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IN SILICO TOXIC EFFECTS OF 2-(4-TERT-BUTYLPHENYL) PROPANAL, 3-(4-TERT-BUTYLPHENYL)-2-METHYLPROPANAL AND LILAL MOLECULES AND PREGNANCY

2-(4-TERT-BUTİLFENİL) PROPANAL, 3-(4-TERT-BUTİLFENİL)-2-METİLPROPANAL VE LİLAL MOLEKÜLLERİNİN IN SILICO TOKSİK ETKİLERİ VE GEBELİK

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Abstract
In our daily lives, we are exposed to many chemicals originating from products such as cosmetics, cleaning products, and food. These substances may be potential carcinogens and endocrine disruptors. In recent years, BMHCA (3-(4-(tert-butyl) phenyl)-2-methylpropanal, Lilial) and its derivatives have attracted much attention. These substances are generally used in products for fragrance purposes. BMHCA has been published in the EU Cosmetic Products Regulation and banned in cosmetic products offered in the EU and Northern Ireland markets. In this study, the toxic effects of 2-(4-tert-butylphenyl) propanal, 3-(4-tert-butylphenyl)-2-methylpropanal, and Lilal were determined by in silico analysis. According to the results obtained, it was determined that the molecules had moderate and high toxicity levels. The common features of all these compounds include their ability to cross the blood-brain barrier (BBB) at a high rate and their potential to be moderately to highly carcinogenic. Our study is a preliminary study and needs to be supported by in vitro and in vivo studies.

Keywords: BMHCA(Lilial), Carcinogens, Obstetrics and Gynaecology, Pregnancy, Toxicity.

Öz
Günlük hayatımızda kozmetik, temizlik, gıda gibi ürünler kaynaklı pek çok kimyasal maddeye maruz kalmaktayız. Bu maddeler, potansiyel kanserojen ve endokrin bozucu olabilirler. Son yıllarda BMHCA (3-(4-(tert-butil) fenil)-2-metilpropanal, Lilial) ve türevleri çok dikkat çekmektedir. Bu maddeler genellikle koku verme amacıyla ürünlerde kullanılmaktadır. BMHCA, AB Kozmetik Ürünler Yönetmeliği'nde yayımlanarak AB ve Kuzey İrlanda pazarında sunulan kozmetik ürünlerde yasaklanmıştır. Bu çalışmada 2-(4-tert-butilfenil) propanal, 3-(4-tert-butilfenil)-2-metilpropanal ve Lilal'ın toksik etkileri in silico analiz ile belirlenmiştir. Elde edilen sonuçlara göre, moleküllerin orta ve yüksek toksisite düzeylerine sahip olduğu belirlenmiştir. Tüm bu bileşiklerin ortak özellikleri arasında kan-beyin bariyerini (BBB) yüksek oranda geçebilme ve orta-yüksek düzeyde kanserojen olma potansiyeli de yer almaktadır. Bizim çalışmamız, ön çalışma niteliği taşımakta olup in vitro ve in vivo çalışmalarla desteklenmesi gerekmektedir.

Anahtar Kelimeler: BMHCA (Lilial), Karsinojenler, Kadın Doğum, Gebelik, Toksisite.

1. Introduction

The widespread and increasing use of chemical products in modern daily life has raised significant concerns regarding their potential adverse effects on maternal health and fetal development during pregnancy. Products such as hand and face creams, deodorants, hair dyes, nail polishes, sunscreens, cream-based deodorants, and colognes—particularly those formulated with synthetic floral fragrances—constitute major sources of exposure to potentially harmful chemicals. In particular, the frequent use of colognes has markedly intensified following the global COVID-19 pandemic, further increasing the risk of exposure during pregnancy (Özdemir et al., 2022). The escalating use of chemical products in everyday life has emerged as a significant public health concern,

particularly regarding maternal and fetal well-being during pregnancy. Among these products are hand and face creams, deodorants, hair dyes, nail polishes, sunscreens, cream-based deodorants, and colognes, with particular concern surrounding colognes containing synthetic floral fragrances. Following the onset of the COVID-19 pandemic, the widespread use of colognes has markedly increased, further elevating the potential for chemical exposure among pregnant women (Özdemir et al., 2022).

Table 1: Cosmetic Products (Sade & Özkan, 2020)

Cosmetic Products	Application Area	Purpose of Use	Product Structure
• Applied to the outer part of the human body	• Applied to the skin	• Cleansers	• Solutions
• Applied to hair, fibers, and nails	• Applied to external genital organs	•Moisturizers and emollients	• Suspensions
• Applied to teeth and oral cavity	• Anti-wrinkle agents	• Nourishers	• Emulsions
	• Spot removers	• Tanning agents	• Creams
	• Sunscreens	• Baby cosmetics	• Pastes
			• Gels
			• Powders

Pregnancy is a period characterized by tightly coordinated hormone-mediated events that cause changes in maternal physiology to adapt to the developing fetus, prepare for childbirth, and facilitate breastfeeding. During this time, dramatic changes in the mother's metabolism, reproductive organs, endocrine activity, and immune system increase sensitivity to chemical substances. The literature has reported serious side effects and diseases associated with exposure to chemicals and their impacts on women's health (ACOG Committee Opinion No. 575; Varshavsky et al., 2020; Wang et al., 2016).

Endocrine-disrupting chemicals (EDCs) can enhance biological changes during pregnancy and affect hormone levels by mimicking or blocking cell signaling and interfering with hormone production or degradation (Gore et al., 2015). EDCs are commonly found in consumer and personal care products; however, safety oversight in the United States is limited. It has been reported that EDCs and other chemicals contribute to a high body burden among pregnant women and children in the U.S. (Hendryx et al., 2018; Varshavsky et al., 2020).

Flavors and fragrances are defined as substances that directly stimulate taste and smell receptors in the mouth and nose. They are incorporated into many pharmaceutical and personal care products (PPCPs) — including perfumes, creams, lotions, detergents, and various personal and household products — to enhance or alter their scents and/or flavors and are released into the environment as pollutants due to human activities (Di Sotto et al., 2014).

The number of aromatic compounds used in everyday products is quite extensive. The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has a list containing 2,750 entries for fragrance and aromatic raw materials. The European Food Safety Authority (EFSA) has reported a list of compounds for which additional toxicity data is required to identify safely usable fragrances in food materials (EFSA, 2011; Di Sotto et al., 2014).

Among these compounds, aldehydes are defined as a group of potentially reactive substances due to the presence of polarized carbon-oxygen double bonds in their structures. Because of their reactivity, certain aldehydes can interact with electron-rich biological macromolecules, leading to adverse health effects such as general toxicity, allergic reactions, genotoxicity, and carcinogenicity (Langton et al., 2006).

BMHCA(Lilial), used as a synthetic fragrance in cosmetic products and recently banned in the EU, falls under the aldehyde group. Commonly used chemical substances in daily life include perfumes, colognes, cosmetic products, and cleaning agents. BMHCA (3-(4-(tert-butyl)phenyl)-2-methylpropanal) is utilized in these products as a synthetic fragrance. This substance is also known as lyal, lilac, and lily aldehyde. Today, its use has been banned in most countries due to its classification as a strong allergen (skin sensitizer) and reproductive toxicant (CMR1B classification) (ECHA, 2023; Jablonská et al., 2023).

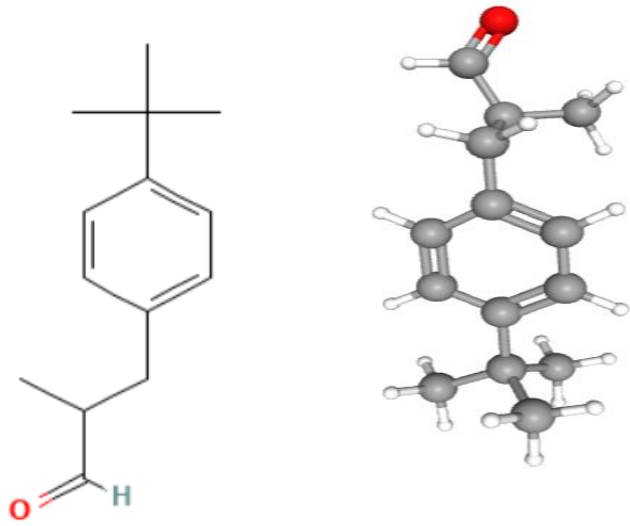

Figure 1. Chemical Structure of 3-(4-(tert-butyl)phenyl)-2-methylpropanal

Lilial®, also known as BPMP and 2-4-tert-butylphenylpropionaldehyde, is a compound within the aldehyde group that includes a structure prone to oxidation and is responsible for cell and DNA damage. It is chemically characterized by a floral scent and is used as an ingredient in personal care and household products, as well as an additive in the food and pharmaceutical industries (Di Sotto et al., 2014; Jablonská et al., 2023).

Hydroxycinnamic aldehydes (HCA) and p-tert-butyl-alpha-methylhydrocinnamic aldehyde (BMHCA) are widely used as flavoring compounds in medical and consumer products, food, beverages and desserts. Chemically, these compounds are synthetic aldehydes characterized by carbonyl groups containing polarized carbon-oxygen double bonds. The electronegativity difference between the oxygen and carbon atoms in the carbonyl group allows these compounds to react with electron-rich biomolecules such as DNA and proteins, thereby causing adverse health effects such as toxicity, allergenic reactions, mutagenicity and carcinogenicity (Garaycoechea et al., 2012). In addition, BMHCA has been reported to pose a potential health risk due to its metabolism to a reactive α,β-unsaturated intermediate (Usta et al., 2013; Di Sotto et al., 2014).

Studies have shown that HCA and BMHCA do not cause genotoxic effects. In the presence of an exogenous metabolic activation system, a lack of point mutations such as frameshifts and oxidative damage was observed in bacteria, indicating that a genotoxic derivative was not produced during CYP450-mediated biotransformations. In some cases, the presence of the metabolic activator reduced the toxicity of the tested substances. No damage was observed at the chromosomal level in mammalian cells (Di Sotto et al., 2014).

In vitro studies report that lilial does not exhibit genotoxicity and mutagenicity. It has been reported that it does not carry genotoxic and mutagenic potential in bacteria such as S. typhimurium, E. coli, CHO(Chinese hamster ovary) cell and Chinese hamster V79 cells (Bernauer et al., 2019). At concentrations up to 500 µM, lilialin did not cause genotoxicity (clastogenicity or aneuploidy) or DNA strand breaks at the chromosomal level (Di Sotto et al., 2014). However, in a study conducted in fertilized eggs of white turkeys (Meleagris gallopavo) in vivo, lilialin caused significant DNA breaks in the comet test (2.0-fold) (Kobets et al., 2018; Jablonská et al., 2023). The endocrine disrupting effects of lilialin have been reported by the European Chemicals Agency (ECHA, 2023). In addition, lilialin has been shown to exhibit estrogenic activity in MCF-7 human breast cancer cells (Charles and Darbre, 2009). However, the effects of lilialin in some sensitive species are thought to be due to toxicity in seminiferous tissues rather than endocrine disrupting effects. Lilialin has also been shown to cause eye and skin irritation in rabbits in various studies (Bernauer et al., 2019; Jablonská et al., 2023). Dermal absorption rates were determined as 13.5% for hydroalcoholic-based fragrances and deodorant/antiperspirant products, 8.9% for oil-in-water-based products, and 10.5% for water-in-oil-based products. Lilialin has been reported to cause skin sensitization reactions at a concentration of 5% (Api et al., 2020; Lalko et al., 2004; Roberts et al., 2007). In an in vitro study with Caco-2 cells, lilialin was reported to be 80% recovered by Caco-2 cells with high solubility and low metabolism (Jablonská et

al., 2023). Lilialin was observed to significantly reduce relative viability in HeLa9903 cells at a concentration of 100 nM. However, the relative viability above 80% at all concentrations (up to 100 μM) indicates that lilial is not cytotoxic (Jablonská et al., 2023).

The European Union initially restricted the use of lilial due to its skin sensitizing properties. Lilial concentrations exceeding 0.001% in skin-retaining products and 0.01% in washable products must be declared on product labels. In 2022, the European Union banned the use of lilial in cosmetic products due to its CMR 1B classification (reproductive health hazardous substance). However, there are several studies in the literature that provide contradictory and inconsistent findings on the toxicological effects of lilial (Jablonská et al., 2023).

Lilial is identified as one of the 26 fragrance ingredients that cause allergic contact dermatitis and must be declared as an ingredient in cosmetic products (Heisterberg et al., 2011).

2. Material and Methods

A comprehensive toxicity assessment study was conducted to investigate the potential toxic effects of Lilial and its structurally similar analog compounds. In this study, the freely accessible online tool available at https://tox.charite.de/ was employed to perform detailed toxicity predictions. To facilitate the analysis, the SMILES (Simplified Molecular Input Line Entry System) notations, which provide a standardized way to represent chemical structures in text form, were retrieved for Lilial and its analog compounds from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Using these SMILES strings, the toxicity profiles of three selected compounds were comparatively examined. Various toxicological parameters, including mutagenicity, carcinogenicity, and potential for skin sensitization, were evaluated and compared across the compounds. This approach provided valuable insights into the toxicological characteristics of Lilial and its analogs, contributing to a better understanding of their potential risks for human health and environmental safety.

3. Results and/or Discussion

In the conducted study, the in silico toxicity of the molecules 2-(4-tert-butylphenyl) propanal, 3-(4-tert-butylphenyl)-2-methylpropanal, and Lilial was investigated.

In the toxicity assessment study conducted on 2-(4-tert-Butylphenyl) propanal using the website https://tox.charite.de/, it was determined that this compound is a moderately to highly toxic substance with a toxicity level of 5 (Figure 2). The estimated LD50 dose was found to be 3500 mg/kg. Additionally, it was identified that 2-(4-tert-Butylphenyl) propanal possesses neurotoxic and carcinogenic properties, and is also a substance that can cross the blood-brain barrier (BBB) (Table 2).

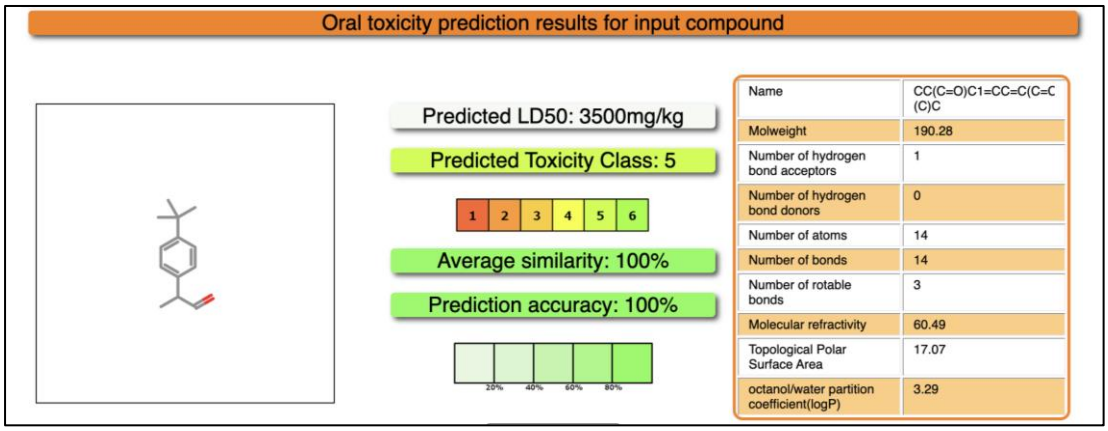

Figure 2. (4-tert-Butylphenyl) propanal

Table 2. Toxicity Results of 2-(4-tert-Butylphenyl) propanal

Toxicity Model Report

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.76
Organ toxicity	Neurotoxicity	neuro	Active	0.54
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.88
Organ toxicity	Respiratory toxicity	respi	Inactive	0.86
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Active	0.54
Toxicity end points	Immunotoxicity	immuno	Inactive	0.97
Toxicity end points	Mutagenicity	mutagen	Inactive	0.94
Toxicity end points	Cytotoxicity	cyto	Inactive	0.87
Toxicity end points	BBB-barrier	bbb	Active	0.99
Toxicity end points	Ecotoxicity	eco	Active	0.67
Toxicity end points	Clinical toxicity	clinical	Inactive	0.74
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.84
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	1.0
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	1.0
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	1.0
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.99
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	1.0
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	1.0
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.93
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.94
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Active	0.51
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.97
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.53
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.95
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	1.0
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	1.0
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Active	0.63
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	0.99
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Active	0.52
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.85
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.88
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.97
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.93
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.65
Metabolism	Cytochrome CYP2C9	CYP2C9	Active	0.53
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.85
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.94
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.97

In the toxicity study conducted on 3-(4-Tert-butylphenyl)-2-methylpropanal, it was determined that this compound is a level 4 toxic substance (Figure 3). The estimated LD50 dose was calculated to be 2000 mg/kg. Additionally, it was found that 3-(4-Tert-butylphenyl)-2-methylpropanal can cross the blood-brain barrier (BBB) and is an ecotoxic substance. This compound also plays an active role on the cytochrome CYP2C9 enzyme (Table 3).

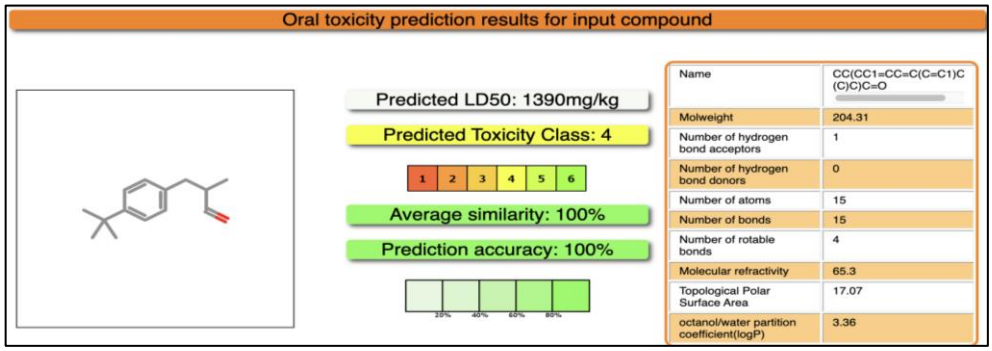

Figure 3. Chemical Structure and Properties of 3-(4-Tert-butylphenyl)-2-methylpropanal

Table 3. 3-(4-Tert-butylphenyl)-2-methylpropanal toxicity report.

Toxicity Model Report

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.69
Organ toxicity	Neurotoxicity	neuro	Inactive	0.53
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.88
Organ toxicity	Respiratory toxicity	respi	Inactive	0.82
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Active	0.53
Toxicity end points	Immunotoxicity	immuno	Inactive	0.99
Toxicity end points	Mutagenicity	mutagen	Inactive	0.96
Toxicity end points	Cytotoxicity	cyto	Inactive	0.86
Toxicity end points	BBB-barrier	bbb	Active	0.99
Toxicity end points	Ecotoxicity	eco	Active	0.65
Toxicity end points	Clinical toxicity	clinical	Inactive	0.77
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.95
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	1.0
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	1.0
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.96
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.99
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	1.0
Molecular Initiating Events	Thyroid hormone receptor alpha (THRa)	mie_thr_alpha	Inactive	0.89
Molecular Initiating Events	Thyroid hormone receptor beta (THRß)	mie_thr_beta	Inactive	0.94
Molecular Initiating Events	Transthyretrin (TTR)	mie_ttr	Active	0.50
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.97
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.52
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.93
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	1.0
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	1.0
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.61
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	1.0
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.53
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.84
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.86
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.98
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.93
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.73
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.50
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.85
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.93
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.97

Investigations regarding Lilial have determined that this substance is moderately toxic (Figure 4). Our analyses conducted on the website https://tox.charite.de/ indicate that Lilial falls into toxicity class 4, with an LD50 value established at 1390 mg/kg. Furthermore, it has been identified as an

active compound that can cross the blood-brain barrier (BBB) and possesses carcinogenic properties (Table 4).

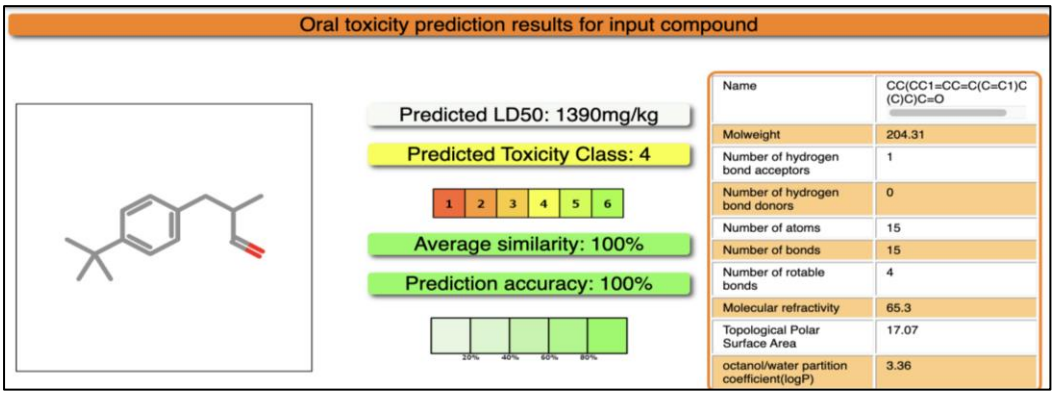

Figure 4. Chemical Structure and Properties of Lilial

Before a cosmetic product is introduced to the EU market, it is evaluated to determine its safety under foreseeable conditions of use (Regulation 1223/2009, Article 3). If the content of the cosmetic poses a risk to human health, safety must be assessed under foreseeable conditions of use. If toxicological concerns are serious for regulatory purposes, this issue is investigated by the Scientific Committee on Consumer Safety (SCCS), an independent advisory body (SCCS, 2021; Bialas, 2023). Moreover, every raw material used in cosmetics must meet the legal requirements of REACH and the Classification, Labelling, and Packaging (CLP) regulations. As of March 1, 2022, BMHCA (Lilial) has been banned in cosmetic products marketed in the EU and Northern Ireland following its publication in the EU Cosmetics Regulation (CPTA).

Hydroxycinnamic aldehydes (HCA) and p-tert-butyl-alpha-methylhydrocinnamic aldehyde (BMHCA) are widely used as flavoring compounds in medical and consumer products, as well as in food, beverages, and sweets. Chemically, these compounds are characterized as synthetic aldehydes containing polarized carbon-oxygen double bonds in carbonyl groups. The electronegativity difference between the oxygen and carbon atoms in the carbonyl group enables these compounds to react with electron-rich biomolecules such as DNA and proteins, potentially leading to adverse health effects including toxicity, allergic reactions, mutagenicity, and carcinogenicity (Garaycoechea et al., 2012). Additionally, it has been reported that BMHCA poses a potential health risk due to its metabolism into a reactive α,β-unsaturated intermediate (Usta et al., 2013; Di Sotto et al., 2014). Studies have shown that HCA and BMHCA do not cause genotoxic effects. In the presence of an exogenous metabolic activation system, a lack of point mutations, such as frameshift and oxidative damage in bacteria, was observed, indicating that no genotoxic derivatives are produced during CYP450-mediated biotransformations. In some cases, the presence of the metabolic activator has reduced the toxicity of the tested substances. Chromosomal-level damage has also not been observed in mammalian cells (Di Sotto et al., 2014).

In Vitro Studies, Reports indicate that lilialin does not exhibit genotoxicity or mutagenicity. It has been documented that it does not carry genotoxic and mutagenic potential in bacteria such as *S. typhimurium* and *E. coli*, as well as in CHO and Chinese hamster V79 cells (Bernauer et al., 2019). It has been observed that lilialin does not lead to chromosomal-level genotoxicity (clastogenicity or aneuploidy) or DNA strand breaks at concentrations up to 500 µM (Di Sotto et al., 2014). However, an in vivo study conducted on fertilized eggs of white turkey (Meleagris gallopavo) showed that lilial caused significant DNA strand breaks (2.0-fold increase) in the comet test (Kobets et al., 2018; Jablonská et al., 2023). Endocrine-disrupting effects of lilialin have been reported by the European Chemicals Agency (ECHA, 2023).

Table 4. Lilial Toxicity Report

Toxicity Model Report

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.69
Organ toxicity	Neurotoxicity	neuro	Inactive	0.53
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.88
Organ toxicity	Respiratory toxicity	respi	Inactive	0.82
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Active	0.53
Toxicity end points	Immunotoxicity	immuno	Inactive	0.99
Toxicity end points	Mutagenicity	mutagen	Inactive	0.96
Toxicity end points	Cytotoxicity	cyto	Inactive	0.86
Toxicity end points	BBB-barrier	bbb	Active	0.99
Toxicity end points	Ecotoxicity	eco	Active	0.65
Toxicity end points	Clinical toxicity	clinical	Inactive	0.77
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.95
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	1.0
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	1.0
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.96
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.99
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	1.0
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.89
Molecular Initiating Events	Thyroid hormone receptor beta (THRß)	mie_thr_beta	Inactive	0.94
Molecular Initiating Events	Transthyretin (TTR)	mie_ttr	Active	0.50
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.97
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.52
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.93
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	1.0
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	1.0
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.61
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	1.0
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.53
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.84
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.86
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.98
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.93
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.73
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.50
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.85
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.93
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.97

Additionally, it has been demonstrated that lilialin exhibits estrogenic activity in MCF-7 human breast cancer cells (Charles and Darbre, 2009). However, it is thought that the effects of lilialin in some sensitive species are more related to toxicity in seminiferous tissues rather than endocrine-disrupting effects. Various studies have also shown that lilialin causes eye and skin irritation in rabbits (Bernauer et al.,

2019; Jablonská et al., 2023). Dermal absorption rates have been determined as 13.5% for hydroalcoholic-based fragrances and deodorant/antiperspirant products, 8.9% for water-in-oil-based products, and 10.5% for oil-in-water-based products. It has been reported that lilialin at a 5% concentration can cause skin sensitivity reactions (Api et al., 2020; Lalko et al., 2004; Roberts et al., 2007). In an in vitro study with Caco-2 cells, it was reported that lilialin was recovered at 80% with high solubility and low metabolism (Jablonská et al., 2023). At a concentration of 100 nM, lilialin significantly reduced relative viability in HeLa9903 cells. However, the relative viability remained above 80% at all concentrations (up to 100 μM), indicating that lilialin is not cytotoxic (Jablonská et al., 2023). The European Union initially restricted the use of lilial due to its skin sensitization properties, requiring that products containing lilial at concentrations exceeding 0.001% in leave-on products and 0.01% in rinse-off products be labeled. In 2022, the European Union banned the use of lilial in cosmetic products due to its CMR 1B classification (substance hazardous to reproductive health). However, there are various studies in the literature that present conflicting and inconsistent findings regarding the toxicological effects of lilial (Jablonská et al., 2023). Lilial is identified as one of the 26 fragrance components that can cause allergic contact dermatitis in cosmetic products (Heisterberg et al., 2011). The European Chemicals Agency's suspicions regarding lilial's potential as an endocrine disruptor remain controversial.

4. Conclusion

There has been a rapid increase in the global use of industrial products, and this rise has led to widespread undesirable effects on pregnant women and fetuses. This article discusses the BMHCA molecule and its derivatives, which were banned in the European Union in 2022 due to their negative effects on the reproductive system and pregnancy. Comparative toxicity studies were conducted using lilial and synonymous compounds obtained from PubChem. In these studies, 2-(4-tert-butylphenyl) propanal, 3-(4-tert-butylphenyl)-2-methylpropanal, and lilial were found to possess moderate to high levels of toxicity. Common characteristics of all these compounds include a high ability to cross the blood-brain barrier (BBB) and a moderate to high potential for carcinogenicity. Due to the conflicting results in the literature and ongoing widespread exposure to this substance in daily life, further experimental studies are needed to clarify the toxicological effects of lilial.

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References

ACOG Committee Opinion No 575. Exposure to toxic environmental agents. Fertil Steril. 2013 Oct;100(4):931-4. doi: 10.1016/j.fertnstert.2013.08.043. PMID: 24070500; PMCID: PMC9060637.

Api, A. M., Belsito, D., Biserta, S., Botelho, D., Bruze, M., Burton, G. A., ... & Tsang, S. (2020). RIFM fragrance ingredient safety assessment, pt-butyl-α-methylhydrocinnamic aldehyde, CAS Registry Number 80-54-6. Food and Chemical Toxicology, 141, 111430.

Bernauer, U. et al. "SCCS OPINION ON the safety of Butylphenyl methylpropional (p-BMHCA) in cosmetic products-Submission II" SCCS/1591/17-Final version, https://health.ec.europa.eu/system/files/2021-08/sccs_o_213_0.pd (2019).

Bialas, I., Zelent-Kraciuk, S., & Jurowski, K. (2023). The Skin Sensitisation of Cosmetic Ingredients: Review of Actual Regulatory Status. Toxics, 11(4), 392. https://doi.org/10.3390/toxics11040392

Charles AK, Darbre PD. Oestrogenic activity of benzyl salicylate, benzyl benzoate and butylphenylmethylpropional (Lilial) in MCF7 human breast cancer cells in vitro. J Appl Toxicol. 2009 Jul;29(5):422-34. doi: 10.1002/jat.1429. PMID: 19338011.

Di Sotto, A., Maffei, F., Hrelia, P., Di Giacomo, S., Pagano, E., Borrelli, F., & Mazzanti, G. (2014). Genotoxicity assessment of some cosmetic and food additives. Regulatory toxicology and pharmacology: RTP, 68(1), 16–22. https://doi.org/10.1016/j.yrtph.2013.11.003

ECHA. Substance Infocard: 2-(4-tert butylbenzyl) propionaldehyde, https://echa.europa.eu/substance-information/-/substanceinfo/100.001.173 (2023).

EFSA, C. (2011). Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011. Scientific opinion on Flavouring group evaluation, 25.

Garaycoechea, J. I., Crossan, G. P., Langevin, F., Daly, M., Arends, M. J., & Patel, K. J. (2012). Genotoxic consequences of endogenous aldehydes on mouse haematopoietic stem cell function. Nature, 489(7417), 571-575.

Gore, A. C., Chappell, V. A., Fenton, S. E., Flaws, J. A., Nadal, A., Prins, G. S., Toppari, J., & Zoeller, R. T. (2015). EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocrine reviews, 36(6), E1–E150. https://doi.org/10.1210/er.2015-1010

Heisterberg, M. V., Menné, T., & Johansen, J. D. (2011). Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. Contact Dermatitis, 65(5), 266-275.

Hendryx, M., & Luo, J. (2018). Children's environmental chemical exposures in the USA, NHANES 2003–2012. Environmental Science and Pollution Research, 25, 5336-5343.

https://tox.charite.de/protox3/index.php?site=compound_search_similarity(Son erişim: 16.04.2025)

Jablonská, E., Míchal, Z., Křížkovská, B., Strnad, O., Tran, V. N., Žalmanová, T., Petr, J., Lipov, J., & Viktorová, J. (2023). Toxicological investigation of lilial. Scientific reports, 13(1), 18536. https://doi.org/10.1038/s41598-023-45598-y

Kobets, T., Duan, J. D., Brunnemann, K. D., Iatropoulos, M. J., Etter, S., Hickey, C., ... & Williams, G. M. (2018). In ovo testing of flavor and fragrance materials in Turkey Egg Genotoxicity Assay (TEGA), comparison of results to in vitro and in vivo data. Food and Chemical Toxicology, 115, 228-243.

Lalko, J., Isola, D., & Api, A. M. (2004). Ethanol and diethyl phthalate: vehicle effects in the local lymph node assay. International Journal of Toxicology, 23(3), 171-177.

Langton, K., Patlewicz, G. Y., Long, A., Marchant, C. A., & Basketter, D. A. (2006). Structure–activity relationships for skin sensitization: recent improvements to Derek for Windows. Contact Dermatitis, 55(6), 342-347.

No, A. C. O. (2013). Exposure to toxic environmental agents. Fertility and sterility, 100(4), 931-934.

Özdemir, R., Şahin, S., & Arıöz, A. (2022). Gebelikte Kozmetik Kullanımı ve Sağlık Riskleri. Hemşirelik Bilimi Dergisi, 5(1), 30-37.

Roberts, D. W., Patlewicz, G., Kern, P. S., Gerberick, F., Kimber, I., Dearman, R. J., ... & Aptula, A. O. (2007). Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chemical Research in Toxicology, 20(7), 1019-1030.

Sade, G., & Özkan, H. (2020). Kozmetik ürünlerdeki bazı kimyasalların gebe, fetüs ve yenidoğan sağlığına etkisi. Jinekoloji-Obstetrik ve Neonatoloji Tıp Dergisi, 17(3), 473-477.

SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation 11th Revision SCCS/1628/21. 2021. [(accessed on 27 March 2023)]. Available online: https://health.ec.europa.eu/system/files/2022-08/sccs_o_250.pdf [PubMed]

UE Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. 2009. [(accessed on 27 March 2023)]. Available online: http://data.europa.eu/eli/reg/2009/1223/2022-12-17

Usta, J., Hachem, Y., El-Rifai, O., Bou-Moughlabey, Y., Echtay, K., Griffiths, D., ... & Makki, R. F. (2013). Fragrance chemicals lyral and lilial decrease viability of HaCat cells' by increasing free radical production and lowering intracellular ATP level: protection by antioxidants. Toxicology in Vitro, 27(1), 339-348.

Varshavsky, J., Smith, A., Wang, A., Hom, E., Izano, M., Huang, H., Padula, A., & Woodruff, T. J. (2020). Heightened susceptibility: A review of how pregnancy and chemical exposures influence maternal health. Reproductive toxicology (Elmsford, N.Y.), 92, 14–56. https://doi.org/10.1016/j.reprotox.2019.04.004

Wang, A., Padula, A., Sirota, M., & Woodruff, T. J. (2016). Environmental influences on reproductive health: the importance of chemical exposures. Fertility and sterility, 106(4), 905-929.