Current Research in Toxicology 1 (2020) 12–24

Contents lists available at ScienceDirect

Current Research in Toxicology

journal homepage: www.elsevier.com/locate/crtox

Sub-chronic dermal exposure to aircraft engine oils impacts the reproductive organ weights and alters hematological profiles of Sprague Dawley rats

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ARTICLE INFO

Article history:
Received 27 August 2019
Received in revised form 15 January 2020
Accepted 7 February 2020

ABSTRACT

There is little data available for the toxicity of used aircraft engine oils relative to their unused (new) versions. This study was conducted to determine if grade 3 (G3) and 4 (G4) aircraft engine oils in their new states (G3-N and G4-N) and their used versions (G3-U and G4-U) have the potential to induce toxicity via dermal application. Male and female Sprague Dawley rats were dermally exposed to water (control), new and used versions of G3 and G4 oils to determine the oil sub-chronic toxicity potentials. A volume of 300 μL of undiluted oil was applied to the pad of the Hill Top Chamber System©. Then the chamber was attached to a fur-free test site located at the back of the rat for 6 h/day for 5 consecutive days/week for 21 days (15 total exposures). Recovery rats also received similar treatments and were kept for 14 days post-exposure to screen for reversibility, persistence, or delayed occurrence of toxic effects. Both G3 and G4 oils had a significant impact on the weight of male and female reproductive organs: testes weights for recovery rats exposed to G3-N significantly decreased (12%) relative to controls; G3-N and G3-U decreased uterus weights by 23% and 29%, respectively; G4-N decreased uterus weights by 32% but were resolved at the end of the recovery period; G4-N increased the weight of the adrenals and spleen for females by 34% and 27%, respectively, during the recovery period. G3 and G4 induced more changes in female blood indices than in those for males. Of all versions of oils, G4-N induced the most changes in profiles of female blood. G4-N significantly decreased the white blood cells, lymphocytes, neutrophils, eosinophils and increased the mean platelet volumes. Interestingly, males were not affected by exposure to G4-N oil. While G3-N decreased the white blood cells and lymphocytes for females it slightly increased those for males. In summary, G3 and G4 oils impacted the weights for male and reproductive organs. This study highlights the health risks that aircraft maintenance workers may be exposed to if precautions are not taken to minimize exposure to these oils.

1. Introduction

Human toxicity associated with organophosphate compounds has been known at least since 1899, when it was reported that they induce neurotoxicity (Echobion, 1993). Currently, the use of organophosphate compounds is not just limited to pesticides used to control pests on crops in fields, but they are also used in aircraft engine oils and hydraulic fluids as additives, and as flame retardants as well. In recent decades, concerns have arisen regarding aircraft crew members who have developed various symptoms consistent with exposure to organophosphates and toxic fumes (Abou-Donia et al., 2014; Harrison and Mackenzie Ross, 2016; Liyasova et al., 2011). Concerns have also been raised regarding the possibility that the aircraft maintenance workers may be exposed to organophosphate esters that are in aircraft engine oils through inhalation and/or dermal contact (Denola et al., 2011). Since the1960s, ingredients for aircraft lubricating oils have barely changed and their compositions include approximately 95% synthetic esters with roughly 1% phenyl-α-naphthalamines and 3% tricresyl phosphates (TCP) (Winder and Balouet, 2002). Other organophosphate ingredients of aircraft engine oils that potentially pose health risks include phenol isopropylated phosphate (3:1) (TIPP) and triphenyl phosphate (TPP) (Winder and Balouet, 2002). The manufacturers for jet engine oils have been reluctant to modify their ingredient formulations and substitute the toxic TCP additives with non-toxic ingredients. They continue to use TCP isomer mixtures because they perform so well in critical applications (Winder and Balouet, 2002; Mackerer et al., 1999). A TCP molecule comprises three cresyl (methylphenyl and hydroxyl) groups linked to a

Abbreviations: BA, basophils; EO, eosinophils; 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; HCT, hematocrit; Hgb, hemoglobin; IACUC, Institutional Animal Care and Use Committee; LY, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MO, monocytes; MPV, mean platelet volume; NE, neutrophils; NMR, nuclear magnetic resonance; PLT, platelets; RBC, red blood cells; RDW, red blood cell distribution width; SDS, safety data sheet; TCP, tricresyl phosphate; TIPP, phenol isopropylated phosphate (3:1); TOCP, tri-*ortho*-cresyl phosphate; TPP, triphenyl phosphate; WBC, white blood cells; WPAFB, Wright-Patterson Air Force Base.

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http://dx.doi.org/10.1016/j.crtox.2020.02.001

phosphate group. The methyl group attached to each cresol can be in the ortho- (o), meta- (m) or para- (p) positions relative to the hydroxyl group on the cresol and the combination gives rise to 10 structural TCP isomers. Megson et al. (2016) analyzed fresh and used aircraft engine lubricants (Mobil Jet Oil II) and found that only four TCP isomers (mmm-TCP; mmp-TCP; mpp-TCP and ppp-TCP) were present at detectable levels in both versions of oils.

Exposure to TCP has been associated with various adverse health effects (Leon-S et al., 1996; Rosenstock et al., 1991; Steenland, 1996) that include toxicity to the reproductive system (Asmathbanu and Kaliwal, 1997; Carlton et al., 1987; Chapin et al., 1988; Dhondup and Kaliwal, 1997; Sortur and Kaliwal, 1999). Animal studies have provided limited details about TCP effects on the rat's reproductive system (Carlton et al., 1987; Latendresse et al., 1994a; Latendresse et al., 1995; Latendresse et al., 1994b; Somkuti et al., 1991). Several studies have suggested that the mechanisms by which organophosphates in general induce reproductive toxicity in animals involve alterations in the release of neurotransmitters, thus impairing hypothalamo-putuitary gonadal regulation of reproduction (Baligar and Kaliwal, 2002; Muller et al., 1977). Histopathologic changes in testes, epididymides and ovaries have been observed in rats exposed to TCP (Carlton et al., 1987). Latendresse et al. (1994a, 1994b, 1995) reported that exposure to TCP significantly decreased testicular and epididymal weights, increased ovarian weights, caused degeneration of the seminiferous tubular epithelium and severe lesions in ovary in F344 rats. Somkuti et al. (1991) have shown that male F344 rats exposed to the TOCP isomer of TCP have decreased sperm density and increased necrotic spermatids.

TCP and other organophosphate ingredients in lubricants such as hydraulic fluids and extreme pressure fluids are used as anti-wear and anti-corrosive additives designed to enhance the load bearing properties and improve tolerance to high temperatures (Solbu et al., 2010). While aircraft engine oils are known to contain organophosphate toxic ingredients at a very low level, little is currently known about oil transformations occurring in running engines. The oil in a jet engine may be heated to several hundred degree Celsius (Ramsden, 2013). The extremely high temperatures in running engines may be conducive to the breakdown of oil's ingredients and/or the release of worn engine particles into the oil, thus, altering the composition of the original oil and yielding a cocktail of chemicals that could potentially change the oil's properties. This could also increase the oil's hazard profile. Currently, there is no data available on hematological toxicity of used aircraft engine oils relative to their unused (new) versions and their effects on organ weights. In this study, we dermally exposed male and female Sprague Dawley rats with unused (new) and used versions of grade 3 and 4 aircraft engine oils over a period of 21 days (total 15 exposures) and assessed their potential effects on organ weights and hematological indices since these oils contain organophosphate additives that are known to be associated with adverse health conditions. This study was conducted in accordance with the ARRIVE guidelines.

2. Materials and methods

2.1. Chemicals

Grade 3 and 4 aircraft engine oils in their new states were obtained from the Air Force Petroleum Office, Wright-Patterson Air Force Base (WPAFB), Ohio. The used version of grade 3 oil was removed from a C-17 aircraft at WPAFB, Ohio. The used version of grade 4 oil was removed from F-22 aircraft at Langley Air Force Base, Virginia. The Hill Top chamber system© dermal delivery (full system, 25 mm diameter each) were purchased from Hill Top Research (St. Petersburg, Florida, USA). FS-PAK multispecies reagent kit (Product No. 200106), MULTI-TROL quality control standards (Product No. 600070), and MD Calibration kit (Product No. CDC0317) were purchased from ERBA Diagnostics, Inc. (Miami, Florida, USA) to measure hematological parameters. Deuterochloroform ($CDCl_3$) was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Animal care

All Sprague Dawley rats (*Rattus norvegicus*) involved in this study were obtained from Charles River Laboratories (640 N. Elizabeth Street, Spencerville, OH 45887). Virological tests were negative. All rats were housed individually in polycarbonate cages with cell sorb bedding, with ad libitum access to standard pellet feed (Purina #5008) and reverse osmosis drinking water in chew-proof clear water glass bottles. The room was maintained under standard environmental conditions of 12:12 h light: dark cycle, an ambient temperature of 25–28 °C and with a relative humidity of 40% to 60%. The study protocol was approved by the Wright-Patterson Air Force Base 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 (National Research Council, 2011), and in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

2.3. Experimental design and animal treatment

All animals were acclimatized to laboratory conditions for 14 days prior to the start of the study. The study involved male and female Sprague-Dawley rats assigned to the main study groups (n = 50 per sex) and recovery groups (n = 25 per sex). These rats were 8–9 weeks of age, weighing 200–325 g and 150–225 g, respectively, at the time of treatment. For treatments and necropsy procedures to be performed in a timely manner, the study was carried out in two phases. Each phase involved 50 rats (n = 25 per sex) for the main study and 25 rats (12 males and 13 females for phase 1; 13 males and 12 females for phase 2) for the recovery groups. Each phase comprised three replicates (n = 25 per replicate, rats for main study and recovery groups combined). For the main study, the three replicates involved 16, 17 and 17 rats, respectively. For the recovery groups, these replicates involved 9, 8 and 8 rats, respectively. Each replicate involved both males and females randomly assigned to one of five treatment groups consisting of 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). Rats were randomly placed one per cage by the vivarium staff. They did not know which treatment each rat would receive when they assigned them to cages. The cage cards identifying the group were randomly assigned to cages by the principle investigator. He did not know which rat was in each cage when he assigned the rats to groups. Schedules for the start of replicates in each phase were spaced by one day. Each treatment group involved 10 rats in total per sex for the main study, except for G3-N and G3-U groups for males which involved 9 and 11 rats, respectively. For the recovery groups, each treatment involved 5 rats per sex. A volume of 300 μL of undiluted engine oil was evenly applied to the pad of the Hill Top Chamber System© dermal delivery (full system, 25 mm diameter). These oils were kept in a chemical cabinet at the room temperature (22–23 °C). For controls, a volume of 300 μL of reverse osmosis deionized water was applied to the pad of the Hill Top chamber. Then the chamber was attached to a fur-free test site located at the back of the rat for 6 h. On each treatment day, application of the chambers on the rat's test sites started at 7 am. After 6 h of application each day, the chamber was removed, and each test site gently cleansed as thoroughly as possible using a soft pad soaked in Dawn® soapy water to eliminate the residual engine oil. The site was then rinsed with a water-soaked soft pad to remove the soap. Rats were dermally exposed to treatments for 5 days a week (except weekends) for a period of 21 days (15 total exposures). Rats in the recovery groups also received similar treatments and were kept for 14 days post-exposure to screen for reversibility, persistence, or delayed occurrence of toxic effects. The initial body weight for each rat was determined

Table 1
Organophosphate contents of grade 3 and 4 of aircraft engine oils.

Organophosphates	Versions of grade 3 and 4 aircraft engine oils			
	G3-N	G3-U[a]	G4-N	G4-U[a]
TCP (mM)	30.0	22.2 (−26.0%)	Not found	Not found
TPP (mM)	Not found	Not found	12.7	9.9 (−22.0%)
TIPP (mM)	Not found	Not found	37.6	35.2 (−6.4%)

[a] Changes (%) relative to new oils; TCP isomers were not found in G4 oils; TPP and TIPP were not found in G3 oils.

before the first exposure (day 0). It was followed by weekly body weight measurements and at the end of the study prior to necropsy. Food and water intake were also measured weekly. At the end of exposure and recovery period, all rats were fasted for 12 h prior to their blood draw and gross necropsy by withdrawing food at the appropriate time on the day before euthanasia. There was no restriction of water. All rats for each replicate were randomly euthanized by deeply anesthetizing them with a ketamine/xylazine cocktail through intraperitoneal injection at a dose of 80–100 mg/kg ketamine and 10 mg/kg xylazine. The body organs that were collected and weighed included brain, testes, epididymis, uterus, ovary, liver, right and left kidneys, adrenals, spleen, thymus, and heart.

2.4. Hematological parameters

Blood was drawn following deep anesthesia. Whole blood for hematological analysis was collected in 2 mL BD vacutainer blood collection tubes (Cat. # 02-687-98) with spray-coated anticoagulant ethylene diamine tetra-acetic acid (K_2EDTA). Blood samples were analyzed immediately after collection. Hematological parameters determined included white blood cells (WBC#), neutrophils (NE# and NE%), lymphocytes (LY# and LY%), monocytes (MO# and MO%), eosinophils (EO# and EO%); basophils (BA# and BA%); total red blood cells (RBC), hemoglobin (Hgb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW%), platelets (PLT) and mean platelet volume (MPV). Each blood sample was analyzed using a Drew Scientific (ERBA) HEMAVET 950 Multispecies Hematology Analyzer and multispecies software (Drew Scientific). Each day of analysis, quality control was assessed according to the manufacturer's protocols.

2.5. Phosphorus-31 NMR (^{31}P NMR) of grade 3 and 4 aircraft engine oils

Aircraft engine Oil samples were diluted with deuterochloroform ($CDCl_3$) and the final composition of $CDCl_3$:oil ratio in NMR tube was set to 60:40. A 280 μL aliquot of undiluted oil was mixed with 320 μL of $CDCl_3$ and the mixture was transferred into a 5 mm NMR tube. A 100 μL aliquot of phosphatidylcholine (PC) solution (34 mM of PC prepared using $CDCl_3$ was transferred to the NMR tube containing oil to serve as a chemical shift reference and an internal standard for quantification of the phosphorus metabolites. The ^{31}P NMR spectra of oils were acquired using a 5 mm broadband probe operating at 242.8 MHz on a Varian Inova 600 NMR Spectrometer, applying a 90° pulse (10.4 μs pulse width) and 0.8 s acquisition time with a 15.8 s interpulse delay (which was long enough to allow full T1 relaxation). The acquisition signal was averaged for ≥2 h to achieve a spectrum with sufficient signal-to-noise ratio (S/N). No proton (^{1}H) decoupling was needed. Spectral ^{31}P data were Fourier transformed and the baseline corrected (flattened) using a 5th order polynomial and spline peak-fitting routine. The chemical shifts were referenced to PC set at 0.0 ppm. NMR peaks were fit using a Lorentzian function fitting routine (deconvolution tool) in the Varian software. Quantitative measures of each organophosphate were determined based on the peak area of PC and on its known concentration in each ^{31}P NMR run sample.

2.6. Statistical analyses

Levine's and Welch's tests were performed to assess the equality of variances between experimental groups for each measurement using JMP® software, version 11.0 for Windows (SAS Institute, Cary, NC, USA). If Levine's test was significant ($p \leq 0.05$), then a Welch's test was conducted to assess if there were significant differences in the mean values between groups for each measurement. If the Levine's test was not significant, then the data were considered to have equal variances (Welch's test was not applicable), and significance was tested using one-way analysis of variance (ANOVA). If both Levine's and Welch's tests were significant ($p \leq 0.05$), then a pairwise Welch test was performed for all pairs of groups. Results shown in figures and tables are expressed as mean ± standard error of the mean (S.E.) and are considered statistically significant at $p \leq 0.05$.

We also calculated the effect size or Cohen's d (magnitude of change) (Cohen, 1988) induced by each treatment subtracting the values obtained for the control group from those obtained for the oil treated groups and assessing the difference relative to the pooled standard deviations for oil

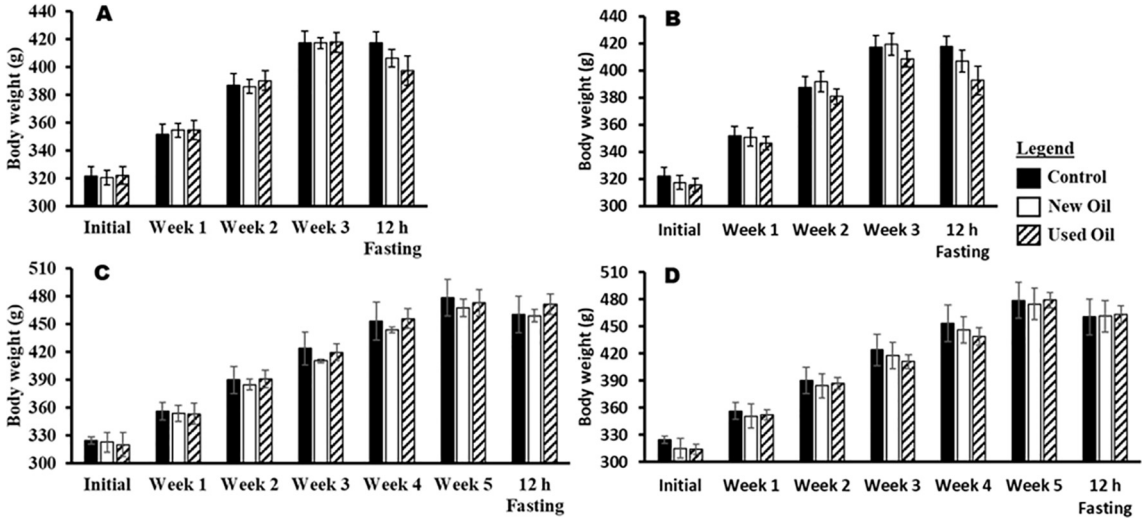

Fig. 1. Body weights for male rats dermally exposed to unused (new) and used versions of grade 3 and grade 4 aircraft engine oils. Body weight for males exposed to (A) grade 3 and (B) grade 4 engine oils over a period of 21 days (15 total exposures) (Mean ± S.E.; N = 9–11). Body weight for males in the recovery groups exposed to (C) grade 3 and (D) grade 4 engine oils over a period of 21 days (15 total exposures) and were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects (Mean ± S.E.; N = 5).

treated groups and control group as shown in Eq. (1),

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

where $(X)_T$ and $(X)_C$ are the blood parameter values for oil treated and control groups, respectively, while $(\sigma)_T$ and $(\sigma)_C$ are the standard deviations values for the means in oil treated and control groups, respectively. Effect size values were graded as large ($d = |0.8|$) based on Cohen's effect size (d) classification (Cohen, 1988).

3. Results

3.1. Organophosphate contents of aircraft engine oils

The ^{31}P NMR analyses were performed on unused and used versions of grade 3 (G3-N and G3-U) and grade 4 (G4-N and G4-U) aircraft engine oils and the results of these analyses are shown in Table 1. Four different TCP isomers were present in both versions of grade 3 oil at detectable levels. The concentration for all the four TCP isomers combined in G3-N was 30.0 mM while that in G3-U amounted to 22.2 mM, representing a 26% decrease relative to G3-N (Table 1). Both versions of grade 4 contained TPP and TIPP. The concentration of TPP in G4-N was 12.7 mM while that in G4-U was 9.9 mM, representing a 22% decrease relative to G4-N. G4-N contained 37.6 mM of TIPP while G4-U comprised 35.2 mM, representing a 6.4% decrease relative to G4-N. These data show that TPP in G4-U was depleted to a greater extent than TIPP.

3.2. Effects of aircrafts engine oils on body weights, feed intake and water consumption

Prior to oil exposure, rats involved in this study were 8–9 weeks old. As shown in Figs. 1A–B & 2A–B, the initial weights for male and female controls were 322.0 ± 6.8 g and 196.6 ± 7.6 g, respectively. At the end of the treatment period, they were about 11–12 weeks old and weighed 417.8 ± 7.6 g and 234.9 ± 4.8 g, respectively. The initial weights for control rats assigned to the recovery assessment were 324.8 ± 4.2 g and 203.7 ± 10.7 g, respectively (Figs. 1C–D & 2C–D). At the end of the recovery period, they were about 13–14 weeks old and weighed 460.7 ± 19.9 g and 252.5 ± 10.8 g, respectively. Exposure to grades 3 and 4 aircraft engine oils in both new and used states over a period of 21 days (15 total exposures) did not have a significant impact on the body weights for both male and female rats (Figs. 1A–B & 2A–B). Over the three week period, male rat controls gained an overall mean ± SE of 27 ± 4% relative to their initial body weight (prior to treatments). Males exposed to G3-N gained 25 ± 3% in body weight. Similarly, those exposed to G3-U gained 25 ± 3% in body weight while those exposed to G4-N and G4-U increased their weights by 29 ± 2% and 25 ± 3%, respectively. Female rat controls gained 14 ± 3% in body weight during the three week period. Female rats treated with G3-N and G3-U increased their weights by 13 ± 3% and 9 ± 5%, respectively. Those exposed to G4-N gained 13 ± 2% in body weight as did those exposed to G4-U. It is noteworthy to highlight that rats exposed to these oils and fasted for 12 h tended to lose more weight when compared to controls, regardless of their sex (Figs. 1A–B & 2A–B). Interestingly, this trend was not observed for rats assigned to the recovery groups and subjected to similar treatments (Figs. 1C–D & 2C–D). Over a period of five weeks, male rat controls in the recovery group gained 42 ± 5% of their initial body weights. Males exposed to G3-N and G3-U gained 43 ± 5% and 48 ± 4%, respectively. Those exposed to G4-N and G4-U increased their body weight by 44 ± 2% and 47 ± 2%, respectively. Female rat controls gained 24 ± 3% in body weight during the five weeks period. Females exposed to G3-N and G3-U gained 29 ± 2% and 22 ± 2% of their initial body weights, respectively. Those exposed to G4-N and G4-U gained 21 ± 7% and 25 ± 3%, respectively. Both males and females did not show any delayed effect on body weights that could be associated with exposure to these oils (Figs. 1C–D & 2C–D). It is also worth noting that exposure to oils did not significantly affect the weekly measured feed intake and water consumption (data are not shown).

3.3. Aircraft engine oils impact the reproductive organ weights of Sprague Dawley rats

As shown in Fig. 3A, exposing male rats to both versions of G3 (G3-N and G3-U) oil over a period of 21 days did not impact the brain weights. Additionally, keeping these rats for 14 days post-treatment did not indicate any delayed treatment effect on brain weights (Fig. 3D). Similar results were also obtained for female rats (Fig. 4A & D). Therefore, all organ weights were normalized to brain weights and the data are herein shown as organ/brain weights (Figs. 3 & 4; Tables 2–5; see supplemental Tables S1–S4 for non-normalized organ weights). Data obtained at the

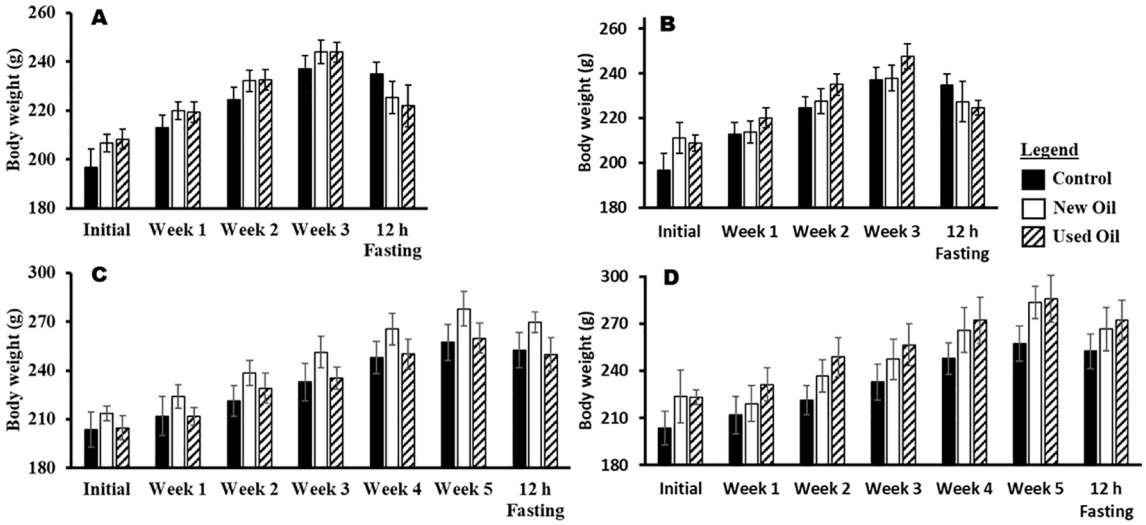

Fig. 2. Body weights for female rats dermally exposed to unused (new) and used versions of grade 3 and grade 4 aircraft engine oils. Body weight for females exposed to (A) grade 3 and (B) grade 4 engine oils over a period of 21 days (15 total exposures) (Mean ± S.E.; N = 10). Body weight for females in the recovery groups exposed to (C) grade 3 and (D) grade 4 engine oils over a period of 21 days (15 total exposures) and were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects (Mean ± S.E.; N = 5).

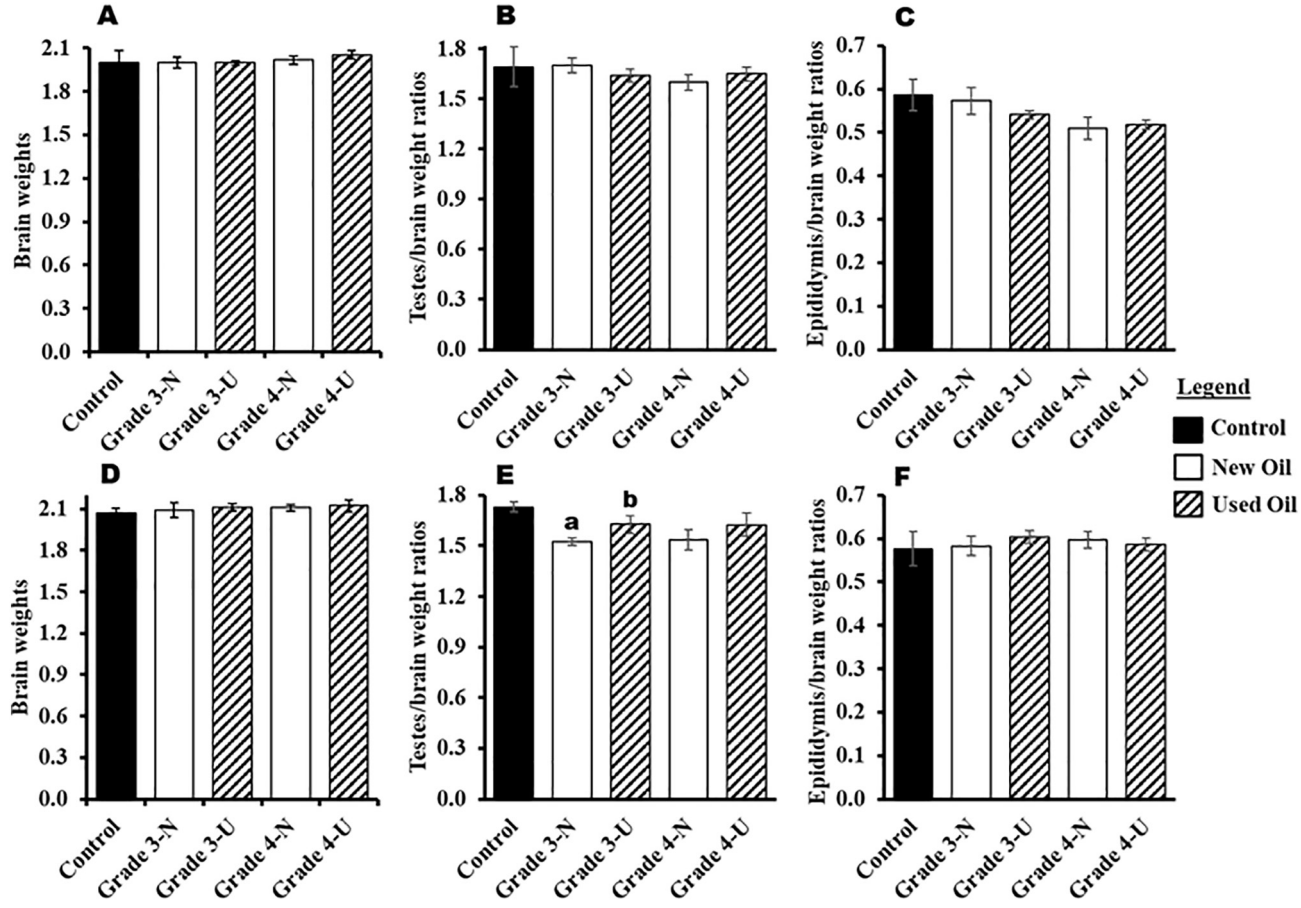

Fig. 3. Brain weights and organ weight/brain weight ratios for male rats assigned to main study and recovery groups and dermally exposed to aircraft engine oils. (A) Brain weights, (B) testes/brain weight and (C) epididymis/brain weight ratios for male rats exposed to unused and used versions of grade 3 and 4 aircraft engine oils over a period of 21 days (15 total exposures) (Mean ± S.E.; N = 9–11). (D) Brain weights, (E) testes/brain weight and (F) epididymis/brain weight ratios for male rats in the recovery groups that received similar treatments and were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects (Mean ± S.E.; N = 5). Significant differences between groups are indicated by letters: a, differs from control group; b, differs from G3-N treated group; $p \leq 0.05$.

end of 21-day treatment period indicate that exposure to grade 3 and 4 oils did not affect testes and epididymis weights (Fig. 3B & C). A delayed treatment effect was observed during the recovery period that was only associated with exposure to G3-N (Fig. 3E). Male rats treated with this oil had significantly decreased testes weights relative to controls. Although the testes weights for rats exposed to G3-U were similar to those obtained for the controls (Fig. 3E), they were statistically higher relative to those for G3-N-treated rats. No delayed effect on epididymis weights was observed with G3 or G4 treated rats (Fig. 3F).

As shown in Fig. 4B, all versions of G3 significantly decreased the uterus weights at the end of 21 days exposure period relative to controls. During the recovery period, the uterus weights for rats treated with G3-N were resolved. Those for rats exposed to G3-U remained decreased as compared to controls and G3-N-treated group (Fig. 4E). Although G4-N induced a significant decrease in uterus weights during the exposure period (Fig. 4B), this effect was resolved at the end of the recovery period (Fig. 4E). G4-U did not have an impact on the uterus weights (Fig. 4B & E). Exposure to both versions of G3 and G4 oils had no impact on ovary weights, regardless of the time of data collection (Fig. 4C & F).

3.4. Aircraft engine oils affect weights of liver, adrenals and spleen of female Sprague Dawley rats

The weights for liver, right and left kidneys, adrenals, spleen, thymus and heart were also taken at the end of 21 days of exposure to G3 and G4 oils and at the end of the 14 days recovery period (Tables 2–5). Data obtained at the end of the treatment period indicate that G3 and G4 oils did not significantly influence the weights for these organs when compared to controls, regardless of sex (Tables 2–5). It is worthy to point out that G4-U slightly increased the weight of the adrenals for females during treatment period as compared to those obtained for G4-N group (Table 5). There was no delayed effect associated with exposing males to G3 and G4 oils (Tables 2 & 4). However, exposure to G3-N elevated the liver weights for females during the recovery period compared to both control and G3-U groups (Table 3). During this period, G4-N increased the weight of the adrenals and spleen for females relative to those obtained for controls (Table 5). The adrenal weights for G4-N group were also elevated when assessed against those obtained with G4-U group. G4-U exposure yielded an increase in spleen weights relative to those obtained for controls (Table 5).

3.5. Effects of grade 3 and 4 aircraft engine oils on hematological parameters of Sprague Dawley rats

Data describing hematological indices for male and female Sprague Dawley rats following a 21-day exposure to G3 and G4 engine oils and 14 days recovery period are shown in Tables 6–9. To more clearly illustrate the levels of changes in blood indices induced by exposure to these oils, we calculated the effect size or Cohen's *d* (magnitude of change induced by each treatment relative to control). Contrary to *p* values that are dependent on the sample size and are less likely to be statistically significant when the sample size is small and more likely if the sample size is large, effect sizes are not influenced by sample size and provide information about the direction (decrease or increase) and strength of the relationship between

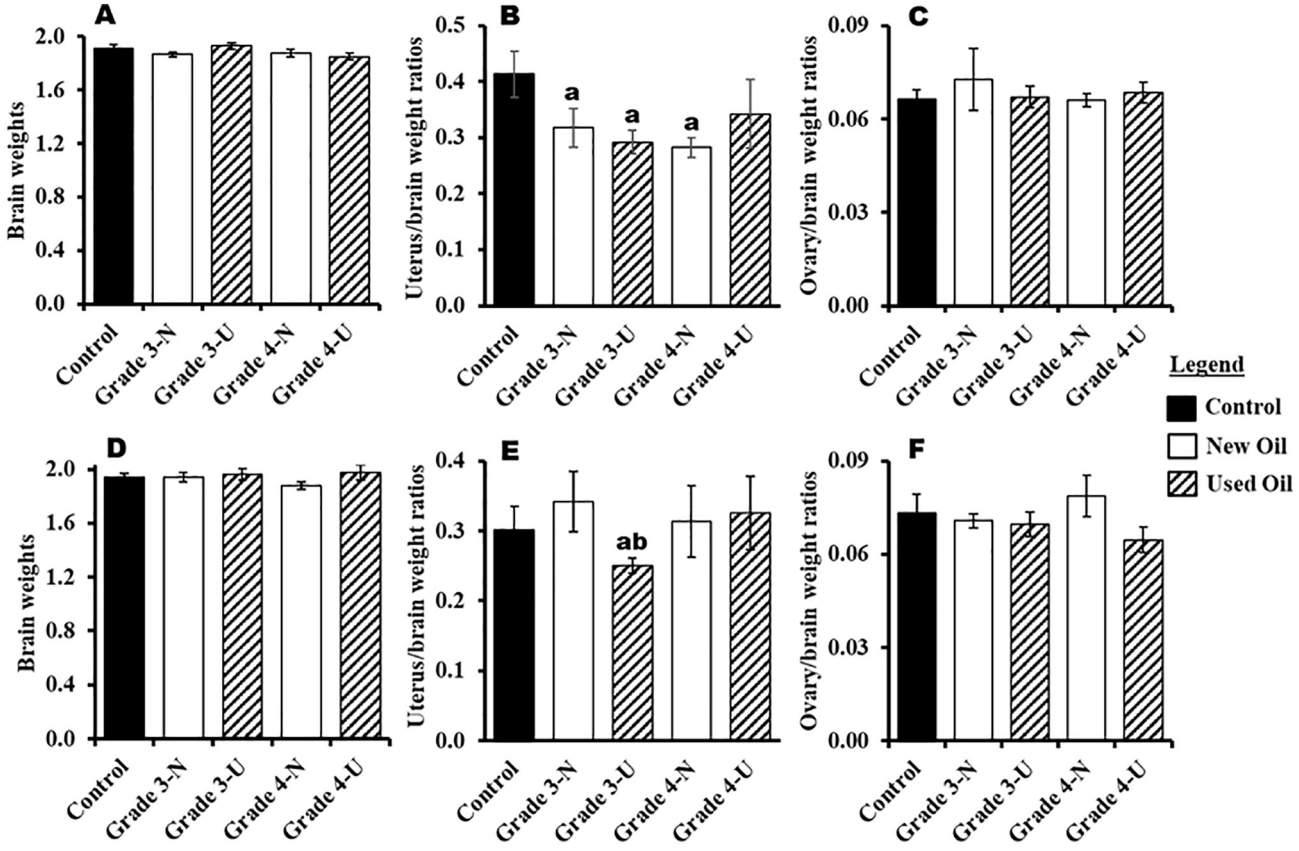

Fig. 4. Brain weights and organ weight/brain weight ratios for female rats assigned to main study and recovery groups and dermally exposed to aircraft engine oils. (A) Brain weights, (B) uterus/brain weight and (C) ovary/brain weight ratios for female rats exposed to unused and used versions of grade 3 and 4 aircraft engine oils over a period of 21 days (15 total exposures) (Mean ± S.E.; N = 10). (D) Brain weights, (E) uterus/brain weight and (F) ovary/brain weight ratios for female rats in the recovery groups that received similar treatments and were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects (Mean ± S.E.; N = 5). Significant differences between groups are indicated by letters: a, differs from control group; b, differs from G3-N treated group; $p \leq 0.05$.

variables (Berben et al., 2012). Equation used for calculation of effect sizes for each blood parameter is shown in Methods and results are shown in Tables 6–9 (in parentheses).

3.5.1. Effects of grade 3 aircraft engine oil exposure on blood indices for male and female Sprague Dawley rats

Hematology data for male Sprague Dawley rats following a 21-day exposure to G3 engine oils and those obtained after 14 days recovery period are shown in Table 6. G3-N significantly increased ($p \leq 0.05$) the levels of eosinophil count and eosinophil % for males compared to those for controls (Table 6). As shown in this table (in parentheses) this treatment induced large effect sizes for eosinophils ($d = 1.1$ and $d = 1.0$ for eosinophil count and eosinophil %, respectively), hematocrit ($d = 0.8$) and platelets ($d = -0.8$). The impact of G3-U exposure on blood parameters for male rats was relatively moderate as compared to that induced by G3-N treatment. G3-U did not induce significant changes in blood parameters and no large effect size ($d \geq |0.8|$) was obtained with this treatment. Although the levels of all blood parameters in G3-U treated group were similar to those obtained for controls, eosinophil levels were significantly decreased ($p \leq 0.05$) relative to those obtained for male rats exposed to G3-N. During the recovery period, the levels of eosinophils and basophils for G3-N group felt below the instrument's limit of detection and were not measured (Table 6). As shown in this table, a delayed effect induced by G3-N treatment was a significant increase ($p \leq 0.05$) in white blood cell and lymphocyte counts for males. This treatment also induced large effect sizes for the numbers of white blood cells, lymphocytes and monocytes

Table 2
Organ weight to brain weight ratios for male Sprague-Dawley rats assigned to the main study and recovery groups. All rats were dermally exposed to new (G3-N) and used (G3-U) versions of grade 3 of the aircraft engine oils over a period of 21 days. Rats in the recovery groups were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects (Mean ± S.E.).

Organs	Main study groups			Recovery groups		
	Control (n = 10)	G3-N[a] (n = 9)	G3-U[a] (n = 11)	Control (n = 5)	G3-N (n = 5)	G3-U (n = 5)
Liver	6.00 ± 0.37	5.82 ± 0.21	5.97 ± 0.22	6.63 ± 0.61	6.52 ± 0.42	6.34 ± 0.27
Left kidney	0.73 ± 0.02	0.70 ± 0.02	0.73 ± 0.02	0.75 ± 0.05	0.72 ± 0.03	0.76 ± 0.03
Right kidney	0.75 ± 0.03	0.71 ± 0.02	0.75 ± 0.02	0.77 ± 0.05	0.73 ± 0.04	0.79 ± 0.03
Adrenals	0.027 ± 0.002	0.025 ± 0.002	0.024 ± 0.002	0.023 ± 0.003	0.023 ± 0.002	0.024 ± 0.002
Spleen	0.42 ± 0.02	0.37 ± 0.01	0.38 ± 0.02	0.42 ± 0.03	0.41 ± 0.02	0.39 ± 0.02
Thymus	0.26 ± 0.02	0.23 ± 0.03	0.23 ± 0.02	0.19 ± 0.04	0.26 ± 0.03	0.23 ± 0.03
Heart	0.60 ± 0.03	0.59 ± 0.02	0.59 ± 0.02	0.61 ± 0.05	0.65 ± 0.06	0.59 ± 0.02

[a] Sample sizes for G3-N and G3-U groups were 9 and 11 rats, respectively.

Table 3
Organ weight to brain weight ratios for female Sprague-Dawley rats assigned to the main study and recovery groups. All rats were dermally exposed to new (G3-N) and used (G3-U) versions of grade 3 of the aircraft engine oils over a period of 21 days. Rats in the recovery groups were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects (Mean ± S.E.).

Organs	Main study groups			Recovery groups		
	Control (n = 10)	G3-N (n = 10)	G3-U (n = 10)	Control (n = 5)	G3-N (n = 5)	G3-U (n = 5)
Liver	3.61 ± 0.11	3.88 ± 0.20	4.00 ± 0.22	3.92 ± 0.19	4.44 ± 0.10[a]	3.96 ± 0.13[b]
Left kidney	0.46 ± 0.01	0.47 ± 0.02	0.48 ± 0.02	0.45 ± 0.01	0.49 ± 0.02	0.48 ± 0.01
Right kidney	0.47 ± 0.01	0.48 ± 0.02	0.50 ± 0.02	0.49 ± 0.02	0.52 ± 0.02	0.50 ± 0.01
Adrenals	0.035 ± 0.002	0.032 ± 0.002	0.034 ± 0.002	0.032 ± 0.002	0.036 ± 0.003	0.034 ± 0.003
Spleen	0.27 ± 0.02	0.29 ± 0.01	0.29 ± 0.02	0.26 ± 0.02	0.33 ± 0.03	0.27 ± 0.03
Thymus	0.20 ± 0.02	0.22 ± 0.02	0.21 ± 0.03	0.18 ± 0.03	0.27 ± 0.04	0.20 ± 0.02
Heart	0.41 ± 0.01	0.43 ± 0.02	0.41 ± 0.01	0.43 ± 0.01	0.45 ± 0.01	0.41 ± 0.02

[a] Significant differences relative to control group ($p \leq 0.05$).
[b] Significant differences relative to G3-N group ($p \leq 0.05$). See Materials and Methods for details on statistical analyses performed on the data.

Table 4
Organ weight to brain weight ratios for male Sprague-Dawley rats assigned to the main study and recovery groups. All rats were dermally exposed to new (G4-N) and used (G4-U) versions of grade 4 of the aircraft engine oils over a period of 21 days. Rats in the recovery groups were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects (Mean ± S.E.).

Organs	Main study groups			Recovery groups		
	Control (n = 10)	G4-N (n = 10)	G4-U (n = 10)	Control (n = 5)	G4-N (n = 5)	G4-U (n = 5)
Liver	6.00 ± 0.37	6.30 ± 0.40	5.48 ± 0.26	6.63 ± 0.61	6.84 ± 0.76	6.56 ± 0.46
Left kidney	0.73 ± 0.02	0.70 ± 0.02	0.68 ± 0.03	0.75 ± 0.05	0.72 ± 0.03	0.76 ± 0.05
Right kidney	0.75 ± 0.03	0.74 ± 0.03	0.69 ± 0.02	0.77 ± 0.05	0.73 ± 0.04	0.79 ± 0.05
Adrenals	0.027 ± 0.002	0.024 ± 0.001	0.025 ± 0.002	0.023 ± 0.003	0.026 ± 0.002	0.030 ± 0.002
Spleen	0.42 ± 0.02	0.40 ± 0.02	0.38 ± 0.02	0.42 ± 0.03	0.41 ± 0.02	0.41 ± 0.02
Thymus	0.26 ± 0.02	0.23 ± 0.02	0.22 ± 0.03	0.19 ± 0.04	0.23 ± 0.03	0.20 ± 0.04
Heart	0.60 ± 0.03	0.58 ± 0.01	0.51 ± 0.05	0.61 ± 0.05	0.61 ± 0.01	0.57 ± 0.02

($d = 2.0$, $d = 2.7$, and $d = 0.9$, respectively). Although males exposed to G3-U did not show a significant delayed impact on their hematological indices when compared to controls, this treatment induced large changes for white blood cells ($d = 1.0$), lymphocyte count ($d = 0.9$) and eosinophil % ($d = 0.9$) cells. Additionally, the numbers of lymphocytes under this treatment was significantly reduced ($p \leq 0.05$) relative to that obtained for G3-N group.

Hematology data for female Sprague Dawley rats following a 21-day exposure to G3 engine oils and those obtained after 14 days recovery period are shown in Table 7. Females exposed to G3-N had significantly decreased ($p \leq 0.05$) levels of white blood cells, lymphocyte count and hematocrit relative to controls following 21 day treatment period (Table 7). Under this treatment, six blood indices were characterized by large effect sizes. These included white blood cells ($d = -1.6$), neutrophil count ($d = -0.8$), lymphocyte count ($d = -1.5$), red blood cells ($d = -0.9$), hemoglobin ($d = -0.8$) and hematocrit ($d = -1.0$). However, all the blood indices that were affected by G3-N treatment returned to normal at the end of the recovery period (Table 7). Delayed effects associated with this treatment included large effect sizes that were observed for mean corpuscular hemoglobin concentration ($d = 0.9$) and platelets ($d = -1.1$). Females exposed to G3-U significantly increased ($p \leq 0.05$) the levels of monocyte count and monocyte % relative to controls (Table 7). Under G3-U treatment, these blood indices also had large effect sizes ($d = 1.2$ and $d = 1.5$, respectively) together with lymphocyte % ($d = -1.3$) and mean platelet volume ($d = 1.1$). It is worth noting that the numbers of white blood cells, neutrophils, lymphocytes and monocytes, as well as monocyte % and hemoglobin were statistically elevated ($p \leq 0.05$) for G3-U treated group relative to those obtained for G3-N group. In general, G3-N treatment reduced the levels of most blood indices for females compared to G3-U (Table 7). Under G3-N treatment, twelve blood parameters scored negative effect sizes compared to only five that were recorded for G3-U treatment. Although the levels of all measured blood indices for both versions of G3 oil were statistically similar to those obtained for the control group during the recovery period, large effect sizes were observed for mean corpuscular hemoglobin concentration ($d = 0.9$) and platelets ($d = -1.1$) for G3-N treated group and neutrophil % ($d = -1.4$), lymphocyte % ($d = 1.0$), hematocrit ($d = 0.9$) and platelets ($d = -1.3$) for G3-U group (Table 7).

Table 5
Organ weight to brain weight ratios for female Sprague-Dawley rats assigned to the main study and recovery groups. All rats were dermally exposed to new (G4-N) and used (G4-U) versions of grade 4 of the aircraft engine oils over a period of 21 days. Rats in the recovery groups were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects (Mean ± S.E.).

Organs	Main study groups			Recovery groups		
	Control (n = 10)	G4-N (n = 10)	G4-U (n = 10)	Control (n = 5)	G4-N (n = 5)	G4-U (n = 5)
Liver	3.61 ± 0.11	3.75 ± 0.16	3.72 ± 0.25	3.92 ± 0.19	4.47 ± 0.27	4.13 ± 0.15
Left kidney	0.46 ± 0.01	0.46 ± 0.02	0.48 ± 0.01	0.45 ± 0.01	0.51 ± 0.02	0.47 ± 0.01
Right kidney	0.47 ± 0.01	0.48 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	0.54 ± 0.01	0.50 ± 0.01
Adrenals	0.035 ± 0.002	0.030 ± 0.002	0.036 ± 0.002[b]	0.032 ± 0.002	0.042 ± 0.004[a]	0.033 ± 0.002[b]
Spleen	0.27 ± 0.02	0.28 ± 0.01	0.28 ± 0.02	0.26 ± 0.02	0.33 ± 0.02[a]	0.34 ± 0.04[a]
Thymus	0.20 ± 0.02	0.20 ± 0.02	0.18 ± 0.02	0.18 ± 0.03	0.19 ± 0.02	0.24 ± 0.03
Heart	0.41 ± 0.01	0.41 ± 0.01	0.43 ± 0.02	0.43 ± 0.01	0.45 ± 0.01	0.46 ± 0.03

[a] Significant differences relative to control group ($p \leq 0.05$).
[b] Significant differences relative to G4-N group ($p \leq 0.05$). See Materials and Methods for details on statistical analyses performed on the data.

Table 6
Hematological indices for male Sprague-Dawley rats assigned to the main study and recovery groups. All rats were dermally exposed to new (G3-N) and used (G3-U) versions of grade 3 of the aircraft engine oils over a period of 21 days (Mean ± S.E.; effect sizes or Cohen's *d* (Cohen, 1988) (magnitude of changes) estimated relative to controls are shown in parentheses). Rats in the recovery groups were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects.

Blood parameters	Main study groups			Recovery groups		
	Control (n = 6)	G3-N (n = 6)[a]	G3-U (n = 7)[a]	Control (n = 4)	G3-N (n = 4)[a]	G3-U (n = 4)[a]
WBC# ($\times 10^3$ cells/μL)	11.90 ± 2.11	12.79 ± 1.49 (0.2)	11.54 ± 0.94 (−0.1)	10.24 ± 0.77	13.96 ± 1.09 (2.0)[b]	11.82 ± 0.76 (1.0)
NE# ($\times 10^3$ cells/μL)	2.43 ± 0.45	2.13 ± 0.14 (−0.4)	2.37 ± 0.26 (−0.1)	2.43 ± 0.50	3.01 ± 0.60 (0.5)	2.72 ± 0.37 (0.3)
LY# ($\times 10^3$ cells/μL)	8.65 ± 1.70	9.65 ± 1.39 (0.3)	8.28 ± 0.67 (−0.1)	7.44 ± 0.38	10.27 ± 0.64 (2.7)[b]	8.51 ± 0.77 (0.9)[c]
MO# ($\times 10^3$ cells/μL)	0.81 ± 0.16	0.98 ± 0.14 (0.4)	0.88 ± 0.12 (0.2)	0.36 ± 0.10	0.52 ± 0.06 (0.9)	0.53 ± 0.21 (0.5)
EO# ($\times 10^3$ cells/μL)	0.008 ± 0.002	0.022 ± 0.007 (1.1)[b]	0.007 ± 0.001 (−0.1)[c]	0.013 ± 0.001	–	0.039 ± 0.017
BA# ($\times 10^3$ cells/μL)	–	0.005 ± 0.003	–	0.003 ± 0.001	–	–
NE%	20.39 ± 3.53	17.57 ± 1.77 (−0.4)	20.47 ± 1.45 (0)	23.17 ± 3.15	21.22 ± 2.94 (−0.3)	23.16 ± 3.32 (0)
LY%	72.89 ± 3.86	74.14 ± 2.88 (0.1)	71.88 ± 1.13 (−0.1)	73.06 ± 2.24	74.07 ± 3.66 (0.2)	71.74 ± 2.86 (−0.3)
MO%	6.63 ± 0.80	8.10 ± 1.28 (0.6)	7.59 ± 0.63 (0.5)	3.63 ± 1.07	3.77 ± 0.59 (0.1)	4.58 ± 1.84 (0.3)
EO%	0.08 ± 0.02	0.15 ± 0.04 (1.0)[b]	0.06 ± 0.01 (−0.6)[c]	0.12 ± 0.02	–	0.32 ± 0.16 (0.9)
BA%	–	0.037 ± 0.024	0.009 ± 0.006	0.03 ± 0.01	–	–
RBC ($\times 10^6$ cells/μL)	7.72 ± 0.29	7.99 ± 0.16 (0.5)	7.74 ± 0.23 (0)	8.14 ± 0.16	8.06 ± 0.29 (−0.2)	8.15 ± 0.33 (0)
Hgb (g/dL)	15.50 ± 0.51	16.10 ± 0.31 (0.6)	15.69 ± 0.47 (0.1)	15.75 ± 0.38	15.29 ± 0.28 (−0.7)	16.05 ± 0.59 (0.3)
HCT%	48.41 ± 1.49	50.59 ± 0.55 (0.8)	48.19 ± 1.23 (−0.1)	47.03 ± 1.83	46.68 ± 1.12 (−0.1)	47.38 ± 1.77 (0.1)
MCV (fL)	62.89 ± 1.41	63.45 ± 0.98 (0.2)	62.38 ± 0.88 (−0.2)	57.79 ± 1.95	58.13 ± 2.37 (0.1)	58.26 ± 1.95 (0.1)
MCH (pg/cell)	20.10 ± 0.26	20.19 ± 0.46 (0.1)	20.29 ± 0.34 (0.2)	19.38 ± 0.59	19.01 ± 0.51 (−0.3)	19.71 ± 0.24 (0.4)
MCHC (g/dL)	32.02 ± 0.40	31.82 ± 0.43 (−0.2)	32.52 ± 0.24 (0.6)	33.59 ± 0.75	32.79 ± 0.46 (−0.6)	33.91 ± 0.80 (0.2)
RDW%	16.15 ± 0.42	16.31 ± 0.10 (0.2)	16.59 ± 0.23 (0.5)	16.66 ± 0.38	16.86 ± 0.56 (0.2)	16.85 ± 0.35 (0.3)
PLT ($\times 10^3$ cells/μL)	924.9 ± 22.7	852.6 ± 55.6 (−0.8)	906.1 ± 37.3 (−0.2)	968.0 ± 11.6	873.2 ± 140.8 (−0.5)	873.2 ± 138.3 (−0.6)
MPV (fL)	6.24 ± 0.33	6.16 ± 0.15 (−0.1)	5.93 ± 0.10 (−0.5)	6.11 ± 0.20	6.29 ± 0.22 (0.4)	6.18 ± 0.45 (0.1)

[a] Changes (effect sizes) relative to controls; "–" indicates a missing value because the level of the parameter in the sample was below the instrument's limit of detection.
[b] Significant differences relative to control ($p \leq 0.05$).
[c] Significant differences relative to G3-N group ($p \leq 0.05$). See Materials and Methods for details on statistical analyses performed on the data. The sample sizes in the table are different from those indicated in Materials and Methods because not all blood samples were analyzed due to a malfunction of the hematology analyzer.

3.5.2. Effects of grade 4 aircraft engine oil exposure on blood indices for male and female Sprague Dawley rats

Hematology data for male Sprague Dawley rats following a 21-day exposure to G4 engine oils and those obtained after 14 days recovery period are shown in Table 8. All hematological parameters for males were not significantly influenced by G4-N. Although G4-N induced considerable increases in eosinophil count and eosinophil % levels (157% and 93.8%, respectively) compared to controls, these changes were not statistically significant due to large variations in data. Eosinophil count and eosinophil % were also the only blood parameters associated with large effect sizes ($d = 1.2$ and ($d = 1.0$, respectively). Male rats exposed to G4-U significantly increased ($p \leq 0.05$) hematocrit levels (by 7.2%) relative to controls (Table 8). Under this treatment, hematocrit and hemoglobin were the only blood parameters that were associated with a large effect sizes ($d = 1.0$ and $d = 0.8$, respectively). Although the levels of all hematological indices for both versions of G4 oil were statistically similar to those obtained for the control group during the recovery period (Table 8), large effect sizes were observed for monocyte

Table 7
Hematological indices for female Sprague-Dawley rats assigned to the main study and recovery groups. All rats were dermally exposed to new (G3-N) and used (G3-U) versions of grade 3 of the aircraft engine oils over a period of 21 days (Mean ± S.E.; effect sizes or Cohen's *d* (Cohen, 1988) (magnitude of changes) estimated relative to controls are shown in parentheses). Rats in the recovery groups were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects.

Blood parameters	Main study groups			Recovery groups		
	Control (n = 7)	G3-N (n = 7)[a]	G3-U (n = 7)[a]	Control (n = 3)	G3-N (n = 4)[a]	G3-U (n = 3)[a]
WBC# ($\times 10^3$ cells/μL)	10.39 ± 0.54	7.37 ± 0.86 (−1.6)[b]	11.42 ± 1.23 (0.4)[c]	7.83 ± 1.24	7.07 ± 0.51 (−0.5)	8.83 ± 1.11 (0.5)
NE# ($\times 10^3$ cells/μL)	1.91 ± 0.27	1.35 ± 0.29 (−0.8)	2.33 ± 0.27 (0.6)[c]	1.22 ± 0.03	1.34 ± 0.32 (0.3)	1.09 ± 0.19 (−0.5)
LY# ($\times 10^3$ cells/μL)	7.86 ± 0.28	5.59 ± 0.76 (−1.5)[b]	7.96 ± 0.86 (0.1)[c]	6.37 ± 1.30	5.40 ± 0.53 (−0.6)	7.48 ± 0.87 (0.6)
MO# ($\times 10^3$ cells/μL)	0.57 ± 0.10	0.41 ± 0.10 (−0.6)	1.06 ± 0.20 (1.2)[b,c]	0.22 ± 0.08	0.27 ± 0.07 (0.3)	0.24 ± 0.04 (0.2)
EO# ($\times 10^3$ cells/μL)	0.044 ± 0.019	0.018 ± 0.006 (−0.7)	0.059 ± 0.026 (0.2)	–	0.055 ± 0.039	–
BA# ($\times 10^3$ cells/μL)	0.016 ± 0.009	0.004 ± 0.003 (−0.7)	0.013 ± 0.007 (−0.2)	–	–	–
NE%	17.93 ± 1.81	18.98 ± 3.25 (0.2)	20.45 ± 0.98 (0.7)	16.29 ± 2.15	18.85 ± 4.24 (0.4)	12.22 ± 0.93 (−1.4)
LY%	76.10 ± 2.14	75.13 ± 4.32 (−0.1)	69.69 ± 1.67 (−1.3)	80.35 ± 3.57	76.42 ± 4.73 (−0.5)	84.93 ± 1.19 (1.0)
MO%	5.44 ± 0.86	6.13 ± 0.94 (0.3)	9.29 ± 1.08 (1.5)[b,c]	3.33 ± 1.43	3.85 ± 1.09 (0.2)	2.73 ± 0.21 (−0.3)
EO%	0.39 ± 0.17	0.30 ± 0.14 (−0.2)	0.47 ± 0.19 (0.2)	–	–	0.10 ± 0.10
BA%	0.15 ± 0.08	0.13 ± 0.08 (−0.1)	0.11 ± 0.05 (−0.2)	–	0.11 ± 0.09	–
RBC ($\times 10^6$ cells/μL)	7.59 ± 0.17	7.05 ± 0.25 (−0.9)	7.42 ± 0.15 (−0.4)	7.49 ± 0.25	7.18 ± 0.42 (−0.5)	7.77 ± 0.38 (0.5)
Hgb (g/dL)	14.97 ± 0.30	14.21 ± 0.37 (−0.8)	15.37 ± 0.40 (0.4)[c]	14.23 ± 0.37	13.93 ± 0.77 (−0.3)	14.73 ± 0.75 (0.5)
HCT%	47.26 ± 0.75	44.39 ± 1.28 (−1.0)[b]	46.78 ± 1.01 (−0.2)	42.53 ± 0.84	40.63 ± 2.74 (−0.5)	44.47 ± 1.51 (0.9)
MCV (fL)	62.40 ± 0.83	63.05 ± 0.65 (0.3)	63.05 ± 0.84 (0.3)	56.80 ± 0.93	56.56 ± 1.80 (−0.1)	57.37 ± 0.83 (0.4)
MCH (pg/cell)	19.75 ± 0.35	20.18 ± 0.26 (0.5)	20.75 ± 0.63 (0.7)	19.00 ± 0.21	19.41 ± 0.41 (0.6)	18.97 ± 0.07 (−0.1)
MCHC (g/dL)	31.69 ± 0.49	32.02 ± 0.30 (0.3)	32.91 ± 0.91 (0.6)	33.43 ± 0.48	34.35 ± 0.55 (0.9)	33.10 ± 0.57 (−0.4)
RDW%	14.53 ± 0.29	14.24 ± 0.19 (−0.4)	14.66 ± 0.30 (0.2)	15.47 ± 0.22	15.16 ± 0.31 (−0.6)	15.37 ± 0.33 (−0.2)
PLT ($\times 10^3$ cells/μL)	735.3 ± 121.1	693.3 ± 83.2 (−0.2)	770.1 ± 99.3 (0.1)	928.0 ± 26.9	809.8 ± 81.4 (−1.1)	769.3 ± 94.6 (−1.3)
MPV (fL)	5.69 ± 0.08	5.89 ± 0.29 (0.4)	0.14 (1.1)	5.80 ± 0.06	6.31 ± 0.60 (0.6)	5.87 ± 0.29 (0.2)

[a] Changes (effect sizes) relative to controls; "–" indicates a missing value because the level of the parameter in the sample was below the instrument's limit of detection.
[b] Significant differences relative to control ($p \leq 0.05$)
[c] Significant differences relative to G3-N group ($p \leq 0.05$). See Materials and Methods for details on statistical analyses performed on the data. The sample sizes in the table are different from those indicated in Materials and Methods because not all blood samples were analyzed due to a malfunction of the hematology analyzer.

Table 8
Hematological indices for male Sprague-Dawley rats assigned to the main study and recovery groups. All rats were dermally exposed to new (G4-N) and used (G4-U) versions of grade 4 of the aircraft engine oils over a period of 21 days (Mean ± S.E.; effect sizes or Cohen's *d* (Cohen, 1988) (magnitude of changes) estimated relative to controls are shown in parentheses). Rats in the recovery groups were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects.

Blood parameters	Main study groups			Recovery groups		
	Control (n = 6)	G4-N (n = 7)[a]	G4-U (n = 7)[a]	Control (n = 4)	G4-N (n = 3)[a]	G4-U (n = 4)[a]
WBC# ($\times 10^3$ cells/μL)	11.90 ± 2.11	12.11 ± 0.34 (0.1)	10.73 ± 0.65 (−0.3)	10.24 ± 0.77	11.38 ± 0.98 (0.7)	8.73 ± 1.03 (−0.8)
NE# ($\times 10^3$ cells/μL)	2.43 ± 0.45	2.51 ± 0.27 (0.1)	2.27 ± 0.16 (−0.2)	2.43 ± 0.50	2.69 ± 0.20 (0.3)	1.90 ± 0.26 (−0.7)
LY# ($\times 10^3$ cells/μL)	8.65 ± 1.70	8.58 ± 0.25 (0)	7.60 ± 0.77 (−0.3)	7.44 ± 0.38	8.09 ± 1.13 (0.4)	6.40 ± 0.83 (−0.8)
MO# ($\times 10^3$ cells/μL)	0.81 ± 0.16	1.00 ± 0.21 (0.4)	0.83 ± 0.12 (0.1)	0.36 ± 0.10	0.59 ± 0.19 (0.8)	0.44 ± 0.06 (0.4)
EO# ($\times 10^3$ cells/μL)	0.008 ± 0.002	0.019 ± 0.005 (1.2)	0.015 ± 0.006 (0.7)	0.013 ± 0.001	0.007 ± 0.003 (−1.3)	–
BA# ($\times 10^3$ cells/μL)	–	0.003 ± 0.002	0.006 ± 0.004	0.003 ± 0.001	–	0.003 ± 0.001 (0)
NE%	20.39 ± 3.53	20.61 ± 1.86 (0)	21.66 ± 2.01 (0.2)	23.17 ± 3.15	23.86 ± 2.45 (0.1)	21.76 ± 1.81 (−0.3)
LY%	72.89 ± 3.86	71.04 ± 2.05 (−0.2)	69.95 ± 2.90 (−0.3)	73.06 ± 2.24	70.46 ± 4.31 (−0.4)	72.95 ± 1.55 (0)
MO%	6.63 ± 0.80	8.17 ± 1.59 (0.5)	8.18 ± 1.45 (0.5)	3.63 ± 1.07	5.59 ± 2.12 (0.6)	5.20 ± 0.87 (0.8)
EO%	0.08 ± 0.02	0.16 ± 0.04 (1.0)	0.15 ± 0.05 (0.7)	0.12 ± 0.02	0.07 ± 0.04 (−0.8)	0.06 ± 0.03 (−1.2)
BA%	–	0.027 ± 0.018	–	–	–	–
RBC ($\times 10^6$ cells/μL)	7.72 ± 0.29	7.88 ± 0.09 (0.3)	8.21 ± 0.28 (0.7)	8.14 ± 0.16	8.01 ± 0.22 (−0.4)	8.19 ± 0.09 (0.2)
Hgb (g/dL)	15.50 ± 0.51	15.84 ± 0.33 (0.3)	16.36 ± 0.28 (0.8)	15.75 ± 0.38	16.03 ± 0.32 (0.4)	15.63 ± 0.30 (−0.2)
HCT%	48.41 ± 1.49	48.89 ± 0.71 (0.2)	51.89 ± 1.15 (1.0)[b]	47.03 ± 1.83	48.08 ± 0.91 (0.4)	46.94 ± 1.39 (0)
MCV (fL)	62.89 ± 1.41	62.04 ± 0.67 (−0.3)	63.41 ± 1.12 (0.2)	57.79 ± 1.95	60.08 ± 0.52 (0.8)	57.24 ± 1.10 (−0.2)
MCH (pg/cell)	20.10 ± 0.26	20.12 ± 0.36 (0)	20.02 ± 0.43 (−0.1)	19.38 ± 0.59	20.03 ± 0.55 (0.6)	19.09 ± 0.27 (−0.3)
MCHC (g/dL)	32.02 ± 0.40	32.39 ± 0.50 (0.3)	31.57 ± 0.48 (−0.4)	33.59 ± 0.75	33.35 ± 0.65 (−0.2)	33.31 ± 0.50 (−0.2)
RDW%	16.15 ± 0.42	16.41 ± 0.31 (0.3)	15.98 ± 0.33 (−0.2)	16.66 ± 0.38	16.13 ± 0.74 (−0.5)	17.01 ± 0.57 (0.4)
PLT ($\times 10^3$ cells/μL)	924.9 ± 22.7	976.6 ± 55.4 (0.5)	880.6 ± 51.0 (−0.5)	968.0 ± 11.6	889.7 ± 24.4 (−2.3)	961.0 ± 85.8 (−0.1)
MPV (fL)	6.24 ± 0.33	6.69 ± 0.60 (0.4)	6.25 ± 0.41 (0)	6.11 ± 0.20	5.92 ± 0.16 (−0.6)	6.09 ± 0.29 (−0.1)

[a] Changes (effect sizes) relative to controls; "–" indicates a missing value because the level of the parameter in the sample was below the instrument's limit of detection.
[b] Significant differences relative to control ($p \leq 0.05$). See Materials and Methods for details on statistical analyses performed on the data. The sample sizes in the table are different from those indicated in Materials and Methods because not all blood samples were analyzed due to a malfunction of the hematology analyzer.

count ($d = 0.8$) and eosinophil count ($d = -1.3$), eosinophil % ($d = -0.8$), mean corpuscular volume ($d = 0.8$) and platelets ($d = -2.3$) for G4-N treated group and white blood cells ($d = -0.8$), lymphocytes ($d = -0.8$), monocyte % ($d = 0.8$) and eosinophil % ($d = -1.2$) for G4-U group.

Hematology data for female Sprague Dawley rats following a 21-day exposure to G4 engine oils and those obtained after 14 days recovery period are shown in Table 9. Female rats exposed to G4-N had significantly decreased ($p \leq 0.05$) levels of white blood cells, neutrophil count, lymphocyte count, eosinophil count, eosinophil % and increased levels of mean platelet volume compared to controls (Table 9). All these blood parameters scored large effect sizes ($d = -1.5$; $d = -1.2$; $d = -1.4$; $d = -1.0$; $d = -0.9$ and $d = 1.2$, respectively) together with red blood cell distribution width ($d = 0.8$) and platelets ($d = 0.8$). Although changes induced by G4-N were all resolved during the recovery period, large effect sizes were observed for hemoglobin ($d = 0.8$), hematocrit ($d = 1.0$), mean corpuscular volume ($d = 0.9$), mean corpuscular hemoglobin ($d = 1.4$), red blood cell distribution width ($d = -1.2$) and mean platelet volume ($d = -1.2$) at

Table 9
Hematological indices for female Sprague-Dawley rats assigned to the main study and recovery groups. All rats were dermally exposed to new (G4-N) and used (G4-U) versions of grade 4 of the aircraft engine oils over a period of 21 days (Mean ± S.E.; effect sizes or Cohen's *d* (Cohen, 1988) (magnitude of changes) estimated relative to controls are shown in parentheses). Rats in the recovery groups were kept for 14 days post exposure to screen for reversibility, persistence, or delayed occurrence of toxicity effects.

Blood parameters	Main study groups			Recovery groups		
	Control (n = 7)	G4-N (n = 7)[a]	G4-U (n = 6)[a]	Control (n = 3)	G4-N (n = 4)[a]	G4-U (n = 3)[a]
WBC# ($\times 10^3$ cells/μL)	10.39 ± 0.54	7.65 ± 0.81(−1.5)[b]	9.28 ± 1.22 (−0.5)	7.83 ± 1.24	7.40 ± 0.88 (−0.2)	7.45 ± 1.11 (−0.2)
NE# ($\times 10^3$ cells/μL)	1.91 ± 0.27	1.24 ± 0.14 (−1.2)[b]	1.64 ± 0.08 (−0.5)	1.22 ± 0.03	1.26 ± 0.08 (0.3)	1.32 ± 0.35 (0.2)
LY# ($\times 10^3$ cells/μL)	7.86 ± 0.28	5.94 ± 0.65 (−1.4)[b]	6.98 ± 1.11 (−0.4)	6.37 ± 1.30	5.94 ± 0.85 (−0.2)	5.98 ± 0.78 (−0.2)
MO# ($\times 10^3$ cells/μL)	0.57 ± 0.10	0.46 ± 0.08 (−0.5)	0.64 ± 0.10 (0.3)	0.22 ± 0.08	0.19 ± 0.03 (−0.3)	0.15 ± 0.02 (−0.7)
EO# ($\times 10^3$ cells/μL)	0.044 ± 0.019	0.006 ± 0.002 (−1.0)[b]	0.004 ± 0.003 (−1.1)[b]	–	–	0.013 ± 0.003
BA# ($\times 10^3$ cells/μL)	0.016 ± 0.009	–	–	–	–	–
NE%	17.93 ± 1.81	16.32 ± 1.14 (−0.4)	19.16 ± 2.29 (0.2)	16.29 ± 2.15	17.52 ± 1.55 (0.4)	17.01 ± 2.10 (0.2)
LY%	76.10 ± 2.14	77.55 ± 1.80 (0.3)	73.66 ± 2.77 (−0.4)	80.35 ± 3.57	79.43 ± 2.34 (−0.2)	80.61 ± 1.65 (0.1
MO%	5.44 ± 0.86	6.01 ± 0.95 (0.2)	7.08 ± 0.93 (0.7)	3.33 ± 1.43	2.92 ± 0.98 (−0.2)	2.14 ± 0.50 (−0.6)
EO%	0.39 ± 0.17	0.11 ± 0.03 (−0.9)[b]	0.09 ± 0.04 (−0.9)[b]	–	0.09 ± 0.06	0.22 ± 0.06
BA%	0.15 ± 0.08	–	–	–	0.05 ± 0.03	–
RBC ($\times 10^6$ cells/μL)	7.59 ± 0.17	7.43 ± 0.09 (−0.4)	7.44 ± 0.21 (−0.3)	7.49 ± 0.25	7.51 ± 0.06 (0.1)	7.52 ± 0.16 (0.1)
Hgb (g/dL)	14.97 ± 0.30	14.49 ± 0.29 (−0.6)	14.83 ± 0.37 (−0.2)	14.23 ± 0.37	14.66 ± 0.20 (0.8)	14.40 ± 0.51 (0.2)
HCT%	47.26 ± 0.75	46.32 ± 0.74 (−0.5)	47.30 ± 1.08 (0)	42.53 ± 0.84	43.94 ± 0.74 (1.0)	43.27 ± 1.70 (0.3)
MCV (fL)	62.40 ± 0.83	62.32 ± 0.77 (0)	63.66 ± 0.87 (0.6)	56.80 ± 0.93	58.54 ± 1.13 (0.9)	57.57 ± 1.59 (0.3)
MCH (pg/cell)	19.75 ± 0.35	19.53 ± 0.43 (−0.2)	19.94 ± 0.19 (0.3)	19.00 ± 0.21	19.51 ± 0.19 (1.4)	19.17 ± 0.29 (0.4)
MCHC (g/dL)	31.69 ± 0.49	31.34 ± 0.66 (−0.2)	31.34 ± 0.32 (−0.3)	33.43 ± 0.48	33.44 ± 0.96 (0)	33.30 ± 0.67 (−0.1)
RDW%	14.53 ± 0.29	15.05 ± 0.21 (0.8)	14.34 ± 0.12 (−0.3)[c]	15.47 ± 0.22	14.98 ± 0.21 (−1.2)	15.80 ± 0.40 (0.6)
PLT ($\times 10^3$ cells/μL)	735.3 ± 121.1	907.3 ± 58.1 (0.8)	868.3 ± 35.6 (0.6)	928.0 ± 26.9	907.0 ± 45.0 (−0.3)	859.0 ± 74.9 (−0.7)
MPV (fL)	5.69 ± 0.08	6.84 ± 0.55 (1.2)[b]	5.91 ± 0.14 (0.8)	5.80 ± 0.06	5.69 ± 0.04 (−1.2)	6.00 ± 0.12 (1.3)[c]

[a] Changes (effect sizes) relative to controls; "–" indicates a missing value because the level of the parameter in the sample was below the instrument's limit of detection.
[b] Significant differences relative to control ($p \leq 0.05$).
[c] Significant differences relative to G4-N group ($p \leq 0.05$). See Materials and Methods for details on statistical analyses performed on the data. The sample sizes in the table are different from those indicated in Materials and Methods because not all blood samples were analyzed due to a malfunction of the hematology analyzer.

the end of this period. During oil exposure period, G4-U significantly decreased ($p \leq 0.05$) the levels of eosinophils and eosinophil % relative to those obtained for controls (Table 9). These blood parameters also scored large effect sizes ($d = -1.1$ and -0.9, respectively) together with mean platelet volume ($d = 0.8$). The level of red blood cell distribution width measured for G4-U group was statistically decreased ($p \leq 0.05$) compared to that obtained for G4-N group. Although changes induced by G4-U were all resolved during the recovery period (Table 9), a large effect size was observed for mean platelet volume ($d = 1.3$). Females exposed to G4-U had significantly higher ($p \leq 0.05$) levels of mean platelet volume (3.4% increase) compared to those obtained for G4-N group (Table 9).

4. Discussion

To the best of our knowledge, this is the first study to assess the potential dermal subchronic toxicity of grade 3 and 4 aircraft engine oils in unused states relative to their used versions. G3 and G4 oils are chemical mixtures. Since there may be synergistic interactions between ingredients that can enhance their hazardous status, the study assessed their potential toxicity as whole products instead of evaluating their individual components.

4.1. Effects of aircraft engine oils on rat's body weights, feed and water intake

This study indicated, as expected, that males gained more body weight during three-weeks of treatments and two weeks of recovery period as compared to females. Exposure to G3 and G4 oils did not significantly influence rat's body weight, regardless of sex. However, it was interesting to note that rats exposed to oils tended to lose more body weight at the end of the treatment period following a 12 h fasting when compared to controls. Fasting is known to induce body weight loss in rodents (Dietze et al., 2016). Our data suggest that the fasting-induced body weight loss may have been associated with treatment effects. There may have been minor changes occurring in body weights due to treatments which were probably unmasked due to fasting-induced stress. Upon fasting, females tended to lose more body weight than males. Female controls and those exposed to G3-N and G3-U oils lost 0.9%, 8% and 9% of their body weight, respectively, while males subjected to similar treatments lost 0%, 3% and 5%, respectively. Females exposed to G4-N and G4-U oils lost 4% and 9%, respectively, while males subjected to these treatments lost 3% and 4%, respectively. Moreover, the body weights for rats exposed to new oils tended to be less affected by treatments as compared to those exposed to their used versions, regardless of sex (Figs. 1A&B; 2A&B). Although we did not measure their fecal and urinary outputs during the fasting challenge, fasting may have increased the rat's metabolic functions, thus increasing their fecal and urinary excretion and contributing to weight loss. Food deprivation or restriction for animals has been associated with increasing metabolic and psychological stress (Toth and Gardiner, 2000). Limiting water intake and/or increasing fecal and urinary outputs can have a noticeable effect on an animal's body weight. The weekly food and water consumption measurements taken during treatments and before fasting indicate that treatments did not influence the food and water intake. Although we did not assess the water consumption during the fasting period, we cannot rule out that oil treated rats reduced their water intake, which may also have contributed to the body weight loss trend observed at the end of the treatment period. Studies have indicated that rats exposed to organophosphates can significantly lose weight. The USA National Toxicology Program (National Toxicology Program, 1994) has reported that male and female F344/N rats that received (by gavage) the doses of mixed isomer preparation of 79% TCP esters (consisting of 21% tri-*m*-cresyl, 4% tri-*p*-cresyl, 1% tri-*o*-cresyl and other unidentified tri-cresyl phosphate esters) ranging between 1450 mg/kg and 5800 mg/kg body weight, 5 days per week, for a total of 13 or 14 doses in a 16-day period have mean body weights significantly lower than those of the controls. It has also been shown that exposure to TPP can alter the rat's body weight gain (Sutton et al., 1960). When male Holtzman rats were fed with 0–0.5% TPP (equivalent to 0–3632 mg/kg body weight) for 35 days, the body weight gain for rats that received the highest dose decreased by 7% relative to controls (Sutton et al., 1960). Taib et al. (2013) reported that exposing (orally by gavage) male Sprague-Dawley rats to a 20 mg/kg body weight of an organophosphate compound (fenitrothion) for 28 days induced significant adverse effects, including a decrease in body weight gain and attributed this decrease to systemic toxicity. Thus, the organophosphates in the oil mixtures may also have contributed to decrease in body weight. It is interesting to note that the trend in fasting-induced body weight loss observed for rats exposed to G3 and G4 oils at the end of the treatment period was not observable with rats in the recovery groups. This may suggest that the treatment residues were fully cleared from the rat's body systems.

4.2. Alteration of reproductive organ weights of Sprague Dawley rats

Our findings indicate that only G3-N decreased the weights for testes. We analyzed the organophosphate contents of these oils and observed that both versions G3 oils contained a mixture of four TCP isomers. The concentration of these isomers combined in G3-U decreased by 26% relative to those in G3-N (Table 1), suggesting that the potential toxicity of TCP in G3 oil is weakened as the oil ages. This may explain the observation that the unused version of G3 affected the weights of testes while the used version of this oil did not significantly affect this reproductive organ. These results are in agreement with Latendresse et al.'s findings (Latendresse et al., 1994a; Latendresse et al., 1994b) who reported that F344 rats exposed to TCP decreased testicular weights. The mechanisms of testicular degeneration caused by TCP exposure are not well known. Thus, more studies are needed to elucidate the TCP-induced testis weight decrease.

Results for this study show that both versions of G3 and the unused version of G4 oils reduced uterus weights relative to controls. Since our study did not control the estrous cyclicity for females, we cannot rule out that it may have contributed to the alteration of uterine weight. None of the oils had a significant impact on epididymis and ovarian weights. The effects of organophosphate compounds on testes, epididymis, uterus and ovaries have been extensively studied in animals. Some of the results for these studies are in agreement with our findings. For instance, Choudhary et al. (2008) reported a significant reduction in the weight of testes for male Wistar rats orally dosed with malathion ranging from 50 mg/kg to 250 mg/kg body weight per day for 60 days. A study carried out by Dhondup and Kaliwal (1997) indicates that the IP administration of 5 mg/kg body weight of methyl parathion to normal hemicastrated virgin rats for 15 consecutive days induced a decrease in uterine weight. Although G3 and G4 oils did not have significant effects on epididymis and ovarian weights, other investigators have reported an alteration in their weights following rat exposure to organophosphates (Dhondup and Kaliwal, 1997; Latendresse et al., 1994a; Latendresse et al., 1995; Latendresse et al., 1994b; Choudhary et al., 2008; Prashanthi et al., 2006). Although our study did not investigate the effects of G3 and G4 oils on rat fertility, other studies have highlighted the adverse effects of organophosphates on the fertility of mice and rats (Mitra and Maitra, 2018; Ngoula et al., 2007). Mathew et al. (1992) have reported an increase in sperm shape abnormalities for Swiss albino mice after 1 week and 5 weeks following a single acute oral exposure to four different doses of methyl parathion, an organophosphate pesticide used in agriculture. A relatively small dose of this compound (i.p. injection of 0.5–1.5 mg/kg/day for 12–25 days) induced the formation of abnormal sperms of male Wistar rats and a reduction in the sperm count (Narayana et al., 2005). Exposing rat females to organophosphate compounds is associated with a decrease in healthy follicles, follicular dynamics, estrous cycle and reproductive performances (Asmathbanu and Kaliwal, 1997; Dhondup and Kaliwal, 1997; Sortur and Kaliwal, 1999; Choudhary et al., 2008). Kaur and Dhanju (2005) have also reported that exposure to a low dose of methyl parathion for 90 days decreased the phospholipids, protein, total lipids and cholesterol levels in the ovaries for albino rats. All these observations highlight various adverse effects associated with exposure to organophosphate compounds. Although the rats in our study were dermally exposed, we cannot rule out that

changes in the reproductive organ weights that we observed for male and female Sprague Dawley rats exposed to G3 and G4 engine oils were inherent to toxicity associated with the oil's organophosphates or any other ingredients.

4.3. Alteration of liver, adrenal and spleen weights of female Sprague Dawley rats

Data taken at the end of both treatment and recovery periods indicate that the weights for brain, liver, left kidney, right kidney, adrenals, spleen, thymus and heart of male Sprague-Dawley rats were not affected by G3 (Table 2) and G4 (Table 4) oils. Similar observations were noted with females only at the end of the treatment period (Tables 3 & 5). During the recovery period, G3-N elevated the liver weights for females (Table 3) while the unused version of G4 increased adrenal and spleen weights (Table 5). Other researchers have also observed changes in liver weights for animals exposed to organophosphate compounds (Sutton et al., 1960; Xu et al., 2016). Sutton et al. (1960) reported a 25% increase in liver weight for male Holtzman rats exposed to a sub-chronic low dose of TPP. Our data suggest that females are more likely to experience adverse effects resulting from exposure to G3 and G4 oils. Hinton et al. (1987) reported that when male and female Sprague Dawley rats were fed with a diet containing TPP doses ranging from 0 to 1% daily for 120 days, these treatments did not alter the weights for their thymus and spleens. Although the route of administration of TPP and doses applied in the Hinton et al. study and ours were different, their findings are in agreement with our data for G4 oil which contains TPP and did not impact the weights for thymus. However, these data raise two questions that remain to be answered: (1) why the delayed treatment effects were only observed with females and (2) which molecular mechanisms are involved in altering the female's weights of liver, adrenals and spleen.

4.4. Alteration of hematological parameters of Sprague Dawley rats

Hematological assessment provides useful information about the extent of toxicity of drugs or toxicants. Alterations in hematological parameters are considered as crucial biomarkers of physiological stress (Akinrotimi et al., 2009). In this study, we report the profiles of hematological parameters for male and female Sprague Dawley rats dermally exposed to new and used versions of grade 3 and 4 aircraft engine oils over a period of 21 days (total 15 exposures). We found noticeable changes in hematological profiles, regardless of rat sex (Tables 6–9). In general, new oils induced more changes in blood parameters for both males and females when compared to the performance of their used versions. Males exposed to G3-N were characterized by an increase in eosinophil levels (both eosinophil count and eosinophil %) while blood indices for those treated with G3-U were not significantly affected (Table 6). G4-N did not influence blood parameter levels while G4-U increased hematocrit (Table 8). Although the G3 and G4-induced exposure effects were resolved during the recovery period, G3-N increased the levels of white blood cells and lymphocytes for males during this period (Tables 6 & 8). Overall, females were more susceptible to oil exposure than males. Their response to G3-N was to decrease the levels of white blood cells, lymphocytes and hematocrit while G3-U increased monocytes and monocyte % (Table 7). Female response to G4 oils was more pronounced relative to G3 performance. Those treated with G4-N had decreased levels of white blood cells, neutrophils, lymphocyte count, eosinophils, eosinophil % and increased mean platelet volume while those exposed to G4-U had decreased levels of eosinophils and eosinophil % (Table 9). All these effects were resolved during the recovery period (Table 9).

It is interesting to note that all the blood parameters that were affected by G3 and G4 oils in males were increased while those affected by these treatments in females were decreased except mean platelet volume which was elevated for G4-N. Both unused versions of G3 and G4 oils significantly influenced the levels of white blood cells regardless of sex. G3-N increased the levels of white blood cells in males which decreased in females. However, this effect in males was not observed until the end of the recovery period while the response in the females was noticeable at the end of treatments. The differences in response to oil exposure between males and females may derive from their differences in hormones. White blood cells, neutrophils, eosinophils, monocytes, basophils and lymphocytes are essential for the immune system involved in body's protection against infections and foreign invaders. Any adverse effect on these parameters might be regarded as an alteration of the immune system. Kumar et al. (2018) have suggested that leukocyte levels in blood could serve as a simple marker for assessment of organophosphorus poisoning. Although the mechanisms by which aircraft engine oils significantly altered the levels of white blood cells, neutrophils, lymphocytes, eosinophils, eosinophil % and monocyte % in Sprague Dawley rats are not yet known, it is worthy to suggest that organophosphate components of these oils may have been a key player in the alteration process. This assertion is supported by various studies which reported adverse changes in blood indices for farm workers occupationally exposed to organophosphate pesticides (Al-Sarar et al., 2009; Andreadis et al., 2013; Fareed et al., 2013; Nassar et al., 2016). Hematocrit is another blood parameter that was affected by engine oil exposure. Our data on this parameter in males exposed to G4-U oil are in disagreement with Lox's (1983) findings who reported a decrease in hematocrit and platelet counts for Sprague Dawley rats which were treated with 1 mL of malathion (99% pure), 1750 ppm given orally via gavage. Nevertheless, Lox's findings corroborate with our data for females exposed to G4-N oil. This oil also elevated mean platelet volume levels in females. Mean platelet volume is the most accurate measure of size of platelets and is inversely associated with platelet count (Levin and Bessman, 1983; Thompson and Jakubowski, 1988). An increased mean platelet volume is an indicator of larger and more reactive platelets resulting from an elevated turnover (Martin et al., 1983) which represents a risk factor for overall vascular mortality (Slavka et al., 2011). Some studies have reported a decrease in mean platelet volume levels for farm workers exposed to pesticides (Nassar et al., 2016; Varol et al., 2014). Although it is may be worthy to insinuate that male rats resisted the G4-N-induced biochemical events that led to alteration of mean platelet volume levels in females, the mechanisms at play remain to be elucidated. Although the results show only limited toxicity they suggest the need for additional more focused studies. Additional endpoints are still being evaluated and will be reported in the future.

In general, new oils induced more changes in blood parameters for both males and females when compared to the performance of their used versions, suggesting that the potential toxicity associated with the unused versions of the oils was reduced as they aged. A possible explanation could be that during the aircraft engine operations, the extremely high temperatures in running engines may have been conducive to the breakdown of the oil's ingredients into products that were less toxic relative to their parents. This assertion is supported by our data on organophosphate contents of oils which decreased their levels in used oils relative to those measured in their unused versions (Table 1). Overall, females were more susceptible to oil exposure than males. These differences may have resulted from sex difference and/or treatment dose since females weighed less than males and all animals were exposed to the same dose of 300 μL of oil regardless of their weights. In other words, females may have received a higher dose compared to males. There probably was a difference in plasma exposure between males and females. Although it would be nice to know the concentration of organophosphates in the blood of the rats, it would be difficult to measure because the small percent of each organophosphate compound in the oil slowly being absorbed through the skin is then diluted into the total blood volume of the rat. It may only be possible to detect in blood by using a radioactive organophosphate. The use of radiotracers were beyond the scope of this study. The justification of applying the same dose of oil to all animals regardless of sex and body weights was to mimic what may happen in a real world environment where male and female aircraft maintenance workers may similarly be exposed to engine oils. Further studies of this nature in which sex and body weights are considered when dosing animals and determining extrapolation to humans are needed. The purpose of this study was limited to assessing the potential toxicity of G3 and G4 oils in their new and used states to organ weights and hematology parameters. In the future studies, we will include other endpoints and report about the full

consequences for the animals' health and how the results translate to the occupational setting. Conducting detailed analytical assessment of both versions of these oils may throw light on the status of their ingredient composition, especially the organophosphate degradation by-products such as xylenyl and ethylphenyl phosphates which have been reported to exhibit a similar toxicity to ortho substituted TCP isomers (Winder and Balouet, 2002; Bondy et al., 1960).

5. Conclusion

This study revealed that a subchronic exposure to G3 and G4 oils in both their new or used states altered the weights of the reproductive organs and hematological indices for male and female Sprague Dawley rats. Some of these alterations are similar to changes that have been observed with agricultural workers occupationally exposed to organophosphate pesticides. All these observations resulting from just three weeks of exposure to G3 and G4 oils raise concerns about the magnitude of impact related to prolonged exposure in the real world environment; aircraft maintenance workers may be exposed for many years.

Author's contributions

Dr. Isaie Sibomana and Dr. David R. Mattie were involved in the study design and all aspects related to the implementation of the study and reporting the findings.

CRediT authorship contribution statement

Isaie Sibomana: Conceptualization, Methodology, Validation, Investigation, Supervision, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing.**David R. Mattie:**Conceptualization, Methodology, Project administration, Funding acquisition, Resources, Validation, Investigation, Supervision, Visualization, Writing - review & editing.

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.

Acknowledgements

The authors wish to thank (1) Naval Medical Research Unit (NAMRU) – Dayton, Ohio, for assisting in hematological analyses; (2) the staff of the Research Support Center, Air Force Research Laboratory (AFRL)/711 HPW/RHDV, Wright-Patterson Air Force Base (WPAFB), Ohio, for animal work support; (3) Dr. Lewis Rosado and Mr. Patrick T. Hellman, AFRL, Aerospace Systems Directorate, Turbine Engine Division, Engine Mechanical Systems Branch (AFRL/RQTM), WPAFB, Ohio, for consultation and operational justification for the study and (4) AFPET for providing unused aircraft engine oils. Dr. Isaie Sibomana also wishes to thank the staff of the Consortium Research Fellow Program, Consortium of Universities of the Washington, DC Metropolitan Area, 1100H Street NW, Washington, DC 20005, USA, for supporting his post-doctoral fellowship. This manuscript was approved for public release (PA Case No. 88ABW-2019-3733, 15 Aug 2019).

Funding

The funding for this work was provided through the Aerospace Toxicology Program in the Air Force Research Laboratory, 711 HPW/RHBB (formerly Molecular Bioeffects Branch, 711 HPW/RHXD and RHXJ).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crtox.2020.02.001.

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