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# Toxicological Evaluation of Siloxanes by *In Silico* Approaches

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**Abstract**

**Background and Aims:** Silicones are widely used in household items, personal care products, and medical devices. They are especially preferred for products used directly on humans, as they are biocompatible, biologically stable, and unlikely to cause allergic reactions. It has been estimated that the global production capacity of siloxanes has reached 400,000 tons annually. Humans in all age groups are exposed to siloxanes from various products by oral, inhalation, dermal, or parenteral routes. For many years, siloxanes have been widely regarded as non-toxic substances, fostering a sense of safety in their use across various applications. However, emerging scientific research reveals a more nuanced perspective, indicating that this assumption of safety cannot be uniformly applied to all siloxanes. This study aimed to evaluate the in silico toxicological profile of sixteen different siloxanes, commonly regarded as safe, thereby questioning their potential risks and the validity of the current safety perception.

**Methods:** This study employed in silico toxicological evaluation of sixteen cyclic and linear siloxanes using VEGA (v.1.2.3), VEGA NRMEA (v.1.1.1), US EPA TEST (v.5.1.2 and 4.2.1), US EPA CompTox Chemicals Dashboard (v.2.5.3), PanScreen, ProTox (v.3.0), and Deep-PK models.

**Results:** Our results suggested that siloxanes may affect the endocrine system through the oestrogen receptor (ER) pathway.

**Conclusion:** There is a growing need for new toxicity models that focus on silicone compounds. As our understanding advances, it becomes evident that the diverse chemical structures and behaviours of these compounds may pose potential risks that were previously overlooked.

**Keywords** Siloxanes · In Silico Toxicity · Acute Toxicity · Genotoxicity · Developmental Toxicity · Endocrine Disrupting Effect

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## INTRODUCTION

Siloxanes (also known as silicones) are organosilicon compounds that contain carbon-silicon bonds. More than 150,000 applications have been registered, covering areas such as pharmaceuticals, medicine, cosmetics, and food production (Clewell et al., 2024). Siloxanes are generally classified as cyclic ($D_n$) or linear ($L_n$), where "n" indicates the number of silicon atoms (Si-O). Cyclic siloxanes are individual-chain length cyclic dimethyl polysiloxane compounds that contain repeating units of Si and O atoms in a closed loop, giving them a cyclic structure (Johnson et al., 2011; Krenczkowska et al., 2020; Wang et al., 2009). Octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) are the most common cyclic siloxanes, containing repeating units of $[(CH_3)_2SiO]$ (Johnson et al., 2011). Linear siloxanes are viscous polymeric silicone compounds containing repeating units of $[(CH_3)_2SiO]$ (ECETOC, 2011). Hexamethyldisiloxane (HMDS; L2), octamethyltrisiloxane (L3), decamethyltetrasiloxane (L4), and dodecamethylpentasiloxane (L5) are low-molecular-weight volatile linear siloxanes (GSC, 2023).

The widespread use of siloxanes in everyday products leads to significant population exposure primarily through direct skin contact with cosmetics and personal care items, as well as dermal exposure in industrial settings. Inhalation is another potential route of exposure, particularly from evaporating or aerosolized siloxanes in personal care and cleaning products and certain industrial processes. There is also a risk of oral exposure from the accidental ingestion of products containing siloxanes. Children are especially vulnerable to these risks. In general, siloxanes are tolerated by humans and are considered to be non-toxic or only slightly toxic to human organisms and nature. The molecular weight, chemical groups, particle size, and shape of the siloxanes determine their physicochemical characteristics, which directly affect their safety and risks (Mojsiewicz-Pieńkowska et al., 2016). Despite their versatility, some siloxanes exhibit adverse biological effects. Notably, siloxane D4 has been categorised as an endocrine disrupting substance by the European Union (ECHA, 2018). Endocrine disruptors can cause hormone imbalance. This can lead to various adverse health effects, such as impaired reproductive function and development, neurological disorders, obesity, and cancer (WHO, 2012). Similar to siloxane D4, siloxane D5 has been found to induce uterine tumours and negatively affect the reproductive and immune systems (Klaunig et al., 2016). The endocrine system plays a vital role in our overall health, as it governs the synthesis and regulation of hormones that are essential for the development, growth, and proper operation of various bodily systems. Therefore, evaluating a chemical's potential to disrupt this intricate hormonal balance is a crucial component of safety assessments. Such evaluations are essential for safeguarding individual and community well-being by ensuring substances do not interfere with the hormonal pathways governing metabolism and reproductive health (WHO, 2012).

Given the widespread use of cyclic and linear siloxanes, this work aims to contribute to the knowledge of their potential adverse effects on human health. To this end, the absorption, distribution, metabolism, elimination (ADME) properties of the selected siloxanes were initially investigated. Then, in silico approaches utilising VEGA (v.1.2.3), US EPA (v.5.1.2 and 4.2.1), PanScreen, ProTox (v.3.0), and Deep-PK platforms were employed to predict the toxicological effects on the reproductive and endocrine systems, as well as the mutagenicity, carcinogenicity, systemic organ toxicity, and acute oral toxicity ($LD_{50}$) potential. These in silico models utilise computational methods, including molecular docking, machine learning-driven quantitative structure-activity relationships (QSAR), and expert rule-based approaches, to assess a chemical's likelihood of interacting with specific endocrine receptors and triggering or suppressing signalling pathways. Figure 1 illustrates the schematic diagram of our study.

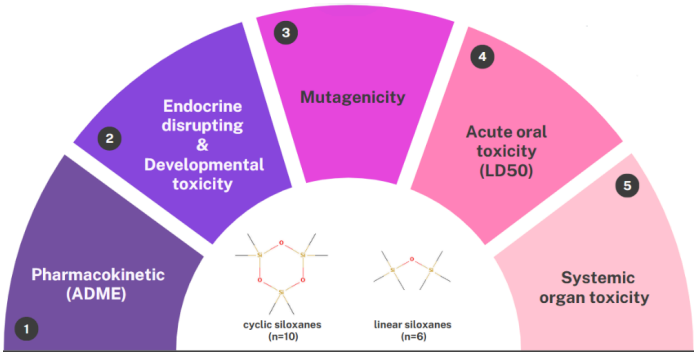


**Figure 1.** Study scheme

## METHODS

### Dataset

Our dataset comprised sixteen siloxanes: 10 cyclic and six linear compounds. The cyclic siloxanes included: hexamethylcyclotrisiloxane (D3), D4, D5, D6, tetradecamethylcycloheptasiloxane (D7), hexadecamethylcyclooctasiloxane (D8), octadecamethylcyclononasiloxane (D9), eicosamethylcyclodecasiloxane (D10), docosamethylcycloundecasiloxane (D11), and tetracosamethylcyclododecasiloxane (D12). The linear siloxanes included: L2, L3, L4, L5, tetradecamethylhexasiloxane (L6), and hexadecamethylheptasiloxane (L7). See Supplemental Table S1 for detailed compound information. The canonical simplified molecular input line entry system (SMILES) and CAS

numbers were investigated and verified using ChemSpider (2024) and PubChem (2024).

## Physicochemical and Pharmacokinetic (ADME) Prediction

The experimental data on the physicochemical properties of the selected siloxanes were determined using the US EPA CompTox Chemicals Dashboard (v.2.5.3) (Williams et al., 2017; 2021; US EPA, 2025). The absorption, distribution, metabolism, and excretion (ADME) parameters of the selected siloxanes were analysed using Deep-PK, which is a new version of pkCSM. This web server uses deep learning to predict, analyse, and optimise pharmacokinetics and toxicity (Myung et al., 2024).

## Endocrine-Disrupting Effects and Developmental Toxicity Prediction

The following tools were utilised to predict the endocrine-disrupting effects and developmental toxicity of the siloxanes: VEGA Nuclear Receptor-Mediated Endocrine Activity (NRMEA) (v.1.1.1), VEGA QSAR (v.1.2.3), the United States Environmental Protection Agency (US EPA) Toxicity Estimation Software Tool (TEST) (v.5.1.2 and 4.2.1), PanScreen (VirtualToxLab), and ProTox (v.3.0). The following are the details regarding these methods:

### *VEGA Platform*

VEGA NRMEA (v.1.1.1) is a python application that offers a qualitative assessment of 12 classical nuclear receptor-mediated endocrine disruptions. Models for the androgen receptor (AR), oestrogen receptor alpha (ERα), ER beta (β), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), thyroid hormone receptor α (TRα), and TRβ models were used to evaluate siloxanes. Developmental toxicity model (CAESAR v.2.1.8), developmental/reproductive toxicity library (PG v.1.1.2), ER-mediated effect (IRFMN-CERAPP v.1.0.1), ER relative binding affinity model (IRFMN v.1.0.2), AR-mediated effect (IRFMN-COMPARA v.1.0.1), TRα effect (NRMEA v.1.0.1), TRα/β effect (NRMEA v.1.0.1), GR (OBERON v.1.0.0), thyroperoxidase (TPO) inhibitory activity (OBERON v.1.0.1), and endocrine disruptor activity screening (IRFMN v.1.0.0) methods were performed to determine the endocrine disrupting and developmental effects of the selected siloxanes.

### *US EPA Toxicity Estimation Software Tool (TEST)*

TEST is a tool that estimates the toxicity of chemicals via QSAR methodologies (US EPA, 2020). TEST v.5.1.2 includes hierarchical, nearest neighbour, and consensus methods, while TEST v.4.2.1 includes the FDA model in addition to these three methods. Both versions of TEST employed specialised developmental toxicity assessment methods, allowing researchers and regulatory agencies to evaluate the potential impacts of chemicals on developmental processes and reproductive health.

### *PanScreen (VirtualToxLab)*

PanScreen is the successor to VirtualToxLab (Sellner et al., 2023). VirtualToxLab is an in silico virtual screening tool that yields binding affinities to nuclear receptors. The tool employs an innovative flexible docking protocol that accommodates both full ligand flexibility and the dynamic movements of protein side chains, enabling the generation of viable binding modes at targeted nuclear receptors. A meticulously refined and thoroughly validated scoring function was employed to evaluate and quantify the free energy associated with a diverse ensemble of docking poses. This scoring function considers various molecular interactions and conformational adjustments, providing a comprehensive assessment of the stability and feasibility of each docking configuration. The resulting analysis aids in identifying the most energetically favourable binding modes, thereby enhancing the accuracy of the docking predictions. Predictions are deemed successful-termed hits-when the estimated inhibition constant (Ki) falls below the threshold of $10^{-5}$ M, highlighting their potential efficacy in binding interactions (Vedani et al., 2012; Vedani et al., 2015; Vedani & Smiesko, 2009).

### *ProTox*

ProTox (v.3.0), a virtual lab, was used to predict AR, AR Ligand Binding Domain (AR-LBD), ERα, ER-LBD from Tox21-Nuclear receptor signalling pathways, and TRα-TRβ as molecular initiating events. This advanced tool employs a combination of molecular similarity metrics, fragment trend analysis, and an examination of the most frequently occurring structural features to assess the toxicity of chemical compounds. By utilising machine learning techniques, specifically CLUSTER cross-validation rooted in fragment similarity, the tool systematically evaluates how closely the compounds resemble known toxicological profiles. ProTox's multifaceted approach not only improves the accuracy of toxicity predictions but also allows for the identification of new compounds that may have similar risk characteristics based on their structural features (Drwal et al., 2014; Benarjee et al., 2016; Benarjee et al., 2018).

## Acute Oral Toxicity ($LD_{50}$), Mutagenicity, Carcinogenicity, and Systemic Organ Toxicity Predictions

The VEGA Acute Toxicity ($LD_{50}$) model (KNN v.1.0.0), US EPA TEST (v.5.1.2 and 4.2.1) and ProTox (v.3.0) $LD_{50}$ models were used

to determine experimental (if there is) and predicted oral rat acute toxicity values (The median lethal dose ($LD_{50}$) in mg/kg) of the selected siloxanes.

VEGA individual and Consensus (v.1.0.4) mutagenicity models were used to determine the mutagenicity potential of the siloxanes. The individual models were CAESAR (v.2.1.14) (Hansen et al., 2009), ISS (v.1.0.3) (Honma, 2020; Manganelli et al., 2016), SarPy-IRFMN (v.1.0.8) (Ferrari et al., 2013), and KNN (v.1.0.1) (Mombelli et al., 2022), which were developed based on experimental data derived from the Ames Test in *Salmonella typhimurium* and *Escherichia coli* strains. The US EPA TEST (v.5.1.2 and 4.2.1) mutagenicity consensus models (US EPA, 2020), ProTox (v.3.0) (Banerjee et al., 2024), and Deep-PK mutagenicity models were also performed (Myung et al., 2024).

Organ toxicity endpoints, including hepatotoxicity, neurotoxicity, nephrotoxicity, and cardiotoxicity, as well as toxicity endpoints, such as immunotoxicity, mutagenicity, cytotoxicity, and carcinogenicity of the siloxanes were predicted by ProTox (v.3.0) (Banerjee et al., 2024).

## RESULTS and DISCUSSION

Siloxanes are polymeric compounds consisting of silicon and oxygen units with organic side chains. They are used in cosmetic and household products, medical and electrical devices, and technical applications because of their outstanding physicochemical properties (Clewell et al., 2024). The siloxane bond in silicone exhibits remarkable flexibility coupled with strength (Bains & Kaur, 2023). These compounds can be formed in cyclic and linear forms, spanning a range of low and high molecular weights. Cyclic siloxanes, which are dimethyl polysiloxanes of different chain lengths, are commonly represented by siloxane D4, D5, and D6 (Kumari et al., 2023). Low molecular weight linear siloxanes are commonly used as constituents in the production of silicone liquids and consumer products. These compounds have highly lipophilic structures and release significant amounts of vapour at nominal temperatures and pressures. Moreover, they are routinely found in air samples (Chen et al., 2023). The increasing use of these substances, coupled with the potential for chronic exposure, has raised concerns regarding their potential toxicity to humans, other living things, and the environment (Kumari et al., 2023). In light of this widespread exposure and the increasing scrutiny from prominent regulatory agencies such as the US EPA and the European Chemicals Agency (ECHA), as highlighted in our study's rationale, we investigated the ADME properties and toxicity potential of sixteen selected siloxanes using in silico models. These selected cyclic (including D4, D5, D6, and others) and linear (including L2, L3, L4, L5, and others) siloxanes exhibited significant structural diversity within this chemical class. These substances are currently under close scrutiny by the relevant authorities owing to concerns regarding their environmental persistence, bioaccumulation potential, and in particular their endocrine-disrupting properties (ECHA, 2018; Klaunig et al., 2016).

The physicochemical properties (i.e., boiling point, melting point, vapour pressure, and water solubility), based on the experimental values of the selected siloxanes using the US EPA CompTox Chemicals Dashboard (v.2.5.3), are given in Supplemental Table S2. The absorption levels were predicted using the Caco-2 permeability model, the Madin-Darby canine kidney cells (MDCK) model, the human intestinal absorption rate, the human oral bioavailability rate, the skin permeability, and the P-glycoprotein substrate or inhibitor activity. Siloxanes (**1-16**) were predicted to have low permeability with a Caco-2 Papp (apparent permeability) value of less than $8\times10^{-6}$ cm/s, with the predicted permeability values being <0.9. All siloxanes exhibited human intestinal absorption, with linear siloxanes (**11-16**) having high confidence and cyclic siloxanes (**1-10**) having moderate to low confidence. The oral bioavailability were predicted to be less than 50% for all patients. Based on the MDCK model, siloxanes were found to have low permeability, and the predicted values were less than 4 nm/s. The logKp (cm/h) values of siloxanes (**1-16**) indicate that all the compounds have a skin permeability with a logKp higher than −2.5. The ADME properties of the siloxanes are shown in Supplemental Tables S3 and S4. Krenczkowska et al. (2019) provided evidence supporting our findings, noting that cyclic siloxanes can penetrate the epidermis and dermis, where blood and lymphatic vessels are present. P-glycoprotein (P-gp), also known as multidrug resistance protein 1 (MDR1), is a crucial ATP-binding cassette (ABC) transporter that plays a significant role as a biological barrier in cellular function. P-gp limits oral bioavailability and intestinal absorption of a xenobiotic (Nguyen et al., 2021). None of the tested siloxanes (**1-16**) were predicted to be substrates of P-gp. Except for siloxane D3 (**1**), L2 (**11**), and L3 (**12**), the others were found to be P-gp inhibitors. Inhibition of P-gp by siloxanes may cause toxic substances to accumulate intracellularly, increasing the risk of toxicity. The distribution volume (VD) reflects how a compound is spread throughout the body's tissues compared to its presence in the plasma. Higher VD values indicate a greater distribution in the tissues. The logarithm of VD is categorised as low if it is below −0.15 and high if it is above 0.45. All siloxanes (**1-16**) were predicted to have values higher than −0.45, indicating that the VD was high. The blood-brain barrier (BBB) acts as a defence mechanism for the central nervous system (CNS), preventing the entry of external compounds. The ability of a compound to penetrate brain tissue

is a critical consideration, as it can significantly influence its pharmacological or toxicological impacts on the CNS. A compound with a log BBB higher than 0.3 is readily crossing the BBB, whereas a log BBB below −1.0 indicates poor distribution into the brain. The Deep-PK predicted that all siloxanes (**1-16**) have values less than −1.0, indicating a low CNS distribution. All the tested siloxanes were estimated to be penetrable to the BBB. However, the ability to be penetrable to the BBB does not necessarily correlate with the extent of CNS distribution. The toxicological activity of a compound can be affected by the strength of its binding affinity to plasma proteins. A higher fraction of unbound (FU) indicates that the compound can more efficiently cross cellular membranes or diffuse through tissues. All the siloxanes tested were predicted to have an FU ratio greater than 0.50, with siloxane D12 (**10**) being the highest and siloxane L2 (**11**) the lowest. Siloxane D12 (**10**) also had the highest plasma protein binding rate (83%), while siloxane D3 (**1**) and D4 (**2**) had the lowest (with a ratio of 28% and 38%, respectively).

The liver is the main organ of metabolism, and the liver tissue commonly contains CYP450 enzymes. This enzyme family is crucial for the metabolism and detoxification of many xenobiotics. None of the siloxanes were predicted to be substrates of CYP2C9. Except for L2 (**11**), the tested siloxanes were also not substrates of CYP2D6. In contrast, all the siloxanes (**1-16**) were identified as substrates of CYP1A2. Except for siloxane D3 (**1**) and L2 (**11**), all the compounds were found to be substrates of CYP3A4. Siloxanes **1-11** were predicted to be inhibitors of CYP1A2, with siloxane D3 (**1**) , L2 (**11**), L3 (**12**), and L4 (**13**) being inhibitors of CYP2C19, and siloxane D3 (**1**), D4 (**2**), and L2 (**11**) being inhibitors of CYP2C9. Siloxane D4 (**2**) and D5 (**3**) are known to induce liver CYP450 enzymes and constitutively activated/androstane receptor expression in rodents, suggesting that both compounds have the potential to act on important xenobiotic metabolic pathways (Johnson et al., 2011). None of the siloxanes were found to be inhibitors of either organic anion-transporting polypeptides (OATP) 1B1 or OATP1B3. Excretion parameters were predicted from the total clearance, half-life ($t_{1/2}$), and renal OCT2 substrate activity. OCT2, or the organic cation transport protein 2, is a critical transporter located in the kidneys. It plays a pivotal role in the renal uptake and clearance of various endogenous substances, pharmaceuticals, and other substances. The interaction between OCT2 substrates and OCT2 inhibitors can lead to significant pharmacokinetic implications and adverse effects. None of the siloxanes were renal OCT2 substrates. The total clearances were predicted similarly, with an average of 11.88±1.22 (10.73-13.89) mL/min/kg. The half-life of all siloxanes, or the time required for the concentration in plasma/serum to halve, was estimated to be less than 3 hours.

The individual in silico predictions generated by the VEGA models for the effects of siloxanes on oestrogen, androgen, thyroid, and glucocorticoid receptors are displayed in Supplemental Table S5. None of the siloxanes (**1-16**) were predicted to have AR, ER, TRα, TRβ, GR, TP-related, or endocrine disruptor effects in any of the tested models employed in this study. This suggests that siloxanes do not interact with these hormonal pathways at the levels tested, indicating a lack of potential endocrine disruption or hormonal activity. Supplemental Table S6 exhibits androgen, oestrogen, glucocorticoid, mineralocorticoid, progesterone, and thyroid receptor-mediated pathways of siloxanes as identified by VEGA NMREA models. All the tested siloxanes (**1-16**) were predicted to be inactive in AR, ERα, GR, MR, PR, TRα, and TRβ receptor interactions but were found to have a role in the ERβ-mediated pathway. ERα and ERβ are proteins that bind to oestrogen hormones and trigger various biological effects inside the cell. These receptors are involved in many important processes such as reproductive health, bone density, and cardiovascular and central nervous system functions. The role of ERβ has been linked to various conditions such as cancer, Alzheimer's disease, and autoimmune diseases (Jia et al., 2015). The receptor interactions of the siloxanes were predicted by PanScreen software and are shown in Supplemental Table S7. Siloxane L2 (**11**), L3 (**12**), L4 (**13**), and L5 (**14**) were found to have low affinity for the AR, while the others either showed no affinity or had uncertain binding affinity. AR acts as a key regulator of downstream signalling pathways that depend on androgens (Matsumoto et al., 2013). This transcription factor, which requires a ligand to function, influences gene expression by recruiting various co-regulator complexes, promoting chromatin reorganisation, and facilitating epigenetic modifications, such as histone alterations, at specific genomic regions. When androgen/AR signalling is dysregulated, it disrupts normal reproductive development and can lead to a variety of health issues, including androgen insensitivity syndrome, prostate cancer, and spinal bulbar muscular atrophy (Matsumoto et al., 2013). Siloxane L4 (**13**) exhibited low affinity for ERα, while the others were predicted to have uncertain binding. D4 (**2**), L2 (**11**), L3 (**12**), and L4 (**13**) showed low binding affinity for ERβ, whereas the remaining siloxanes showed no affinity. Siloxanes with high affinity for GR were L6 (**15**) and L7 (**16**). GR is the receptor to which cortisol and other glucocorticoids bind. Glucocorticoids are steroid hormones that affect many important processes in the body such as carbohydrate, fat and protein metabolism, immune system functions and stress response (Kadmiel & Cidlowski, 2013). Siloxane D3 (**1**) L2

(**11**), L3 (**12**), L4 (**13**), and L5 (**14**) exhibited low affinity for the β2 adrenergic receptor (β2AR), whereas the others showed no binding affinity. Similarly, siloxane L2 (**11**), L3 (**12**), L4 (**13**), and L5 (**14**) had low affinity for the dopamine receptor D2 (D2R), whereas the binding affinity of the remaining siloxanes was uncertain. β2AR and D2R lead neurons to respond to both adrenergic (adrenaline/noradrenaline) and dopaminergic signals. Klaunig et al. (2016) proposed that siloxane D5 may alter the pituitary control of the oestrous cycle, potentially through a mechanism similar to that of a dopamine receptor agonist. Additionally, oral exposure to D4 in mice caused weak oestrogenic activity, which was mediated by ERα (He et al., 2003). This finding is corroborated by Quinn et al. (2007), who reported that D4 binds to ERα but not to ERβ. Notably, D5 did not exhibit binding to either ERα or ERβ in their study.

In Table 1, except for siloxane L2 (**11**), all siloxanes were predicted as toxicants in the Developmental/Reproductive Toxicity Library (PG)-Prediction. The developmental toxicity model CAESAR predicted all the tested siloxanes (**1-16**) as toxic. Siloxane D3 (**1**) and L2 (**11**) were predicted to be developmental toxicants by the US EPA TEST Consensus Model (v.5.1.2). In the earlier version (v.4.2.1), siloxane D3 (**1**), L2 (**11**), L3 (**12**), and L4 (**13**) were identified as developmental toxicants. Siloxane D4 is classified under the hazard class of reproductive toxicity, specifically categorised as category 2 (hazard statement H361f) by the ECHA (2018). Taken together, the available studies on cyclic and linear siloxanes (D4, D5, D6, L2, L4, L5) in rodents consistently demonstrate that the reproductive system is the principal target following repeated exposure. This classification indicates a suspicion that exposure to siloxane D4 may adversely affect fertility in humans. Research suggests that substances in this category can disrupt normal reproductive functions, thereby raising concerns about both male and female reproductive health. Additionally, the Danish Centre on Endocrine Disrupters has classified siloxane D4 as an endocrine disruptor because of a study showing that D4 exhibits both weak oestrogenic and anti-oestrogenic effects (DEPA, 2021). However, siloxane D4 was compared with ethinylestradiol, showing that its potency was reduced by a factor of 585,000 in rats and 3.7 million in the Fisher-344 strain (ECHA, 2023a). The toxic effect of D4 on the reproductive system was investigated in a two-generation inhalation study. The D4-exposed F1 generation exhibited extended oestrous cycles along with decreased mating and fertility rates. However, there were no unfavourable outcomes on anogenital distance, vaginal patency, or preputial separation at any level of exposure. In addition, no negative impacts were reported for male reproductive parameters, spermatogenic endpoints, or histopathologic assessment of male reproductive tissues. When F1 males exposed to D4 were mated with unexposed

**Table 1.** Developmental and reproductive effects of siloxanes by VEGA and US EPA TEST models

| No | Siloxane | VEGA (v.1.2.3) | | US EPA TEST | |
|---|---|---|---|---|---|
| | | Developmental Toxicity Model (CAESAR) (v.2.1.8) | Developmental/ Reproductive Toxicity Library (PG) (v.1.1.2) | Developmental Toxicity Consensus Model (v.5.1.2) | Developmental Toxicity Consensus Model (v.4.2.1) |
| 1 | D3 | Toxicant (LR) | Toxicant (MR) | Toxicant (0.97) | Toxicant (0.94) |
| 2 | D4 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 3 | D5 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 4 | D6 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 5 | D7 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 6 | D8 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 7 | D9 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 8 | D10 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 9 | D11 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 10 | D12 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 11 | L2 | Toxicant (LR) | Non-toxicant (LR) | Toxicant (0.95) | Toxicant (1.01) |
| 12 | L3 | Toxicant (LR) | Toxicant (LR) | Non-toxicant (0.50) | Toxicant (0.70) |
| 13 | L4 | Toxicant (LR) | Toxicant (MR) | Non-toxicant (0.49) | Toxicant (0.65) |
| 14 | L5 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 15 | L6 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |
| 16 | L7 | Toxicant (LR) | Toxicant (MR) | N/A | N/A |

LR: Low reliability; MR: Moderate Reliability; N/A: Not applicable. The prediction reliability and predicted values are given in brackets in VEGA and US EPA TEST models, respectively.

females, no adverse effects were found. This indicates that the observed reproductive toxicity was primarily associated with female exposure (Siddiqui et al., 2007). D4 showed only a weak oestrogenic response in vivo, but no progestagenic, androgenic, or anti-androgenic activity (Quinn et al., 2007). Siloxane D4 and D5 have been shown to exert toxic effects on the endocrine, reproductive, immune, and nervous systems (Dekant & Klaunig, 2016; Klaunig et al., 2016; King et al., 2020; Hoang et al., 2022). Moreover, the European Commission classified siloxane D4, D5, and D6 in the Candidate List of Substances of Very High Concern (SVHC) for Authorisation (ECHA, 2018). Since siloxane D4 is persistent, bioaccumulative, and toxic, and D5 is highly persistent and highly bioaccumulative, both have the potential to accumulate in the environment and lead to unforeseen, difficult-to-reverse long-term effects. The European Commission has officially sanctioned the limitation of siloxane D4 and D5 in personal care products designed for "wash-off" applications (ECHA, 2023b). This important decision reflects growing concerns over the environmental and health impacts associated with siloxane compounds, signalling a significant step towards more sustainable and responsible product formulations in the cosmetics industry.

Table 2 presents the detailed predictions regarding the mutagenicity of the siloxanes tested in this study. Notably, all the siloxanes evaluated were consistently classified as non-mutagenic by a variety of predictive models, including CAESAR (v.2.1.14), ISS (v.1.0.3), SarPy-IRFMN (v.1.0.8), and KNN-Read-Across (v.1.0.1). All predictions of the CAESAR, ISS and SarPy-IRFMN models were with low reliability. The KNN-Read-Across provided the experimental value for siloxane L3 and L4, predicted 68.75% of the siloxanes as non-mutagen with moderate reliability, but failed to predict D10, D11 and D12. Additionally, the mutagenicity Consensus Model (v.1.0.4), which synthesises the results from multiple sources, also corroborated these findings. It assigned a consensus score between 0.2 and 0.3 to the tested siloxanes, reinforcing their classification as non-mutagenic. This consensus score was derived from the predictive outputs of the VEGA mutagenicity models: CAESAR, SarPy, ISS, and KNN, providing a comprehensive assessment of the mutagenic potential of these siloxane compounds. The US EPA TEST models have provided experimental values for siloxane L3 and L4 and stated that these compounds are mutagenicity negative. 31.25% of the compounds were predicted mutagenicity negative in the US EPA TEST v.5.1.2, while the model failed to estimate 56.25% of the compounds. The previous model (v.4.2.1) predicted all the compounds as mutagenicity negative. The deep-PK mutagenicity model predicted all the siloxanes tested as safe, ranging from medium to high confidence. Although high reliability in the predictions of the models cannot be obtained, the literature supports these results. According to Vergnes et al. (2000), the exposure of CHO cells to siloxane D4 (at a maximum concentration of 0.003 mg/mL without metabolic activation-S9 and 0.03 mg/mL with S9) did not induce sister chromatid exchange. Furthermore, no chromosomal aberrations were observed in the in vivo studies. Consequently, siloxane D4 was reported to lack a genotoxic potential. According to the Danish EPA report, there are no relevant data on siloxane D5's mutagenicity, gene mutation, chromosome abnormalities, other genotoxic effects, and cancer review (Lassen et al., 2005). Exposure to D5 at 160 ppm led to an increased incidence of endometrial adenocarcinoma in the uterus of Fischer 344 rats after 24 months. However, earlier studies assessing mutagenicity, genotoxicity, and acute, subacute, and subchronic toxicity did not identify the uterus as a potential target organ. The specificity of both the target organ and tumour type suggests that this effect may be related to alterations in the oestrous cycle (Jean et al., 2016). Exposure of adult female F-344 rats to D4 and D5 by inhalation at 700 ppm for 35 days was associated with an increase in large follicles in the animals due to sustained oestradiol secretion. Endometrial hyperproliferation and the stimulation of carcinogenic processes are predicted to increase in tandem with the cumulative number of days of endogenous oestrogen exposure (Dekant et al., 2017; Jean & Plotzke, 2017; Molinier et al., 2022).

Table 3 exhibits the rat oral acute toxicity ($LD_{50}$ as mg/kg bw) of the tested siloxanes using the VEGA KNN, US EPA TEST, and ProTox models. The VEGA Acute Toxicity ($LD_{50}$) model (KNN v.1.0.0) predicted seven of the compounds from 1,168 to 2,410 mg/kg bw, all with low reliability. The US EPA TEST v.5.1.2 predicted $LD_{50}$ values of the five compounds, and these values were found to be between 1,095 and 3,686 mg/kg bw. The US EPA TEST v.4.2.1 predicted 13 compounds with $LD_{50}$ values ranging from 649.22 to 4,839 mg/kg bw. The US EPA TEST models provided a broader range of $LD_{50}$ predictions, with some values suggesting a slightly higher acute toxicity potential for certain siloxanes compared with VEGA. However, similar to VEGA, the predictions generally indicate a low to moderate acute oral toxicity profile. Siloxane L6 (**15**) had the highest $LD_{50}$ value according to VEGA, while siloxane L4 (**13**) showed the highest $LD_{50}$ value in both TEST versions 5.1.2 and 4.2.1. In the VEGA and TEST models, the $LD_{50}$ values of the linear siloxanes were estimated to be higher than those of the cyclic structures. The predicted toxicity classes and prediction accuracy (%) of the compounds were also identified in the ProTox model. Among sixteen siloxanes, the $LD_{50}$ of 13 was predicted to be 1,540 mg/kg bw (with prediction accuracy of 100%), referring to the Globally Harmonised System (GHS) classification (UNECE, 2011) of class IV (acute oral toxicity),

**Table 2.** Mutagenicity predictions of the siloxanes using VEGA, US EPA TEST, and Deep-PK models

| No | Siloxane | VEGA (v.1.2.3) | | | | | US EPA TEST | | Deep-PK |
|---|---|---|---|---|---|---|---|---|---|
| | | CAESAR (v.2.1.14) | ISS (v.1.0.3) | SarPy-IRFMN (v.1.0.8) | KNN-Read-Across (v.1.0.1) | Consensus Model (v.1.0.4)* | Consensus Model (v.5.1.2) | Consensus Model (v.4.2.1) | |
| 1 | D3 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | Mutagenicity negative (0.09) | Mutagenicity negative (0.04) | Safe (MC) |
| 2 | D4 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | N/A | Mutagenicity negative (0.04) | Safe (MC) |
| 3 | D5 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | Mutagenicity negative (0.12) | Mutagenicity negative (0.15) | Safe (MC) |
| 4 | D6 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | Mutagenicity negative (0,03) | Mutagenicity negative (-0.05) | Safe (MC) |
| 5 | D7 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | N/A | Mutagenicity negative (0.12) | Safe (MC) |
| 6 | D8 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | N/A | Mutagenicity negative (0.04) | Safe (MC) |
| 7 | D9 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | N/A | Mutagenicity negative (0.15) | Safe (MC) |
| 8 | D10 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | N/A | Non-mutagenic (CS=0.2) | N/A | Mutagenicity negative (0.02) | Safe (MC) |
| 9 | D11 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | N/A | Non-mutagenic (CS=0.2) | N/A | Mutagenicity negative (0.01) | Safe (MC) |
| 10 | D12 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | N/A | Non-mutagenic (CS=0.2) | N/A | Mutagenicity negative (0.01) | Safe (MC) |
| 11 | L2 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (EV) | Non-mutagenic (CS=1.0) | Mutagenicity negative (EV) | Mutagenicity negative (EV) | Safe (HC) |
| 12 | L3 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (EV) | Non-mutagenic (CS=1.0) | Mutagenicity negative (EV) | Mutagenicity negative (EV) | Safe (HC) |
| 13 | L4 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | Mutagenicity negative (0.02) | Mutagenicity negative (0.02) | Safe (HC) |
| 14 | L5 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | N/A | Mutagenicity negative (-0.04) | Safe (HC) |
| 15 | L6 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | Mutagenicity negative (0,00) | Mutagenicity negative (0.10) | Safe (HC) |
| 16 | L7 | Non-mutagenic (LR) | Non-mutagenic (LR) | Possible non-mutagenic (LR) | Non-mutagenic (MR) | Non-mutagenic (CS=0.3) | N/A | Mutagenicity negative (0.11) | Safe (HC) |

* Consensus value; CS: Consensus score; EV: Experimental value; HC: High confidence; LR: Low reliability; MC: Medium confidence; MR: Moderate reliability; N/A: Not applicable. The predicted values are given in brackets.

harmful if swallowed (300<$LD_{50}$≤2,000). The siloxane L2 (**11**) was found to have an $LD_{50}$ value of 3,000 mg/kg bw, with a prediction accuracy of 100%. This places it in class V, which may be harmful if swallowed (2,000<$LD_{50}$≤5,000). The siloxane D3 (**1**), L3 (**12**), and L4 (**13**) were found to have the highest predicted $LD_{50}$ value of 24,134 mg/kg bw, with an estimated prediction accuracy of approximately 70%. Based on this value, these siloxanes are classified as class VI (non-toxic), as their $LD_{50}$ exceeds 5,000 mg/kg. The acute toxicity of siloxanes is generally regarded as low, indicating that these compounds are not likely to cause significant harm through short-term exposure (Lassen et al., 2005). The cyclic siloxanes D4 and D5 have not been reported to produce any gross adverse effects after oral administration in rats. The oral $LD_{50}$ of siloxane D4 and D5 is reported to be >2,000 mg/kg bw and 4,800 mg/kg bw, respectively, in male and female rats (SCCS, 2010). The acute oral $LD_{50}$ values for siloxane D5 are reported to be greater than 2,000 mg/kg bw (unnamed, 1999; cited by the ECHA). The short-linear siloxane L2 has an oral $LD_{50}$ of >5,000 mg/kg bw in rats (Lassen et al., 2005). Overall, the $LD_{50}$ predictions from VEGA and ProTox show a reasonable alignment with these literature values, suggesting a low to moderate acute oral toxicity for most tested siloxanes. While some discrepancies exist, particularly the high $LD_{50}$ predictions for D3 (**1**), L3 (**12**), and L4 (**13**) by ProTox, the majority of the in silico results support the generally accepted low acute toxicity of these compounds.

**Table 3.** Acute rat oral toxicity ($LD_{50}$) of the siloxanes using US EPA TEST, and ProTox

| No | Siloxane | VEGA KNN (v.1.0.0) | US EPA TEST $LD_{50}$ (mg/kg) | | ProTox (v.3.0) | | |
|---|---|---|---|---|---|---|---|
| | | $LD_{50}$ (mg/kg) | (v.5.1.2) | (v.4.2.1) | $LD_{50}$ (mg/kg) | GHS class | PA (%) |
| 1 | D3 | 1,167.85 (LR) | 1,413.77 | 1,302.42 | 24,134 | 6 | 70.97 |
| 2 | D4 | 1,557.13 (EV) | 1,094.67 | 1,139.96 | 1,540 | 4 | 100 |
| 3 | D5 | 1,946.41 (LR) | N/A | 2,591.65 | 1,540 | 4 | 100 |
| 4 | D6 | 2,335.69 (LR) | N/A | 1,046.23 | 1,540 | 4 | 100 |
| 5 | D7 | N/A | N/A | 649.22 | 1,540 | 4 | 100 |
| 6 | D8 | N/A | N/A | 1,719.98 | 1,540 | 4 | 100 |
| 7 | D9 | N/A | N/A | N/A | 1,540 | 4 | 100 |
| 8 | D10 | N/A | N/A | N/A | 1,540 | 4 | 100 |
| 9 | D11 | N/A | N/A | 1,446.97 | 1,540 | 4 | 100 |
| 10 | D12 | N/A | N/A | N/A | 1,540 | 4 | 100 |
| 11 | L2 | N/A | 3,272.43 | 3,832.27 | 3,000 | 5 | 100 |
| 12 | L3 | N/A | 3,084.59 | 3,241.44 | 24,134 | 6 | 69.26 |
| 13 | L4 | 1,631.03 (LR) | 3,685.62 | 4,839.15 | 24,134 | 6 | 72.90 |
| 14 | L5 | 2,020.31 (LR) | N/A | 1,082.48 | 1,540 | 4 | 100 |
| 15 | L6 | 2,409.59 (LR) | N/A | 3,775.90 | 1,540 | 4 | 100 |
| 16 | L7 | N/A | N/A | 2,662.74 | 1,540 | 4 | 100 |

The acute toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals (GHS). Class I: Death after swallowing ($LD_{50}$≤5); Class II: Death after swallowing (5<$LD_{50}$≤50); Class III: Toxic after swallowing (50<$LD_{50}$≤300); Class IV: Harmful after swallowing (300<$LD_{50}$≤2,000); Class V: May be harmful after swallowing (2000<$LD_{50}$≤5,000) and Class VI: Non-toxic ($LD_{50}$>5,000). EV: Experimental value; PA: Prediction accuracy; N/A: Not applicable.

In Figure 2, ProTox (v.3.0) predicted that the 16 siloxanes were inactive with regard to the tested organ toxicity endpoints, including hepatotoxicity, neurotoxicity, nephrotoxicity, and cardiotoxicity; as well as toxicity endpoints such as immunotoxicity, mutagenicity, cytotoxicity, and carcinogenicity. The probability scores were less than 0.70 for nephrotoxicity and carcinogenicity in general and mutagenicity in cyclic siloxanes.

Short- and long-term repeated-dose toxicity tests in animals revealed that the liver is the crucial target organ for siloxane D4 and D5 (SCCS, 2010). Inconsistent with these findings, our analysis using the ProTox model indicated a high probability of inactivity for hepatotoxicity across all siloxanes (**1**-**16**), suggesting no potential for liver toxicity. A long-term inhalation study found only minor effects on the rats' respiratory system. However, there were noticeable increases in the liver weight, along with a heightened occurrence of uterine endometrial epithelial hyperplasia. Additionally, a clear dose-dependent rise was observed in the development of endometrial adenomas, indicating a significant correlation between the dosage and these pathological changes. The increase in liver weight, linked to the activation of hepatic metabolising

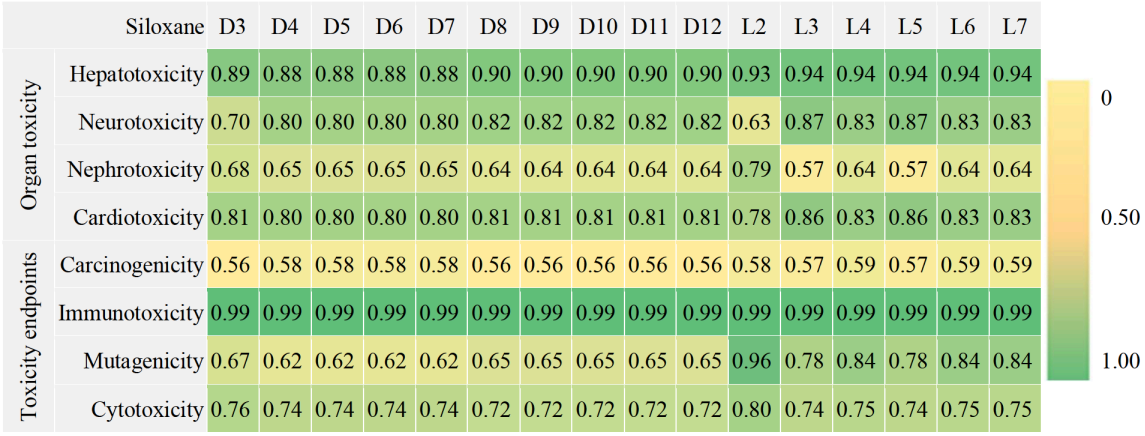


**Figure 2.** Organ toxicity and toxicity end point results of the siloxanes using ProTox (v.3.0). Prediction result given with a probability score

enzymes, is comparable to that caused by phenobarbital (Franzen et al., 2018). Based on a two-year chronic inhalation study in rats, the Danish EPA has categorised siloxane D4 as a reproductive toxicant (Lassen et al., 2014). Initial findings suggest that siloxane D5 may have carcinogenic potential in rats. D5 has been identified as a weak inducer of cytochrome P450 enzymes in rats, exhibiting effects similar to those of phenobarbital. The mode of action data suggest that siloxane D5 alters the pituitary regulation of the oestrous cycle, possibly through a dopamine receptor agonist-like mechanism. Comprehensive investigations into the mechanisms by which endometrial adenocarcinomas could arise from D5 indicate a tumorigenic pathway that is unlikely to be applicable to humans (Klaunig et al., 2016). Siloxane D6 underwent a comprehensive 90-day repeated-dose inhalation toxicity study in rats, conducted in accordance with the OECD Test Guideline (TG) 413 and following the Good Laboratory Practice (GLP) standards (Dow Corning Corporation, 2013; cited by the ECHA). The inhalation route was chosen to accurately assess the potential toxic effects of D6, considering its possible exposure scenarios in occupational and environmental settings. No systemic adverse effects were found in either sex at the maximum administered dose of 30 ppm, which corresponds to 546 mg/m³. The key repeated-dose oral toxicity study on siloxane D6, conducted by Dow Corning Corporation in 2005 (as cited by ECHA), was a 4-week study involving rats. This study combined the assessment of repeated-dose toxicity with a screening test for reproductive and developmental toxicity. The study was conducted in accordance with OECD TG 422 and complied with GLP. The No Observed Adverse Effect Level (NOAEL) was established as at least 1,000 mg/kg bw/d, which is the highest dose tested. The observed liver effects (increased absolute and/or relative liver weight in all treated groups and periportal lipidosis at all doses in females) were described as of minimal toxicological significance, and the thyroid effects (follicular cell hypertrophy, incidence possibly treatment-related in both sexes) were considered secondary and adaptive to the liver changes. In a subchronic (28-day) repeated oral gavage study (Shin-Etsu, 1994; cited by the ECHA), the NOAEL for siloxane L2 was reported as 160 mg/kg bw/d. This conclusion was based on decreased food intake, lower body weight gain, increased liver weight, and variations in white blood cell counts and other blood parameters observed in male rats (ECHA). In a subchronic repeated-dose oral toxicity study (Dow Corning, 2010; cited by the ECHA), no treatment-related significant effects were determined in rats administered siloxane L5 via oral gavage at doses of 25, 250, or 1,000 mg/kg bw/d for 28 days. A NOAEL for systemic toxicity of ≥1,000 mg/kg bw/d was reported (ECHA). A 28-day repeated-dose toxicity study in rats was conducted with animals receiving oral doses of siloxane L4 at 25, 250, and 1,000 mg/kg bw/d via gavage. The NOAEL was determined to be 25 mg/kg bw/d. This conclusion was based on the significantly elevated mean absolute liver weights, liver-to-body weight ratios, and liver-to-brain weight ratios observed in both males and females treated with doses of 250 and 1,000 mg/kg bw/d. Furthermore, the presence of brown pigment accumulation was considered an adverse finding, linked to secondary periportal chronic inflammation and bile duct proliferation (ECHA).

The carcinogenicity evaluation was part of a two-year chronic toxicity study conducted on rats, in which a polydimethylsiloxane mixture was administered orally at doses up to 1,000 mg/kg bw/d. No neoplastic or pre-neoplastic lesions were reported (Mertens, 2003; cited by ECETOC, 2011). In a separate two-year chronic toxicity and carcinogenicity assessment conducted on Fischer 344 rats (Research & Consulting Comp. Ltd., 2005; cited by the ECHA), whole-body exposure to siloxane L2 resulted in an increase in Leydig cell tumours. However, this was deemed a spontaneous finding that is frequently observed in Fischer 344 rats, including those in the control group. It has been suggested that siloxane L2 may promote tumour progression (ECHA). Regarding genotoxicity and cancer potential, Figure 2 provides in silico predictions for carcinogenicity and mutagenicity. The carcinogenicity probability scores for siloxanes (**1**-**16**) range from 0.56 to 0.59, suggesting a moderate potential for carcinogenicity according to the ProTox model. This contrasts with the in vitro and in vivo findings for D4 and D5, which showed no signs of genotoxic effects (SCCS, 2010), and the lack of mutagenicity for linear polydimethylsiloxane in other in vitro studies (ECETOC, 2011). The IARC classification of silicone breast implants as Group 3 indicates that their carcinogenicity to humans is not classifiable, and current evidence does not support a link to breast carcinoma in humans (IARC, 1999). Our in silico receptor binding predictions (Supplemental Table S6 and S7) indicated affinity for ERα and/or ERβ for several siloxanes including D4 (**2**), L2 (**11**), L3 (**12**), and L4 (**13**), which could provide a mechanistic basis for the observed hormonally mediated uterine effects in animal studies.

## CONCLUSION

Cyclic and linear siloxanes are widely used across various industries, including pharmaceuticals, medical, cosmetics, and food manufacturing. This study focuses on assessing the potential toxic effects of siloxanes using in silico methods. Our analysis involved comparing our findings with existing data for commonly used cyclic siloxanes and linear siloxanes. Our results indicate that siloxanes may impact the endocrine system through the ER pathway. Further in vitro and in vivo studies are needed to elucidate the potential toxic effects

of these compounds. The development of innovative toxicity models incorporating silicone compounds is essential to enhance our understanding of their effects on human health and the environment. Although siloxanes are reported to be non-mutagenic and non-genotoxic, the potential effects of long-term exposure, particularly via food, cosmetics, and industrial products, warrant consideration, and hormone-mediated carcinogenic mechanisms should be further investigated. As our understanding deepens, it becomes clear that the diverse chemical structures and behaviours of silicone compounds may harbour potential risks that were previously overlooked.

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