



Acute dermal toxicity study of new, used and laboratory aged aircraft engine oils

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ABSTRACT

There is little data available on toxicity levels of used aircraft engine oils relative to their unused (new) versions. This study was conducted to determine if new engine oils and their used versions have the potential to induce dermal irritation. Twelve male New Zealand White rabbits (*Oryctolagus cuniculus*, 19 weeks old) were used to determine the acute dermal toxicity potential of four aircraft turbine oils including MIL-PRF-7808 Grades 3 and 4 and MIL-PRF-23699 Grade 5 High Thermal Stability (HTS) and a Grade 5 experimental aircraft engine oil in their unused and used or laboratory stressed states. Five fur-free test sites (6 cm² each) located lateral to the midline of the back were treated with two undiluted (0.5 ml) new engine oils and their used versions. The fifth site received reverse osmosis deionized (RODI) water as a control. Each treatment was repeated 3 times (3 rabbits/oil type). Each oil was tested under both semi-occluded and occluded conditions. The 4 h exposure was followed by gauze plus wrappings removal, and gentle cleaning of sites prior to scoring for erythema and edema at 0.5–1, 24, 48 and 72 h post exposure based on Draize (1959). E-collars were placed on each animal for at least 72 h to prevent ingestion of the test substance and/or gauze and wrappings and/or disturbance of site recovery. Additional observations were made on days 7, 10 and 14 to determine recovery. Exposure to both used and new oils produced dermal irritation consisting of no more than very slight to well-defined erythema and very slight edema. The calculated Primary Dermal Irritation Index (PDII) indicated that all the oils were slightly irritating (means ranged from 0.42 to 1.08). Although the PDII values for new oils and their used versions were not significantly different from each other, they were all statistically higher ($p < 0.05$) than those obtained for the control regardless of the type of occlusion binding applied. The used oils under semi-occlusion conditions yielded larger size effects (Cohen's d) relative to their unused versions suggesting an enhancement in irritation when the oil is aging. Grade 4 in the used state yielded the largest size effect which was $d = 5.9$ versus 2.6 for its unused version. The slight dermal irritation resulting from four hours of exposure to oils raises concerns about the magnitude of impact related to prolonged and/or repeated exposure.

1. Introduction

The current aircraft engines for military operations are designed to operate at higher speeds and temperatures [1]. These requirements have led to developing high performance lubricants and additives that

are capable of withstanding higher temperatures [2]. Some of the engine oil additives are organophosphate based compounds such as phenol isopropylated phosphate (3:1) (TIPP; CAS No. 68937-41-7), triphenyl phosphate (TPP; CAS No. 115-86-6), and tricresyl phosphate (TCP, CAS No. 1330-78-5) which may pose health risks [3]. Dermal

Abbreviations: EG5, experimental grade 5; EG5-N, experimental grade 5 in an unused and unstressed state; EG5-S, experimental grade 5 in a stressed state; G3, grade 3; G3-N, grade 3 in an unused state; G3-U, grade 3 in a used state; G4, Grade 4; G4-N, grade 4 in an unused state; G4-U, grade 4 in a used state; G5, grade 5 HTS; G5-N, grade 5 HTS in an unused and unstressed state; G5-S, grade 5 in a stressed state; HTS, high thermal stability; PDII, primary dermal irritation index; RODI, reverse osmosis deionized water; TAN, total acid number; VPC, vapor phase coker; WPAFB, wright-patterson air force base

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exposure to organophosphates has been associated with skin irritation [4,5]. Skin acts as a two way barrier to (1) minimize the loss of water, electrolytes and other body constituents and (2) lessen the entry of harmful substances from the external environment [6]. The skin barrier efficiency may be altered by various events that may increase stratum corneum hydration such as changes in environmental humidity, chemical, physical, therapeutic and pathological factors [6–13]. Thus, skin irritation induced by exposure to organophosphate compounds may be influenced by various factors effecting the skin barrier. The severity of irritation may differ depending on whether the body surface exposed to these compounds is completely covered (occlusive), semi-occluded or not covered at all. Reports indicate that skin occlusion enhances the hydration of stratum corneum and exacerbates the irritant effects of the applied chemicals [6–11,14]. Currently, it is not known if exposure to engine oils induces skin irritation under occluded conditions.

There is limited information regarding the possible occupational dermal exposure to these oils among individuals who work on aircraft during maintenance operations. The current study was designed to achieve two different goals. First, each aircraft engine oil has ingredients that the user may be exposed to with potential toxicity associated with each ingredient. Although their ingredients are at very low levels, there is no data in the literature about the toxicity associated with exposure to the mixture. Since the overall toxicity of a mixture depends on the proportion and toxicity of each ingredient as well as the synergic interactions between ingredients, an ideal evaluation of the hazardous effects of exposure to the compound mixture requires a toxicity test on the entire mixture not solely on each component. Thus, the first goal for this study was to assess the dermal irritation potential of each engine oil as a mixture of ingredients since the skin is a primary route of exposure. Second, little is currently known about the oil transformations occurring in running engines. Johnson et al. [2] have demonstrated that when three engine lubricants, containing 5 % (m/m) and 10 % (m/m) of TCP as anti-wear/extreme pressure additives, were placed in thick walled sealed glass tubes containing stainless steels and subjected to various temperatures ranging from 300 °C to 350 °C for time durations ranging from 4 h to 96 h, significant decomposition of the lubricant base-stocks and phosphate ester additives occurred resulting in the formation of carboxylic acids and phenols and a deposition of phosphorus onto the stainless steels [2]. In these experiments, the deposits were only formed when stainless steels were exposed to phosphate ester additives but not to ester lubricant base-stock. This suggests that when the engine oils are subjected to higher temperatures, the breakdown products of their ingredients and/or worn engine components may end up in oils. This alteration in oil composition could potentially change the oil properties, yielding a more toxic oil mixture. Thus, the second goal for this study was to determine the dermal irritation potential of used and laboratory stressed (aged) oils relative to their unused or unstressed versions. The study characterized the irritation potential of Grade 3 (G3) and 4 (G4), Grade 5 high thermal stability (HTS) (G5) and Experimental Grade 5 (EG5) aircraft engine oils in their unused and used or laboratory stressed states under occlusive and semi-occlusive wrapping conditions. To our best knowledge, this was the first study designed to examine and compare the dermal irritation associated with exposure to unused engine oils and their used versions. The New Zealand White rabbits were chosen as the animal model for this study since this species is accepted as the non-rodent species for preclinical toxicity testing by regulatory agencies.

2. Materials and methods

2.1. Chemicals

G3 and G4 aircraft engine oils in their new states (G3-N and G4-N) were obtained from the Air Force Petroleum Office, Wright-Patterson Air Force Base (WPAFB), Ohio. The used version G3 (G3-U) oil was removed from a C-17 aircraft at WPAFB, Ohio. The used version of G4

(G4-U) oil was removed from F-22 aircraft at Langley Air Force Base, Virginia. The G5 and EG5 in their new states (G5-N and EG5-N) oils were obtained from the Engine Mechanical Systems Branch, Turbine Engine Division, Aerospace Systems Directorate, Air Force Research Laboratory, Wright-Patterson AFB OH. The used versions of the G5 and EG5 oils were not available as these oils are either not widely used in the USA Department of Defense systems or they have been proposed for future use. To obtain their stressed versions (G5-S and EG5-S) that reflect the properties of used oil with respect to viscosity and total acid number (TAN) change, these oils were laboratory stressed (aged) through the use of SAE ARP5921 "Evaluation of Coking Propensity of Aviation Lubricants in an Air-Oil Mist Environment using the Vapor Phase Coker (VPC)". The VPC was selected for use in this study due to its ability to moderately age approximately a quart of oil in one testing period. To provide a thermal and oxidative environment for oil aging, 900 g oil were subjected to the following conditions: 204 °C sump, dry air 765 mL/minute bubble through oil, oil vapor 371 °C, for 18 h. The oil aging process changed the viscosity for G5 at 40 °C from 26.15–26.69 and TAN from 0.26 mg KOH/g to 0.43 mg KOH/g. Under this process, the viscosity for EG5 changed from 26.82 to 27.49 and TAN from 0.02 mg KOH/g to 0.41 mg KOH/g.

2.2. Animal care

Male New Zealand White rabbits (*Oryctolagus cuniculus*) were received at Charles River Laboratories, Inc. (640 N. Elizabeth Street, Spencerville, OH 45887) from Covance Laboratories, Denver, PA. The animals chosen for study were randomly selected from healthy stock animals. The animals were individually housed throughout the study in suspended stainless steel cages equipped with an automatic watering valve. This housing allowed animals an *ad libitum* access to standard feed and drinking water. The room was maintained under standard environmental conditions of 12:12 h light:dark cycle, with the temperature and relative humidity sustained in the ranges of 21 °C–22 °C and 49%–52%, respectively. Additionally, ten or greater air changes per hour with 100 % fresh air (no air recirculation) were maintained in the animal rooms.

The study protocol was approved by the Charles River Laboratories, Inc. Institute of Research, Institutional Animal Care and Use Committee (IACUC), and the U.S. Air Force Surgeon General's Office of Research Oversight and Compliance. The experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Research, National Research Council, National Academies Press, 2011, and in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (in compliance with DODI 3216.01).

2.3. Experimental design and animal treatment

All animals were acclimatized to the laboratory conditions for 7 days prior to the first day of treatment. The study involved 12 male rabbits of 19 weeks of age, weighing 2.7 kg–3.1 kg on the day before the treatment. Each animal was identified by a subcutaneously implanted electronic identification chip. One day prior to the start of testing, fur was removed from the dorsal area of the trunk using a small animal clipper. On the treatment day, five test sites (6 cm² each) were delineated with an indelible marker from lateral to the midline of the back of the rabbit. Animals were randomly assigned into 4 groups of 3 rabbits each (Table 1). The n = 3 per group was the minimum required by United States Environmental Protection Agency (EPA) [15] test guidelines for acute dermal irritation study to properly characterize the effects of a test substance. Four test sites on each rabbit were treated with two undiluted (0.5 ml) new engine oils and their used/laboratory stressed versions. The first site received reverse osmosis deionized

Table 1

Test articles, exposure time and method, number of test sites per animal. No used versions of Grade 5 HTS and Experimental Grade 5 oils were available. They were laboratory stressed (aged) to obtain the mimics of their used versions.

Group No.	Test Material	Material Status	# of Animals	Test Site	Dose (mL)	Exposure Method	Exposure Time
1	Control	–	3	1	0.5	Semi-occluded	4 h
	G4-U	Used		2	0.5		
	G4-N	New		3	0.5		
	G3-U	Used		4	0.5		
	G3-N	New		5	0.5		
2	Control	–	3	2	0.5	Semi-occluded	4 h
	G5-S	Stressed		3	0.5		
	G5-N	New		4	0.5		
	EG5-S	Stressed		5	0.5		
	EG5-N	New		1	0.5		
3	Control	–	3	3	0.5	Occluded	4 h
	G4-U	Used		4	0.5		
	G4-N	New		5	0.5		
	G3-U	Used		1	0.5		
	G3-N	New		2	0.5		
4	Control	–	3	4	0.5	Occluded	4 h
	G5-S	Stressed		5	0.5		
	G5-N	New		1	0.5		
	EG5-S	Stressed		2	0.5		
	EG5-N	New		3	0.5		

(RODI) water and served as control. A control group of animals was not needed as each animal served as its own control. The treatment sites in groups 1 and 3 (Table 1) received the control, Grade 3 (G3) and 4 (G4) engine oils in their new states (G3-N and G4-N) and their used versions (G3-U and G4-U). The test sites in groups 2 and 4 received the control, Grade 5 HTS (G5) and Experimental Grade 5 (EG5) engine oils in their new states (G5-N and EG5-N) and their laboratory stressed (aged) versions (G5-S and EG5-S). The treatment sites for animals in groups 1 and 2 were covered with a semi-occlusive dressings, consisting of 1 inch x 1 inch 4-ply gauze patch secured in place with a nonirritating surgical tape and a stockinette placed over the test area of the rabbit trunk. The test sites for animals in groups 3 and 4 (Table 1) were covered with occlusive dressings, consisting of similar dressings as in groups 1 and 2 except that a plastic wrap was placed over the gauze patches prior to stockinette application. Each treatment was repeated 3 times (3 rabbits/oil type). E-collars was placed on each animal for at least 72 h to prevent ingestion of the test substance and/or wrappings and to ensure that the site was not disturbed for recovery.

2.4. Erythema and edema scoring

After four hours of treatment, gauze plus wrappings were removed and the corners of each test site delineated by a marker and the treatment sites gently cleaned. Since RODI water was not sufficient to remove the oils, the residual oil was removed using gauze moistened with acetone, followed by dry gauze, then gauze moistened with RODI water, followed by dry gauze. Similar cleaning procedures were applied to control treatment sites. Erythema and edema scorings were performed at 0.5–1, 24, 48 and 72 h post exposure according to the procedure of Draize [16]. Additional observations and scorings were made on days 7, 10 and 14 to determine recovery. All rabbits were euthanized by sodium pentobarbital injection.

2.5. Statistical analyses

Erythema scores were subjected to one-way ANOVA to test the differences in irritation between oil treated and control sites. This test was also run to assess if exposure to used/laboratory stressed versions of engine oils yields enhanced dermal irritation compared to exposure to new oils. Levine’s test was used to check the homoscedasticity of the data, and the Welch test was conducted if the data displayed unequal variance (Levine test, $p \leq 0.05$). Results are expressed as mean \pm

standard error (S.E.) and considered statistically significant at $p \leq 0.05$. A primary dermal irritation index (PDII) was calculated for each oil from erythema and edema scores recorded at 0.5–1, 24, 48, and 72 h post treatment. The total scores for erythema and edema were calculated separately, divided by the number of rabbits (3) x time points (4), rounded to the nearest tenth, and added together. Based on these values, the Draize’s [16] grading system was used to arrive at a PDII for each oil.

To assess the magnitude of irritability for the oils relative to control, we calculated the effect size or Cohen’s *d* [17] (magnitude of changes) induced by each oil treatment by subtracting the averaged PDII values obtained for the control treated sites from those obtained for the oil treated sites and the difference was assessed relative to the pooled standard deviations of both control and oil treated sites as shown in Eq. (1), where $(X)_T$ and $(X)_C$ are the average PDII values for the oil and the control treated sites, respectively, while $(\sigma)_T$ and $(\sigma)_C$ are the standard deviations for PDII values for oil and control treated sites, respectively.

$$d = \frac{(X)_T - (X)_C}{\sqrt{\frac{(\sigma)_T^2 + (\sigma)_C^2}{2}}} \tag{1}$$

To assess the magnitude of irritability between exposure to used or laboratory stressed oils and their new versions, we calculated the effect size induced by used or laboratory stressed oils. The averaged PDII values obtained for the new oil treated sites were subtracted from those obtained for used or laboratory stressed oil treated sites and the difference was assessed relative to the pooled standard deviations of both new and used or laboratory stressed oil treated sites as shown in Eq. (2). The average PDII values for the used or laboratory stressed oils and the new oils are $(A)_U$ and $(A)_N$, respectively, while $(\sigma)_U$ and $(\sigma)_N$ are the standard deviations for PDII values for the used or laboratory stressed oils and the new oils, respectively.

$$d = \frac{(A)_U - (A)_N}{\sqrt{\frac{(\sigma)_U^2 + (\sigma)_N^2}{2}}} \tag{2}$$

Effect size values for the engine oils were graded against the large value ($d \geq |0.8|$) based on Cohen’s *d* classification [17].

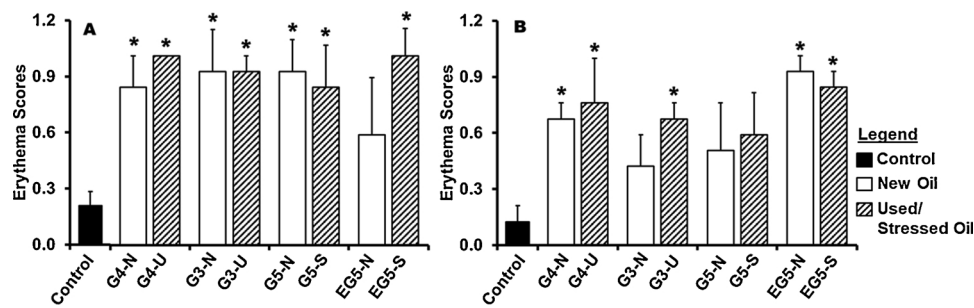


Fig. 1. Rabbit erythema scores induced by a 4 h dermal exposure to new (unused) aircraft engine oils and their used/laboratory stressed versions under (A) semi-occlusive and (B) occlusive wrapping conditions (see methods for details). Asterisk denotes significant differences from control group; $p < 0.05$.

3. Results

3.1. Exposure to aircraft engine oils induces erythema

The averaged erythema scores for treatment groups when semi-occlusive and occlusive dressings were used on the treatment sites are shown in Fig. 1A and B. These figures show that irritation scores for new oils and their used/laboratory stressed versions were not statistically different from each other under both semi-occlusive and occlusive wrapping conditions. However, erythema scores for all oil treated rabbits under semi-occlusive dressing conditions were significantly higher ($p < 0.05$) than those obtained for controls except scores for rabbits exposed to EG5-N (Fig. 1A). Under occlusive wrapping conditions, only erythema scores for both new and used versions of G4, new and laboratory stressed versions of EG5 and G3-U were significantly elevated relative to those obtained for controls.

No animal displayed a sign of edema after exposure to both versions of G3 and G5 and exposure to new versions of G4 and EG5 oils under semi-occlusive and occlusive wrapping conditions. A very slight edema was only observed on 1 animal at 1 h post exposure to EG5-S and 48 h post exposure to G4-U under semi-occlusive and occlusive wrapping conditions, respectively (data not shown). However, this effect was resolved within 24 h.

3.2. Magnitude of skin irritation induced following dermal exposure to aircraft engine oil

Exposure to both used, laboratory stressed and new aircraft engine oils produced dermal irritation consisting of no more than very slight to slight erythema and very slight edema. Calculated PDII values indicate that all the oils were slightly irritating under both semi-occlusive and occlusive wrapping conditions (Table 2). To more clearly illustrate the magnitude difference in irritability between exposure to engine oils and the control, we calculated the effect sizes (magnitude of changes) or Cohen's d [17] induced by oil treatments relative to control under semi-occlusive and occlusive dressing conditions and the results are shown in Fig. 2A and B, respectively. Under both wrapping conditions, all the oils yielded large effect sizes ($d > 0.8$) based on Cohen's d classification [17]. In general, semi-occlusive dressing produced elevated effect sizes relative to the performance of occlusive dressings, except for both versions of EG5 oil. Under semi-occlusive dressing conditions, the highest effect size was obtained with G4-U ($d = 5.9$) while the smallest effect size was produced by EG5-N treated sites ($d = 0.96$) (Fig. 2A). Interestingly, EG5-N yielded the highest effect size under occlusive wrapping conditions ($d = 4.4$) while the smallest effect size was produced by G5-N ($d = 1.1$) (Fig. 2B). The used versions of G3 and G4 oils and the laboratory aged versions of EG5 yielded higher effect sizes than those obtained with the new versions under semi-occlusive conditions (Fig. 2A). Under occlusive dressing conditions, only effect sizes for G3-U and G5-S were higher than those obtained with the new versions (Fig. 2A).

To more clearly illustrate the magnitude difference in irritability between exposure to used or laboratory stressed engine oils and their new versions, we calculated the effect size induced by used or laboratory stressed oils relative to the performance of new oils under semi-occlusive and occlusive dressing conditions and the results are shown in Fig. 3A and B, respectively. These figures show that the performance of used or laboratory stressed oils relative to their new versions depended on the type of dressings applied to treatment sites. Semi-occlusive and occlusive wrapping conditions yielded opposite effects on the strength of skin irritation associated with exposure to G5 and EG5 oils (Fig. 3A and B). Under semi-occlusive dressing conditions, G5-S oil was less irritating than G5-N while under occlusive conditions, G5-S became more irritating than G5-N. Similarly, EG5-S oil was more irritating than EG5-N under semi-occlusive dressing conditions but became less irritating under occlusive dressing conditions. Both versions of G3 oil were equally irritating under semi-occlusive dressing conditions (Fig. 3A) but G3-U became more irritating than G3-N under occlusive dressing conditions (Fig. 3B). Although G4-U was more irritating than G4-N under both dressing conditions ($d = 0.82$), its irritation strength decreased under occlusive wrapping conditions ($d = 0.27$).

4. Discussion

It is important to understand the potential toxicity or consequence of exposure for aircraft fluids. Although many exposures are the results of inhalation, such as hydrazine [18], the dermal route must also be considered. The current study was intended to provide information on the health hazards likely to arise from a short-term exposure to aircraft engine oils by the dermal route. The oils were dermally administered on clipped and intact skin. The treatment sites were covered by semi-occlusive or occlusive dressings, mimicking what may happen in the real world environment when the oil may get trapped under the aircraft maintenance worker's clothes or gloves. All animals were healthy and survived until scheduled euthanasia. Clinical observations were limited to red fur staining and scabs. The findings were normal for animals considering their age and strain. No apparent treatment-related effects on body weights were observed during the study. Results reported in this study highlight three main observations: (1) irritation in control test sites for some rabbits exposed to RODI water (control), (2) exposure to the same oil yielded different responses under semi-occlusive and occlusive wrapping conditions; in general, semi-occlusive dressing conditions tended to produce higher erythema scores and PDII values relative to those obtained under occlusive wrapping conditions and (3) exposure to used or laboratory stressed oils enhanced or decreased skin irritation relative to the performance of their unused or unstressed versions depending on the type of dressing applied to test sites.

Erythema is a redness of the skin or mucous membranes characterized by its reversibility and results from hyperemia of superficial capillaries [19]. Very slight erythema was noted at the early post-exposure observations on control test sites in 4 out of 6 rabbits subjected to semi-occlusive dressing conditions. Applying occlusive dressing was

Table 2
Calculated Primary Dermal Irritation Indices (PDII) for test articles.

Group No.	Test Material	Test Material Status	Exposure Method	PDII values (Mean ± SE)	Irritation Rating
1	Control	–	Semi-occluded	0.21 ± 0.08	Slight Irritant
	G4-U	Used		1.00 ± 0.17	Slight Irritant
	G4-N	New		0.83 ± 0.0	Slight Irritant
	G3-U	Used		0.92 ± 0.08	Slight Irritant
	G3-N	New		0.92 ± 0.22	Slight Irritant
2	Control	–	Semi-occluded	0.21 ± 0.08	Slight Irritant
	G5-S	Stressed		0.83 ± 0.22	Slight Irritant
	G5-N	New		0.92 ± 0.17	Slight Irritant
	EG5-S	Stressed		1.08 ± 0.17	Slight Irritant
	EG5-N	New		0.58 ± 0.3	Slight Irritant
3	Control	–	Occluded	0.13 ± 0.09	Slight Irritant
	G4-U	Used		0.75 ± 0.24	Slight Irritant
	G4-N	New		0.67 ± 0.08	Slight Irritant
	G3-U	Used		0.67 ± 0.08	Slight Irritant
	G3-N	New		0.42 ± 0.17	Slight Irritant
4	Control	–	Occluded	0.13 ± 0.09	Slight Irritant
	G5-S	Stressed		0.58 ± 0.22	Slight Irritant
	G5-N	New		0.50 ± 0.25	Slight Irritant
	EG5-S	Stressed		0.83 ± 0.08	Slight Irritant
	EG5-N	New		0.92 ± 0.08	Slight Irritant

less likely to produce irritation as this was observed for only 2 out of 6 rabbits. The control-induced irritation was rapidly and completely resolved for all affected rabbits. The very low level of irritation in controls may manifest for two reasons. First, acetone which was used to remove the residual oils from treated sites, was also used on controls to match the cleaning for all treatments. Controls that showed irritation may have been sensitive to acetone. Second, gauze dressings applied on clipped sites may have rubbed the skin and also contributed to irritation in controls. Exposure to both used or laboratory stressed and new oils under either semi-occlusive or occlusive wrapping conditions generally produced very slight dermal irritation. Cases of a well-defined erythema were only observed with three animals at Day 3 post treatment. Irritation for the first and second animals was associated with G3-N and EG5-N exposure, respectively. The third animal displayed well-defined erythema for sites exposed to EG5-U and both versions of G5. This irritation was resolved or regressed to very slight erythema for all three animals by Day 7. Edema, which relates to swelling caused by fluid in body's tissue was also evaluated. Two cases of a very slight edema were observed only with EG5-S and G4-U treated sites at 1 h and 48 h post exposure, respectively, but both cases were resolved completely by the following 24 h. No edema case was observed with control test sites.

All oils produced erythema at various observation time points under both semi-occlusive and occlusive wrapping conditions. Averaged erythema scores obtained for the test sites put all oils and controls under the same category of very slight irritation based on Draize's [16] irritation classification report. Since only two cases of edema were observed with oil exposure, calculated PDII values depended on erythema scores. This resulted in a PDII score for each oil being similar to the averaged erythema score. In general, semi-occlusive conditions increased the PDII values for all oil treated sites, regardless the aging

states of oils while occlusive dressings only elevated irritation potential for both versions of EG5. Under semi-occlusive wrapping conditions, all oils yielded higher PDII values ($PDII \geq 0.83$) except EG5-N ($PDII = 0.58$) (Table 2). Under occlusive dressings, only both versions EG5 produced elevated PDII values. Under these treatment site dressing conditions, EG5-N yielded the highest PDII value ($PDII = 0.92$), suggesting that the dressing enhanced skin irritation. Interestingly, the laboratory stressed version of this oil (EG5-S) produced the highest PDII value ($PDII = 1.08$) under semi-occlusive dressings, suggesting that aging enhanced its irritation potential.

Our data clearly demonstrate that exposure to aircraft engine oils can significantly induce skin irritation regardless of the oil's age status as illustrated in Fig. 1 and Table 2. The skin shields the body from an excessive loss of water, electrolytes and other body constituents and minimizes the entry of toxic substances from the external environment [6]. However, various factors such as exposure to chemicals can contribute to perturbation of the skin barrier function, resulting in increased entrance of exogenous substances into the body [20]. In general, occlusive wrapping of test sites lessened dermal irritation for engine oils as compared to semi-occlusive dressings. Investigators have reported that dermal occlusion can improve the hydration of stratum corneum, the principal barrier, thus, progressively decreasing the efficiency in its barrier function [7,8,11] and serving as a reservoir of the chemical for body entry [21]. The compromised skin barrier function leads to impaired transepidermal water loss which aggravates the irritation at the site of the chemical entry [7,8,10,14,22]. The widely accepted dogma is that occlusive dressing may enhance percutaneous absorption and transdermal penetration for compounds [7,8,11]. This suggests that occlusive dressing conditions are more conducive to irritation than semi-occlusive conditions. However, our results contradict

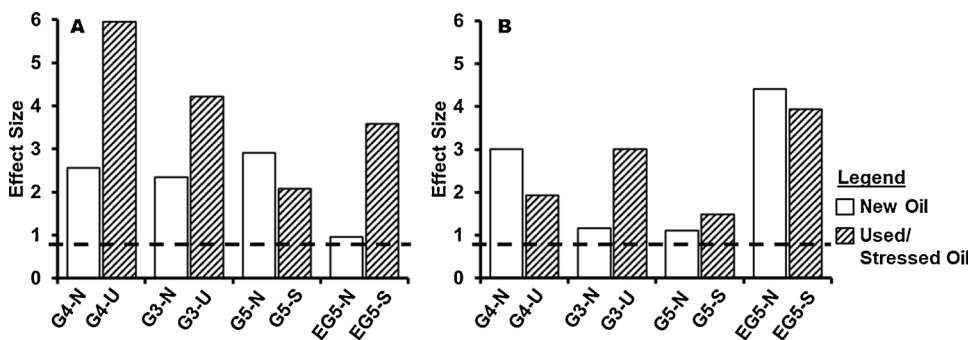


Fig. 2. The magnitude difference in dermal irritability (effect size or Cohen's *d*) between treatment sites on rabbits exposed to aircraft engine oils and control for 4 h under (A) semi-occlusive and (B) occlusive wrapping conditions (see methods for details). Dashed line indicates the level of a large effect size ($d \geq 0.8$) based on Cohen's *d* classification [17].

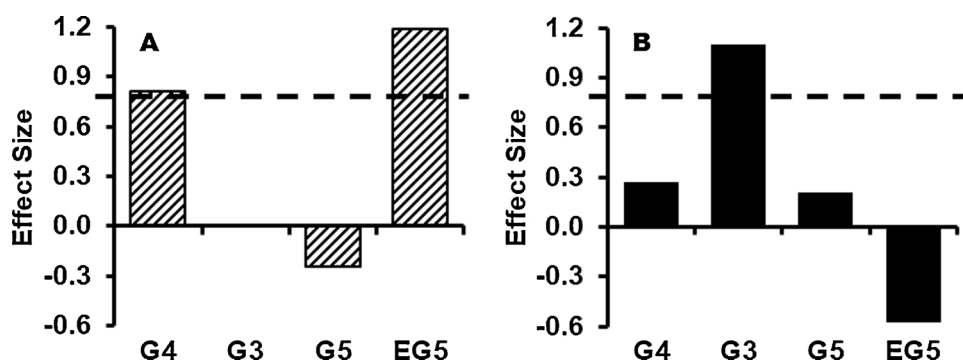


Fig. 3. The magnitude difference in dermal irritability (effect size or Cohen's *d*) between treatment sites on rabbits exposed to new aircraft engine oils and their used or laboratory stressed versions for 4 h under (A) semi-occlusive and (B) occlusive wrapping conditions (see methods for details). Dashed line indicates the level of a large effect size ($d \geq 0.8$) based on Cohen's *d* classification [17].

this dogma. As reports show, occlusion does not increase absorption of all compounds [7–9,11]. This suggests that occlusive dressing may selectively affect the penetration of chemicals into the skin. Occlusion-induced hydration of skin enhances the penetration of non-polar compounds but has a minimal effect on polar molecules [9,11]. Other factors such as the compound's physicochemical properties (aqueous solubility, volatility, partition coefficient, etc.) and anatomy of the test site may also contribute to occlusion's effect on absorption [7,9,11,23,24]. Although we did not assess the physicochemical properties of the oils used in this study, we cannot rule out that these properties may have contributed to differences we observed in irritation potentials of oils under semi-occlusive and occlusive wrapping conditions. Another point that may be taken into consideration is that occlusive dressings may have reduced the amount of oxygen reaching the treated sites, thus limiting the possibility of reaction between oil and oxygen under the warm skin conditions induced by the dressings.

A comparison of the magnitude of difference (effect size) between dermal irritation for rabbits exposed to used/laboratory stressed versions of oils and those treated with the unused versions of these oils under both semi-occlusive and occlusive wrapping conditions indicated that the type of wrapping applied on the test sites has a measurable effect on the strength of skin irritation. G4-U oil tended to be more irritating than G4-N under both wrapping conditions. Similar observations were noted for EG5-S oil subjected to semi-occlusive dressing conditions. Interestingly, applying occlusive wrappings on the test sites exposed to EG5-S oil lessened its irritability potential relative to the performance of EG5-N. Typically, under occlusive dressing conditions, the treatment penetrates the stratum corneum upon skin exposure and after removing the dressing, the stratum corneum dehydrates, absorption of the compound slows resulting in stratum corneum serving as a reservoir for the compound [21]. This may have been the case for G3-U and G5-S since occlusive wrappings of the test sites that received these versions of oils enhanced irritation in comparison to test sites treated with these oils in their new states. It is interesting to note that irritation of the test sites exposed to these oils in their new states was less pronounced. A possible explanation could be that the used oils contained more acids such as carboxylic acids resulting from heat-induced degradation of oil ingredients. Johnson et al. [2] demonstrated that the heat-induced degradation of TCP yields carboxylic acids and phenols. Carboxylic acids are known to significantly increase the human skin irritation [25]. The aging process of G5 and EG5 changed the total acid numbers from 0.26 mg to 0.43 mg KOH/g and from 0.02 mg to 0.41 mg KOH/g, respectively. The observations that G3-N and G5-N oils and their used/laboratory stressed versions (G3-U and G5-S) have different potentialities in dermal irritation clearly suggest that oils go through changes in chemical properties as they age.

In summary, this study shows that a 4 h dermal exposure to aircraft engine oils results in slight skin irritation. This raises concerns about the magnitude of impact related to prolonged exposure as the shifts for aircraft maintenance workers last more than 4 h. It is also unknown what could be the magnitude of impact associated with repeated

exposure that may be happening in the real world environment. Applying occlusive wrappings on test sites tended to provide conditions that lessen irritation levels as compared to semi-occlusive wrappings. In general, used oils tended to enhance the PDII relative to the performance of their unused versions, suggesting an increase in toxicity as the oils age.

5. Conclusion

The slight dermal irritation associated with four hours exposure to aircraft engine oils raises concerns about the magnitude of the impact of prolonged and/or repeated exposure. Our data show that used oils tended to be more irritating as compared to new versions, suggesting that as the oils age, they increase their potential toxicity. While personal protection measures need to always be used when handling the oils, more research is also needed to investigate potential the health problems associated with repeated dermal exposure to both new and used versions, which reflects what happens in a real world environment where the maintenance workers may be repeatedly exposed to engine oils.

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Declaration of Competing Interest

The authors declare that they have no competing financial interest or personal relationships that could have had an influence on the work reported in this paper.

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