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Elucidating the Effect of Estragole: Unlocking the Fine Line between Dose, Risk and Efficiency - A Scientific Narrative Review

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Abstract:

- ***Background:***
Estragole is a constituent of herbs such as tarragon, basil, and fennel. It is known for its anti-inflammatory and antimicrobial activity. There is evidence of its carcinogenic potential in animal models. Estragole carcinogenicity may be linked to its metabolic conversion, forming 1′-sulfoxy metabolites. These metabolites can form covalent DNA adducts, inducing hepatic tumors at high-dose repeated exposure in rodents.

- ***Aim:***
This narrative review aimed to evaluate the carcinogenic potential of estragole by systematically analysing published animal trials and assessing the neoplastic changes.

- ***Materials and Method:***
A systematic review was conducted using Pubmed, Elsevier Science Direct, Wiley Online library, Scopus data bases. The keywords "Estragole" and "Neoplasm" were used as MeSH terms. From the initially identified articles 296, 286 were screened after duplicate removal based on inclusion criteria (English language, Full-text articles, animal trial studies on the carcinogenic effect of estragole). 4 studies were selected for final analysis.

- ***Results:***
The included animal trials investigated estragoles effect on mice and rats at various doses. Estragole was shown to have high carcinogenic potential at high doses. With an increase in estragole dosage, there was an increase in hepatic tumors in both mice and rats.

- ***Conclusion:***
In rat and mice animal models, high dosage of estragole induces tumor formation. The risk of estragole for humans is presently not determined, as it is consumed at low ppm levels. Estragole is a potential carcinogenic agent. Further metabolic, and human related studies are needed to determine carcinogenic risk of estragole to human.

Keywords*: Estragole, Cancer, Hepatocellular Carcinoma.*

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I. INTRODUCTION

One of the biggest issues facing society, public health, and the economy is cancer. It is responsible for nearly one in six deaths worldwide.1 It is a disease that begins with genetic alterations occurring in specific cells, caused by carcinogens.2 These carcinogens can be present in commonly consumed foods and drinks.3 Numerous interconnected elements influence human tumour development, and exposure to small food substances must be taken into account. 4According to the global cancer registry, liver cancer is the third most common cause of cancer-related fatalities and the sixth most common disease overall.5 Its 5-year survival rate is only about 20%. Hepatocellular carcinoma accounts for about 9.2% of all new instances of cancer worldwide. 6 It accounts for 90% of liver cancer cases worldwide. It is the most common cause of all liver cancer cases.

A global health challenge, it has an estimated occurrence of over 1 million cases by 2025.8 The pathogenesis of hepatocellular carcinoma is a complex, multistep process.9 It is closely linked to the prevalence of chronic liver diseases. It also involves genetic susceptibility, interactions between viral and no viral risk factors, cellular microenvironments, and various immune cells. The severity of these factors and an altered microenvironment are key contributing features to cancer.10 Prolonged exposure to compounds that are possibly carcinogenic, particularly during periods of hepatic dysfunction or chronic inflammation, can significantly enhance the risk of hepatocellular carcinogenesis.

Compounds like estragole exhibit potential hepatocarcinogenic properties. Estragole is a compound found in basil and other herbs and has been a staple in traditional medicine for centuries. However, its effects on human health are only now being fully understood. Estragole is a phenylpropanoid. It consists of a benzene ring replaced by a methoxy and a propenyl group, presenting as a constituent of essential oils of many plant species, such as basil, anise, fennel, bay leaves, and tarragon.11,12 Estragole possesses many beneficial medicinal properties, shown through its antimicrobial, antidiabetic, and antioxidant effects.13 It also shows anti-inflammatory and anti-edematogenic activity in acute and chronic inflammation animal models. The mechanism is similar to NSAIDs and corticosteroids.14

It has also been shown that estragole has anti-lipase activity. It can also block neuronal excitability via inhibiting the Na+ channel.15 though estragole has many beneficial properties, it has also been shown to be carcinogenic in animal models, causing hepatocellular carcinoma in mice. After oral exposure, estragole is absorbed from the gastrointestinal tract and undergoes metabolism, mainly in the liver.16 Metabolism of the parent alkenylbenzene by cytochromes P450 and sulfotransferases forms 1′-sulfoxy metabolites. There is much evidence that the 1′-sulfoxy metabolite of estragole can form covalent adducts with guanine or adenine. This compound may raise a health concern because of its DNA adduct formation and carcinogenicity, inducing hepatic tumours at high-dose repeated exposure in rodents.

Formation of DNA adducts may result in mutations and increase the risk of developing cancer. It can also induce DNA repair. Thus, the risk of DNA adduct formation depends on the efficiency of the repair process before repeat exposure.17 DNA adduct repair of covalently bound adducts operates in vivo. This is seen through the rapid decrease in adduct formation following exposure to methyl eugenol or estragole in studies done on mice. However, a study done on eugenol exposure in HepaRG cells or primary hepatocytes showed that the repair of the DNA adducts formed was inefficient. With 80–90% of the adducts still remaining in HepaRG cells or primary hepatocytes after 48 h and/or 4 h repair.18 This systematic review aims to unlock the fine line between the dose, risk, and efficiency of estragole.

II. MATERIALS AND METHODS

This systematic review was conducted to evaluate the carcinogenic potential of estragole by systematically analysing published animal trials and assessing the neoplastic changes. This Systematic review was conducted following PRISMA Guidelines.19 Your text here 1

➢ *Eligibility Criteria*

- Inclusion Criteria

✓ Studies published in English
✓ Full-text articles
✓ Articles on the carcinogenic effect of estragole
✓ Animal Trial studies

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- Exclusion Criteria:

✓ Articles published in other languages
✓ Only abstracts available

➢ *Unrelated Articles Search Strategy*

Electronic databases such as PubMed, Elsevier science direct, Wiley online library, Scopus were used to find published articles on the carcinogenic effect of estragole. The MeSH terms "Estragole" AND "Neoplasm" were used for the search.

III. RESULTS

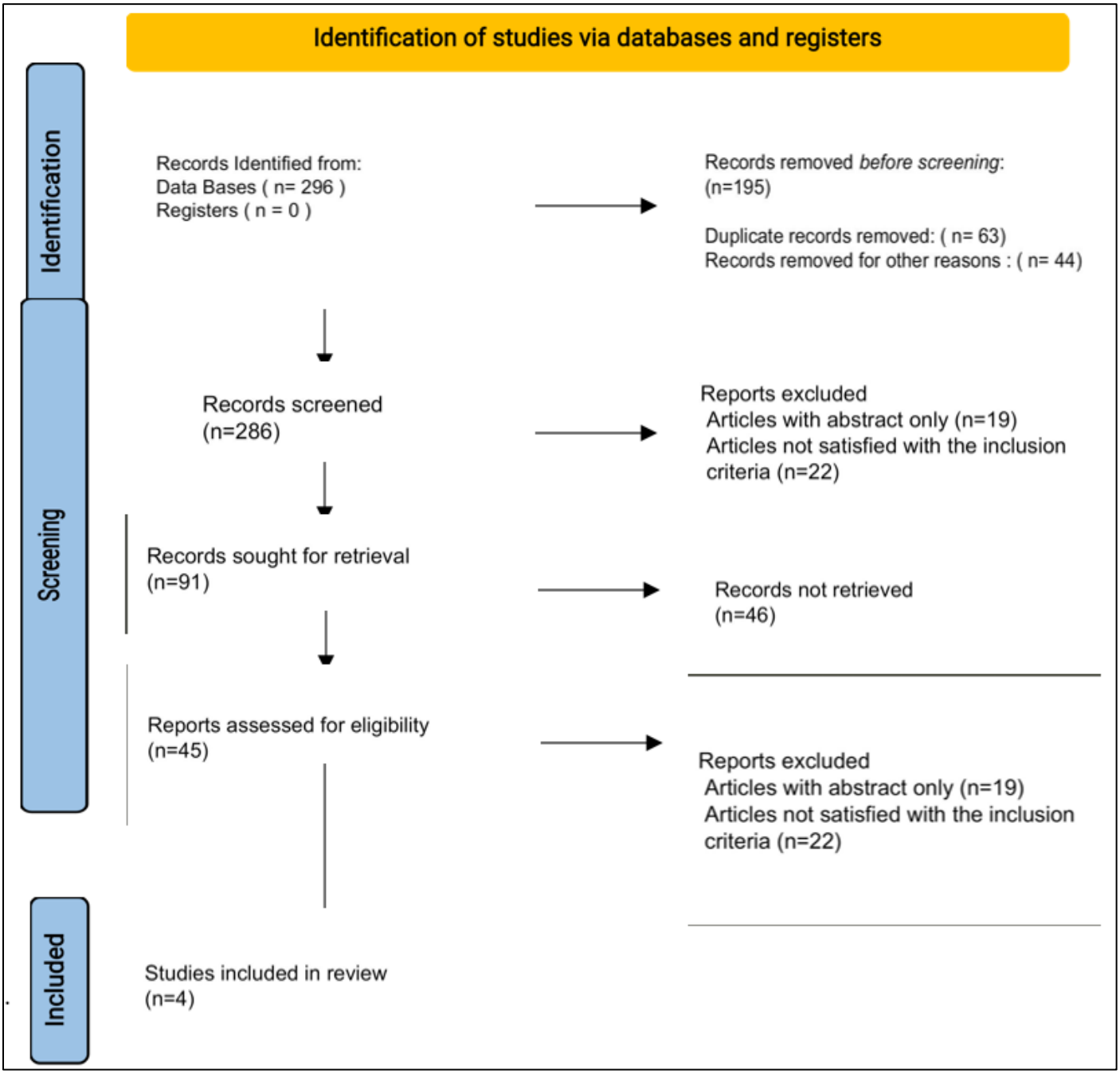

Fig 1 Prisma Flowchart

Figure 1 shows PRISMA flowchart resulted in 45 articles, of which 9 were full-text articles having accessibility and were eligible for review. Ultimately, 4 articles were chosen for inclusion in this systematic review.

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Table 1 Characteristics of Intervention in the Study

S.no	Author Name	Year	Sample Size	Duration	Animal Allocation
1.	Drinkwater, N. R. et al.[22]	1976	There were 79 mice in the group that received the lower doses of estragole; after 12 and 15 months, respectively, the sample size dropped to 60 and 47. There were 19 mice in the group that received the lower doses of estragole; after 12 and 15 months, respectively, the sample number dropped to 18 and 17.	All animals that survived were put to death at 15 months. Each had a routine, thorough autopsy.	Estragole was injected in the nape of the neck of newborn mice according to the following dosage schedule: 0.17µ mole of chemical in 0.025 ml of heat-sterilized trioctanoin Bauer after 24 hours of birth, 0.47µ mole in 0.05 ml on day 8, 0.95µmole in 0.05 ml on day 15, and 2.84µmoles in 0.1 ml on day 22 (total dose=4.43µmoles). Estragole was administered as follows: 0.35 µmole in 0.025 ml on day 1, 0.69 µmole in 0.05 ml on day 8, 1.38 µmoles in 0.05 ml on day 15, and 2.77 µmoles in 0.1 ml on day 22 (total dose=5.19 µmoles).
2.	Wiseman, R.W. *et al.*[23]	1988	This team used breeding stock from The Jackson Laboratory (Bar Harbor, ME) to raise mice. There were 38 male C3H/HeJ mice and 34 female C3H/HeJ mice in one group and 36 male C57BL/6J mice and 36 female C57BL/6J mice in another.	The study lasted for fourteen months. Gross routine autopsies were performed on all animals that died or were killed while moribund and all animals that lived to the end of a study.	On Day 1, male and female C3H/HeJ and CS7BL/6J mice received 0.1 µmol/25 µL sterile trioctanoin/mouse; on days 8, 15, and 22 following birth, they received 0.04 µmol/10 µl/g, 0.04 µmol/5 µl/g, and 0.08 µmol/7 µl/g of 1'-hydroxy estragole.
3.	Bristol DW. et al.[12]	2011	Mice and rats were taken for the students. In rats F344/N rats 20 males and 20 females were taken. In B6C3F1 mice There were ten men and ten females in the bunch.	Mice were fed doses of estragole five days per week for 14 weeks	Rats were gavaged with 5 mL/kg of maize oil at doses of 0, 37.5, 75, 150, 300, or 600 mg/kg. Mice were gavaged with 10 mL/kg of corn oil at doses of 0, 37.5, 75, 150, 300, or 600 mg/kg.
4.	Suzuki,Y. *et al.*[24]	2012	B6C3F1 gpt delta mice, fifty male and fifty female, were divided into five groups at random based on their weight.	For thirteen weeks, mice were given dosages of estragole five days a week.	By gavage, the mice were given dosages of 37.5, 75, 150, or 300 mg/10 mL/kg body weight (250 mg/10 mL/kg bw for females) of estragole in corn oil. Only maize oil was given to vehicle control animals (0 mg/10 mL/kg bw).

Table 1 shows the Characteristics of the studies included in the systematic review with Author name, samples recruited, sample characteristics, duration of the study and sample allocation.

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Table 2 Characteristic of the Primary Outcome and Results of Studies Included in the Current Study

S.no	Author Name	Year	Effect Measure	Result
1.	Drinkwater, N. R. et al.[22]	1976	The number of mice with a single hepatocellular cancer. Mice having numerous hepatocellular carcinomas were counted. How many mice had lung adenomas? Mice having malignant lymphoma in number.	Fourteen of the mice under the lower doses of estragole had single hepatocellular carcinoma, three had multiple hepatocellular carcinomas, three had lung adenomas, and three had malignantly amphora. Seven mice in the higher dose estragole group had a single hepatocellular carcinoma, five had multiple hepatocellular carcinomas, zero had lung adenomas, and zero had malignant lymphoma.
2.	Wiseman, R.W. *et al.*[23]	1988	Susceptibility to Hepatoma Induction in Relation to Mouse Strain, Sex, and Age of Treatment	By 14 months, male C3H/HeJ mice who received cumulative dosages of roughly 1.5 µmol/g body weight of 1'-hydroxy estragole during the first 21 days after birth had five to ten times more hepatomas than male CS7BL/6J mice that received the same treatment. This approach, which delivered the carcinogen only before weaning, made female mice of both strains extremely resistant to hepatoma induction. When the hepatoma responses of male B6C3F mice treated with 1'-hydroxyestragole at 1 or 12 days of age were compared, it was shown that the 12-day-old animals were two to three times more susceptible. Only 73 and 43% of the mice treated with 1'-hydroxyestragole at 1 and 12 days of age, respectively, survived to weaning due to acute toxicity at the 0.15 µmol/g body weight dose.
3.	Bristol DW. et al.[12]	2011	Complete histopathologic analyses, clinical chemistry, and hematology. All groups had their target tissues analyzed. A no-effect level of examination was performed on nontarget tissues. The kidney, liver, lung, mesenteric lymph node, nose, pituitary gland, salivary gland, glandular stomach, and testis were the target tissues in rats, while the kidney, liver, mandibular and mesenteric lymph nodes, nose, spleen, forestomach, and glandular stomach were the target tissues in mice.	In rats, both sexes' livers and male rats' kidneys showed noticeable damage. The 300 and 600 mg/kg groups had the most lesions. Two male rats given 600 mg/kg developed numerous liver cholangiocarcinomas, while a third had a hepatocellul aradenoma. Every male with 600 mg/kg had cholangiofibrosis. Hepatocellular hypertrophy was present in all males given 75 mg/kg or more and all females given 150 mg/kg or more, and the severity increased with increasing dose. In mice, all male dosage groups had higher absolute liver weights. The 75 and 150 mg/kg groups showed a significant increase. Additionally, 300 mg/kg females had a considerably higher absolute liver weight. In those receiving 75 mg/kg or more, relative liver weights increased considerably. Hepatocellular hypertrophy and hepatocellular degeneration were markedly elevated in 75 mg/kg females, 300 and 600 mg/kg males, and 150 and 300 mg/kg females. Males at 300 and 600 mg/kg and females at 75, 150, 300, and 600 mg/kg had significantly higher levels of oval cell hyperplasia in their livers.
4.	Suzuki,Y.*et al.*[24]	2012	Liver weights, body weights, alterations in the liver's histology, Comparing the expression of mRNA in the livers of men and women ES-DNA adduct concentrations in the livers of mice, In vivo tests for mutations, MN test in bone marrow from mice.	Two out of ten, five out of ten, and five out of six female mice in the 75, 150, and 250 mg/kg bw groups, respectively, showed very minor hepatocellular hypertrophy. After 13 weeks of ES treatment, the amounts of ES-DNA adducts, ES-3′-8-dG, 3′-N2-dG, and 3′-N6-dA, in liver DNA were assessed using the LC–MS/MS method. While no ES-DNA adducts were found in the controls, the DNA adducts were found in the livers treated with ES. With the exception of the females in the 250 mg/kg bw group, ES-specific DNA adduct production from ES-treated mice increased in a linear dose-dependent manner.

Table 2 Shows the intervention used in the study included with the outcome.

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Table 3 Quality Assessment of all the Included Studies

Author name	Randomization	Allocation Concealment	Comparison group	Confounding	Experimental conditions	Blinding	Complete outcome data	Exposure Characterization	Outcome Assessment	Outcome Reporting	No other threats
Drinkwater, N. R. et al.											
Wiseman, R.W. *et al.*											
Bristol DW. et al.											
Suzuki, Y. *et al.*											

Risk of Bias	Definitely low risk of bias	Probably low risk of bias	Probably high risk of bias	Definitely high risk of bias
Colour				

Table 3 shows the Risk of bias in all the included studies based on the Office of Health Assessment and Translation (OHAT) Assessment tool.

IV. DISCUSSION

A naturally occurring component of herbs like basil is Estragole. The estimated daily exposure dose for humans is between 0.01 and 0.07 mg/kg. According to reports, Estragole can generate certain DNA adducts in the liver and is hepatocarcinogenic. Estragole's genotoxic testing, however, has yielded conflicting results. It is unknown how Estragole causes hepatocarcinogenicity. When administered at large levels, Estragole may be a potential genotoxic hepatocarcinogen in rats. It has been suggested that the metabolic conversion of Estragole contributes to its carcinogenicity. Cytochrome P450 enzymes primarily convert Estragole to 1-hydroxyestragole, which is then converted by sulfotransferases (SULT) into 1-sulfooxyestragole, the final carcinogenic metabolite.[1] Due to its instability and susceptibility to degradation in an aqueous environment, sulfooxyestragole can generate a reactive carbocation that can bind covalently to DNA.[22] Modest quantities of carcinogens are typically found in food, leading to modest daily intakes. Therefore, when evaluating the results of studies on the carcinogenicity of certain chemicals in animals, it is essential to consider the mechanisms by which mutagenic and other chemicals induce cancer, as well as the extent of human exposure to these chemicals.

In a study conducted by Drinkwater et al.,[22] newborn mice were administered estragole, 1'-hydroxyestragole, or 1'-hydroxysafrole at the nape of the neck according to the following dose schedule: 24 hours following delivery, on days 8, 15, and 22. The male mice that survived the carcinogenicity test were weaned at 22 days of age. There were only 19 mice in the group that received the greater dose of Estragole; there were 60–79 animals in each of the other groups. All animals that survived were put to death at 15 months. Each had a standard gross autopsy that examined the skin, mammary tissues, and the organs of the thoracic and abdominal cavities. The incidence of pulmonary adenomas, malignant lymphoma, and hepatocellular carcinoma in mice increased with estragole exposure. The research acknowledges that Estragole is probably negligible in terms of risks to humans.

In a study conducted by Wiseman, R.W et al.,[23] Methyleugenol appears to be as potent a carcinogen in the liver of mice as Estragole and safrole. Compared to male C57BL/6J mice, male Estragoleice were much more vulnerable to I'-hydroxyestragole. By 14 months, male C3H/HeJ mice had five to ten times as many hepatomas as male C57BL/6J mice given the same treatment. This approach, which delivered the carcinogen only before weaning, made female mice of both strains extremely resistant to hepatoma induction. When the hepatoma responses of male B6C3F mice treated with 1'-hydroxyestragole at 1 or 12 days of age were compared, it

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was shown that the 12-day-old animals were two to three times more susceptible.

In a study by Bristol DW. et al.,[12] Rats and male mice given 300 or 600 mg/kg, and female mice given 75 mg/kg or more, showed a substantial drop in mean body weight. For both male and female rats given Estragole, the liver, glandular stomach, nose, kidney, and salivary gland were the main target organs. Male rats' bone marrow, pituitary gland, testes, and epididymides all showed signs of estragole-related toxicity. The target organs of estragole poisoning in mice were found to be the stomach for females and the liver and nose for both males and females. Estragole therapy was linked to both neoplastic and nonneoplastic liver lesions in rats. One male rat had a hepatocellular adenoma, while two male rats given 600 mg/kg had numerous cholangiocarcinomas. Rats and mice also have a markedly higher incidence of hepatocellular neoplasms of the liver.

In a study done by Suzuki, Y. et al.,[24] The mice were administered ES(Estragole) in corn oil by gavage five days a week for 13 weeks commencing at six weeks of age, at dosages of 37.5, 75, 150, or 300 mg/10 mL/kg body weight (bw) (250 mg/10 mL/kg bw beginning in week two for the females). Only maize oil was given to vehicle control animals (0 mg/10 mL/kg bw). Each mouse was killed twenty-four hours after the last treatment. At doses higher than 75 mg/kg bw in males and 250 mg/kg bw in females, there was a substantial increase in both absolute and relative liver weights. Compared to male mice, female mice exhibited higher amounts of DNA adducts and MFs of the gpt gene due to their higher Sult1a1 expression level. Thus, it is proposed that the carcinogenic mechanism of ES is connected to the genotoxic mechanism. Its genotoxicity is probably caused by SULT-mediated metabolites that produce ES-specific DNA adducts.

When combined, Estragole's genotoxic and carcinogenic potential in rodents is well established; however, because of the significantly lower levels of dietary exposure and interspecies variations in Estragole metabolism, its applicability to human health is still limited. Rather than being a direct-acting genotoxic process at human exposure levels, the evidence suggests that estragole-induced hepatocarcinogenesis is primarily a threshold-dependent, metabolism-mediated event. As a result, the risk to humans under typical dietary conditions seems insignificant, even though high-dose experimental data calls for caution in food safety assessments. To improve quantitative risk assessment models for estragole exposure, future research on human-specific metabolic pathways, dose-response relationships, and DNA repair dynamics will be crucial.

V. CONCLUSION

This narrative review shows estragole as a potential carcinogenic agent. It is seen in mice and rats, that high doses of estragole induces tumor formation, especially in the liver. Estragole occurs naturally and is used in food additives. The risk of estragole for humans is presently not determined, as the level consumed is small at low ppm levels typically estimated to be 0.01–0.07 mg/kg/day. Further metabolic, and human related studies are needed to determine carcinogenic risk of estragole to humans.

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