Contribution to EFSA Public Consultation – Draft Scientific Opinion on Fennel Fruit Preparations Containing Estragole

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Introduction

Fennel is a perennial aromatic plant traditionally used for its therapeutic benefits, including digestive support, carminative activity, lactation promotion, and relief of gastrointestinal discomfort. Its traditional uses , documented over centuries in pharmacopeias and ethnomedicinal records, has been complemented in recent decades by clinical studies that demonstrate its benefits in infant colic, dysmenorrhea, and menopausal symptom alleviation. However, fennel also contains several bioactive constituents, including estragole—a naturally occurring alkenylbenzene that has raised toxicological concerns based primarily on experimental evidence from in vitro studies and animal models. This review synthesizes the available scientific data on the toxicology of estragole, assesses its potential exposure through fennel-based products, describes the methodologies used for risk evaluation, discusses the balance between traditional efficacy and safety, and outlines the current regulatory debates as well as the state of processing or detoxification methods that may reduce estragole content.

Toxicology of Estragole

There is a broad scientific consensus that estragole exhibits genotoxic and carcinogenic potential, particularly when tested under high-dose conditions in animal models. In numerous in vivo studies, estragole has been shown to induce liver tumors in rodents, and these findings are supported by evidence of DNA adduct formation and other genotoxic endpoints. The genotoxicity of estragole has been well characterized using a combination of in vitro assays—such as the Ames test with strains engineered to enhance sulfotransferase activity—and in vivo evaluations in rodent models such as male F344 rats and CD-1 mice ([1.1], [2.1]). Specifically, studies have demonstrated that estragole requires metabolic activation to exert its genotoxic effects. The primary metabolic pathway involves cytochrome P450-mediated oxidation leading to 1′-hydroxyestragole, which can subsequently be conjugated by sulfotransferases to form the electrophilic 1′-sulfooxyestragole. This metabolite is capable of covalently binding to DNA, forming specific adducts that have been quantitatively linked to tumor formation in rodent liver tissues ([1.2], [1.3]).

Dose-response relationships have been established in these studies, showing that DNA adduct formation, oxidative DNA damage, and histopathological alterations such as hepatocyte hypertrophy and necrosis occur in a dose-dependent manner ([3.1], [3.2]). For instance, experiments utilizing the comet assay have reported a statistically significant increase in DNA strand breaks with increasing estragole doses, while 32P-post-labeling techniques have identified dose-related increases in liver DNA adducts ([3.3], [1.4]). Despite the robust mechanistic data from rodent studies, it remains debated to what extent these findings translate into significant human risk given the differences in metabolic rates and detoxification pathways between species ([4.1], [1.5]).

Furthermore, in vitro studies using human-derived models such as the HepG2 cell line have indicated that estragole's genotoxic effects are modulated by the complexity of the herbal matrix.

When present within whole fennel preparations, the genotoxic potential of estragole appears to be mitigated by the presence of other phytochemicals, which may exert anti-genotoxic or chemoprotective effects ([2.2], [2.3]). Consequently, while the mechanistic basis for estragole's genotoxicity and carcinogenicity is well established via metabolic activation pathways, the actual risk in humans—especially at low dietary exposure levels—remains subject to ongoing research and debate ([2.1], [5.1]).

Exposure Assessment

Estragole is naturally incorporated into fennel essential oils along with other constituents such as anethole and fenchone. Analytical studies have employed gas chromatography–mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and high-performance thin-layer chromatography (HPTLC) to quantify estragole in fennel products ([6.1], [6.2]). Reported concentrations in water infusions, herbal teas, and dietary supplements vary widely. For instance, infusions prepared from whole fennel fruits can contain estragole levels ranging from approximately 160 to over 5000 µg/L, with some tea bag infusions averaging around 565 µg/L and reaching maximum values as high as 4644 µg/L under certain preparation conditions ([7.1], [5.1]).

Exposure estimates derived from dietary consumption scenarios suggest that typical daily intakes for adults may be in the low µg/kg body weight (bw) range. For example, adult consumers of fennel herbal teas might ingest estragole at levels of approximately 1–6 µg/kg bw per day under average conditions, which is considerably lower than the doses used in animal carcinogenicity studies that often involve administration of estragole in the mg/kg bw range ([7.2], [7.3]). However, there is variability among population groups: infants who are given fennel teas for colic may have estimated intakes ranging up to 20 µg/kg bw per day in worst-case scenarios, and pregnant women may similarly be exposed to moderately elevated estragole doses ([7.1], [7.3]). Nonetheless, even these higher exposures in sensitive populations remain several orders of magnitude lower than the dosages that produced adverse effects in experimental animal studies ([6.2], [6.3]).

Risk Assessment Methodology

Risk assessment of estragole in fennel preparations typically employs the Margin of Exposure (MOE) approach, which is widely recommended by bodies such as the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The MOE is calculated by comparing the benchmark dose lower confidence limit (BMDL10), which represents a dose associated with a 10% increase in tumor incidence in rodent bioassays, with the estimated human dietary exposure ([7.2], [7.3]). An MOE value exceeding 10,000 is generally considered to indicate a low public health concern for genotoxic carcinogens. In many fennel tea exposure assessments, typical adult consumption yields MOEs well above 10,000; however, in some cases where infant consumption is high relative to body weight, the MOE may fall below this threshold, thereby flagging these groups as requiring further risk management attention ([7.2], [7.3]).

Risk models are also supplemented by physiologically based kinetic (PBK) modeling, which integrates data on absorption, distribution, metabolism, and excretion (ADME) of estragole. These models attempt to account for interspecies differences and interindividual variability in metabolic activation pathways, particularly the formation of reactive intermediates such as 1′-sulfooxyestragole ([3.4], [3.5]). In addition, toxic equivalency factors (TEFs) have been proposed for structurally related alkenylbenzenes, allowing for a cumulative risk assessment when multiple compounds are present in the botanical mixture ([1.6], [1.7]). Safety margins are applied to account for uncertainties associated with interspecies extrapolation and the duration of exposure, and these factors collectively support the inference that typical dietary exposures are far below the levels that elicit adverse outcomes in animal models ([7.3]).

Traditional and Clinical Use of Fennel

Fennel fruit preparations are well documented in traditional medicine for a range of therapeutic indications. Ethnobotanical records and historical accounts date back to Ancient Greece and Rome, with figures such as Hippocrates and Dioscorides citing fennel for its diuretic, carminative, and emmenagogue properties ([6.2]). Modern clinical studies have focused on several endpoints including infantile colic, dysmenorrhea, menopausal symptoms, and gastrointestinal recovery after surgery. For example, randomized controlled trials have reported that fennel oil emulsion alleviates colic in approximately 65% of infants and that fennel preparations perform comparably to conventional nonsteroidal anti-inflammatory drugs in reducing menstrual pain ([6.3]). Traditional usage also extends to promoting lactation and relieving male climacteric symptoms, albeit with the caveat that the safety of prolonged use in vulnerable populations such as pregnant or lactating women remains less well substantiated by clinical data (pqac-ef6b4e2b, [6.2]). Overall, the historical and clinical evidence supports a favorable efficacy profile for fennel preparations when used within traditional dosing regimens while also acknowledging that the presence of estragole necessitates a careful balance between benefit and potential risk ([6.2], [6.3]).

Regulatory and Scientific Debates

Divergent views exist among national and international regulatory bodies regarding the safety of estragole in fennel preparations. The European Medicines Agency (EMA) has raised concerns about the carcinogenic potential of estragole, particularly in formulations intended for sensitive groups such as infants and pregnant women, leading to recommendations to limit exposure ([6.3], [5.1]). In contrast, the Flavor and Extract Manufacturers Association (FEMA) classifies estragole as generally recognized as safe (GRAS) when present at low levels typical of culinary use, emphasizing the dose-dependent nature of its toxicity ([5.1]). EFSA has applied the MOE approach to fennel-based herbal teas, generally finding that traditional consumption levels yield MOEs that are not of immediate concern in adults but may warrant mitigation measures in certain infant consumption scenarios ([7.3]). Additionally, while extensive rodent bioassay data underpin the classification of estragole as a genotoxic carcinogen, there remains ongoing scientific debate regarding the relevance of high-dose animal findings to low-dose human exposures, particularly given interspecies differences in metabolic activation pathways ([2.1], [1.5]). This debate is further complicated by evidence that estragole's genotoxic potency may be mitigated in the presence of other phytoconstituents in the whole herb ([2.2], [2.3]).

Detoxified or Processed Products

Given the concerns over estragole's toxicity, there has been interest in exploring processing methods that can reduce its concentration in fennel preparations without compromising the overall therapeutic properties of the herb. Although various extraction methodologies—including steam distillation, hydrodistillation, supercritical fluid extraction, and ultrasound-assisted extraction—have been investigated for their effect on the chemical profile of fennel essential oils ([6.2], pqac-7fe8fbe6), the available literature does not yet provide robust evidence for technologies or processing methods that consistently reduce estragole content to negligible levels while preserving fennel's bioactive benefits. Studies on extraction methods have primarily focused on the quantification of estragole and its co-occurring compounds rather than on detoxification per se ([5.2]). As of now, no fennel product has been formally authorized with a specific detoxification claim for estragole, and conventional products continue to be marketed based on traditional preparation methods with established safe dosage regimens ([5.2], pqac-764c0962). This aspect remains a potential avenue for future research and product development, especially if regulatory pressures intensify regarding estragole exposure in vulnerable populations.

Discussion and Conclusions

The body of experimental evidence corroborates that estragole is a genotoxic and carcinogenic compound as demonstrated by numerous in vitro assays and in vivo rodent studies. The metabolic activation of estragole—principally via cytochrome P450-mediated 1′-hydroxylation followed by sulfotransferase-catalyzed formation of 1′-sulfooxyestragole—is a critical event driving DNA adduct formation and subsequent tumorigenesis ([1.2], [1.3], pqac-c517aadf). Dose-response assessments in rodent models reveal that as the administered dose increases, so does the extent of DNA damage and tumor incidence, supporting a direct correlation between exposure and adverse outcomes ([3.1], [3.2]). However, it is important to recognize that the doses used in these toxicological studies are typically several orders of magnitude higher than those encountered via traditional consumption of fennel-based herbal teas and dietary supplements ([7.2], [7.3]).

In traditional uses, fennel preparations are consumed at levels that yield estimated estragole intakes in the low microgram per kilogram body weight range. This exposure is significantly lower than the milligram per kilogram doses administered in animal studies that elicited genotoxic effects, resulting in a favorable margin of exposure (MOE) under typical conditions for adult consumers ([7.1], [7.3]). Nevertheless, risk assessment using the MOE approach indicates that in certain sensitive groups, such as infants, the MOE may approach or fall below the critical threshold of 10,000, potentially necessitating further risk management strategies ([7.2], [7.3]). Models incorporating physiologically based kinetic (PBK) data have been instrumental in extrapolating animal data to humans, although inherent uncertainties remain due to interspecies differences in estragole metabolism and detoxification ([3.4], [3.5]).

Clinical evidence supports the therapeutic benefits of fennel preparations, with randomized clinical trials demonstrating efficacy for conditions such as infantile colic and dysmenorrhea ([6.3], [6.2]). These beneficial effects have contributed to fennel's long-standing inclusion in herbal medicine; however, the potential risks arising from estragole content have prompted regulatory agencies to issue guidance on safe usage levels, particularly for vulnerable groups such as infants, pregnant, and lactating women ([6.3], [5.1]). Divergent views among regulatory bodies further complicate the landscape: while EMA and EFSA emphasize the need to minimize estragole exposure, especially in sensitive populations, organizations such as FEMA assert that estragole is safe at the low exposure levels typical of culinary and traditional medicinal use ([6.3], [5.1]).

No current detoxification or processing technology has been definitively proven to reduce estragole content in fennel preparations without affecting the overall pharmacological profile of the herb, meaning that ongoing research in extraction and product standardization remains critical for ensuring consumer safety [5.2]. It is imperative that future work continues to refine both analytical methods for estragole quantification and risk assessment models that incorporate cumulative exposure from a variety of fennel-based products.

In summary, while estragole is clearly a genotoxic and carcinogenic compound in high-dose rodent models owing to its bioactivation to reactive intermediates, the doses to which humans are exposed through typical fennel consumption are many orders of magnitude lower. The MOE approach, supported by physiologically based kinetic modeling, generally indicates that standard dietary usage in adults presents a low risk, whereas some caution is warranted for infants and potentially pregnant women. Traditional and clinical uses of fennel underscore its therapeutic efficacy, but they also necessitate ongoing monitoring and regulatory vigilance to ensure that estragole exposures remain within safe margins. Divergent regulatory positions highlight the need for continued dialogue between scientific researchers and regulatory agencies to better reconcile differences and refine risk management measures. Manufacturers and researchers are encouraged to explore and develop processing methods that may reduce estragole content without compromising the beneficial properties of fennel, thereby further enhancing its safety profile.

Ultimately, while fennel preparations continue to offer significant therapeutic benefits in both traditional and modern medical contexts, their safety evaluation must integrate comprehensive toxicological data with realistic exposure assessments to ensure that the genotoxic and carcinogenic risks associated with estragole are adequately mitigated. Continuous research, improved analytical techniques, and harmonized regulatory assessments will be essential in presenting a balanced view that acknowledges both fennel's efficacy and the importance of minimizing estragole-related risks.

Conclusion and Position of the GFPH

Based on the current body of toxicological and clinical evidence, the GFPH considers that traditional preparations of *Foeniculum vulgare* particularly herbal infusions, do not pose a credible carcinogenic risk to the general population when consumed as traditionally used.
We would like to emphasize that the studies were conducted on estragon and not on fennel. The doses of estragon used in scientific studies do not correspond to the doses of estragon consumed when eating fennel. We would also like to point out that no studies mention a potential link between cancer and fennel.
We recognize the genotoxic potential of estragole at high doses in animal studies, but emphasize that real-world human exposures remain several orders of magnitude lower, as confirmed by both risk models and absence of adverse epidemiological data.

Therefore, the Guild recommends that any regulatory decision maintains proportionality and respects both the historical legitimacy and therapeutic usefulness of fennel in herbal medicine. Precautionary guidance especially for infants and pregnant individuals may be warranted, but prohibition or restriction of fennel-based infusions in the general population would not reflect the actual risk profile nor the benefit–risk balance.

Finally, we encourage EFSA to strengthen collaboration between toxicologists, herbalists, clinicians, and traditional practitioners, in order to produce regulatory frameworks that are both scientifically robust and culturally informed.

References:

[1.1] Level of Alkenylbenzenes in Parsley and Dill Based Teas and Associated Risk Assessment Using the Margin of Exposure Approach. Abdalmajeed M. Alajlouni, Amer J. Al-Malahmeh, Farida Nur Isnaeni, Sebastiaan Wesseling, Jacques Vervoort, Ivonne M. C. M. Rietjens. Journal of Agricultural and Food Chemistry (2016). https://doi.org/10.1021/acs.jafc.6b03650

Context: "The scientific consensus, as reflected in multiple referenced studies, indicates that estragole (1-allyl-4-methoxybenzene) exhibits genotoxic and carcinogenic properties, primarily demonstrated in rodent models. reported hepatocarcinogenicity of estragole and its metabolite 1'-hydroxyestragole in mice, and mutagenicity of 1'-acetoxyestragole in bacterial assays, establishing foundational evidence for estragole's genotoxicity. DNA adduct formation by electrophilic esters derived from estragole metabolites was characterized in vitro and in vivo , supporting the molecular basis for its carcinogenicity. The metabolite 1'-hydroxyestragole is bioactivated via cytochrome P450-mediated hydroxylation of the side chain, which leads to reactive intermediates capable of forming DNA adducts, a key pathway implicated in toxicity. Safety assessments, such as those by EFSA (2005), SCF (2001a,b), and the National Toxicology Program , have applied physiologically based pharmacokinetic (PBPK) and biokinetic modeling approaches to understand dose-response relationships and human relevance, although direct dose-response data in humans remain limited. Animal studies, primarily in mice and rats, using oral administration have elucidated dose-dependent hepatocarcinogenic effects, which support a margin of exposure based risk assessment framework. In vitro bacterial mutagenicity assays and in vivo rodent carcinogenicity bioassays constitute the main experimental models used. Human metabolism studies show similar but slower metabolic processing compared to rodents, suggesting potential but lower risk in humans. Overall, estragole's toxicity involves metabolic activation to DNA-reactive species via side-chain hydroxylation, DNA adduct formation, and subsequent genotoxicity and carcinogenicity observed in rodent models. Regulatory bodies recognize its genotoxic carcinogen classification, advising risk management based on exposure, especially in herbal products like fennel and parsley where estragole is present. »

[1.2] Level of Alkenylbenzenes in Parsley and Dill Based Teas and Associated Risk Assessment Using the Margin of Exposure Approach. Abdalmajeed M. Alajlouni, Amer J. Al-Malahmeh, Farida Nur Isnaeni, Sebastiaan Wesseling, Jacques Vervoort, Ivonne M. C. M. Rietjens. Journal of Agricultural and Food Chemistry (2016). https://doi.org/10.1021/acs.jafc.6b03650

Context: "The scientific consensus identifies estragole, a naturally occurring alkenylbenzene found in fennel and other plants, as a genotoxic and carcinogenic compound in animal models. Its carcinogenicity has been demonstrated primarily in rodent studies, particularly mice, through bioactivation to reactive metabolites capable of DNA binding and adduct formation. Key metabolic pathways involve cytochrome P450-mediated hydroxylation to 1'-hydroxyestragole, which is further activated to DNA-reactive species such as 1'-acetoxyestragole. This bioactivation is a proximate step toward carcinogenicity. Several in vivo animal studies, including mouse liver tumorigenesis models, have established dose-response relationships for estragole, although human data remain scarce. In vitro assays, such as unscheduled DNA synthesis tests in rat hepatocytes and bacterial mutation assays, support these findings by demonstrating genotoxic effects of estragole and its metabolites. Physiologically based biokinetic (PBBK) modeling has been employed to better understand estragole bioactivation and detoxification in both rats and humans, offering insights into species differences in metabolism and risk assessment. The margin of exposure approach is used in risk assessment to link levels in food (e.g., fennel) with potential health risks. Overall, estragole undergoes metabolic activation primarily via cytochrome P450 enzymes leading to genotoxic intermediates, which underlies its carcinogenic potential observed in experimental models. The consensus acknowledges established dose-response data from animal studies and highlights the importance of metabolic pathways in estragole bioactivation for toxicity and carcinogenicity evaluation. »

[1.3] Level of Alkenylbenzenes in Parsley and Dill Based Teas and Associated Risk Assessment Using the Margin of Exposure Approach. Abdalmajeed M. Alajlouni, Amer J. Al-Malahmeh, Farida Nur Isnaeni, Sebastiaan Wesseling, Jacques Vervoort, Ivonne M. C. M. Rietjens. Journal of Agricultural and Food Chemistry (2016). https://doi.org/10.1021/acs.jafc.6b03650

Context: "Estragole, an alkenylbenzene found in herbs like fennel, is metabolized primarily by liver cytochrome P450 enzymes through O-demethylation, yielding hydroxy- or dihydroxyallylbenzene metabolites that are readily excreted. At higher doses, alternative metabolic pathways including 1'-hydroxylation and epoxidation of the allyl side-chain become significant, leading to reactive sulfate conjugates that can form DNA adducts implicated in genotoxicity and carcinogenicity. Experimental studies in rodents have demonstrated dose-dependent shifts in metabolic pathways, with bioactivation occurring more at high doses. Genotoxic effects have been confirmed by DNA adduct formation detected using methods such as 32P-postlabeling in mouse liver and in cultured human HepG2 cells, with estragole, safrole, and methyleugenol showing the highest activity among alkenylbenzenes. Carcinogenicity studies in rodents report liver tumor formation after exposure to safrole, estragole, and related compounds, indicating their genotoxic and carcinogenic potential. The margin of exposure (MOE) approach, recommended by EFSA, is employed for risk assessment, using benchmark dose lower confidence limits (BMDL10) derived from animal tumor incidence data combined with estimated human intake. Because direct carcinogenicity data to establish BMDL10 values for estragole are sometimes unavailable, physiologically based kinetic (PBK) modeling and read-across from related compounds have been proposed to fill data gaps. PBK models incorporate absorption, distribution, metabolism, and excretion parameters to simulate in vivo kinetic and metabolic processes across species, facilitating dose-response analysis and interspecies extrapolation. Collectively, the scientific consensus recognizes estragole as genotoxic and carcinogenic primarily via metabolic activation to DNA-reactive intermediates, with dose-dependent metabolic pathways and robust experimental evidence from in vitro (e.g., HepG2 cell) and in vivo (rodent) models supporting these conclusions. »

[1.4] Level of Alkenylbenzenes in Parsley and Dill Based Teas and Associated Risk Assessment Using the Margin of Exposure Approach. Abdalmajeed M. Alajlouni, Amer J. Al-Malahmeh, Farida Nur Isnaeni, Sebastiaan Wesseling, Jacques Vervoort, Ivonne M. C. M. Rietjens. Journal of Agricultural and Food Chemistry (2016). https://doi.org/10.1021/acs.jafc.6b03650

Context: "The excerpt presents detailed insights into the genotoxicity and carcinogenicity of alkenylbenzenes, including estragole, with a focus on metabolic activation to ultimate carcinogenic metabolites. For estragole and related compounds, toxicity is linked to bioactivation to reactive 1′-sulfoxy metabolites that form DNA adducts in the liver. These DNA adducts are key intermediates in the carcinogenic process. Studies have demonstrated dose-linearity in the formation of these metabolites over a wide dose range from human dietary levels to BMDL10 levels inducing liver tumors in rodents, suggesting a linear dose-response relationship for bioactivation. However, whether this linearity extends to mutagenicity and carcinogenicity remains under discussion and is debated in the risk assessment community. In vitro mutagenicity has been confirmed, for example by Ames tests using Salmonella strains expressing sulfotransferase enzymes. In vivo studies predominantly use rodent carcinogenicity bioassays to determine BMDL10 values for margin of exposure (MOE) calculations, which guide risk management prioritization. Human data includes detection of estragole-related DNA adducts in human liver tissues, confirming relevance of these pathways in humans. Physiologically based kinetic (PBK) modeling has been applied to compare human and rodent species differences, indicating higher formation of ultimate carcinogens in humans for certain alkenylbenzenes, emphasizing the need for compound-specific uncertainty factors. Risk assessment employs MOE approaches based on these models and long-term carcinogenicity data, with efforts to refine decisions accounting for less-than-lifetime exposures using principles such as Haber's Rule, though this remains controversial for genotoxic carcinogens. The metabolic pathway critical for estragole toxicity involves hydroxylation to 1′-hydroxyestragole, followed by sulfation to the ultimate reactive 1′-sulfoxy intermediate that can bind DNA. This pathway and the resulting DNA adducts are central to the mode of action leading to tumor formation. Experimental models used range from in vitro mutagenicity assays, isolated hepatocyte studies (both rat and human), to in vivo long-term feeding studies in rodents, complemented by biomonitoring of DNA adducts in human liver samples. These findings support the conclusion that estragole exerts genotoxic carcinogenic effects through metabolite-mediated DNA adduct formation, with dose-response relationships indicated at the bioactivation level, though uncertainties remain at the mutagenicity/cancer outcome level and in extrapolations to human risk. »

[1.5] Level of Alkenylbenzenes in Parsley and Dill Based Teas and Associated Risk Assessment Using the Margin of Exposure Approach. Abdalmajeed M. Alajlouni, Amer J. Al-Malahmeh, Farida Nur Isnaeni, Sebastiaan Wesseling, Jacques Vervoort, Ivonne M. C. M. Rietjens. Journal of Agricultural and Food Chemistry (2016). https://doi.org/10.1021/acs.jafc.6b03650

Context: "Current scientific consensus recognizes estragole, a naturally occurring alkenylbenzene found in fennel and other plants, as a genotoxic and carcinogenic compound. Estragole's genotoxicity and carcinogenicity have been extensively studied using a variety of experimental models, including in vitro systems (such as primary hepatocytes and Salmonella typhimurium assays), in vivo animal models (notably rats and mice), and physiologically based biokinetic (PBBK) modeling to understand human relevance. Studies have employed DNA adduct analyses (e.g., 32P-post-labeling) and mutagenicity assays to demonstrate estragole's capability to form genotoxic DNA adducts. Dose-response relationships have been investigated both experimentally and through modeling approaches. Physiologically based biodynamic (PBBD) models have been developed for estragole, predicting DNA binding in rat liver and estimating human exposure risks, with some models incorporating Monte Carlo simulations to evaluate interindividual variation in bioactivation and DNA adduct formation. The key metabolic pathways relevant to estragole's toxicity involve its bioactivation by sulfotransferases (SULTs), converting hydroxylated estragole metabolites to reactive sulfate esters that can covalently bind DNA, forming adducts. Humans and rodents express varying levels of these enzymes, influencing toxicity profiles. Detoxification pathways also exist, modulating bioactivation and overall risk. Such metabolic understanding underpins the risk assessment approaches, including margin of exposure calculations for human health. Supporting data include comparative studies of estragole metabolism and DNA adduct formation in humans versus rats and in vitro systems. Overall, the genotoxic and carcinogenic potential of estragole is recognized, with dose-response characterization aided by in vitro, in vivo, and in silico models that incorporate metabolic activation and detoxification pathways. »

[1.6] Level of Alkenylbenzenes in Parsley and Dill Based Teas and Associated Risk Assessment Using the Margin of Exposure Approach. Abdalmajeed M. Alajlouni, Amer J. Al-Malahmeh, Farida Nur Isnaeni, Sebastiaan Wesseling, Jacques Vervoort, Ivonne M. C. M. Rietjens. Journal of Agricultural and Food Chemistry (2016). https://doi.org/10.1021/acs.jafc.6b03650

Context: "The excerpt discusses the genotoxicity and carcinogenicity of alkenylbenzenes, including estragole, which is structurally related to other compounds such as safrole, methyleugenol, elemicin, apiol, and myristicin found in botanicals like parsley and dill. These compounds are of concern because they have shown the potential to induce liver cancer in experimental rodents at high levels of exposure. They are rapidly absorbed and metabolized in the liver, where a key metabolic pathway involves the formation of a reactive 1'-sulfoxy metabolite. This metabolite can form adducts with DNA and proteins, contributing to their genotoxic and carcinogenic properties. The genotoxicity assessment has been performed with in vitro assays such as those using Salmonella typhimurium strains, and biochemical studies on adduct formation have been conducted both in vitro and in vivo in rodents. Studies cited include mutagenicity assays , DNA adduct formation characterization , and dose-dependent metabolism studies in rats and mice . The Margin of Exposure (MOE) approach is used in risk assessment by organizations like EFSA, JECFA, and ILSI to evaluate the risk of genotoxic and carcinogenic compounds such as estragole, considering the dose-response relationship derived from animal studies. The excerpt highlights that the formation of DNA adducts via the reactive metabolite is a key molecular event in their carcinogenic mode of action, pointing to a relatively well established mechanism supported mainly by rodent in vivo models and corroborated by in vitro metabolism and mutagenicity studies. However, human relevant risk assessment also uses physiologically based kinetic modeling and toxic equivalency factors for combined exposure assessment. Thus, the scientific consensus is that estragole is genotoxic and carcinogenic based on animal model evidence via metabolic activation to reactive metabolites forming DNA adducts, and dose-response relationships have been characterized primarily in rodents. »

[1.7] Level of Alkenylbenzenes in Parsley and Dill Based Teas and Associated Risk Assessment Using the Margin of Exposure Approach. Abdalmajeed M. Alajlouni, Amer J. Al-Malahmeh, Farida Nur Isnaeni, Sebastiaan Wesseling, Jacques Vervoort, Ivonne M. C. M. Rietjens. Journal of Agricultural and Food Chemistry (2016). https://doi.org/10.1021/acs.jafc.6b03650

Context: "Alkenylbenzenes, including estragole, are genotoxic and carcinogenic compounds due to their ability to form adducts with cellular macromolecules like proteins and DNA, which underlies their carcinogenicity. The genotoxic and carcinogenic risks of these compounds are assessed using the Margin of Exposure (MOE) approach, which compares a reference point such as the Benchmark Dose Lower confidence limit for 10% tumor incidence (BMDL10) derived from epidemiologic or experimental tumor data to estimated human exposure. MOE values above 10,000 suggest low priority for risk management. To evaluate levels of alkenylbenzenes like estragole, methyleugenol, safrole, myristicin, apiol, and elemicin in botanicals, analytical methods including methanolic and hot water extraction followed by Ultra Performance Liquid Chromatography (UPLC) are used. Studies typically involve spiking samples with known standards for recovery estimation and use tea samples from market sources for analysis. The toxicological assessment includes correction for sample recovery and estimation of human daily intake based on tea consumption representing realistic exposure scenarios. Animal models have been used to establish tumor incidence data for reference points in MOE. Although the excerpt does not specify experimental models for genotoxicity or detailed metabolic pathways for estragole, it references extensive experimental data and experts groups' recommendations on risk assessment approaches. Key components of the approach are the metabolism-dependent bioactivation of alkenylbenzenes into DNA-reactive species causing genotoxic effects, supported by historical studies on protein and DNA adduct formation . Thus, the current scientific consensus recognizes estragole as genotoxic and carcinogenic with dose-response established through experimental tumor incidence data from animal studies and standards recommended by EFSA, JECFA, and ILSI for risk characterization. »

[2.1] In vitro toxicity evaluation of estragole-containing preparations derived from Foeniculum vulgare Mill. (fennel) on HepG2 cells. Sara Levorato, Luca Dominici, Cristina Fatigoni, Claudia Zadra, Rita Pagiotti, Massimo Moretti, Milena Villarini. Food and Chemical Toxicology (2018). https://doi.org/10.1016/j.fct.2017.12.014

Context: "Estragole is a naturally occurring alkenylbenzene found in spices and herbs, including Foeniculum vulgare Mill. (fennel), which can contain high concentrations of estragole in its fruits and essential oils. Estragole has been identified as a genotoxic hepatocarcinogen based on rodent studies where high doses of pure estragole led to liver DNA adduct formation and tumor development. The genotoxicity and carcinogenicity of estragole are attributed to its metabolic activation through a pathway involving cytochrome P450 enzymes producing 1'-hydroxyestragole, which subsequently forms reactive sulfate conjugates (1'-sulfooxyestragole) catalyzed by sulfotransferase enzymes (SULT1A1), leading to DNA adduct formation. The Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) has reviewed toxicological data and concluded that carcinogenic effects in rodents occur at doses (1-10 mg/kg body weight) approximately 100-1000 times higher than human exposure levels from herbal medicinal products containing estragole, suggesting low cancer risk at typical human doses. Nevertheless, it recommends minimizing exposure in sensitive groups such as children, pregnant, and breastfeeding women. Recently, toxicity assessments have emphasized the importance of evaluating estragole within the natural complex mixtures of fennel preparations rather than as a pure compound, as other compounds present may modulate its genotoxic potential. Studies assessing estragole toxicity in fennel preparations had not been conducted prior to this work. The HepG2 human hepatoma cell line, employed in in vitro assays, is used as a relevant model for assessing estragole-mediated toxicity because it expresses relevant xenobiotic metabolizing enzymes, including CYP450 family members involved in estragole bioactivation and sulfotransferases responsible for producing reactive metabolites. HepG2 cells share regulatory gene expression profiles of phase I and II metabolic enzymes with primary hepatocytes, making them suitable for investigating genotoxicity, apoptosis, and cell cycle perturbations induced by fennel-derived estragole-containing preparations. Thus, both in vivo rodent models and human cell-based in vitro models (HepG2) are utilized for toxicity evaluations of estragole, taking into account metabolism-dependent bioactivation pathways. »

[2.2] In vitro toxicity evaluation of estragole-containing preparations derived from Foeniculum vulgare Mill. (fennel) on HepG2 cells. Sara Levorato, Luca Dominici, Cristina Fatigoni, Claudia Zadra, Rita Pagiotti, Massimo Moretti, Milena Villarini. Food and Chemical Toxicology (2018). https://doi.org/10.1016/j.fct.2017.12.014

Context: "The study evaluated the genotoxicity and cytotoxic effects of estragole-containing fennel preparations (FSPw and FSEO) on HepG2 cells using various in vitro assays, including the comet assay for DNA strand breaks, the cytokinesis-block micronucleus (CBMN) test for chromosomal aberrations, apoptosis, and cell cycle analysis. Results showed no significant genotoxic effects from FSPw or FSEO at tested concentrations; there were no increases in DNA damage or micronuclei frequency compared to controls, whereas positive controls confirmed assay sensitivity. FSEO induced a dose-dependent cytostatic effect and increased apoptosis without detectable DNA damage, suggesting selective toxicity against malignant cells rather than genotoxicity. Previous studies cited confirm that low doses of estragole are non-genotoxic in human liver cell models (HepG2 and HepaRG), with higher doses showing some genotoxicity in certain assays. Metabolic pathways of estragole show dose- and species-dependent variation: at low doses, detoxification via O-dealkylation dominates, while higher doses increase bioactivation via 1'-hydroxylation producing toxic intermediates like 1'-sulfoxyestragole, which is implicated in carcinogenicity mainly observed in rodents. Physiologically based kinetic (PBK) modeling indicates humans form less of the toxic metabolite compared to rats. Overall, current scientific consensus suggests that estragole's genotoxic and carcinogenic potential is significantly reduced at low exposure levels and in natural matrices such as fennel, with differences in metabolism accounting for species-specific responses. Experimental toxicological assessments have primarily utilized in vitro human liver cell lines (HepG2, HepaRG), comet and micronucleus assays, and PBK modeling for metabolic analysis; some in vivo data come from rodent studies highlighting interspecies metabolic differences. Dose-response relationships indicate increasing estragole doses shift metabolism from detoxification to bioactivation pathways, influencing toxic outcomes."

[2.3] In vitro toxicity evaluation of estragole-containing preparations derived from Foeniculum vulgare Mill. (fennel) on HepG2 cells. Sara Levorato, Luca Dominici, Cristina Fatigoni, Claudia Zadra, Rita Pagiotti, Massimo Moretti, Milena Villarini. Food and Chemical Toxicology (2018). https://doi.org/10.1016/j.fct.2017.12.014

Context: "The article by assesses the genotoxicity and carcinogenicity of estragole-containing fennel preparations using in vitro models, specifically human hepatic HepG2 cells. Despite extensive literature reporting estragole's genotoxicity and hepatocarcinogenicity in rodent models, this study did not find evidence of DNA damage in human cells exposed to fennel's essential oil (FSEO) or fennel aqueous extracts (FSPw) containing estragole. The study suggests that genotoxic effects of estragole may be reduced or nullified when present within complex herbal mixtures, likely due to additive or synergistic interactions among phytochemicals such as anethole and flavonoids. Anethole, present in higher concentrations than estragole in fennel, has demonstrated anti-genotoxic and chemopreventive activities, potentially mitigating estragole's harmful effects. The essential oil's pro-apoptotic effect on HepG2 cells, involving G2/M cell cycle arrest, occurred without detectable genotoxic stress, indicating a possible non-genotoxic apoptosis induction mechanism. The research highlights differences in metabolic pathways between humans and rodents, noting that rodent data may not reliably predict human toxicity. Metabolic activation producing reactive intermediates like 1'-hydroxyestragole and further sulfo-conjugated carcinogenic species has been implicated in rodent carcinogenicity; however, human metabolism appears to differ significantly, potentially leading to lower formation of these toxic metabolites. The authors used in vitro comet and cytokinesis-block micronucleus (CBMN) assays in HepG2 cells to assess DNA damage and cytogenetic effects, standard methods to detect genotoxicity. Literature also reports anti-genotoxic effects of anethole in vivo in mice, and various studies document estragole carcinogenicity in rodents via metabolic activation. Regarding dose-response, the article does not specifically mention established dose-response relationships for estragole genotoxicity in humans but acknowledges rodent studies showing carcinogenicity at certain doses and metabolism-dependent dose effects. The complexity of natural matrices and metabolic differences complicate extrapolation. The article cites regulatory evaluations from EMA and EFSA that consider these aspects. Overall, the current scientific consensus is cautious: estragole is genotoxic and carcinogenic in rodents, primarily due to metabolic activation pathways producing reactive electrophilic intermediates; however, in humans, when consumed within complex herbal preparations like fennel extracts, genotoxic effects appear greatly reduced or absent due to metabolic differences and phytochemical interactions. The use of HepG2 cells provides a relevant human-derived model for in vitro genotoxicity testing, complementing in vivo rodent data and highlighting limitations in cross-species extrapolation. »

[3.1] In vivo genotoxicity of estragole in male F344 rats. Wei Ding, Dan D. Levy, Michelle E. Bishop, Mason G. Pearce, Kelly J. Davis, Alan M. Jeffrey, Jian-Dong Duan, Gary M. Williams, Gene A. White, Lascelles E. Lyn-Cook, Mugimane G. Manjanatha. Environmental and Molecular Mutagenesis (2015). https://doi.org/10.1002/em.21918

Context: "The study by investigated the in vivo genotoxicity of estragole in male F344 rats, providing evidence of dose-dependent DNA damage in liver tissues following estragole administration at 300, 600, and 1,000 mg/kg body weight doses. DNA damage was measured using the comet assay, focusing on direct strand breaks and oxidative DNA lesions recognized by specific enzymes (EndoIII and hOGG1). Significant increases in oxidative DNA damage were observed in the liver but not in the stomach, suggesting tissue-specific effects correlated with estragole metabolism. The results supported a dose-response relationship with a lowest observed effect level (LOEL) around 300 mg/kg/day for estragole-induced genotoxicity. Peripheral blood micronucleus assays revealed no biologically significant increase in micronucleated reticulocytes, indicating limited systemic genotoxic effects under these conditions. Histopathological analysis showed liver lesions characterized by hepatocyte hypertrophy and mild necrosis at high doses, further confirming estragole's hepatic toxicity. The study emphasized the importance of mechanistic data in risk assessment, noting that estragole requires metabolic activation mainly in the liver, primarily through cytochrome P450 (CYP450)-mediated hydroxylation of the allyl side chain, to form reactive metabolites responsible for DNA damage. This metabolic activation is crucial to understanding estragole's mode of action and its cancer risk. The correspondence between DNA adduct formation from estragole metabolites and similar carcinogens like methyleugenol strengthens the link between metabolic activation, DNA damage, and carcinogenicity. Overall, experimental models employed include in vivo rat studies with the comet assay for DNA damage detection and micronucleus assays for chromosomal damage. The findings underscore a dose-dependent genotoxic and carcinogenic potential of estragole mediated by metabolic bioactivation primarily in the liver, with established dose-response relationships in animal models essential for human risk assessments. »

[3.2] In vivo genotoxicity of estragole in male F344 rats. Wei Ding, Dan D. Levy, Michelle E. Bishop, Mason G. Pearce, Kelly J. Davis, Alan M. Jeffrey, Jian-Dong Duan, Gary M. Williams, Gene A. White, Lascelles E. Lyn-Cook, Mugimane G. Manjanatha. Environmental and Molecular Mutagenesis (2015). https://doi.org/10.1002/em.21918

Context: "The study by provides detailed in vivo evidence concerning the genotoxicity of estragole in male F344 rats, which is relevant to its carcinogenic potential. Using a combination of assays including the alkaline comet assay, enzyme-modified comet assay, micronucleus assay, DNA adduct analysis by 32P-post-labeling, and standard histopathology, the research investigates dose-response relationships and tissue-specific genotoxic effects of estragole. Male F344 rats were administered various doses of estragole , then liver, kidney, stomach tissue, and blood were analyzed. The alkaline comet assay detected a dose-related increase in DNA strand breaks and alkali-labile sites specifically in liver tissue but not stomach, the latter not being a cancer target site. Enzymatic treatments that reveal oxidative DNA damage (with EndoIII and hOGG1 enzymes) further confirmed significant oxidative DNA lesions in the liver across all estragole doses. DNA adduct analyses revealed a dose-dependent formation of multiple DNA adducts exclusively in the liver of treated rats but not controls, supporting direct DNA interaction as a potential carcinogenic mechanism. Safrole, a structurally related compound, also caused dose-dependent liver DNA damage in the same model. These findings point to liver as the primary tissue susceptible to estragole genotoxicity, consistent with its carcinogenicity seen in rodent bioassays. The study employed a robust set of in vivo animal models and modern genotoxicity endpoints to establish clear dose-response relationships. Key experimental techniques included comet assays (for strand breaks and oxidative damage), micronucleus assays in blood samples, 32P-post-labeling for DNA adducts, and histological examination for tissue pathology. While the excerpt does not cover metabolic pathways explicitly, the detection of oxidative DNA lesions and DNA adducts implies the involvement of estragole metabolic activation, likely by liver enzymes generating reactive metabolites capable of DNA binding. This data aligns with the consensus that estragole's carcinogenicity is mediated via metabolic activation producing DNA-reactive species that cause genotoxic effects in target tissues such as liver. The information primarily covers in vivo rodent data (male F344 rats) with no direct mention of in vitro or human studies in this excerpt. The clear dose-response effects on DNA damage and adduct formation provide solid evidence supporting estragole's genotoxic potential in animal models. Further research would be required to detail the metabolic pathways and assess relevance to human risk, but this study contributes substantial in vivo evidence on estragole genotoxicity and its carcinogenic mechanisms. »

[3.3] In vivo genotoxicity of estragole in male F344 rats. Wei Ding, Dan D. Levy, Michelle E. Bishop, Mason G. Pearce, Kelly J. Davis, Alan M. Jeffrey, Jian-Dong Duan, Gary M. Williams, Gene A. White, Lascelles E. Lyn-Cook, Mugimane G. Manjanatha. Environmental and Molecular Mutagenesis (2015). https://doi.org/10.1002/em.21918

Context: "Estragole is not inherently reactive but requires metabolic activation primarily in the liver via CYP450-mediated hydroxylation of its allyl side chain to form 10-hydroxyestragole. This metabolite can be detoxified through glucuronidation or glutathione conjugation, but it can also be sulfonated by sulfotransferases to form the unstable 10-sulfooxyestragole. This reactive metabolite decomposes into a carbocation that binds covalently to DNA, forming DNA adducts—predominantly N2-(trans-isoestragol-3'-yl-2'-deoxyguanosine (E-3'-N2-dGuo)—which are implicated in estragole's genotoxic and carcinogenic effects. In vivo studies in male F344 rats demonstrated dose-dependent induction of DNA strand breaks and DNA adducts in liver tissue measured by comet assay and 32P-postlabeling, supporting direct genotoxicity involvement in estragole hepatocarcinogenicity. Alkali-labile sites formed during DNA adduct repair contribute to DNA strand breaks detected in comet assays. Dose-response relationships have been established in rats across a range of doses, with higher doses showing positive comet results and DNA adduct formation. Experimental models used include in vivo assays in male F344 rats with endpoints such as comet assay for DNA strand breaks, micronucleus assays for chromosomal damage, and nucleotide post-labeling for DNA adducts. Comparisons are made with structurally related compounds like methyleugenol and safrole, which also show genotoxicity through metabolic activation and DNA adduct formation. Histopathological assessments confirmed minimal to mild hepatocyte necrosis in treated rats. The study underscores the importance of metabolism in estragole toxicity, demonstrating a clear mechanistic pathway from metabolic activation to DNA damage, linking genotoxicity to carcinogenic risk. While in vivo rodent data are extensive, data on human or in vitro models are more limited or indirect. The research collectively supports the carcinogenic potential of estragole via metabolically formed DNA-reactive intermediates with established dose-response findings in animal models. »

[3.4] In vivo genotoxicity of estragole in male F344 rats. Wei Ding, Dan D. Levy, Michelle E. Bishop, Mason G. Pearce, Kelly J. Davis, Alan M. Jeffrey, Jian-Dong Duan, Gary M. Williams, Gene A. White, Lascelles E. Lyn-Cook, Mugimane G. Manjanatha. Environmental and Molecular Mutagenesis (2015). https://doi.org/10.1002/em.21918

Context: "The scientific consensus from the cited 2015 study suggests that estragole exhibits genotoxicity through both direct and indirect mechanisms. Direct genotoxicity involves DNA adduct formation and DNA strand breaks, while indirect genotoxicity is linked to oxidative DNA damage. These mechanisms collectively contribute to the cancer mode of action (MOA) in the liver. The dose-response relationship is likely complex because the relative importance of the metabolic pathways involved can change with dose, indicating that each type of DNA damage may have a different response curve. Experimental models used include in vivo studies on male F344 rats to assess DNA adduct formation, strand breaks via comet assay, micro-nuclei assays for clastogenicity, and histopathological examination. Previous research also references gpt-delta transgenic rodent models showing base substitution mutations, which are less efficiently detected by micronucleus assays. Short-term exposure studies have been conducted, but longer-term bioassays (e.g., two-year studies) may reveal additional tumor sites and strengthen evidence for genotoxicity. In vitro assays, such as unscheduled DNA synthesis in cultured rat hepatocytes, have also been used to compare genotoxic effects of related alkenylbenzenes. Regarding metabolic pathways, estragole is bioactivated by cytochrome P450 enzymes, converting it into reactive intermediates such as 1′-hydroxyestragole, which can form DNA adducts. Oxidative metabolites (e.g., 10-hydroxyestragole) and quinone intermediates also contribute to toxicity. Human UDP-glucuronosyltransferases (UGT2B7 and UGT1A9) mediate glucuronidation of 10-hydroxyestragole, aiding detoxification. Overall, estragole toxicity involves bioactivation and metabolic detoxification pathways that are dose-dependent. The current chemico-toxicological evidence supports that estragole is genotoxic and carcinogenic in rodents, but additional longer-term studies and clearer dose-response characterizations are necessary. The evaluation has been primarily in animal models, with in vitro hepatocyte assays supplementing the assessment. These findings indicate complex mechanisms with multiple types of DNA damage contributing to estragole's carcinogenic potential in fennel and related plants. »

[3.5] In vivo genotoxicity of estragole in male F344 rats. Wei Ding, Dan D. Levy, Michelle E. Bishop, Mason G. Pearce, Kelly J. Davis, Alan M. Jeffrey, Jian-Dong Duan, Gary M. Williams, Gene A. White, Lascelles E. Lyn-Cook, Mugimane G. Manjanatha. Environmental and Molecular Mutagenesis (2015). https://doi.org/10.1002/em.21918

Context: "Estragole, an alkenylbenzene compound found in fennel and other spices, is metabolized to reactive intermediates such as 1'-hydroxyestragole and estragole 2',3'-oxide, which are implicated in its genotoxic and carcinogenic effects. Studies including in vivo experiments in rodents (e.g., F344 rats, B6C3F1 mice) have demonstrated the formation of DNA adducts and liver tumors after administration of estragole, indicating its carcinogenic potential. The metabolic activation involves bioactivation to electrophilic metabolites like quinone methides and sulfate conjugates, catalyzed by enzymes such as sulfotransferases (notably SULT1A1), which facilitate DNA binding and consequent mutagenesis. Evidence from in vitro assays and genetically engineered bacterial strains supports the genotoxicity of estragole and its metabolites. Dose-response relationships have been investigated, showing nonlinearities and thresholds in genotoxic and epigenetic effects, as observed in various toxicity and carcinogenicity bioassays. Animal models, particularly rats and mice, have been predominant in assessing estragole’s genotoxicity and carcinogenicity, with some studies focusing on liver and nasal mucosa tissues. Techniques employed include 32P-post-labelling for DNA adduct detection, comet assays for oxidative DNA damage, and mutagenicity assays in microbial systems. Physiologically based biodynamic modeling has also been utilized to predict in vivo DNA adduct formation. The current scientific consensus acknowledges estragole as genotoxic and a liver carcinogen in rodent models, with metabolic activation via sulfation as a key step in the formation of DNA-reactive species. These findings have informed safety evaluations by expert committees, such as JECFA and the National Toxicology Program, highlighting the importance of dose considerations and metabolic pathways in risk assessment. »

[4.1] Safety and efficacy of a feed additive consisting of an essential oil from the fruit of Cuminum cyminum L. (cumin oil) for use in all animal species (FEFANA asbl). Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Josef Schlatter, Dieter Schrenk, Johannes Westendorf, Paola Manini, Fabiola Pizzo, Birgit Dusemund. EFSA Journal (2022). https://doi.org/10.2903/j.efsa.2022.7690

Context: "Estragole, a component found in cumin oil and related botanicals such as fennel, has a well-documented genotoxic and carcinogenic profile in rodents. Experimental studies have shown its metabolism involves demethylation producing proximate metabolites like 10-hydroxyestragole, which can react covalently with DNA. In low dose studies in rodents and humans, estragole is mostly exhaled as 14CO2 after metabolic transformation; however, higher oral doses increase urinary excretion of the reactive metabolite glucuronide conjugate 10-hydroxyestragole. The metabolic pathway includes hydroxylation and epoxidation with ring hydrolysis, similar across rats, mice, and humans. Estragole shares its mode of action with methyleugenol, another p-allylalkoxybenzene, and BMDL10 values have been derived from methyleugenol rodent carcinogenicity studies for risk assessment with estragole, set at 22.2 mg/kg body weight/day. Carcinogenicity has been documented in female CD-1 mice fed diets containing estragole, resulting in hepatic tumors (hepatomas) at doses of 2.3 or 4.6 g/kg diet over 12 months, although the mouse strain has high spontaneous liver tumor incidence complicating precise dose-response modeling. Additional in vivo studies in newborn mice using intraperitoneal injection showed estragole induced hepatomas, whereas dillapiole (structurally related compound) did not. DNA adduct formation studies have demonstrated significant DNA binding with estragole, methyleugenol, and safrole, reinforcing their genotoxic potential, while other related alkenylbenzenes showed lower or no detectable adducts. In vitro genotoxicity data and QSAR analyses have been applied for individual cumin oil constituents, many showing no alerts or discounted structural alerts based on read-across from analogues. The major metabolic pathways involve cytochrome P450-mediated oxidation producing reactive sulfoxymetabolites capable of covalent DNA binding, with ring substitutions influencing the extent of reactive metabolite formation. No human high-dose exposure studies exist, but low dose human metabolism resembles that in rodents. The EFSA FEEDAP Panel applies a component-based approach to assess cumin oil's constituents' genotoxicity, acknowledging the well-characterized genotoxic and carcinogenic risk of estragole, supported by both in vivo carcinogenicity in rodents and mechanistic data including metabolite formation and DNA adducts. »

[5.1] Estragole and anethole as potential hazardous compounds in products: extraction , analysis and pharmacological effects.. Lamiaa El Sayed Dief, Enas Elsayed Eltamany, Ghada Hadad, Amany Kamal Ibrahim. Records of Pharmaceutical and Biomedical Sciences (2025). https://doi.org/10.21608/rpbs.2025.363440.1360

Context: "Estragole, a compound found in fennel and other herbs, has a controversial safety profile regarding its genotoxicity and carcinogenicity. The European Medicines Agency (EMA) has restricted estragole use to the lowest levels due to concerns about its carcinogenic and genotoxic potential, based on toxicokinetic and genotoxicity mechanisms that reveal formation of DNA adducts through metabolic activation. This suggests that estragole's metabolites can bind DNA, leading to genotoxic effects. On the other hand, the Flavor and Extract Manufacturers Association (FEMA) classifies estragole as generally recognized as safe (GRAS), highlighting a discrepancy in regulatory stances. The metabolism of estragole produces reactive intermediates implicated in genotoxicity, and recent findings indicate that trans-anethole, a structurally related compound, also forms DNA adducts but with lower toxicity potential than estragole. Experimental models for assessing estragole toxicity include in vitro studies detecting DNA adduct formation and in vivo studies in animals evidencing carcinogenicity, although explicit dose-response relationships are not detailed in the excerpt. Analytical methods such as GC-MS, HPLC, UPLC, and HPTLC have been employed to quantify estragole and anethole in various fennel preparations, supporting toxicological assessments. Overall, while mechanistic and experimental evidence supports potential genotoxicity and carcinogenicity of estragole, regulatory opinions differ, and detailed dose-response data and comprehensive human studies are limited or not reported here. »

[5.2] Estragole and anethole as potential hazardous compounds in products: extraction , analysis and pharmacological effects.. Lamiaa El Sayed Dief, Enas Elsayed Eltamany, Ghada Hadad, Amany Kamal Ibrahim. Records of Pharmaceutical and Biomedical Sciences (2025). https://doi.org/10.21608/rpbs.2025.363440.1360

Context: "Estragole and trans-anethole are primary components in the essential oils of common edible herbs such as basil, fennel, anise, and star anise, which have widespread use in traditional and pharmacological applications. Extraction methods and analysis techniques notably affect the measured concentration of estragole in these herbal products, with various extraction techniques like steam distillation, hydrodistillation, supercritical fluid extraction, and ultrasound-assisted extraction being employed. Studies have conducted risk assessments for estragole's presence in these products due to its potential toxicity. Scientific investigations, including those cited within the provided excerpt, have explored estragole's genotoxicity and carcinogenic potential, particularly through in vitro and in vivo models, along with animal studies. These studies emphasize metabolic activation pathways of estragole, whereby estragole is bioactivated leading to DNA and hemoglobin adducts, indicating a mechanistic basis for genotoxicity. Such bioactivation involves metabolic conversion pathways producing reactive intermediates that interact with cellular macromolecules, potentially driving toxicity and carcinogenicity. Dose-response relationships have been examined in various experimental contexts, highlighting the concern over high exposure levels and toxicological risk associated with estragole consumption. However, there is an implied caution that herbal products containing estragole and trans-anethole should not be overconsumed, and additional comprehensive studies are called for to fully evaluate product safety, including toxicological thresholds. Overall, the consensus stresses the need for careful consideration of estragole's hazardous properties in herbal preparations, with extraction and analysis method variability affecting exposure assessment, and supported by mechanistic studies involving metabolic bioactivation analyzed through both chemical and biological assays. »

[6.1] Foeniculum vulgare Mill. (Apiaceae/Umbelliferae). Shahid Akbar. Handbook of 200 Medicinal Plants (2020). https://doi.org/10.1007/978-3-030-16807-0_100

Context: "Foeniculum vulgare Mill. (fennel) fruits and seeds have extensive traditional and clinical uses across various cultures and medicine systems. Traditionally, fennel preparations are used as carminatives, digestive stimulants, and for reducing flatulence and colic, especially in infants and nursing babies in Europe. A hot infusion is used to treat amenorrhea and improve lactation. In Unani medicine, fennel is utilized for opening liver and spleen obstructions, relieving colic, and inducing diuresis and menstruation. Iranian traditional medicine employs it for inflammatory bowel diseases, while Mexican traditional medicine uses decoctions as galactagogues and to treat respiratory diseases. In East Africa, boiled or roasted roots treat gonorrhea. Chinese traditional medicine uses fennel to alleviate chills, vomiting, diarrhea, and abdominal distension. Leaves and seeds are used in Guyana and Surinam for infant stomachache and adult gas expulsion respectively. European regulatory bodies approved fennel for gastrointestinal disorders since 2005. Phytochemically, fennel fruit contains high concentrations of anethole (~50-85%), fenchone, estragole, and other terpenes, flavonoids, and phenolic compounds contributing to its biological activities. Pharmacologically, aqueous and essential oil extracts demonstrate gastroprotective effects against ulceration, anti-inflammatory, analgesic, antioxidant, diuretic, estrogenic, hepatoprotective, immunomodulatory, hypotensive, and antithrombotic activities in various animal models. It also shows memory enhancement and anxiolytic effects. Fennel preparations improve antioxidant enzyme activities and exhibit NO scavenging abilities. Topical applications reduce intraocular pressure. Safety-wise, fennel is traditionally used in infants to prevent colic and flatulence. Animal studies reveal benefits without notable toxicity at tested doses. The use in pregnant and lactating women is supported by traditional use to improve lactation and modulate estrus cycles in rats suggesting estrogenic activity, but clinical safety data in humans is limited. Overall, fennel fruit preparations have a broad safety and efficacy profile in traditional and experimental settings, with clinical use supporting digestive health, lactation, and respiratory conditions, especially in infants and women, though careful consideration is advised due to phytoestrogenic compounds. »

[6.2] Foeniculum vulgare Mill. (Apiaceae/Umbelliferae). Shahid Akbar. Handbook of 200 Medicinal Plants (2020). https://doi.org/10.1007/978-3-030-16807-0_100

Context: "Foeniculum vulgare Mill., commonly known as fennel, is an aromatic perennial herb whose fruit (seeds) and preparations have been traditionally used worldwide for various medicinal purposes. Historically, fennel has been recognized since ancient times and mentioned by figures like Hippocrates and Dioscorides, mainly as a diuretic and emmenagogue. It has been regarded as a magic herb, credited with powers such as curing ailments and safeguarding against evil spirits. Traditionally, fennel fruit or seed preparations are employed as carminatives and stomachics to relieve colic and flatulence, commonly used in infants and children as diuretics and diaphoretics. In pregnant women, particularly in Italy, fennel consumption is widespread for at least three months during pregnancy, reputed to promote lactation (galactagogue effect) and facilitate menstruation and birth. It is also used to alleviate symptoms related to the male climacteric and to increase libido. Across various traditional medical systems, including Unani, Iranian traditional medicine, and Traditional Chinese Medicine, fennel preparations have been used to treat digestive complaints, inflammatory bowel diseases, liver and spleen obstructions, bronchitis, diabetes, kidney stones, and chronic coughs. The fruits contain bioactive compounds such as anethole, fenchone, and estragole, contributing to their pharmacological effects (antispasmodic, anti-inflammatory, analgesic, antioxidant). Fennel seed powders have topical applications such as poultices for snakebites. Clinically, fennel infusion is approved in Europe since 2005 for gastrointestinal disorder treatments by the European Medicines Agency's HMPC, reflecting recognized safety and efficacy for this use. The safety profile in infants, pregnant, and lactating women seems supported by traditional uses and widespread consumption, but detailed clinical safety studies are not described in the excerpt. Overall, fennel fruit preparations balance traditional therapeutic benefits with recognized clinical applications for digestive and reproductive health, making it a commonly used herbal remedy among diverse populations. »

[6.3] Foeniculum vulgare Mill. (Apiaceae/Umbelliferae). Shahid Akbar. Handbook of 200 Medicinal Plants (2020). https://doi.org/10.1007/978-3-030-16807-0_100

Context: "Foeniculum vulgare (fennel) contains estragole, which has raised concerns due to carcinogenicity observed in rodent studies. However, the risk of estragole carcinogenicity in rodents is suggested to be overestimated for humans because of differences in metabolism and natural inactivation of estragole by other substances in fennel preparations. Typical herbal preparations recommended by the European Medicine Agency include infusions of 1-5 g of crushed fennel fruits per day for children and 1.5–2.5 g three times daily for adults, brewed in boiling water. These traditional doses result in estragole intakes significantly lower than those used in toxicological rodent studies, which often use pure estragole at much higher concentrations. The comprehensive clinical and pharmacological data on fennel advocate traditional safe doses, noting that adverse effects in humans are rare and mainly observed in special populations (e.g., infants developing methemoglobinemia or seizure risk in epileptic patients). Due to the complexity in phytochemical profiles and presence of inhibitory compounds, including in vivo estragole detoxification, the typical intake via herbal teas and dietary supplements is considered safe and far below toxicological exposure levels used in animal models. Nonetheless, recommendations call for caution and further studies to better establish safe usage, particularly during pregnancy and infancy. »

[7.1] Levels of estragole in fennel teas marketed in Austria and assessment of dietary exposure. Daniela Mihats, Leopold Pilsbacher, Robert Gabernig, Martin Routil, Martin Gutternigg, Reinhard Laenger. International Journal of Food Sciences and Nutrition (2017). https://doi.org/10.1080/09637486.2016.1262334

Context: "Typical estragole contents in fennel-based infusions and teas vary widely depending on preparation and plant material. Estragole content ranges from 160 to 5336 µg/L in water infusions made from whole fennel fruits, with a maximum of 4644 µg/L observed in the current study. Infusions from fine cut fennel teas contain 64–1340 µg/L estragole. Granules for instant teas generally did not contain detectable estragole. Estimated daily estragole intake from consumption of fennel teas prepared from tea bags ranges from about 0.24 to 6.42 µg/kg body weight (bw) per day depending on population group: children, women, men. For infants (assumed body weight 5 to 12 kg) consuming fennel teas marketed for infants, estimated daily intake ranges from 0.08 to

20.78 µg/kg bw/day. Margin of Exposure (MOE) values calculated relative to toxicological benchmark doses (BMDL10 values of 9–33 mg/kg bw/day based on hepatoma incidence in mice) range widely, from below 1000 in some cases up to over 50,000, indicating varying risk levels depending on consumption scenario. The study notes that MOE values below 10,000 for some tea bag preparations suggest higher priority for risk management. Toxicological doses from animal studies (9–33 mg/kg bw/day) are several orders of magnitude higher than the estimated human exposures from traditional fennel tea consumption. Past exposure assessments (e.g., Raffo et al., EFSA) used theoretical estragole content assumptions based on fennel fruit essential oil content (5%), extraction efficiency (25–30%), and estragole proportion in oil (3.5–12%). The preparation methods used in the current study reflect consumer typical preparation at home, allowing more relevant exposure estimates. Overall, estragole exposure from typical fennel teas is lower than toxic doses but varies with product type, preparation, and population group, with infant exposures often higher on a body weight basis. »

[7.2] Levels of estragole in fennel teas marketed in Austria and assessment of dietary exposure. Daniela Mihats, Leopold Pilsbacher, Robert Gabernig, Martin Routil, Martin Gutternigg, Reinhard Laenger. International Journal of Food Sciences and Nutrition (2017). https://doi.org/10.1080/09637486.2016.1262334

Context: "The current scientific consensus indicated in the study suggests that estragole, present in fennel tea, was not found to be cytotoxic, genotoxic, or apoptotic in human cells, implying limited genotoxicity in vitro. The toxicological reference values used for risk assessment, such as BMDL10 values, were derived from long-term rodent studies using high concentrations of pure estragole. This animal model data may not fully represent human dietary exposure due to differences in dosing and exposure context, potentially leading to an overestimation of human risk. Additionally, the study notes that dietary flavonoids present in the botanical matrix may modulate the bioactivation or cancer risk posed by estragole, indicating that matrix effects need consideration in risk assessment. The estragole metabolism relevant for toxicity involves bioactivation pathways leading to potentially carcinogenic metabolites; however, the presence of other plant compounds could influence these pathways. Established dose-response relationships are based largely on animal data, with MOE (Margin of Exposure) values developed from rodent BMDL10 data to estimate human risk. Risk estimates show variable MOEs, with some infant exposures from fennel teas being below 10,000, indicating potential priorities for risk management. Overall, the evidence points to estragole having carcinogenic potential in animal models at high doses, but human risk at dietary levels, especially in fennel tea consumption, is likely lower due to metabolic and matrix interactions, and the lack of genotoxicity in human cells in vitro. The study emphasizes the importance of case-by-case assessment of botanical matrix effects and dose-dependent responses in evaluating estragole-related risk. »

[7.3] Levels of estragole in fennel teas marketed in Austria and assessment of dietary exposure. Daniela Mihats, Leopold Pilsbacher, Robert Gabernig, Martin Routil, Martin Gutternigg, Reinhard Laenger. International Journal of Food Sciences and Nutrition (2017). https://doi.org/10.1080/09637486.2016.1262334

Context: "Estragole, a constituent of fennel essential oil, has been evaluated as genotoxic and carcinogenic by the Scientific Committee on Food (SCF), which concluded that a safe exposure threshold could not be established. Consequently, reductions in exposure and usage restrictions have been suggested. However, the Flavor and Extract Manufacturers Association (FEMA) Expert Panel disagrees, indicating that dietary intake of estragole does not pose a health risk at low exposure levels, due to dose-dependent differences in metabolism, metabolic activation, and covalent binding observed in studies. These differences suggest that the relative importance of toxic metabolic pathways greatly diminishes at low doses. The European Food Safety Authority (EFSA) recommends the use of the Margin of Exposure (MOE) approach derived from animal studies—using the BMDL10, a benchmark dose lower confidence bound associated with 10% extra cancer incidence—to assess risk for genotoxic and carcinogenic botanicals like estragole.

An MOE above 10,000 is generally considered low risk from a public health perspective. The key to risk assessment is estimating human exposure through dietary consumption of fennel tea, which can vary widely. Experimental assessment of genotoxicity and carcinogenicity referenced includes animal studies (from which BMDL10 values are derived), although specific details on in vitro models and human studies are not detailed in this excerpt. Metabolic pathways relevant to estragole toxicity involve metabolic activation leading to DNA binding and cancer risk; the dose dependency of these pathways affects the risk profile. Observations indicate that at low exposure levels, the pathways leading to genotoxicity and carcinogenicity are less prominent. Due to the genotoxic and carcinogenic potential of estragole, accurate dietary exposure estimates and risk management priorities are informed through such assessments."