressors, such as pesticides, is regarded to be hazardous to health and a substantial contributor to the majority of public health diseases. Numerous physiological activities have been demonstrated to alter the pesticide's hepatotoxicity. Hepatotoxicity is a term that refers to a pesticide's intrinsic propensity to cause harm or death, demonstrating how deadly the chemical is. Hepatotoxicity leads to many diseases with preliminary examination shows inflammation in liver when pesticide residues more in the blood than the liver is able to manage. Pesticides such as Endosulfan, Phorate and Fenvalerate causes harmful effects in mammalian system. The liver of rats treated with Endosulfan exhibited leukocyte infiltration, activated Kupffer cells, sinusoidal dilation of the circulation. Phorate treated rats causes bile pigmentation and necrotic granulomas. Fenvalerate treated rats' liver section shows hepatocellular necro inflammation and hepatocellur damage.

Keywords: Pesticides, Endosulfan, Phorate, Fenvalerate, Pesticide Residue, Hepatotoxicity.

1 Introduction

Pest management and the eradication of diseases caused by insects are two ways that pesticides have significantly increased agricultural productivity. In Silent Spring, the toxicological consequences of pesticide use were revealed, including the destruction of animals and tainted food for humans. Pesticide use in agriculture has also been linked to the "green revolution."

Pesticides were one of the most essential parts of agricultural practices during the Green Revolution. For agricultural pesticide management, the green revolution required more pesticides than conventional agricultural systems since most high-yield varieties were not generally resistant to pests and diseases, in part because of the monoculture method used by the revolution [1,2,3,4]. Pesticides have improved the efficiency of plant cultivation and the amount of food that can be produced. Consumer organisations have expressed tremendous worry about the possible impact of trace quantities of leftover pesticide residue on food items, generating a need that the food we eat be safe.

1.1 Objectives of this Study

Fuzzy based application of Endosulfan, Phorate and Fenvalerate as pesticide residues and their hepatotoxic potentials in animals are the aims of this study. This research aims to:

1. Fuzzy logic Facing uncertainty and variability in the incidence of toxicity assessments, a fuzzy logic model to quantify and model the signs of liver toxicity that result from exposure to pesticides.
2. Use of fuzzy logic to delineate the complex and graded hepatotoxic responses expressed by mice

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across a broad range of pesticide doses to enable determination of safe dosage levels for animal studies.
3. Fuzzy logic-based dose estimation framework in toxicology studies—Fuzzy logic provides an intelligent method for classification of hepatotoxicity (high, medium, low) and provides fuzzy classifications for dose-response relationships.
4. Advance the accuracy and dependability of toxicity assessments, as applicable to risk assessments of pesticides and other toxic agents for both animal and, possibly, human health, providing a significant benefit to public health and safety.

Ultimately, we hope this research will contribute to the development of an approach for more robust toxicity assessment of pesticide residues in microorganisms, with greater flexibility and accuracy, to inform the risks posed by pesticides in biological systems.

1.2 About Fuzzy Logics

A mathematical paradigm known as fuzzy logic addresses ambiguity and imprecision in thinking and decision-making. It is a sort of logic that, unlike classical or binary logic, which is based on rigid true or false values, allows for intermediate or partial degrees of truth.

At the core of fuzzy logic, there is fuzzy sets, which can represent information about uncertainty and vagueness. In classical sets, an element either has nothing to do with a set, or completely belongs to it; in a given fuzzy set an element present can only be a member or may be non-member of the set to a certain amount of degree. Fuzziness, or degree of membership is represented here as a assigned membership function, which assigns a value only between 0 and 1 to each element of the corresponding set [5,6,7,8].

Fuzzy logic has been widely applied in engineering, computer science, decision making, control systems, and artificial intelligence, among others. Fuzzy logic is particularly useful when the information is ambiguous, vague, or incomplete, and traditional binary logic may not fully capture the complexity of the problem.

This is one of the most-biggest advantages of fuzzy logic that which can handle uncertainty and ambiguity with high flexibility and intuitiveness. It allows for visualisation of uncertain or qualitative data and provides a framework for inference in situations of partial or ambiguous information. Fuzzy logic can also be used in combination with other mathematical processes and methods to develop hybrid systems that exploit the benefits of several approaches [9,10,11].

In summary, the fuzzy logics is a powerful and flexible mathematical numerical framework that provides decision-makers and reasoners with a method of dealing with uncertainty and imprecision. This has widespread and now growing application in many other industries as means for coping with a multi-faceted dynamic and volatile environment. Future research directions may include establishing fuzzy logic theory and applications or discovering new contexts in which fuzzy logic can be applied to help solve real-life problems.

1.3 Literature Review of Fuzzy Logic Applications in Toxicology

Fuzzy logic has received increasing attention in toxicology because it is able to deal with uncertainty and ambiguity in ways that other traditional quantitative models may not be capable of handling with complex biological systems. Originally introduced by Zadeh [12], fuzzy logic has become a robust and effective tool across scientific disciplines, particularly in toxicology, where the complex interaction of exposure levels, individual susceptibility, and other factors dictate the variability of toxic responses. The flexibility is crucial when evaluating toxicity in biological systems where no clear lines exist between parameters or when they overlap [12,13,14].

Fuzzy logic is used extensively in the field of toxicological studies, especially in dose-response analysis settings. Established approaches tend to use sharp thresholds for toxicity and do not account for the gradual transition of toxic effects with exposure doses. Previous studies, e.g. Sugeno [15] and Jang [16], showed us that fuzzy logic is a powerful tool for modelling this continuum, therefore it is possible to assess toxicity in such a way that rather than representing substances as simply toxic or non-toxic, degrees of harm can be included in the assessment. These methods become vital when the change in effect is gradual over the doses/concentrations (i.e., dose-response curves) instead of passing a straight threshold [15,17,18,19].

Fuzzy logic is also adopted in toxicity assessment of a specific organ damage like hepatotoxicity, as these toxicities have multiple interacting physiological parameters. For example, Dubois and Prade [20] modelled cellular responses (absence/presence and/or level of molecular expression) under toxic exposure using fuzzy sets in order to define intermediary states of liver damage as a response to indicators such as enzyme levels and histopathological changes. Using fuzzy logic, these responses can be categorized as mild, moderate, or severe depending on partial membership (rather than absolute membership) and thus allow a relative assessment and more realistic appraisal of toxicity levels [21,22].

Recently, the various application of fuzzy logic has been widened in the very much use of descriptors for many other toxicological phenomena. For example, the important study conducted by Bagchi et al. [23] employed a fuzzy logic approach to assess liver toxicity in rats subjected to pesticides by measuring oxidative stress markers. Due to the flexibility of fuzzy sets, the authors managed to consider parameters such as change in enzyme activity, an important parameter for the

assessment of the response of liver to toxicants, Similarly Geluso et al. [24]. Fuzzy models have also been used to examine the effect of pesticide-induced oxidative stress on non-target species [4], which demonstrates the power of fuzzy logic as a tool to express the continuous and graded nature of toxic responses [24,25,26,27].

Additionally, fuzzy logic approaches in conjunction with other computational methods, such as artificial neural networks have demonstrated potential improvements in predictive performance in toxicology. In the terms of the ANFIS proposed by Jang [16], an adaptive network presented unique combination of neural network and fuzzy logic concepts to find its application in estimating complex toxicity data, allowing better interpretation of different hepatotoxicity levels via integrated fuzzy logic and neural network algorithms. Such a hybrid approach provides the powerful combination of empirical biological insights with computational modelling that together can reliably predict dose-dependent responses in liver tissues exposed to counterfactual toxic agents, and thus addresses the challenges posed for plausible applicability to biomedical research [14,16].

Fuzzy logic in toxicology summarizes the basic principles of fuzzy logic and some of its applications in toxicology by providing a sophisticated method for processing the uncertainty and variability associated with biological responses to toxins. As a fuzzy rule can accommodate continuous changes in toxicity and is efficiently combined with other computational methods, the application of fuzzy logic increases the accuracy and interpretability of toxicity evaluations, especially in the fields of hepatotoxicity and environmental toxicology [28].

Membership Function Definition: Introduce membership functions to handle uncertainty in toxicity levels. Membership functions are used to define fuzzy sets for pesticide residue levels (L: Low, M: Medium, H: High):

$$\mu_L(x) = \begin{cases} 1, & x \le a \\ \frac{b-x}{b-a}, & a < x \le b \\ 0, & x > b \end{cases}$$

$$\mu_M(x) = \begin{cases} 0, & x \le a \\ \frac{x-a}{b-a}, & a < x \le b \\ \frac{c-x}{c-b}, & b < x \le c \\ 0, & x > c \end{cases}$$

where a,b,c define thresholds for residue levels (e.g., a=0,b=1,c=10).

Fuzzy Entropy for Uncertainty Quantification

To assess classification uncertainty, fuzzy entropy is calculated:

$$H = -\sum_i \mu_i log(\mu_i)$$

where μ_i are membership values. Higher entropy indicates greater overlap among classes. For example, with membership values μ=[0.7,0.3]:

$$H = -(0.7log(0.7) + 0.3 + log(0.3)) = 0.61$$

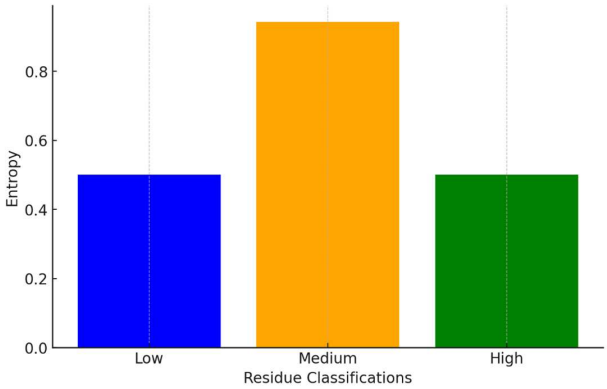

Fig. 1: Fuzzy Entropy for Residue Classifications

This bar chart displays the entropy values for ”Low,” ”Medium,” and ”High” residue classifications, quantifying the uncertainty in each classification.

2 Hepatotoxicity

2.1 Acute and chronic hepatotoxicity

Hepatotoxicity refers to the potential of a substance to cause liver damage. This can be categorized into two main types: acute and chronic.

Acute Hepatotoxicity: Acute hepatotoxicity refers to liver damage induced by a drug or toxin after a single dose or brief period of exposure. Hurt can be evident inside a number of hours to a number of days after publicity. Acute hepatotoxicity is often associated with rapid onset liver cell injury or failure, therefore leading to supportive signs such as jaundice, abdominal pain and abnormal liver enzymes. However, certain medications may have acute liver toxicity as side effect especially in overdose situations [29].

Chronic Hepatotoxicity: Chronic hepatotoxicity occurs from persistent or repeated contact with a toxicant for a long duration. Unlike an early response, there is a gradual impact on the liver that can go on to cause fibrosis (scarring of the liver) or cirrhosis (extensive scarring and loss of liver function). You may see this type of toxicity if you drink alcohol heavily over the long term, take certain medications for an extended period, or come in contact with certain industrial chemicals. Early signs of chronic

liver injury could take everything from psychological fatigue to weight loss, jaundice, and liver failure.

In both cases, the severity of hepatotoxicity is influenced by factors such as the dose, duration of exposure, individual susceptibility, and pre-existing liver conditions. Recognizing the type of hepatotoxicity is important for the evaluation, management, and prevention of liver health risks from diverse agents. Qualitatively defined, it is the capacity of a chemical to damage after a single exposure or dose. The chronic hepatic toxicity, which is the ability of the substance to exert its effects over time owing to repeated doses or exposures [30].

2.2 LD50 value

The lethal dosage, or LD50 value, is a term used to describe a pesticide's relative amount of hepatotoxicity. This value indicates the dosage necessary to eliminate 50% of a test population.

2.3 Liver as a target organ

The liver is a main location of detoxification and a target organ. It is the primary location of intensive metabolism and is thus prone to a variety of illnesses as a result of exposure to extrinsic and intrinsic toxins. The liver is important for metabolism because it assists the body in maintaining an adequate supply of energy and structural stability [31]. Additionally, at the biotransformation site, the process of hazardous chemical is transformed into a less toxic form to minimise hepatotoxicity [28]. This, however, will result in liver cell destruction and hepatotoxicity. Acid phosphatase-ACP, Aspartate transaminase-AST, Alkaline phosphatase-ALP & Alanine transaminase-ALT are all indications of hepatotoxicity, and variations in their activity in the liver may suggest damage to the liver. One of enzyme in mitochondria is Aspartate transaminase found in skeletal muscle, liver, and kidneys. Cytosolic enzyme is Alanine transaminase found only in the liver.

3 Pesticide Induced Hepatotoxicity

3.1 Endosulfan induced hepatotoxicity

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide) (Figure 2) is classified as a polycyclic chlorinated hydrocarbon insecticide. Endosulfan, classified as yellow label (highly toxic) pesticide by the Central Insecticides Board, is also the biggest producer and end user of endosulfan in the world. Endosulfan is a potent neurotoxin highly toxic to insects as well as to larger animals, including humans. From the 30 mg/kg rat LD50, it is classed as Class I: "Highly Acutely Toxic," by the United States Environmental Protection Agency, while from the 80 mg/kg rat LD50 WHO classifies as Class II: "Moderately Hazardous".

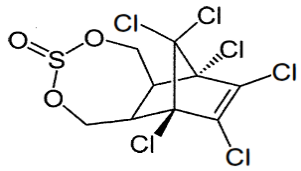

Fig. 2: Structure of Endosulfan

Endosulfan is commonly utilised in the majority of India's plantation crops. Endosulfan's hepatotoxicity and health consequences of bioaccumulation came to public notice when health problems in Kasargod area in the state of Kerala was heralded, and the pesticide was subsequently prohibited. Because of its severe hepatotoxicity, endocrine disruptor and bioaccumulation potential, endosulfan became a very contentious agrichemical. Endosulfan is rapidly metabolised by microsomal enzymes in animals, first to endosulfan sulphate and then to endosulfan diol, finally eliminated in the faeces & urine. Also discovered that it is dispersed throughout the adipose, muscle tissues, heart, liver, prostate, spleen, testes, milk, kidney, epididymis and seminal vesicle.

3.2 Phorate induced hepatotoxicity

Phorate (Figure 3) is the O, O-diethyl S-[(ethylthio) methyl] ester of respective phosphorodithioic acid. (Thimet; Timet). Phorate is an organophosphorus (OP) systemic insecticide that is widely applied to chewing pests & control sucking in agriculture. Additionally, used on root and field crops such as coffee, maize & cotton, as well as a variety of ornamental plants and bulbs, as well as pine forests [32].

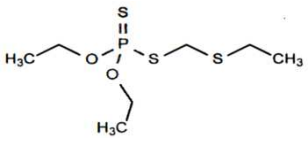

Fig. 3: Structure of phorate

The biotransformation of phorate into a phosphorothiolate ester involves oxidation of the thioether moiety to the corresponding sulphoxide and sulphone, and desulphurizsation of the P = S moiety to P

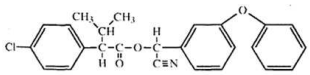

Fig. 4: Structure of Fenvalerate

= O. Phorate (OT) is a high-risk hazardous OP chemical with a median lethal dose (LD50) of 1.1-3, and is classified as Class I. rat body weight (7 mg/kg). Everyone knows that if the hydroxyl group of serine of the substrate binding domain is phosphorylated, the action of acetylcholinesterase will be inhibited, resulting in the accumulation of acetylcholine and neurotoxicity [33].

3.3 Fenvalerate induced hepatotoxicity

Fenvalerate (Figure 4), a synthetic type II like pyrethroid insecticide, is a mixture of four(4) optical isomers: cyano (3-phenoxyphenyl) methyl 4-chloro-alpha-(lmethylethyl) benzene acetate -22 percent of the [2S, aS] isomer; 28% of the [2S, aR] isomer; 22% of the [2R, aR] isomer; and 28% of the [2R, aS] isomer. The liver is the primary location of fenvalerate metabolism, with hepatic microsomal esterases hydrolyzing fenvalerate to produce fenvaleric acid. In mammals, fenvalerate causes considerable hepatotoxicity; oral LD50 values range from 100 to over 3000 mg/kg for a range of rodent species.

Synthetic pyrethroids, a new class of agricultural pesticides, are more effective as insecticides than organophosphate and organochlorine. Among the pyrethroid insecticides, fenvalerate and cypermethrin are highly hepatotoxic to a wide range of insects including resistant strains, but are low in hepatotoxicity in mammals and birds and are rapidly biodegradable [35]. The extensive use of pyrethroids is not risk-free, however, as it creates a risk for non-target species (birds, animals, soil and waterborne organisms). Although the metabolism of pyrethroids in animals occurs rapidly, a potential role of oxidative stress has not been explored. Study data is used to determine the total residues that can buildup in daily food. This allows for the calculation of total residual concentrations of three pesticides from each insecticide class, which are effectively ubiquitous in all samples. Calculate the cumulative residual concentration for each day value to year values and then to life time values. By providing equal dose in terms of food to rats and comparing various liver hepatotoxicity tests, the hepatotoxic potential of the pesticides may be derived [36].

3.4 LD50 Estimation

To calculate the median lethal dose for a pesticide:

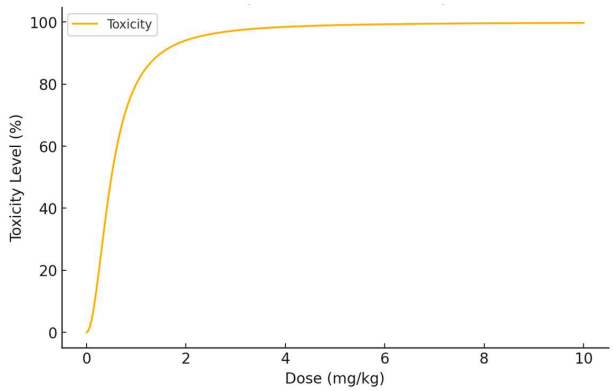

Fig. 5: Dose-Response Curve (Hill Equation)

$$LD50 = \frac{\sum_{i=1}^{n} D_i}{n}$$

Where: D_i is the dose administered to the i^{th} test subject, n is the total number of test subjects.

For example, if the doses D_i=30,50,70,90mg/kg were tested across 4 rats (n=4), then:

$$LD50 = \frac{30+50+70+90}{4} = 60mg/kg$$

Residue Accumulation

The cumulative pesticide residue over time:

$$R(t) = R_0 t$$

Where: R_0 is the annual residue (13mg/kg/ year for Endosulfan), t is the time in years.

For a 15-year period:

$$R(15) = 13 * 15 = 195mg/kg$$

Nonlinear Regression for Dose-Response Curves

The relationship between dose (D) and toxicity (T) is modeled using a Hill equation:

$$T(D) = T_{max}\frac{D^n}{K^n + D^n}$$

Where: T_{max}=100: maximum toxicity, K=0.5: half-maximal dose, n=2: Hill coefficient.

Example Fit: For a dose range of D=[0,10], the toxicity curve reflects a gradual increase.

4 Physiological activities of pesticides

Dosage estimation for animal experiments. Using the information acquired from the daily residual concentration, the quantity of residue that may accumulate over the course of one year, fifteen years, and thirty years is calculated. Finding the comparable values

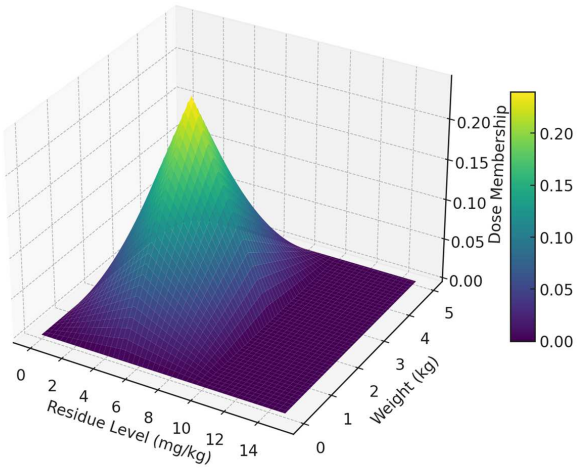

Fig. 6: Fuzzy Surface Plot: Dose as a Function Residue and Weight

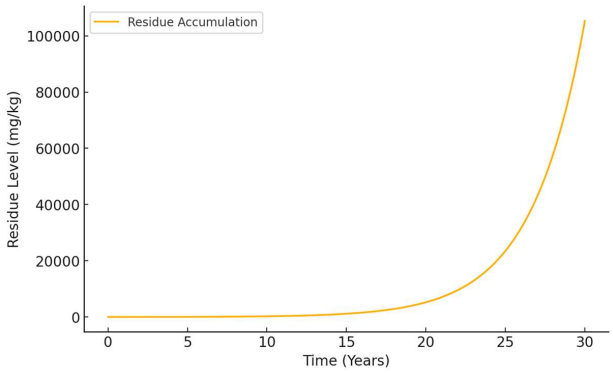

Fig. 7: Time-Dependent Residue Accumulation

for animals allows one to establish the dose for animal therapy in various studies.

This 3D surface illustrates how dose membership depends on residue levels and animal weight. It provides a visual tool for understanding dose allocation in a fuzzy logic system.

Endosulfan

–The maximum amount of residue that can build up in a year is 13 milligram/kg.
–Animals' corresponding respective value (b.w.0.250) is (13 x 0.250)/60, or 0.054 milligram/kg.
–The amount/number of residues acquired after 15-years is equal to 0.81 milligram/kg (0.054 x 15).
–The amount/number of residues that accumulated over 30-years is 1.62 milligram/kg (0.054 x 30).

Phorate

–The maximum amount/number of residue that can build up in a year is 31.7 milligram/kg.
–Animals' corresponding value (b.w. 0.250) is equal to (31.7 x 0.250)/60, or 0.13 milligram/kg.
–The amount/number of residues accumulated after 15-years is equal to 0.13 x 15 = 1.95 milligram/kg.
–The amount/number of residues that have built up over 30-years is equal to 0.13 x 30 = 3.9 milligram/kg.

Fenvalerate

–The maximum amount/number of residue that can build up in a year is 144 milligram/kg.
–The corresponding figure for animals with a body weight of 0.250 is (144 x 0.250)/60, or 0.6 milligram/kg.
–The amount/number of residues that accumulated after 15 years is equal to 0.6 x 15= 9 milligram/kg.
–The amount/number of residues accumulated after 30 years is equal to 0.6 x 30 = 18 mg/kg.=18 milligram/kg

The doses are very much fixed based on the above-mentioned values and accordingly respective animals are grouped as follows.

Table 1: Group of animals with pesticide concentration

Groups	Concentration of Pesticide (mg/kg) (groundnut oil)
C	-
E1	0.05 (Endosulfan)
E2	0.1 (Endosulfan)
E3	1.5 (Endosulfan)
P1	0.1 (Phorate)
P2	2.0 (Phorate)
P3	4.0 (Phorate)
F1	0.5 (Fenvalerate)
F2	10.0 (Fenvalerate)
F3	20.0 (Fenvalerate)

The considered rats were treated/exposed with various dosages of chosen type of pesticides to determine the required antioxidant state of the cells, the expression of certain enzyme markers, and the histology of liver tissues. Various tissues are analysed to evaluate the extent to which residues are distributed and accumulate in various organs.

Time-Dependent Residue Accumulation Model

The dynamics of residue accumulation are modeled as:

$$\frac{dR(t)}{dt} = kR(t) - \lambda R(t)$$

Where: R(t): residue concentration, k: input rate, λ: elimination rate.

Solution:

$$R(t) = R_0 e^{(k-\lambda)t}$$

For example, with R_0=13mg/kg/ year, k=0.5, and λ=0.2:

$$R(t) = 13e^{(0.5-0.2)t}$$

4.1 Pesticide administration

The pesticides levels in the heart, brain, kidney, lungs, & liver of rats following daily oral dosing for 90 days are shown in Table 1. Endosulfan was found to be spread as follows in rat tissues: liver>kidney>lungs>brain>heart. As seen in the table, endosulfan, like other organochlorine pesticides, has a practice to collect in these organs due to its lipotropic nature. phorate was distributed as follows in rat tissues: liver>kidney>lungs>heart>brain. Phorate was shown to accumulate most heavily in the liver and least heavily in the brain. Similarly, fenvalerate was distributed as follows in rat tissues: liver>kidney>lungs>heart. Fenvalerate also accumulated most abundantly in the liver and least abundantly in the heart, but the residue was found to be absent in the brain.

Table 2: The tissue residual concentration in various organs following oral administration of endosulfan, phorate and fenvalerate for 90 days

Substrates	Concentration in mg/kg		
	Endosulfan	Phorate	Fenvalerate
Liver	2.45±0.11	1.92±0.09	0.33
Kidney	1.28±0.05	0.71±0.14	0.21±0.42
Lungs	0.78±0.12	0.42±0.03	0.19±0.22
Brain	0.46±0.38	0.11±0.08	0
Heart	0.21±0.03	0.35±0.05	0.08±0.09

4.2 Histopathology

Figures 8, 9 and 10 illustrate histopathological examinations of liver tissues from rats treated with Endosulfan, Phorate, and Fenvalerate. The results showed that pesticides had a moderate to significant effect on the liver. The liver of rats treated with endosulfan exhibited leukocyte infiltration, activated Kupffer cells, sinusoidal dilatation of the circulation, and significant cytoplasmic vacuolization (Figure 8). All of these characteristics refer to hepatocellular injury.

C: Control group liver slices showing normal histology, including hepatic cells-HC, central vein-CV, Kupffer cells-KC, blood sinusoids-BS, and centrally placed nuclei. (N). EI-E3: endosulfan-treated liver sections show pyknotic nuclei-PN, fatty infiltrations-FD, activated Kupffer cells-KC, dilatation in blood sinusoids-DBS, and cytoplasmic vacuoles-VC.

Phorate-treated rat livers revealed widespread granular degeneration from moderate to medium, large vesicular fatty alteration, and minor periportal multifocal lymphocytic/leucocytic infiltration. (Figure 9). Bile discoloration and necrotic granulomas were evident. This validated pesticide hepatotoxicity-induced hepatocellular damage.

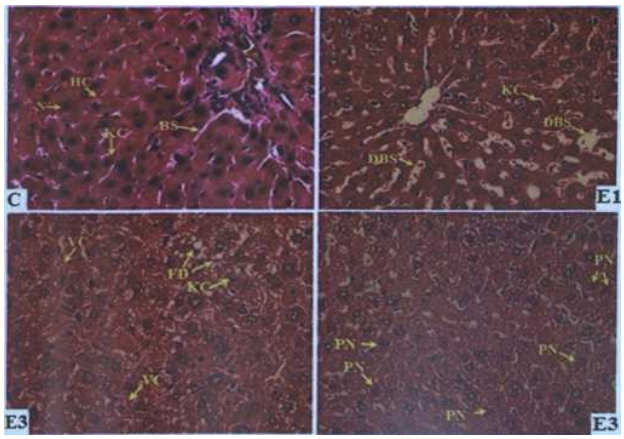

Fig. 8: A Photomicrograph of treated rat liver after 90 days of daily oral endosulfan at varied dosages

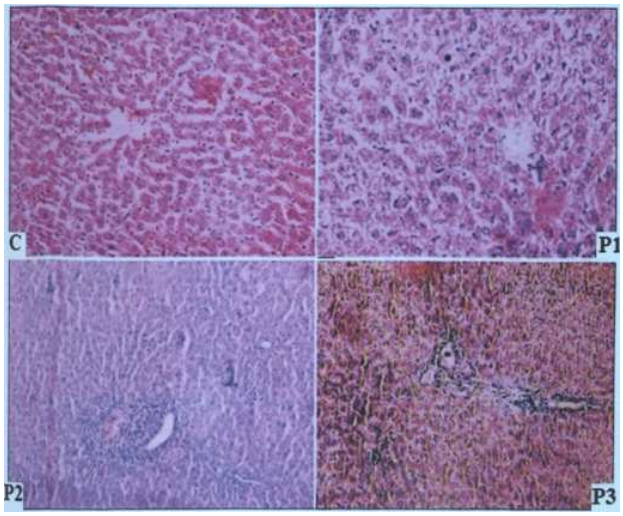

Fig. 9: Rat liver after 90 days of daily oral phorate treatment at varying dosages

C- Control rat liver, P1–P3: In phorate-treated rats, liver samples revealed mild to moderate diffuse granular degeneration and macro vesicular fatty alteration and focal periportal multifocal lymphocytic/leucocytic infiltration. P3, showing features of necrosis, to bile colour and necrotic granulomas.

Leukocyte infiltrations, blood sinusoids dilating, activated Kupffer cells, significant cytoplasmic vacuolization, hepatocellular necro-inflammation, mild to strong multifocal fatty degeneration, and necrosis were all seen in fenvalerate-treated rat liver slices. (Figure 10). All of these characteristics point to moderate to severe hepatocellular injury.

C: Hepatic cells (”HC”), blood sinusoids (”BS”), Kupffer cells (”KC”), centrally positioned nuclei (N), and the central vein are all visible in the liver sections of

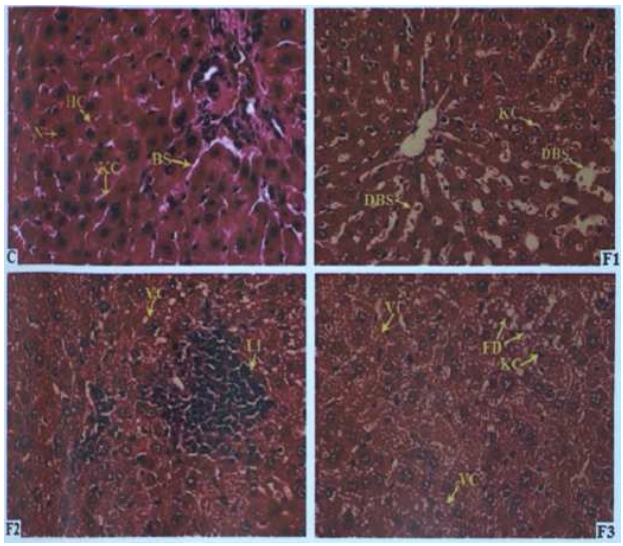

Fig. 10: Fenvalerate-treated rat liver after 90 days of oral treatment

control animals, demonstrating the organ's typical histological appearance. (CV). F1-F3: Pyknotic nuclei-PN, inflammatory leukocyte infiltrations-LI, activated Kupffer cells-KC, fatty infiltrations-FD, cytoplasmic vacuoles-YC, and dilatation in blood sinusoids are seen in the liver sections of fenvalerate-treated rats (F1-0.05, F2-0.1, and F3-1.0 mg/kg). (DBS).

4.3 Physiological Activities of Pesticides

Dose Estimation for Animals

Calculate doses for animals based on residue accumulation:

$$D = \frac{R * W}{CF}$$

Where: R is the residue concentration (13mg/kg for Endosulfan), W is the animal's weight (0.25kg), CF is the conversion factor (60).

For a rat:

$$D = \frac{13 * 0.25}{60} = 0.054 mg/kg$$

Fuzzy Set Definitions for Dose Levels
Categorize doses into fuzzy sets:
Low Dose: D $\leq$ 0.1mg/kg,
Medium Dose: 0.1<D$\leq$1.0mg/kg,
High Dose: D>1.0mg/kg.

5 Determination for animal studies based on fuzzy logic

To apply fuzzy logic approach for the calculation of dose determination for animal studies based on the data provided, one can use fuzzy sets to represent or identify the uncertainty and variability in the data. Fuzzy sets allow us to represent values as linguistic variables, such as "low", "medium", and "high", instead of precise numerical values. This also comes in handy when the data is uncertain and variable which is the case with concentration of pesticide residues [36].

Optimization Techniques for Dose Determination

To optimize dose levels, we minimize a cost function:

$$J(D) = \sum_{i=1}^{n} [\mu_{Toxicity}(D_i) - \mu_{Safe}(D_i)]^2$$

Using evolutionary algorithms: Initialize a population of dose candidates, Evaluate J(D) for each candidate, Apply selection, crossover, and mutation to evolve better solutions.

Fuzzy Inference System

Use fuzzy rules to infer dose levels. For example:

$$\mu_{Dose} = max(min(\mu_{Residue}(x) - \mu_{Weight}(y))$$

Where: $\mu_{Residue}$(x): membership value for residue level x, μ_{Weight}(y): membership value for animal weight y.

Fuzzy Logic Rule Example

1. IF Residue Level is Low AND Weight is Low THEN Dose is Low.
2. (IF Residue Level is Medium AND Weight is Medium THEN Dose is Medium.
3. IF Residue Level is High AND Weight is High THEN Dose is High.

Fuzzy Membership Values

For a rat weighing 3.5 kg with a residue level of 5.0mg/kg :

$\mu_{Residue}$(5.0)=0.5 (Medium)
μ_{Weight}(3.5)=0.7 (Medium)
Output Dose:

$$\mu_{Dose} = min(0.5, 0.7) = 0.5(Medium)$$

Fuzzy Membership Function Definition and Adjustment

To classify pesticide residue levels and doses, fuzzy membership functions are defined. For residue levels, we use triangular membership functions:

Membership Function for Residue Levels:

$$\mu_L(x) = \begin{cases} 1, & x \leq a \\ \frac{b-x}{b-a}, & a < x \leq b \\ 0, & x > b \end{cases}$$

Where: a=0,b=1: thresholds for low residue levels.

Similarly, for doses:

$$\mu_{Low}(D) = \begin{cases} 1, & D \leq 0.1 \\ \frac{1-D}{1-0.1}, & 0.1 < D \leq 1 \\ 0, & D > 1 \end{cases}$$

Adaptive Membership Adjustment: To refine the classification accuracy, adaptive gradient-based tuning is applied:

$$\acute{\mu}(x) = \mu(x) - \eta \frac{\partial L}{\partial \mu(\acute{x})}$$

Where: L is the error between predicted and observed classifications.

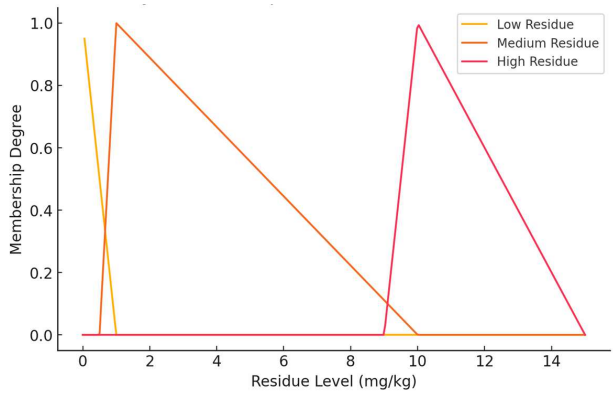

Fig. 11: Fuzzy Membership Functions for Residue Levels

We consider the fuzzy sets over the ranges for pesticide residue concentrations in the following ranges:

–Low (pesticide residue): 0 to 1 mg/kg
–Medium (pesticide residue): 1 to 10 mg/kg.
–High (pesticide residue): from 10 mg/kg and above.

After calculating these fuzzy sets, we can then use fuzzy logic rules to find the fuzzy output for animals in mapped values. For example, we are able to establish the fuzzy logic rules as follows for Endosulfan:

–Animals are assigned a ”Low” value if the amount/number of residues that can be accumulated in 1-year is categorized as ”Low”.
–Animals are assigned a ”Medium” value if the amount/number of residues that can be accumulated in 1-year is categorized as ”Medium”.
–Animals are assigned a ”High” value if the amount/number of residues that can be accumulated in 1-year is categorized as ”High”.

We can use similar fuzzy logic rules for Phorate and Fenvalerate as well.

Using these fuzzy logic rules, we can derive systematic information for animals for other time periods (1 year, 15 years and 30 years) using the residue concentrations. Example calculation based on the fuzzy logic approach in a fuzzy logic framework for Endosulfan is provided in this section [37].

–The fuzzified value for residues that could be accumulated in 1 year is classified as ”Low” based on the fuzzy logic rule when the maximum quantity of residues which may be cumulated in 1 year is 13 milligram/kg. As a result, the equivalent figure for identified animals with a body weight of 0.250 is similarly labelled as ”Low”.
–The fuzzified value for residues accumulated in 15 years is classified as ”Low” based on the fuzzy logic rule when the number of residues accumulated in 15 years is estimated as 0.054 multiplied by 15, resulting in 0.81 mg/kg. As a result, the equivalent figure for animals with a body weight of 0.250 is similarly labelled as ”Low”.
–The fuzzified value for residues accumulated in 30 years is classified as ”Low” based on the fuzzy logic rule when the number of residues accumulated in 30 years is computed as 0.054 multiplied by 30, resulting in 1.62 mg/kg. As a result, the equivalent figure for animals with a body weight of 0.250 is similarly labelled as ”Low”.

We can apply similar calculations using fuzzy logic approach for Phorate and Fenvalerate as well, based on the fuzzy logic rules defined for those pesticides.

Based on the data provided in tabular form, let’s assume we have the following data for animal studies:

Table 3: Tabular data of dose of pesticides on animals with respect to age

Weight (kg)	Age (months)	Dose (mg)
2	1	5
3	2	10
4	3	15
5	4	20
6	5	25

Whereas we can get fuzzy logic approach to dose derive for the animals according to their weight and age. Let define fuzzy sets for weight and age as follow:
Weight

–Low: 0 - 2 kg
–Medium: 2 - 4 kg
–High: 4 - 6 kg

Age

–Young: 0 - 2 months
–Adult: 2 - 4 months
–Elderly: 4 - 6 months

Now, let's define the fuzzy sets for the dose:
Dose:

–Low Dose: 0 to 10 milligrams
–Medium Dose: 10 to 20 milligrams
–High Dose: 20 to 30 milligrams

We can use membership functions to represent the fuzzy sets. Let's assume triangular membership functions for this example.

Subsequently, we take the fuzzy sets obtained from the weight and age of the patient and use the fuzzy rules to calculate the dose. Fuzzy rules can be defined as follows:

1.Dose = Low, IF Weight = Low AND Age = Young
2.Dose = Low, IF Weight = Low AND Age = Adult
3.Dose = Medium, IF Weight = Low AND Age = Elderly
4.Dose = Low, IF Weight = Medium AND Age = Young
5.Dose = Medium, IF Weight = Medium AND Age = Adult
6.Dose = High, IF Weight = Medium AND Age = Elderly
7.Dose = Medium, IF Weight = High AND Age = Young
8.Dose = High, IF Weight = High AND Age = Adult
9.Dose = High, IF Weight = High AND Age = Elderly

We can use these rules to determine the appropriate dose for animals based on their weight and age using fuzzy logic inference.

Once we have the fuzzy inference system defined, we can use it to calculate the dose for a given weight and age of an animal. For example, if we have an animal with weight of 3.5 kg and age of 3 months, we can input these values into the fuzzy inference system and obtain the appropriate dose as the output.

6 Conclusion

6.1 Summary of the impact of pesticides on liver health

Pesticide residue levels in various tissues have been measured and analysed. The results indicate that various tissues collect residues at varying quantities. The accumulation was highest in the liver is endosulfan, phorate, and fenvalerate administered groups.

6.2 Implications for public health

Finally, data from eosin and hematoxylin-stained liver sections have been validated, with the exception of biochemical alterations. Hepatocytes showed significant inflammation, indicating that the incidence of hepatocellular injury increased with increasing pesticide doses in rats. Histopathological examinations also revealed the compounds' severe toxicological effects on the liver [38].

Molecular analyses in rat liver have revealed that the pesticides under research can induce apoptosis. Pesticides were indicated to be triggered by intrinsic ROS formation, follow-on in oxidative damage to the entire cell. Our work showed that pesticides may greatly boost p53 expression in vivo, implying that p53 expression take part in major apoptosis process. The inhibitory impact that these compounds may have on the DNA repair mechanism might be read as the genotoxic effect (DNA damage) of the pesticide treatments discussed in this paper. Measurement of DNA single-strands confirms the damage induced by pesticide treatment to DNA (DNA-SSB). The pesticides mutagenic or genotoxic properties in rat liver are thus proven. Both phosphorylation status and p53 expression levels can thus show a crucial role in pesticide-induced apoptosis. To evaluate the pesticide function in caspase pathway activation, the caspase 3 activity in rat liver was estimated. Caspase 8 or 10 is known to be triggered on the cell surface by death-receptor mediated activation, following caspase 3. This route can boost caspase 9, which is one of the most important initiators, and caspase 3, which is an effector caspase. As a result, the induction of caspase 3 verifies pesticide-induced apoptosis in mitochondria [12].

The high levels of Bax expression, which were linked to a reduction in Bcl-2 protein expression, added to the evidence that pesticides had an impact. In the treated groups, cytochrome C levels are also found to be high. Pesticide-exposed cells showed a rise in Bax protein translocation to mitochondria as well as the cytochrome-c protein from cytosol. These findings support the introduction of cytoplasmic p53 and its function in mitochondrial membrane permeability in cells. The impact of endosulfan, phorate, and fenvalerate on molecular cell death has been confirmed by improvements in prapoptotic proteins. We speculated that oxidative stress, damage to DNA, and the intrinsic apoptotic pathway in the rat liver may be explained by pesticide exposure based on our experimental data.

The health effects of the fuzzy logic dosage estimation method are varied. Firstly it can make it more accurate and safe to give drugs to animals which then means it improves animal health. This can be especially important in veterinary medicine as proper dosing for different species, breeds, and ages of animals can be challenging to discover.

Second, fuzzy logic strategy could also be applied to human health. There are tons of ambiguities or variations in the data, particularly with children, the elderly, or patients with complicated medical problems, therefore when establishing dosage in human research fuzzy logic may be applied. This can help in enhancing the molecular efficacy of the drug among various socio-economic backgrounds enhancing the health and ameliorating the therapeutic outcome of the entire population.

6.3 Limitations and Challenges of Fuzzy Logic in Toxicology Studies

Fuzzy logic is a very robust architectural tool to respond to the phenomenon of uncertainty in toxicology, particularly in gradual dose-response relationships. The defining characteristic of fuzzy logic is the use of membership functions, but the need for reliable data and expertise to define accurate membership functions is one of the main limitations of the method–fuzzy logic systems are often not bio–realistic due to the simplification of biological responses. The toxicological responses that these functions define are highly variable, and may not even exist (there may be no clear threshold of a toxicological change); designing these functions can be subjective. Moreover, the process of creating rule-based systems to interpret toxicity uses subjective language-based rules, which influences the consistency and reliability across studies, especially with limited or noisy data. Moreover, fuzzy logic sometimes has limited ability to model highly nonlinear interactions, as toxicological processes may contain complex biochemical pathways and interdependent variables, that fuzzy logic may not capture fully on its own.

The second important limitation relates to the difficulty at validating and standardizing fuzzy logic models in toxicology where most of other traditional approaches possess established validation criteria. Lack of standard protocols makes benchmarking fuzzy models or ensuring their robustness among studies very hard. Furthermore, fuzzy logic's graded or linguistic outputs (such as "moderate risk") face the challenge of lacking the standardization by regulatory agencies (which yield zero tolerance and preference to quantifiable levels/thresholds) leading to unwillingness for acceptance by regulatory agencies. In addition, when it comes to treating high-dimensional data the fuzzy logic models typically require significant computation and may therefore present a limitation for scale with the more significant toxicology studies. However, the combination of fuzzy logic with other computational methods (eg, machine learning) may potentially address some of these limitations and expand its application in toxicology.

6.4 Future Research Directions

A possible model pathway was proposed. The pesticides might induce oxidative damage by causing intrinsic ROS generation, as illustrated in the mechanism. An increase in ROS levels can cause DNA damage, which can cause the p53 protein to be tummed, Bax to be elevated, and Bcl-2 to be attenuated, resulting in improved Bcl-2/Bax ratio. Cytochromium-c was released when the Bcl-2/Bax ratio was changed. Finally, Apaf-l and caspase-9 were produced by cytochrome c, which activated the apoptosome complex caspase-3. This mechanism might be explained by the molecular mechanism implicated in pesticide hepatotoxicity. This route might be explained by the molecular mechanism behind pesticide hepatotoxicity.

Potential future research directions of fuzzy logic method of dosage estimation in animal experiments A few of these are:

Enhancement of fuzzy membership functions: The accuracy of a fuzzy logic method hinges on the proper establishment of fuzzy membership functions, as they define the structure and behaviour of the fuzzy sets. Future research could further investigate alternate methodologies for designing membership functions, including data driven methods or expert knowledge into the model.

Validation of fuzzy logic technique: Future work can focus on validating the accuracy and performance of the fuzzy logic system used for dosage calculation in animal studies through extensive laboratory-based experiments or clinical trials. This could show the reliability and robustness of the method in different animal populations and with different treatments.

Building a decision support systems: The next step in achieving more accurate and practical dose parameterization in animals is to further investigate the potential of developing a decision support system that combines fuzzy logic approach with other computational methods, such as machine learning or artificial intelligence.

Use in real-world settings: Further study can focus on use of the fuzzy logic method in the field and in clinical practice and determine its impact on clinical outcomes, patient safety, and public health. This can provide useful insights into the benefits and effectiveness of the fuzzy logic approach in drug delivery.

Hybrid Models: Propose integrating fuzzy logic with neural networks for improved dose estimation:

$$O = f(ANN(\mu_{Dose}, \mu_{Wight}, \mu_{Residue}))$$

Where O is the optimized dose output, and ANN is an adaptive neural network.

The fuzzy logic method to dose determination is a valuable public health tool due to its capacity to improve the precision and safety of medicine dosage in animal experiments. It also provides suggestions for future research to continue developing and applying it in many contexts.

All of these methods, as shown here by using data collected in tabular form and represented graphically, are based on fuzzy logic for dosage determination in animal studies and provide a flexible and rationale for animal weight and age specific dosages. The convenience of the graph is that it shows graphically the fuzzy membership values of the dose categories (Low, Medium, and High) corresponding to different combinations of a particular animal that could be easily identified to obtain an optimum dosage unit.

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