ORIGINAL ARTICLE

GHS Acute Toxicity Hazard Class of the Selected Car Air Fresheners Sold in Major Hypermarkets, Penang, Malaysia Without Ingredient Information

Hazirah Pengiran[1], Jahangir Kamaldin[2], Siti Farhanah Mohd-Salleh[2], Adibah Rahman[2]

[1] Civil Department, Faculty of Civil Engineering & Technology, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia.
[2] Department of Toxicology, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Penang, Malaysia.

ABSTRACT

Introduction: A car air freshener (CAF) often contains hazardous volatile organic compounds associated with health hazards. Some of the CAF products' labels sold in Malaysia lacked ingredient descriptions and GHS hazard communication symbols/signal words, which creates concerns about the potential hazard. The study aims to determine the acute toxicity health hazard of selected CAFs sold in Penang, Malaysia based on GHS classification. **Materials and methods:** Two CAFs of locally manufactured are selected, and their acute oral toxicity is evaluated based on OECD test guideline 423. Twelve female Sprague Dawley rats are dosed with the 2000 mg/kg body weight of the CAFs in a stepwise procedure, followed by 14 days of observation for signs of toxicity (clinical signs, severe pain/ distress, moribund, and mortality). **Results:** The observation did not show rats experiencing severe pain/ distress or moribund or mortality during the 14 days of the post-dosing, thus both CAFs are unlikely to cause acute toxicity upon oral exposures. However, both CAFs caused the rats to exhibit multiple clinical signs of toxicity concurrently that are reversible which include piloerection, inactiveness, decreased food intake, restless turning of the head from side to side, decreased grooming, salivation, sneezing, porphyrin secretion and blood around nose. **Conclusion:** Both selected CAFs are under category 5 of the GHS acute toxicity hazard class (LD50 > 2000 to 5000 mg/kg body weight). The reversible clinical signs observed strongly justify the determination of the GHS hazard class of specific target organ toxicity via repeated exposure.
Malaysian Journal of Medicine and Health Sciences (2024) 20(6): 227-234. doi:10.47836/mjmhs20.6.30

Keywords: Fragrance, Volatile organic compounds, GHS, Acute toxicity, Good health

Corresponding Author:
Jahangir Kamaldin, PhD
Email: jahangir@usm.my
Tel : +6019-9817922

INTRODUCTION

Car air freshener (CAF) is a common product used in Malaysia to emit fragrance, get rid of unpleasant odours and freshen up the air in the car. It includes liquids, gels, sprays/aerosols, sticks and potpourri (1). CAF usage is high and the worldwide market for CAF is expected to reach the value of USD 22.44 billion in the next five years (2). In Malaysia, the air freshener market size is expected to grow by USD 38 million with a 7.5% annual growth rate for the same forecast period (3). The CAF products are known to contain hazardous volatile organic compounds namely phthalates, limonene, linalool, benzaldehyde and β-citronellol (1, 9, 11). Some of the volatile organic compounds are known to cause toxic effects to insects which normally could only be affected by insecticides that are also toxic to humans (7, 8). CAF can be source of volatile organic compounds (VOCs) emission such as xylene, pinenes, benzene, toluene, ethylbenzene and others (22).

Most of the CAF products sold in the Malaysian market are not regulated and lack safety information as recommended by the United Nations Globally Harmonized System for Classification and Labelling of Hazardous Substances aka GHS (4). Lack of ingredient description and warning signs on the label may encourage common belief among consumers that CAF is a non-hazardous product. According to the European Chemicals Agency (ECHA), manufacturers shall place warning signs on the label of dangerous products for hazard communication to consumers on the chemical hazards and their potential threat to health especially to vulnerable groups (5). In a toxicity assessment of consumer exposure against ingredients in air fresheners

through inhalation and dermal, most of the ingredients were found poses health risk (20). The margin of exposure (MOE) from the air freshener was higher than target MOE even though the risk assessment indicated no health risk at maximum concentration (20). In another study to evaluate the cancer risk, similar result has been reported where no cancer risk was found among consumers who exposed with air freshener (22). Conversely, exposure to air fresheners through inhalation caused an increase of oxidative stress in exposed rat groups compared to control group (21). Nonetheless, there is limited study on the effect of air freshener upon oral exposure if there is accidental ingestion of the consumer product as risk of spillage may occurs during refilling event, emergency or leaks upon prolonged expose with direct sunlight (23).

The GHS labelling rule is intended to ensure essential information about chemical hazards is communicated to protect human health and the environment from the point of production, handling, transportation, use by consumers and disposal. Consumers especially car drivers and occupants in Malaysia cities are exposed to the airborne chemical substances of the CAF at prolonged hours, because of constant heavy traffic. Leaks and spills from the CAF tend to occur due to refilling event, emergency, manufacturing defects or heating of the CAF during parking under direct sunlight. Hence, they are also exposed through dermal contact during handling and eventually oral ingestion of the CAF contents tainted on their fingers (6).

The study aimed to conduct a preliminary evaluation of whether the CAF that didn't carry ingredients description or acute toxicity symbol/signal word (danger/warning) poses health threats to the consumer from hand contact and accidental ingestion of the CAF contents on their fingers. Therefore, the objective of this study is to evaluate acute oral toxicity of the three selected local CAF products sold at hypermarkets in Penang, Malaysia. Subsequently, the selected CAF health hazard will be classified based on the acute oral toxicity result following the GHS health hazard classification guide (4).

MATERIALS AND METHODS

Car air freshener (CAF)

CAFs in bottles with diffuser wicks were chosen for this study since this type of CAF was represented by most brands based on the market survey conducted at the hypermarket. Two brands were selected for the study based on previous research which found the selected CAF brands were positive in causing mosquitoes to knockdown, indicating apparent neurotoxicity against the mosquito (7,8). The selected CAFs were conveniently named Brand X and Brand Y, which were locally manufactured and did not have the ingredients description and GHS acute toxicity hazard symbol/ warning sign.

Methods

The study employed a standard operating procedure of the acute oral toxicity – acute toxic class method (AOTATC) TG 423 published by OECD (10, 12, 13) that requires the experiment to be conducted in two or three steps, whereby the treatment dose of the subsequent step depends on the outcome of the previous step. Each step involved the sequential processes of animal acclimatization, animal selection, dose preparation, dose administration and post-dosing animal observation.

Animal ethics approval for the study was obtained from the Universiti Sains Malaysia (AECUSM Approval No. 2014/532) with care and use of the rats followed according to the guideline established by the Animal Research Section of the Advanced Medical and Dental Institute, Universiti Sains Malaysia. Twelve healthy young female rats (Rattus norvegicus) of the Sprague Dawley strain that was nulliparous and non-pregnant, aged between 8 and 12 weeks at the commencement of dosing were utilized in the study. The female rats were utilized because they were slightly more sensitive (12). The rats were kept in the experimental room at a temperature of 19 to 25 °C, relative humidity of 30 to 70% and an illumination cycle of 12 hours light:12 hours dark. The rats were fed with conventional grain pellets and an unlimited supply of drinking water. The environmental conditions of temperature, relative humidity and illumination cycle in the breeding/holding room and experimental room were recorded daily with minimum and maximum measurements.

Rats were acclimatized for a minimum of five days. Rats aged 7 to 8 weeks of similar sizes by the inspection of naked eyes were selected by convenience-random from the breeding room, and each rat was transferred into an individual experimental cage. The mean weight of female rats was 261.8 ± 15.3 g. The variation of rat's weight within the same batch was less than ± 20% of the mean weight. Each rat was given a unique identification and marked to differentiate between numbers of rats, doses and selected CAFs. The rats were divided into two groups representing for each brand and marked as X or Y for CAFs brand and no. 1/2/3 for step 1 and 4/5/6 for step 2 for their identification (ID).

Dose preparation

The selected CAFs were in liquid form and thus, dose was prepared as undiluted liquid at constant concentration of 75 %w/w of solvents and 25 %w/w of fragrances. The dose was adjusted accordingly based on the volume of administration (12). Dose preparation was performed in the procedure room without the presence of test animals to prevent accidental contamination. The prepared doses were stored in air-tight vials, isolated from the test animal and placed under a ventilated environment at temperatures 18°C to 25°C. The initial starting dose (Step 1) was 2000 mg/kg body weight selected based

on the literature on the LD50 value of VOCs commonly contained in the CAF, which is equal to or more than 2000 mg/kg body weight (14, 15, 16).

Dose administration (Dosing)

The oral administration (dosing) was selected in view the focus of this preliminary study is evaluating the severity effect should any accidental oral exposure occur via finger contamination from the CAF content leaks or spills during handling. Before the administration of the dose (dosing procedure), the rats were not fed for 10 to 12 hours while maintaining a supply of drinking water, except for 1 to 2 hours before the administration of the dose. A total of six rats from the holding room were taken to the procedure room, subsequently, the weight and rectal temperature of each rat were recorded. Then, the six rats were administered separately with the test item in aqueous solution in a single dose by gavage using an oral dosing needle. After dosing, the rats were transferred to the experimental room which had a similar environment condition to the holding room, for post-treatment observations. Feeding of the rats was continued after 4 hours of post-dose administration.

Animal observations

Each rat was physically observed for signs of toxicity (clinical signs, severe pain/ distress, moribund and mortality) at the intervals 0.5, 1, 2, 3, 4, 6, 8, 12 hours and daily up to 14 days after dosing. The signs of toxicity were distinguished based on the animal behaviour or condition as described by the OECD guidance document on the recognition, assessment, and use of clinical signs as human endpoints for experimental animals used in safety evaluation (17). The weight of each rat was recorded on the day of dosing, day-7 and 14 after dosing. The signs of toxicity are considered as not reversible if the signs are persistent for more than 24 hours and/or regularly seen for more than 7 days out of the 14 days of observation. During the observation period, none of the rats showed severe pain or distress that required early euthanization. Therefore, the rats were humanely euthanized on day 14 using carbon dioxide with 99% purity at 1.3 L/min. The emphasis of the study is to ascertain the acute toxic class of selected CAFs based on the number of rats that died within 14 days of observation as described in AOTATC TG 423 (12). Thus, a necropsy was performed only on the rats found dead or showing clinical signs of severe pain/ distress or moribund within 14 days to verify that the cause is related to selected CAFs.

Statistical analysis

The findings of the toxicity signs are analysed qualitatively based on the description given by the OECD guidance document No. 19 on the recognition, assessment and use of clinical signs as human endpoints for experimental animals used in safety evaluation (17) and the acute toxicity category are determined descriptively based on the criteria provided in the OECD AOTATC TG423 and GHS guidance (4, 11), which have been validated by the OECD working groups. An Independent T-test was conducted to calculate mean difference for food and water consumption among the rats between the CAF brands.

Ethical Clearance

The study was indirectly funded by equipment, apparatus and consumables purchased under several grants namely MOSTI IRPA EAR Project No. 06-02-05-00031, USM RUI Project No: 1001/CIPPT/817040, MOHE KTP Project No: 203/CIPPT/6750060, and postgraduate student incentive fund under the Advanced Medical and Dental Institute, Universiti Sains Malaysia.

RESULTS

Table I and Table II show the animal physical observations of the toxicity signs (clinical signs, severe pain/ distress, moribund and mortality) observed at step 1 and step 2 of the experiments. The tables also show the onset and duration of the signs persisted. In view none of the three rats for each brand showed severe pain/ distress or moribund or death during the 14 days of the post-dosing observation, the experiments were only conducted up to step 2 with the same dose level of the 2000 mg/kg body weight. For the oral dosing with the brand X at the first step, the clinical signs of “restless turning of the head from side to side” (RTH), “piloerection” (PE) and “inactive” (IA) were immediately exhibited on the 1st day of the post-dosing by all three rats. The duration was less than 24 hours which reflects its reversibility. Whereas for brand Y, the same PE and IA behaviour were also shown by all three rats on the 1st day of the post-dosing. Additionally, two of the three rats dosed with the brand Y exhibited nasal “porphyrin secretion” (PS), one (rat ID: Y-1) on the 1st day while the other (rat ID: Y-3) on the 11th day after the dosing. The other rat of the same group (rat ID: Y-2) still showed a “decrease of food intake” (DFI) on the 1st day but recovered the following days.

Table I: Signs of toxicity (clinical signs, signs onset, signs duration, reversibility, severe pain/ distress and, moribund/mortality) shown by Sprague Dawley female rats at step 1 (one) upon oral dosing of brand X and brand Y of the local car air freshener at the dose of 2000 mg/kg body weight.

Step	Animal Identification	Dose (mg/kg body weight)	Clinical sign [a]	Sign Onset (hour)	Sign Duration (hour)	Reversibility [b]	Severe pain/ distress	Moribund/ Mortality
1	Brand X Rat 1	2000	RTH	1st	1	Reversible	None	Alive until day-14
			PE	2nd	4	Reversible		
			IA	1st	8	Reversible		
	Brand X Rat 2	2000	RTH	1st	1	Reversible	None	Alive until day-14
			PE	2nd	4	Reversible		
			IA	1st	12	Reversible		
	Brand X Rat 3	2000	RTH	1st	1	Reversible	None	Alive until day-14
			PE	2nd	4	Reversible		
			IA	1st	8	Reversible		
	Brand Y Rat 1	2000	PE	1st	1	Reversible	None	Alive until day-14
			IA	1st	6	Reversible		
			SN	2nd	24	Reversible		
	Brand Y Rat 2	2000	PS	1st	1	Reversible	None	Alive until day-14
			PE	1st	1	Reversible		
			IA	1st	1	Reversible		
			DFI	12th	24	Reversible		
	Brand Y Rat 3	2000	RTH	3rd	1	Reversible	None	Alive until day-14
			PE	1st	2	Reversible		
			IA	1st	2	Reversible		
			PS	264th (Day 11)	24	Reversible		

[a] Clinical sign

AV	= Abnormal vocalisation	DG	= Decreased grooming	PS	= Porphyrin secretion
BN	= Blood around nose	ESL	= Excessive salivation	RSA	= Reduced spontaneous activity
CSN	= Continuous sneezing	EC	= Eyelid closure	RTH	= Restless turning of head from side to side
CWL	= Continuous weight loss	HT	= Head tremor	SL	= Salivation
CG	= Coughing	IA	= Inactive	SN	= Sneezing
DFI	= Decreased food intake	PE	= Piloerection	WL	= Weight loss

[b] Reversibility; The signs of toxicity are considered as not reversible if the signs persistent for more than 24 hours and/or regularly seen for more than 7 days out of the 14 days of observation.

Table II: Signs of toxicity (clinical signs, signs onset, signs duration, reversibility, severe pain/ distress and, moribund/mortality) shown by Sprague Dawley female rats at step 2 (two) upon oral dosing of brand X and brand Y of the local car air freshener at the dose of 2000 mg/kg body weight.

Step	Animal identification	Dose (mg/kg body weight)	Clinical sign [a]	Sign onset (hour)	Sign duration (hour)	Reversibility [b]	Severe pain/ distress	Moribund/ Mortality
2	Brand X Rat 4	2000	RTH	1st	1	Reversible	None	Alive until day-14
			PE	1st	6	Reversible		
			IA	1st	3	Reversible		
			SN	216th	24	Reversible		
			DFI	12th	24	Reversible		
	Brand X Rat 5	2000	PE	1st	6	Reversible	None	Alive until day-14
			IA	1st	3	Reversible		
			PS	3rd	1	Reversible		
			DFI	12th & 192th	24 & 24	Reversible		
	Brand X Rat 6	2000	PE	1st	6	Reversible	None	Alive until day-14
			IA	1st	3	Reversible		
			PS	3rd	1	Reversible		
			DFI	12th & 168th	24 & 24	Reversible		
			BN	11th & 336th	24 & 24	Reversible		

CONTINUE

Table II: Signs of toxicity (clinical signs, signs onset, signs duration, reversibility, severe pain/ distress and, moribund/mortality) shown by Sprague Dawley female rats at step 2 (two) upon oral dosing of brand X and brand Y of the local car air freshener at the dose of 2000 mg/kg body weight. (CONT.)

Step	Animal identification	Dose (mg/kg body weight)	Clinical sign [a]	Sign onset (hour)	Sign duration (hour)	Reversibility [b]	Severe pain/ distress	Moribund/ Mortality
2	Brand Y Rat 4	2000	PE	1st	6	Reversible	None	Alive until day-14
			IA	1st	3	Reversible		
	Brand Y Rat 5	2000	RTH	1st	1	Reversible	None	Alive until day-14
			PE	1st	6	Reversible		
			IA	1st	1	Reversible		
	Brand Y Rat 6	2000	PE	1st	6	Reversible	None	Alive until day-14
			IA	1st	3	Reversible		
			DG	1st	1	Reversible		
			SL	1st	1	Reversible		

[a] Clinical sign

AV	= Abnormal vocalisation	DG	= Decreased grooming	PS	= Porphyrin secretion
BN	= Blood around nose	ESL	= Excessive salivation	RSA	= Reduced spontaneous activity
CSN	= Continuous sneezing	EC	= Eyelid closure	RTH	= Restless turning of head from side to side
CWL	= Continuous weight loss	HT	= Head tremor	SL	= Salivation
CG	= Coughing	IA	= Inactive	SN	= Sneezing
DFI	= Decreased food intake	PE	= Piloerection	WL	= Weight loss

[b] Reversibility; The signs of toxicity are considered as not reversible if the signs persistent for more than 24 hours and/or regularly seen for more than 7 days out of the 14 days of observation.

Subsequently, during the experiment at the 2nd step (Step 2) as shown in Table II, all three rats dosed with brand X at the dose of 2000 mg/kg body weight, also exhibited the same three clinical signs as seen in the first step i.e. PE, IA, and DFI. However, the RTH is only seen in one of the rats (rat ID: X-4). Further, rat ID X-4 and X-6 showed new clinical signs namely "sneezing" (SN) and "blood around nose" (BN). For the dosing of the brand Y at the 2nd step, the PE and IA have been consistently exhibited as observed at the first step. However, two new clinical signs of toxicity namely "decreased grooming" (DG) and "salivation" (SL) were expressed by rat ID Y-6. Nevertheless, all the clinical signs were reversible, and no rats were found moribund or dead during the 14 days of the post-dosing.

Table III presents the individual rats' body weight before dosing (day-0), day-7 and day-14 post-dosing. The weight of the females before dosing was within a typical range of 200 - 300 g Sprague Dawley rat (18). The dosing of the rats with the brand-X showed a positive increase of body weights not exceeding 9% at week 1 in both steps of the experiment. For brand-Y, one of the rats (rat ID: Y-4) experienced a 4% decrease in weight at week-1 whereas other rats showed an increase of weight within 10%. In the subsequent week 2, three of the six rats dosed with brand-X at step 1 and step 2, also experienced a drop in weight as low as 7% and 1%, respectively. While the other three rats showed a positive increase in weight at least 5%. For the brand-Y dosing, the rats showed an increase of weight at week 2 in both steps of the experiment with two out of three rats (rat ID: Y-5 and Y-6) at step 2 exhibiting relatively higher weight increases of 19% and 35%. Overall, in two weeks, most of the rats (5 out of 6) dosed with either brand-X or brand-Y exhibited an increase in weight, but brand-X seems to restrict the increase, which is consistent with the decrease of food intake behaviour observed more among rats dosed with brand-X at step 2 as shown in Table IV. While rats in brand-X showed slightly low food intake (18.4 ± 4.9 g) compared to rats in brand-Y (19.7 ± 4.5 g), Independent T-test analysis showed no significant difference in food consumption among the rats in both groups with $p \geq 0.05$. As for water consumption, rats in both groups showed a mean intake of 31 to 35 ml throughout the 14 days of observation.

Table III: Body weight and its weekly percentage of changes shown by Sprague Dawley female rats at step 1 (one) and 2 (two), before and after oral dosing of brand X and brand Y of the local car air freshener (CAF) at the dose of 2000 mg/kg body weight.

Step	Animal identification	Dose (mg/kg body weight)	Individual body weight (g)			% of Body weight changes		
			Prior dosing Day 0	Post dosing Day 7	Post dosing Day 14	Week-1 (7 days)	Week-2 (7 days)	2 Weeks (14 days)
1	Brand X – Rat 1	2000	232	234	228	1	-3	-2
	Brand X – Rat 2	2000	240	261	243	9	-7	1
	Brand X – Rat 3	2000	252	268	281	6	5	12
	Brand Y – Rat 1	2000	267	270	277	1	3	4
	Brand Y – Rat 2	2000	276	280	282	1	1	2
	Brand Y – Rat 3	2000	270	273	275	1	1	2
2	Brand X – Rat 4	2000	277	302	300	9	-1	1
	Brand X – Rat 5	2000	276	282	298	2	6	8
	Brand X – Rat 6	2000	275	282	303	3	7	10
	Brand Y – Rat 4	2000	269	258	274	-4	6	2
	Brand Y – Rat 5	2000	266	281	335	6	19	26
	Brand Y – Rat 6	2000	242	266	360	10	35	49

DISCUSSION

The clinical signs of toxicity of PE, IA and DFI exhibited in most of the rats in this study are similar to a study done by de Almeida reported in 2012 (25) where mice exposed to a dose of more than 2000 mg/kg body weight of VOC exhibited several signs of toxicity effect such as PE, decreased spontaneous activity and loss of appetite. Thus, it supports the likelihood that the selected CAFs contain VOC which usually triggers several toxicity signs, concurrently. The SN behaviour exhibited by one of the rats indicates the capability of the selected CAFs to cause adverse effects on the respiratory system even when exposed via the oral route. In the adsorption, distribution, metabolism, and excretion (ADME) studies of limonene in rats, the substance was found distributed to various organ tissues including in the lung upon oral exposure with peak concentration 1-2 hours post administration (26, 27, 32). Restless turning of the head from side to side and head tremors shown by several rats dosed with selected CAFs suggest these rats suffered from neurotoxicity (28). As the PS was commonly observed among the rats, it indicates the selected CAFs caused stress leading to the endocrine system disruption similar to the clinical signs due to VOC exposures (29, 30, 31, 32). It was suggested that the selected CAFs contained VOC such as linalool, limonene, and aldehyde groups as possible factors to the presented clinical signs of toxicity (20). Nevertheless, all the clinical signs were reversible and did not require early euthanization. In terms of body weight, food, and water consumption, the results were within the normal range of Sprague Dawley rats (18).

CONCLUSION

In summary, presence of the severe pain/ distress or moribund or death during the 14 days of the post-dosing observation, both CAFs are unlikely to cause immediate effects of acute toxicity upon oral exposure, which suggests the acute toxicity hazard class category 5 (LD50 >2000 – 5000 mg/kg body weight) of non-hazardous substance based on the OECD AOTATC TG423 test guideline and the GHS classification. There are differences in the clinical signs observed between the brand-X and brand-Y dosed rats suggest, that these two CAFs are likely to have different content compositions (14, 15, 16). However, both CAFs i.e. brand-X and brand-Y are capable of causing the rats to exhibit eight types of clinical signs of toxicity namely PE, IA, and DFI. RTH, DG, SL SN, PS and BN but reversible. These clinical signs suggest the rats were experiencing temporary disruption of its nervous system coordination with possible alteration of the cardiovascular system due to temporary endocrine disruption (27, 30, 32). If a single dose experiment can elicit the temporary clinical signs, there is a possibility the clinical signs may become regular if the rats were dosed repeatedly daily reflecting exposure to the consumer on daily use (24). Therefore, GHS determination of the health hazards for specific target organ toxicity via repeated exposure, whereby necropsy, gross examination and histopathology should be conducted on all relevant organs of all the surviving

animals using other OECD test guidelines.

ACKNOWLEDGEMENT

The authors are grateful for the support provided by the Animal Research Section, Advanced Medical and Dental Institute, Universiti Sains Malaysia in the care and use of the laboratory animals. Special thanks to Prof. Madya Razak Lajis of the National Poison Center, Universiti Sains Malaysia and Prof. Dr. Noordin Mohamed Mustapha of the Faculty of the Veterinary Medicine, Universiti Putra Malaysia for their technical guidance.

REFERENCES

1. Steinemann A, Nematollahi N, Weinberg JL, Flattery J, Goodman N, Kolev SD. Volatile chemical emissions from car air fresheners. Air Qual Atmos Health.2020;13(11):1329–34. doi: 10.1007/s11869-020-00886-8
2. Precedence Research. Air Freshener Market [Internet]. Canada: Precedence Research; 2023 [cited 2023 Oct 12]. Available from: https://www.precedenceresearch.com/air-freshener-market#:~:text=The%20global%20air%20freshener%20market,9.40%25%20from%202023%20to%202032.
3. Market Research. Air Freshener Market in Malaysia [Internet]. United States: Market Research.com; 2022 [cited 2023 Oct 12]. Available from: https://www.marketresearch.com/Gen-Consulting-Company-v4078/Air-Freshener-Malaysia-Snapshot-32117961/https://www.marketresearch.com/Gen-Consulting-Company-v4078/Air-Freshener-Malaysia-Snapshot-32117961/
4. United Nations. Globally harmonized system of classification and labelling of chemicals (GHS). Eighth edi. Vol. 10. New York and Geneva: United Nations; 2019. 1–553 p.
5. European Chemicals Agency. Guidance on labelling and packaging in accordance with Regulation (EC) No. 1272/2008; 2021.
6. Besis A, Katsaros T, Samara C. Concentrations of volatile organic compounds in vehicular cabin air – Implications to commuter exposure. Environmental Pollution. 2023;330:121763. doi: 10.1016/J.ENVPOL.2023.121763
7. Kamaldin J, Jaal Z. Susceptibility of female Aedes aegypti mosquitoes to selected hazardous chemical substances (acetone, chlorobenzene, diethyl ether, methanol, n-hexane & xylene). In: The 49th Annual Scientific Conference of Malaysian Society of Parasitology & Tropical Medicine. Kuala Lumpur, Malaysia.2013.
8. Kamaldin J, Jaal Z. Exploring beneficial use of mosquito as an environmental friendly detector of hazardous volatile organic compounds. In: Euro Analysis 2009 Conference. Innsbruck, Austria.2009.
9. Santos Rodrigues B, de Ɓvila RI, Benfica PL, Bringel LP, de Oliveira CMA, Vandresen F, et al. 4-Fluorobenzaldehyde limonene-based thiosemicarbazone induces apoptosis in PC-3 human prostate cancer cells. Life Sci. 2018;203:141–9. doi: 10.1016/J.LFS.2018.04.024
10. Toukourou H, Sounouvou H, Catteau L, Toukourou F, Van Bambeke F, Gbaguidi F, et al. Chemical composition, direct and indirect antibacterial activity against multidrug resistant bacteria and toxicity assessment of essential oil of *Cymbopogon giganteus* leaves. In: 67th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA) in cooperation with the French Society of Pharmacognosy AFERP [Internet]. 2019. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0039-3400015
11. Owyed Al-Jumayi HAR. Amelioration of gentamicin-induced nephrotoxicity using essential oil extract from *Otostegia fruticosa* leaves in albino rats. Plant Cell Biotechnol Mol Biol. 2020;21:37–38.
12. OECD. Acute oral toxicity - acute toxic class method. OECD Guideline for Testing of Chemicals. Paris: OECD; 2001. p. 1–14.
13. Kamaldin J. Manual for GLP workshop on TG 423 Acute Oral Toxicity-Acute Toxic Class Method testing of chemical substance. Advanced Medical and Dental Institute. Penang: Universiti Sains Malaysia; 2013.
14. INCHEM. SIDS initial assessment report for SIAM 14 – Linalool. 2002 [cited 2023 Sep 28]. Available from: http://www.inchem.org/documents/sids/sids/78706.pdf
15. INCHEM. Concise International Chemical Assessment Document No. 5 - Limonene. 1998 [cited 2023 Sep 28]. Available from: http://www.inchem.org/documents/cicads/cicads/cicad05.htm
16. INCHEM. Environmental Health Criteria 131 - Diethylhexyl Phthalate. 1992 [cited 2023 Sep 28]. Available from: http://www.inchem.org/documents/ehc/ehc/ehc131.htm
17. OECD. Guidance document on the recognition, assessment, and use of clinical signs as human endpoints for experimental animals used in safety evaluation. OECD Series on Testing and Assessment, No. 19. Paris: OECD Publishing; 2000.
18. Johnson MD, GSC and KCJ. The rat. In: Gad SC, editor. Animal Models in Toxicology. 3rd ed. Florida: CRC Press; 2016. p. 155–266.
19. Lin KH, Tsai JH, Cheng CC, Chiang HL. Emission of volatile organic compounds from consumer products. Aerosol Air Qual Res. 2022;22(9):220250. doi: 10.4209/aaqr.220250
20. Kim JH, Lee D, Lim H, Kim T, Suk K, Seo J. Risk assessment to human health: Consumer exposure

to ingredients in air fresheners. Regul Toxicol Pharmacol. 2018;98:31–40. doi: 10.1016/j.yrtph.2018.05.015
21. Gabriel-Brisibe, C. U., Odinga, T., Wokocha, P. G., Agara, H.N., & Adinde, T. C. (2020). Exposure to air freshner and its distresses on the antioxidant biomarkers of male Wistar rats. Journal of Toxicology and Environmental Health Sciences, 12(3), 22-26. DOI:10.5897/JTEHS2020.0460
22. Wickliffe JK, Stock TH, Howard JL, Frahm E, Simon-Friedt BR, Montgomery K, et al. Increased long-term health risks attributable to select volatile organic compounds in residential indoor air in southeast Louisiana. Sci Rep. 2020;10(1):21649. doi: 10.1038/s41598-020-78756-7.
23. NIH. Air Fresheners Fact Sheet. 2021 [cited 2023 Sep 28]. Available from https://www.ncbi.nlm.nih.gov/books/NBK579083/.
24. Bayati M, Vu DC, Vo PH, Rogers E, Park J, Ho TL, et al. Health risk assessment of volatile organic compounds at daycare facilities. Indoor Air. 2021;31(4):977–88. doi: 10.1111/ina.12801
25. de Almeida AAC, Costa JP, de Carvalho RBF, de Sousa DP, de Freitas RM. Evaluation of acute toxicity of a natural compound (+)-limonene epoxide and its anxiolytic-like action. Brain Res. 2012;1448:56–62. doi: 10.1016/j.brainres.2012.01.070
26. Igimi H, Nishimura M. Studies on the metabolism of d-limonene (p-Mentha-l,&diene) I. The absorption, distribution and excretion of d-Limonene in rats. Xenobiotica. 1974;4. doi: 10.3109/00498257409049347
27. Ravichandran C, Badgujar PC, Gundev P, Upadhyay A. Review of toxicological assessment of d-limonene, a food and cosmetics additive. Food Chem.Toxicol. 2018; 120:668-80. doi: 10.1016/j.fct.2018.07.052
28. Umukoro S. Neurobehavioral Effects of Prolonged Exposure to Solid Air Freshener in Mice. Iran. J. Toxicol. 2019;13(3):45–51. doi: 10.32598/IJT.13.3.595.1
29. Mason G, Wilson D, Hampton C, Wьrbel H. Non-invasively assessing disturbance and Stres in laboratory rats by scoring chromodacryorrhoea. ATLA. 2004;32:153–159. doi: 10.1177/026119290403201s25
30. Everds NE, Snyder PW, Bailey KL, Bolon B, Creasy DM, Foley GL, et al. Interpreting stress responses during routine toxicity studies. Toxicol Pathol. 2013;41(4):560–614. doi: 10.1177/0192623312466452
31. Wang F, Liu F, Liu H, Chen W, Si X, Ma X. Effects of immunological and hematological parameter in mice exposed to mixture of volatile organic compounds. Inhal Toxicol. 2016;28(4):164–9. doi: 10.3109/08958378.2016.1148087
32. Jameson CW. NTP Technical report on the toxicology and carcinogenesis studies of d-limonene (CAS No.:5989-27-5) in F344/N rats and B6C3F1 mice (gavage studies). 1990. National Toxicology Program.
33. James VB, Satheesh AS, D. Alan W. Toxic effects of solvents and vapours. In: Curtis DK, editor. Casarett & Doull's Toxicology. The Basic Science of Poisons. 7th edition. Kansas: McGraw-Hill; 2008. p. 981–1052.