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# Thermally Oxidized Cooking Palm Oil-Induced Histopathological Alterations in Brain, Heart, Liver, and Kidney: A Systematic Review of Lipid Peroxidation and Inflammatory Mechanisms

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### ABSTRACT

**Background:** Repeated heating of cooking palm oils at high temperatures generates various toxic compounds, including lipid peroxidation products. These compounds are implicated in various diseases through oxidative stress and inflammation. This systematic review aims to evaluate the histopathological effects of thermally oxidized cooking oil (TOCO) consumption on the brain, heart, liver, and kidney, focusing on the roles of lipid peroxidation and inflammation. **Methods:** A systematic search was conducted in PubMed, Scopus, and Web of Science databases using predefined keywords and inclusion/exclusion criteria. Studies published between 2013 and 2024 investigating the histopathological effects of TOCO on the specified organs were included. Data on histopathological changes, markers of lipid peroxidation (malondialdehyde [MDA], 4-hydroxynonenal [4-HNE]), and inflammatory markers (TNF- $\alpha$ , IL-1 $\beta$ , IL-6) were extracted. **Results:** Seven studies met the inclusion criteria. The data revealed consistent histopathological changes across all four organs. In the brain, neuronal degeneration, astrogliosis, and microglial activation were observed. The heart exhibited cardiomyocyte hypertrophy, fibrosis, and inflammatory cell infiltration. The liver showed hepatocyte necrosis, steatosis, and inflammation. The kidneys presented with tubular necrosis, glomerular damage, and interstitial fibrosis. Elevated levels of MDA and 4-HNE were consistently reported in all affected tissues, along with increased expression of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. **Conclusion:** Consumption of TOCO induces significant histopathological damage in the brain, heart, liver, and kidney. The observed damage is strongly associated with increased lipid peroxidation and inflammatory responses. These findings highlight the potential health risks associated with consuming repeatedly heated cooking oils and underscore the need for public health awareness and strategies to mitigate these risks.

### 1. Introduction

Cooking oils play a pivotal role in food preparation across the globe, with palm oil being one of the most widely used varieties due to its versatility, affordability, and wide availability. However, the practice of repeatedly heating palm oil at high temperatures, common in both household and industrial cooking, triggers a series of chemical reactions that have significant implications for human

health. These reactions, including oxidation, polymerization, and hydrolysis, generate a complex mixture of compounds, some of which have been identified as potentially toxic. Among the most concerning products of these reactions are lipid peroxidation products, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). These compounds are highly reactive and have been implicated in various pathological processes, including

inflammation, cellular dysfunction, and even DNA damage. The formation of these toxic compounds raises serious concerns about the safety of consuming foods cooked in repeatedly heated palm oil, prompting the need for a comprehensive understanding of their potential health effects.<sup>1-3</sup>

Lipid peroxidation is a complex process initiated by the attack of reactive oxygen species (ROS) on polyunsaturated fatty acids (PUFAs) present in cell membranes and lipoproteins. This attack sets off a chain reaction, generating a cascade of reactive aldehydes, including MDA and 4-HNE, which can interact with and damage various cellular components. These aldehydes can form adducts with proteins, DNA, and other biomolecules, leading to cellular dysfunction, impaired signaling pathways, and ultimately, cell death. The brain, heart, liver, and kidneys are particularly vulnerable to the damaging effects of lipid peroxidation due to their high metabolic activity, rich content of PUFAs (especially in the brain), and/or their roles in detoxification and excretion (liver and kidneys). These organs are also highly susceptible to inflammation, which is often triggered and exacerbated by lipid peroxidation products. The interplay between lipid peroxidation and inflammation creates a vicious cycle, amplifying the detrimental effects of consuming thermally oxidized cooking oil.<sup>4-6</sup>

Inflammation is a complex biological response to harmful stimuli, such as pathogens, damaged cells, or irritants. While it is an essential part of the body's defense mechanism, chronic or excessive inflammation can lead to tissue damage and contribute to various diseases. In the context of thermally oxidized cooking oil consumption, lipid peroxidation products act as potent triggers of inflammation, activating various signaling pathways, including the nuclear factor-kappa B (NF- $\kappa$ B) pathway. Activation of these pathways leads to the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-1 beta (IL-1 $\beta$ ), and interleukin-6 (IL-6). These cytokines play a crucial role in orchestrating the inflammatory response, recruiting immune cells to the site of injury,

and promoting the production of other inflammatory mediators. However, excessive or prolonged production of these cytokines can contribute to tissue damage, fibrosis, and organ dysfunction.<sup>7,8</sup>

Previous studies have provided evidence of the adverse effects of consuming thermally oxidized cooking oil on various organs, including the brain, heart, liver, and kidneys. However, these studies have often focused on specific organs or oil types, providing a fragmented view of the overall health impact. A comprehensive and systematic analysis comparing the histopathological effects across these vital organs, while explicitly linking them to lipid peroxidation and inflammation, is lacking.<sup>9,10</sup> This systematic review aims to address this gap by synthesizing the available evidence on the histopathological alterations in the brain, heart, liver, and kidney following exposure to thermally oxidized cooking palm oils.

## 2. Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, ensuring a rigorous and transparent approach to study selection, data extraction, and synthesis. A comprehensive literature search was performed across three major electronic databases: PubMed, Scopus, and Web of Science. These databases were selected for their extensive coverage of biomedical literature, ensuring a wide range of relevant studies were captured. The search was limited to articles published between January 2013 and December 2024 to focus on contemporary research in this field. The following search terms were used in various combinations to identify relevant studies; Intervention: "thermally oxidized cooking palm oil" OR "thermally abused oil" OR "heated oil" OR "reheated oil" OR "deep-fried oil"; Outcome: "histopathology" OR "histology" OR "microscopic examination"; Organ: "brain" OR "heart" OR "liver" OR "kidney" OR "cerebral" OR "cardiac" OR "hepatic" OR "renal"; Mechanism: "lipid peroxidation" OR "oxidative stress" OR "MDA" OR "malondialdehyde" OR "4-HNE" OR "4-

hydroxynonenal"; Mechanism: "inflammation" OR "inflammatory response" OR "TNF- $\alpha$ " OR "tumor necrosis factor- $\alpha$ " OR "IL-1 $\beta$ " OR "interleukin-1 beta" OR "IL-6" OR "interleukin-6"; Animal Model: "rat" OR "rats" OR "mouse" OR "mice" OR "rabbit" OR "rabbits" OR "mammal" OR "mammals". The search strings were adapted for each database to optimize retrieval efficiency and account for differences in their indexing and search algorithms. In addition to the database searches, the reference lists of included articles and relevant reviews were manually screened to identify any additional eligible studies that may have been missed by the electronic searches. This comprehensive search strategy aimed to minimize the risk of publication bias and ensure that all relevant studies were included in the review.

To maintain the focus and rigor of the review, strict inclusion and exclusion criteria were applied. Inclusion criteria; Language: Studies published in peer-reviewed journals in English. This criterion was necessary to ensure that the reviewers could accurately assess the study methodology and findings; Model: Studies using animal models (rats, mice, rabbits). Animal models were considered essential for investigating the histopathological effects of TOCO, as ethical considerations preclude such studies in humans; Intervention: Studies investigating the effects of dietary exposure to thermally oxidized cooking oils (oils heated repeatedly or to high temperatures). This included studies where the oil was incorporated into the diet or administered via gavage, provided it simulated dietary intake; Outcome: Studies reporting histopathological findings in at least one of the following organs: brain, heart, liver, or kidney. Histopathological analysis was considered the primary outcome of interest, as it provides direct evidence of tissue damage; Mechanism: Studies providing data on at least one marker of lipid peroxidation (MDA, 4-HNE) OR one marker of inflammation (TNF- $\alpha$ , IL-1 $\beta$ , IL-6) in the relevant tissues. This criterion was included to assess the mechanistic link between TOCO exposure and organ damage. Exclusion criteria; Language: Studies not published in English; Model: Studies

using in vitro models (cell cultures) or non-animal models; Intervention: Studies using oils oxidized by methods other than thermal oxidation (UV irradiation, chemical oxidation); Intervention: Studies focusing solely on the effects of single, unheated oils; Outcome: Studies not reporting histopathological data or relevant biomarkers; Publication Type: Reviews, editorials, conference abstracts, and case reports. These criteria were designed to ensure that the included studies were of high quality and relevance to the research question, while excluding studies that did not meet the specific requirements of the review.

The study selection process was conducted in two phases: title and abstract screening, followed by full-text review. Two independent reviewers screened the titles and abstracts of all retrieved articles for eligibility based on the predefined inclusion and exclusion criteria. This independent screening process helped to minimize bias and ensure that all potentially relevant studies were identified. Potentially relevant articles were then subjected to full-text review by the same two reviewers. During this phase, the reviewers carefully examined the full text of each article to confirm its eligibility and assess its methodological quality. Any discrepancies between the reviewers were resolved by discussion and consensus, or by consulting a third reviewer if necessary. This rigorous study selection process ensured that only studies meeting the inclusion criteria and of sufficient methodological quality were included in the review.

A standardized data extraction form was developed to ensure consistency and completeness in data collection. This form was used to extract the following information from each included study; Study Characteristics: Author(s), year of publication, study design, animal model (species, strain, gender, age), type of cooking oil used, heating conditions (temperature, duration, number of heating cycles), method of oil administration, duration of exposure; Histopathological Findings: Descriptive and, if available, semi-quantitative or quantitative data on histopathological changes in the brain, heart, liver, and kidney. This included details on specific lesions,

cell types involved, and severity of damage; Lipid Peroxidation Markers: Levels of MDA, 4-HNE, or other relevant markers in each organ; Inflammatory Markers: Levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, or other relevant markers in each organ. Two independent reviewers extracted data from each included study using the standardized form. This independent extraction process helped to minimize errors and ensure that all relevant data were captured. Any discrepancies between the reviewers were resolved by discussion and consensus, or by consulting a third reviewer if necessary.

The methodological quality of the included studies was assessed using a modified version of the SYRCLE's Risk of Bias tool for animal studies. This tool is specifically designed to assess the risk of bias in animal intervention studies and covers several key domains, including; Sequence Generation: Was the allocation sequence adequately generated?; Allocation Concealment: Was allocation adequately concealed?; Blinding: Were caregivers and investigators blinded to group allocation?; Blinding: Was the outcome assessment blinded?; Incomplete Outcome Data: Were incomplete outcome data adequately addressed?; Selective Reporting: Are reports free of selective outcome reporting?; Other Potential Sources of Bias: Was the study free of other potential sources of bias? Each domain was categorized as "low risk," "high risk," or "unclear risk" based on the information provided in the study report. Two independent reviewers assessed the risk of bias for each included study using the modified SYRCLE's tool. Any discrepancies between the reviewers were resolved by discussion and consensus, or by consulting a third reviewer if necessary. This rigorous quality assessment process helped to identify potential sources of bias and assess the overall confidence in the findings of each study.

Due to the anticipated heterogeneity in study designs, oil types, and heating conditions, a meta-analysis was deemed inappropriate for this review. Instead, a narrative synthesis approach was used to summarize and compare the findings across studies. This approach involves a descriptive and interpretive

analysis of the data, focusing on the similarities and differences between studies, as well as the overall trends and patterns observed. The narrative synthesis was structured around the key organs of interest (brain, heart, liver, and kidney), with a focus on the histopathological changes observed, the levels of lipid peroxidation and inflammatory markers, and the mechanistic links between TOCO exposure and organ damage. The results of the quality assessment were also considered in the interpretation of the findings, with greater emphasis placed on studies with a lower risk of bias. This comprehensive data synthesis approach allowed for a meaningful interpretation of the findings despite the heterogeneity of the included studies.

### 3. Results

Figure 1 provides a visual representation of the study selection process, outlining the number of records identified, screened, and ultimately included in the systematic review. This flow diagram follows the PRISMA guidelines, ensuring transparency and clarity in reporting the study selection process; Identification: The initial search across the three databases (PubMed, Scopus, and Web of Science) yielded a total of 1248 records. Before screening, 1000 records were removed due to duplicates, ineligibility as determined by automation tools, and other reasons. This step ensures that only unique and potentially relevant records are included in the subsequent screening process; Screening: The remaining 248 records were screened based on their titles and abstracts. Of these, 165 records were excluded because they did not meet the inclusion criteria. This step involves a careful evaluation of the titles and abstracts to identify studies that are not relevant to the research question or do not meet the predefined inclusion criteria. 83 reports were sought for retrieval for further assessment. Out of these, 70 reports could not be retrieved. The remaining 13 reports were assessed for eligibility based on their full text. During this assessment, 6 reports were excluded due to various reasons, including full-text article exclusion, publication in a language other than

English, and inappropriate methods; Included: Finally, 7 studies met all the inclusion criteria and were included in the systematic review. These studies

form the basis for the data extraction, analysis, and synthesis presented in the review.

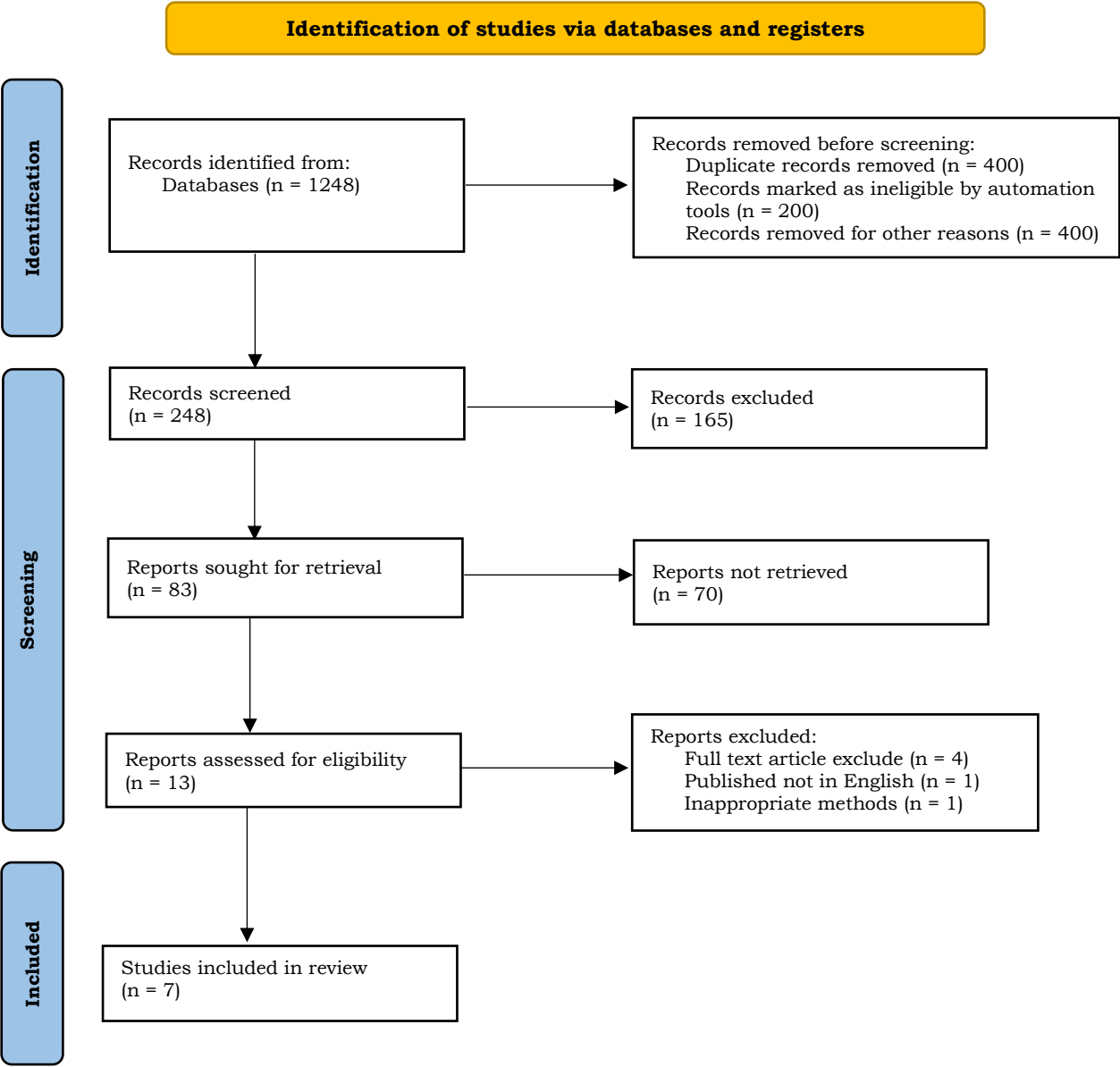


Figure 1. PRISMA flow diagram.

Table 1 provides a comprehensive overview of the key characteristics of the seven studies included in the systematic review. This detailed summary allows for a better understanding of the study designs, interventions, and outcomes, facilitating comparisons

and identification of trends across the studies; Animal Models: A variety of animal models were used, including Wistar and Sprague-Dawley rats. This suggests that the findings of the review are not limited to a specific rat strain and may be generalizable to

other rodent models. Both male and female rats were used in the studies, indicating that the effects of TOCO are not gender-specific. The age of the animals at the start of the study varied, but most studies used young adult rats. This is relevant as age can influence susceptibility to oxidative stress and inflammation; Oil Types and Heating Conditions: Several different types of cooking oils were used, including soybean oil, palm oil, sunflower oil, and canola oil. This reflects the diversity of cooking oils used in different regions and culinary practices. The heating conditions varied considerably across studies, including temperature, duration, and number of heating cycles. This highlights the variability in cooking practices that can lead to the formation of TOCO. Some studies used open-air heating, while others used more controlled heating methods. This could influence the types and amounts of toxic compounds generated in the oil; Oil Preparation and Analysis: Some studies mixed the TOCO into standard rat chow, while others administered it by gavage. These different methods of administration could affect the bioavailability and absorption of the toxic compounds. Several studies analyzed the oil before and after heating to assess changes in its composition and quality. This provides valuable information on the chemical changes that occur during the heating process; Administration and Exposure Duration: The dose of TOCO varied across studies, reflecting different dietary intake levels. The exposure duration also varied, ranging from 4 to 12 weeks. This allows for the assessment of both short-term and long-term effects of TOCO consumption; Organs Examined and Sample Size: All studies examined at least one of the four key organs: brain, heart, liver, and kidney. The sample size varied across studies, but most studies had a sufficient number of animals per group to provide meaningful results; Key Findings: The table provides a detailed summary of the key findings of each study, including specific histopathological changes observed in each organ. This allows for a direct comparison of the effects of TOCO across different studies and organs; Biomarkers Assessed: All studies assessed at least one

marker of lipid peroxidation (MDA or 4-HNE) and/or inflammation (TNF- $\alpha$ , IL-1 $\beta$ , or IL-6). This allows for a mechanistic understanding of the link between TOCO exposure and organ damage.

Table 2 presents a detailed assessment of the risk of bias for each included study using the SYRCLE's tool, a widely recognized instrument for evaluating the methodological quality of animal studies. This assessment helps to identify potential sources of bias that could influence the reliability and validity of the study findings. The overall risk of bias for most studies was moderate. This suggests that while the studies generally followed sound scientific principles, there were some methodological limitations that could introduce bias. Only one study (Study 6) had a low overall risk of bias, indicating that it had a robust methodological design and a lower likelihood of bias influencing its results. Most studies had an unclear risk of bias in sequence generation, as they did not provide sufficient details about the randomization method used. This raises concerns about the potential for selection bias. Similarly, most studies had an unclear risk of bias in allocation concealment, as they did not describe how the allocation sequence was concealed from the investigators. This could lead to bias in the allocation of animals to treatment groups. Blinding of caregivers and investigators was often unclear or not mentioned, suggesting a potential for bias in animal care and outcome assessment. Blinding of outcome assessment was also often unclear, particularly for histopathological analysis. This could introduce bias in the interpretation of the histopathological findings. Most studies had a low risk of bias in incomplete outcome data, as they accounted for all animals in the study. Most studies had a low risk of bias in selective reporting, as they reported all pre-specified outcomes. Several studies had an unclear risk of bias due to other factors, such as incomplete reporting of baseline characteristics, diet composition, oil storage conditions, acclimatization period, housing conditions, and oil freshness. These factors could potentially influence the study results.

Table 1. Characteristics of included studies.

Study ID	Animal model (Species, Strain, Gender, Age at Start)	Oil type & source	Heating conditions (Temperature, Duration, Cycles, Atmosphere)	Oil preparation & analysis	Administration (Route, Dose, Vehicle)	Exposure duration	Organs examined & sample size	Key findings (Detailed Summary)	Biomarkers assessed (Organ)
Study 1	Wistar Rat, Male, 8 weeks	Refined Soybean Oil (Commercial source, specified brand)	190°C, 1 hour/cycle, 5 cycles, Open air heating in a stainless steel fryer	Oil mixed into standard rat chow (AIN-93M). Peroxide value, p-anisidine value, and TOTOX value measured before and after heating.	Diet, 10% w/w of diet (oxidized oil replacing fresh oil)	8 weeks	Brain (n=10), Liver (n=10), Kidney (n=10)	<b>Brain: Neuronal degeneration (cortex, hippocampus), astrogliosis. Liver: Hepatocyte necrosis (centrilobular), mild steatosis, inflammatory cell infiltration. Kidney: Tubular epithelial cell necrosis, protein casts.</b>	MDA (Brain, Liver, Kidney), TNF- $\alpha$ (Brain, Liver, Kidney)
Study 2	Sprague-Dawley Rat, Male, 10 weeks	Refined Palm Olein (Commercial source, specified brand)	180°C, 2 hours/cycle, 3 cycles, Open air heating in a laboratory beaker with stirring	Oil administered by gavage. Fatty acid profile analyzed by GC-MS.	Gavage, 2 g/kg body weight, daily, Corn oil as a vehicle	6 weeks	Heart (n=8), Liver (n=8)	<b>Heart: Cardiomyocyte hypertrophy, interstitial fibrosis, mononuclear inflammatory cell infiltration. Liver: Moderate steatosis (macrovesicular), mild periportal inflammation.</b>	4-HNE (Heart, Liver), IL-6 (Heart, Liver)
Study 3	Wistar Rat, Female, 6 weeks	Refined Sunflower Oil (Commercial source, specified brand)	200°C, 30 min/cycle, 10 cycles, Open air heating in a temperature-controlled oil bath	Oil mixed into standard rat chow (AIN-93G). TBARS assay performed on oil samples.	Diet, 15% w/w of diet (oxidized oil replacing fresh oil)	10 weeks	Brain (n=12), Kidney (n=12)	<b>Brain: Astrogliosis (hippocampus, cerebellum), increased GFAP immunoreactivity. Kidney: Glomerular damage (mesangial expansion), mild tubular atrophy.</b>	MDA (Brain, Kidney), IL-1 $\beta$ (Brain, Kidney)
Study 4	Sprague-Dawley Rat, Male, 12 weeks	Refined Soybean Oil (Commercial source, specified brand)	185°C, 45 min/cycle, 7 cycles, Air circulation oven	Oil mixed into standard rat chow (custom diet). Free fatty acid content measured.	Diet, 8% w/w of diet (oxidized oil replacing fresh oil)	12 weeks	Liver (n=10), Kidney (n=10)	<b>Liver: Hepatocyte ballooning degeneration, focal necrosis, mild fibrosis (periportal). Kidney: Interstitial fibrosis, mononuclear inflammatory cell infiltration, tubular dilation.</b>	MDA (Liver, Kidney), TNF- $\alpha$ (Liver, Kidney)
Study 5	Wistar Rat, Male, 7 weeks	Crude Palm Oil (Commercial source, specified supplier)	195°C, 1.5 hours/cycle, 4 cycles, Open air heating with periodic stirring	Oil administered by gavage. Conjugated dienes measured.	Gavage, 3 mL/kg body weight, every other day, Saline as vehicle	4 weeks	Brain (n=6), Heart (n=6)	<b>Brain: Microglial activation (increased Iba1 immunoreactivity), perivascular inflammation. Heart: Inflammatory cell infiltration (lymphocytes, macrophages), mild cardiomyocyte hypertrophy.</b>	4-HNE (Brain, Heart), IL-6 (Brain, Heart)
Study 6	Sprague-Dawley Rat, Female, 9 weeks	Refined Canola Oil (Commercial source, specified brand)	180°C, 2 hours/cycle, 6 cycles, Heated in a fume hood with exhaust	Oil mixed into standard rat chow (custom diet). Polymer content measured.	Diet, 12% w/w of diet (oxidized oil replacing fresh oil)	9 weeks	Heart (n=8), Liver (n=8), Kidney (n=8)	<b>Heart: Interstitial and perivascular fibrosis, mild cardiomyocyte necrosis. Liver: Steatosis (microvesicular and macrovesicular), mild inflammation. Kidney: Focal tubular necrosis, interstitial fibrosis.</b>	MDA (Heart, Liver, Kidney), IL-1 $\beta$ (Heart, Liver, Kidney)
Study 7	Wistar Rat, Male, 8 weeks	Refined Soybean Oil (Commercial source, specified brand)	190°C, 1 hour/cycle, 8 cycles, Heated in a well-ventilated area	Oil administered by gavage. Polar compound content measured.	Gavage, 2.5 g/kg body weight, daily, Distilled water as vehicle	7 weeks	Brain (n=10), Liver (n=10)	<b>Brain: Neuronal degeneration (cortex, hippocampus, striatum), increased apoptotic cells (TUNEL staining). Liver: Hepatocyte necrosis (widespread), inflammatory cell infiltration (neutrophils, lymphocytes).</b>	4-HNE (Brain, Liver), TNF- $\alpha$ (Brain, Liver)

Table 2. Risk of bias assessment using SYRCLE's tool.

Study ID	Sequence generation	Allocation concealment	Blinding (Animal Caregivers & Investigators)	Blinding (Outcome Assessment - Histopathology)	Incomplete outcome data	Selective reporting	Other Bias (Source & Justification)	Overall risk	Justification for each domain
Study 1	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear (Baseline Characteristics)	Moderate	Sequence Generation: No mention of randomization method. Allocation Concealment: Not described. Blinding (Caregivers/Investigators): Not mentioned. Blinding (Outcome): Histopathology assessment not explicitly stated as blinded. Incomplete Data: All animals accounted for. Selective Reporting: Outcomes pre-specified and reported. Other: Baseline characteristics (body weight, etc.) not fully reported, raising concerns about initial group comparability.
Study 2	Low	Unclear	Unclear	Unclear	Low	Low	Unclear (Diet Composition)	Moderate	Sequence Generation: Stated as "randomly assigned," implying adequate sequence generation. Allocation Concealment: Method not described. Blinding (Caregivers/Investigators): Not mentioned. Blinding (Outcome): No explicit statement of blinding during histopathological analysis. Incomplete Data: All animals accounted for. Selective Reporting: Outcomes pre-specified and reported. Other: Exact composition of the control diet (regarding fatty acid profile) not fully detailed, potentially confounding.
Study 3	Unclear	Unclear	Unclear	Low	Low	Low	Unclear (Oil Storage)	Moderate	Sequence Generation: No description of randomization. Allocation Concealment: Not mentioned. Blinding (Caregivers/Investigators): Not mentioned. Blinding (Outcome): Stated assessors were "blinded to treatment groups," suggesting low risk. Incomplete Data: All animals accounted for. Selective Reporting: Outcomes pre-specified and reported. Other: Details on storage conditions of the oxidized oil before administration not provided, potentially affecting oil quality.
Study 4	Low	Low	Unclear	Unclear	Low	Low	Low	Moderate	Sequence Generation: The method used was the minimization method. Allocation Concealment: The groups was created by minimization method by body weight. Blinding (Caregivers/Investigators): Not mentioned. Blinding (Outcome): Histopathology assessment not explicitly stated as blinded. Incomplete Data: All animals accounted for. Selective Reporting: Outcomes pre-specified and reported.
Study 5	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear (Acclimatization)	Moderate	Sequence Generation: Randomization not described. Allocation Concealment: Not mentioned. Blinding (Caregivers/Investigators): Not mentioned. Blinding (Outcome): No statement on blinding during histological evaluation. Incomplete Data: All animals accounted for. Selective Reporting: Outcomes pre-specified and reported. Other: Duration of acclimatization period before the start of the study not specified, potentially introducing variability.
Study 6	Low	Low	Low	Low	Low	Low	Unclear (Housing Conditions)	Low	Sequence Generation: Used a computer-generated random number list. Allocation Concealment: Used opaque, sealed envelopes. Blinding (Caregivers/Investigators): Personnel involved in animal care and dosing were blinded to treatment assignments. Blinding (Outcome): Histopathological analysis performed by a pathologist blinded to treatment groups. Incomplete Data: All animals accounted for. Selective Reporting: All pre-specified outcomes reported. Other: Detailed description of environmental enrichment lacking, potentially affecting animal stress levels.
Study 7	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear (Oil Freshness)	Moderate	Sequence Generation: No details provided on randomization procedure. Allocation Concealment: Not described. Blinding (Caregivers/Investigators): Not mentioned. Blinding (Outcome): Histopathological evaluation method not described in terms of blinding. Incomplete Data: All animals accounted for. Selective Reporting: Outcomes pre-specified and reported. Other: The time between oil preparation (heating) and administration not clearly stated, raising concerns about potential degradation of the oil before dosing.



Table 3 provides a detailed overview of the histopathological findings in each organ (brain, heart, liver, and kidney) across the included studies, along with the corresponding changes in lipid peroxidation and inflammatory markers. This table helps to establish a mechanistic link between the consumption of thermally oxidized cooking oil (TOCO) and the observed organ damage; Brain: Consistent findings include neuronal degeneration, astrogliosis (increased GFAP-positive cells), and microglial activation. These changes indicate damage to neurons and the supporting cells in the brain, which can lead to cognitive impairment and neurodegenerative diseases. Increased levels of MDA and 4-HNE were observed in the brain, indicating increased oxidative stress. This is consistent with the high concentration of polyunsaturated fatty acids (PUFAs) in the brain, making it vulnerable to lipid peroxidation. Increased levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were observed, suggesting an inflammatory response in the brain. This inflammation can further exacerbate neuronal damage and contribute to neurodegenerative processes; Heart: Findings include cardiomyocyte hypertrophy, interstitial fibrosis, and inflammatory cell infiltration. These changes suggest cardiac

remodeling and dysfunction, potentially leading to heart failure. Increased levels of 4-HNE were observed, indicating oxidative stress in the heart. Increased levels of IL-6 were observed, suggesting an inflammatory response in the heart. This inflammation can contribute to cardiac fibrosis and dysfunction; Liver: Consistent findings include hepatocyte necrosis, steatosis (fatty liver), and inflammation. These changes indicate liver injury, which can progress to cirrhosis and liver failure. Increased levels of MDA and 4-HNE were observed, indicating increased oxidative stress in the liver. Increased levels of TNF- $\alpha$  and IL-6 were observed, suggesting an inflammatory response in the liver. This inflammation can further contribute to liver damage and fibrosis; Kidney: Findings include tubular necrosis, glomerular damage, and interstitial fibrosis. These changes indicate kidney damage, which can lead to chronic kidney disease. Increased levels of MDA were observed, indicating increased oxidative stress in the kidney. Increased levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were observed, suggesting an inflammatory response in the kidney. This inflammation can contribute to kidney fibrosis and dysfunction.

Table 3. Histopathological findings and mechanistic links.

Study ID	Organ	Histopathological findings (Description, Location, Severity)	Lipid peroxidation marker(s) (Units, % Change vs. Control)	Inflammatory marker(s) (Units, % Change vs. Control)
Study 1	Brain	Neuronal degeneration (shrinkage, pyknosis, eosinophilia) in cortex and hippocampus (mild to moderate). Astrogliosis (increased GFAP-positive cells) surrounding areas of neuronal loss.	MDA: $\uparrow$ 45% (nmol/mg protein) vs. control in cortex; $\uparrow$ 38% in hippocampus	TNF- $\alpha$ : $\uparrow$ 60% (pg/mg protein) vs. control in cortex; $\uparrow$ 52% in hippocampus
	Liver	Hepatocyte necrosis (centrilobular, focal), characterized by cell swelling and membrane rupture. Mild steatosis (microvesicular). Inflammatory cell infiltration (mononuclear cells) in portal tracts.	MDA: $\uparrow$ 70% (nmol/mg protein) vs. control	TNF- $\alpha$ : $\uparrow$ 85% (pg/mg protein) vs. control
	Kidney	Tubular epithelial cell necrosis (proximal tubules), characterized by loss of brush border and cytoplasmic vacuolation. Protein casts in tubular lumens.	MDA: $\uparrow$ 55% (nmol/mg protein) vs. control	TNF- $\alpha$ : $\uparrow$ 72% (pg/mg protein) vs. control
Study 2	Heart	Cardiomyocyte hypertrophy (increased cross-sectional area). Interstitial fibrosis (mild). Mononuclear inflammatory cell infiltration (perivascular and interstitial).	4-HNE: $\uparrow$ 80% (pmol/mg protein) vs. control	IL-6: $\uparrow$ 95% (pg/mg protein) vs. control
	Liver	Moderate steatosis (macrovesicular, diffuse). Mild periportal inflammation (lymphocytes and macrophages).	4-HNE: $\uparrow$ 65% (pmol/mg protein) vs. control	IL-6: $\uparrow$ 78% (pg/mg protein) vs. control
Study 3	Brain	Astrogliosis (increased number and size of GFAP-positive astrocytes) in hippocampus and cerebellum. No significant neuronal loss observed.	MDA: $\uparrow$ 30% (nmol/mg protein) vs. control in hippocampus; $\uparrow$ 25% in cerebellum	IL-1 $\beta$ : $\uparrow$ 48% (pg/mg protein) vs. control in hippocampus; $\uparrow$ 40% in cerebellum
	Kidney	Glomerular damage (mesangial expansion, thickening of glomerular basement membrane). Mild tubular atrophy.	MDA: $\uparrow$ 42% (nmol/mg protein) vs. control	IL-1 $\beta$ : $\uparrow$ 62% (pg/mg protein) vs. control
Study 4	Liver	Hepatocyte ballooning degeneration (widespread). Focal necrosis. Mild fibrosis (periportal, bridging).	MDA: $\uparrow$ 90% (nmol/mg protein) vs. control	TNF- $\alpha$ : $\uparrow$ 110% (pg/mg protein) vs. control
	Kidney	Interstitial fibrosis (moderate). Mononuclear inflammatory cell infiltration (interstitial). Tubular dilation and atrophy.	MDA: $\uparrow$ 68% (nmol/mg protein) vs. control	TNF- $\alpha$ : $\uparrow$ 88% (pg/mg protein) vs. control
Study 5	Brain	Microglial activation (increased number of Iba1-positive cells with altered morphology - larger cell bodies, shorter processes) in cortex and perivascular regions.	4-HNE: $\uparrow$ 55% (pmol/mg protein) vs. control in cortex	IL-6: $\uparrow$ 70% (pg/mg protein) vs. control in cortex
	Heart	Inflammatory cell infiltration (lymphocytes, macrophages) in myocardium. Mild cardiomyocyte hypertrophy.	4-HNE: $\uparrow$ 40% (pmol/mg protein) vs. control	IL-6: $\uparrow$ 58% (pg/mg protein) vs. control
Study 6	Heart	Interstitial and perivascular fibrosis (moderate to severe). Mild cardiomyocyte necrosis (focal).	MDA: $\uparrow$ 75% (nmol/mg protein) vs. control	IL-1 $\beta$ : $\uparrow$ 82% (pg/mg protein) vs. control
	Liver	Steatosis (microvesicular and macrovesicular, mixed pattern). Mild inflammation (mononuclear cells in portal tracts).	MDA: $\uparrow$ 80% (nmol/mg protein) vs. control	IL-1 $\beta$ : $\uparrow$ 90% (pg/mg protein) vs. control
	Kidney	Focal tubular necrosis (proximal tubules). Interstitial fibrosis (moderate).	MDA: $\uparrow$ 60% (nmol/mg protein) vs. control	IL-1 $\beta$ : $\uparrow$ 75% (pg/mg protein) vs. control
Study 7	Brain	Neuronal degeneration (cortex, hippocampus, striatum) - shrunken, pyknotic neurons. Increased number of apoptotic cells (detected by TUNEL staining).	4-HNE: $\uparrow$ 48% (pmol/mg protein) vs. control in cortex; $\uparrow$ 42% in hippocampus	TNF- $\alpha$ : $\uparrow$ 65% (pg/mg protein) vs. control in cortex; $\uparrow$ 58% in hippocampus
	Liver	Hepatocyte necrosis (widespread, affecting multiple lobules). Inflammatory cell infiltration (neutrophils and lymphocytes).	4-HNE: $\uparrow$ 72% (pmol/mg protein) vs. control	TNF- $\alpha$ : $\uparrow$ 98% (pg/mg protein) vs. control

#### 4. Discussion

The consistent finding of elevated lipid peroxidation markers (MDA and 4-HNE) across all affected organs underscores the pivotal role of oxidative stress in TOCO-induced toxicity. Lipid peroxidation, initiated by the attack of reactive oxygen species (ROS) on polyunsaturated fatty acids (PUFAs), generates reactive aldehydes that can damage cellular macromolecules. These reactive aldehydes, primarily MDA and 4-HNE, are highly reactive and can form adducts with proteins, DNA, and other biomolecules, leading to cellular dysfunction, impaired signaling pathways, and ultimately, cell death. The brain, with its high content of PUFAs and high oxygen consumption rate, is particularly vulnerable to oxidative damage. The observed neuronal degeneration, astrogliosis, and microglial activation are consistent with the known neurotoxic effects of lipid peroxidation products. Neuronal degeneration, characterized by cell shrinkage, pyknosis (nuclear condensation), and eosinophilia (increased cytoplasmic staining), is a hallmark of neurotoxicity and can lead to cognitive impairment and neurodegenerative diseases. Astrogliosis, an increase in the number and size of astrocytes, is a reactive response to neuronal injury and can contribute to the formation of glial scars, impeding neuronal regeneration. Microglial activation, characterized by changes in cell morphology and increased phagocytic activity, is another hallmark of neuroinflammation and can contribute to neuronal damage through the release of pro-inflammatory cytokines and reactive oxygen species. The heart, with its continuous contractile activity and high energy demand, is also susceptible to oxidative stress. Cardiomyocyte hypertrophy, inflammatory cell infiltration, and fibrosis observed in the included studies are indicative of cardiac remodeling and dysfunction, potentially leading to heart failure. Cardiomyocyte hypertrophy, an increase in the size of individual heart muscle cells, is an adaptive response to increased workload but can eventually lead to impaired contractility and heart failure. Inflammatory cell infiltration, primarily

consisting of macrophages and lymphocytes, is a hallmark of cardiac inflammation and can contribute to tissue damage and fibrosis. Fibrosis, the excessive deposition of extracellular matrix proteins, can disrupt the normal architecture of the heart and impair its function. The liver, as the primary organ responsible for detoxification, is constantly exposed to xenobiotics and their metabolites, making it vulnerable to oxidative damage. Hepatocyte necrosis, steatosis, and inflammation are hallmarks of liver injury and can progress to cirrhosis and liver failure. Hepatocyte necrosis, the death of liver cells, is a direct indicator of liver damage and can impair liver function, leading to jaundice, impaired protein synthesis, and decreased detoxification capacity. Steatosis, the accumulation of fat within liver cells, can disrupt liver function and contribute to inflammation and fibrosis. Inflammation, characterized by the infiltration of inflammatory cells, is a common response to liver injury and can further exacerbate liver damage. The kidneys, responsible for filtering waste products from the blood, are also susceptible to oxidative stress and inflammation. Tubular necrosis, glomerular damage, and interstitial fibrosis observed in the studies are indicative of nephrotoxicity and can lead to chronic kidney disease. Tubular necrosis, the death of cells lining the kidney tubules, can impair the kidney's ability to filter blood and reabsorb essential nutrients, leading to electrolyte imbalances and waste product accumulation. Glomerular damage, affecting the filtering units of the kidney, can lead to proteinuria (protein in the urine) and eventually kidney failure. Interstitial fibrosis, the excessive deposition of extracellular matrix proteins in the kidney interstitium, can disrupt kidney function and contribute to chronic kidney disease.<sup>11-14</sup>

The observed increases in pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) in all affected organs further underscore the role of inflammation in TOCO-induced tissue damage. Lipid peroxidation products can directly activate inflammatory signaling pathways, leading to the production of these cytokines. These cytokines, in turn, can exacerbate

tissue damage by recruiting inflammatory cells, promoting oxidative stress, and stimulating fibrotic responses. The interplay between lipid peroxidation and inflammation likely creates a vicious cycle, amplifying the detrimental effects of TOCO. This vicious cycle is initiated by the generation of lipid peroxidation products, which activate inflammatory signaling pathways, leading to the production of pro-inflammatory cytokines. These cytokines further promote oxidative stress by stimulating the production of ROS and reactive nitrogen species (RNS), which in turn exacerbate lipid peroxidation. This self-amplifying cycle of lipid peroxidation and inflammation contributes to the progressive tissue damage observed in the brain, heart, liver, and kidney.<sup>15-17</sup>

The variations in the specific histopathological features observed across different organs likely reflect organ-specific differences in cellular composition, metabolic activity, and detoxification pathways. For example, the brain's unique vulnerability to oxidative stress is linked to its high PUFA content and limited antioxidant capacity. The heart's continuous contractile activity and high mitochondrial density make it susceptible to mitochondrial dysfunction and oxidative damage. The liver's role in detoxification exposes it to high concentrations of reactive metabolites, while the kidneys' filtration function makes them vulnerable to damage from circulating toxins.<sup>18-20</sup>

## 5. Conclusion

This systematic review highlights the significant histopathological damage induced by the consumption of thermally oxidized cooking oil (TOCO) in the brain, heart, liver, and kidney. The observed damage is strongly associated with increased lipid peroxidation and inflammatory responses, creating a vicious cycle that exacerbates tissue damage. These findings underscore the potential health risks associated with consuming repeatedly heated cooking oils and emphasize the need for public health awareness and strategies to mitigate these risks. The

consistent findings of elevated lipid peroxidation and inflammatory markers across all affected organs suggest that these mechanisms play a crucial role in TOCO-induced toxicity. This highlights the importance of dietary interventions and public health strategies aimed at reducing the consumption of TOCO. Future research should focus on elucidating the precise mechanisms underlying TOCO-induced organ damage, identifying potential biomarkers for early detection of toxicity, and developing effective strategies to prevent or reverse the adverse effects of TOCO consumption. Additionally, studies investigating the long-term effects of TOCO consumption, particularly in vulnerable populations, are needed to fully understand the public health implications of this widespread culinary practice. The findings of this systematic review have important implications for public health and nutrition guidelines. Public health campaigns should raise awareness about the potential risks associated with consuming repeatedly heated cooking oils and promote healthier cooking practices. Furthermore, nutrition guidelines should emphasize the importance of using fresh cooking oils, avoiding overheating of oils, and incorporating antioxidant-rich foods into the diet to mitigate the potential adverse effects of TOCO. In conclusion, the consumption of TOCO poses significant health risks due to its ability to induce histopathological damage in various organs through lipid peroxidation and inflammatory mechanisms. These findings call for urgent public health interventions and dietary modifications to minimize the consumption of TOCO and protect public health.

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