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Comparative 90-day dietary study of paraffin wax in Fischer-344 and Sprague–Dawley rats ☆

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ABSTRACT

Highly refined mineral hydrocarbons (MHCs) such as low melting point paraffin wax (LMPW) and low viscosity white oils can cause inflammatory changes in the liver and mesenteric lymph nodes (MLNs) of the Fischer-344 (F-344) rat. In contrast, only minimal MLN changes are seen in the Sprague–Dawley (S–D) rat with no changes in the liver. In this study, the response of female F-344 and S–D rats was compared after 90 days dietary treatment with 0%, 0.2% or 2% LMPW. Effects in the F-344 rats were significantly greater than in the S–D rats: increased liver and splenic weights and inflammatory changes (hepatic microgranulomas) in these tissues were observed only in the F-344 rats. Microgranulomas in the MLNs were observed in both strains but the effects were substantially greater in the F-344 rats. Cellular markers of inflammation were examined in a subset of rats from each group using immunohistochemical staining. An increase in staining for CD3 (T-cells), CD8a (suppresser/cytotoxic T-cells) and CD4 (helper T-cells) correlated with an increase in lymphoid cells in the livers of treated F-344 rats. The majority of macrophages in the hepatic microgranulomas of treated F-344 rats were negative for the ED2 marker, indicating a likely origin from non-resident macrophages. Electron microscopy showed Kupffer cell hypertrophy and hyperplasia in treated F-344 rats. However, lysozyme staining (indicating activation of epithelioid macrophages) decreased with increasing granuloma size. Non-ED2 expressing cells may have been recruited but not sufficiently activated to be lysozyme positive. Inflammatory changes in the cardiac mitral valve noted in previous studies of LMPW were also seen in the F-344 rats in this study but not in the S–D rats. Chemical analysis showed that MHC accumulated in livers from treated F-344 but not S–D rats and the concentration was more than 2-fold greater in MLNs from the F-344 than from the S–D rats. The F-344 appears to be more immunologically sensitive to a number of agents than other rat strains and the results of this study suggest that this may contribute, along with pharmacokinetic differences, to the inflammatory response of F-344 rats to dietary MHCs.

Abbreviations: ALT, alanine aminotransferase; ALKP, alkaline phosphatase; AST, aspartate aminotransferase; cSt, centistokes; CRD, certified rodent diet; EM, electron microscopy; F-344, Fischer-344; GC/MS, gas chromatography/mass spectroscopy; GGT, gamma-glutamyl transferase; H&E, hematoxylin and eosin; LMPW, low melting point paraffin wax; LOQ, limit of quantitation; LVWO, low viscosity white oil; MHC, mineral hydrocarbon; MLN, mesenteric lymph node; RBC, red blood cell; S–D, Sprague–Dawley; SD, standard deviation; WBC, white blood cell.

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1. Introduction

Highly refined mineral hydrocarbon (MHC) products (white oils and petroleum waxes) are widely used in foods, food packaging, cosmetics and pharmaceutical products. Historically, the safe use of these products for human consumption has been supported by a number of subchronic and chronic feeding studies in dogs and various rat strains (Hulse et al., 1992; Smith et al., 1995; Shubik et al., 1962). The studies of Shubik et al. involved feeding high concentrations (10%) of different waxes to rats with no significant toxic effects after a lifetime exposure. In later studies, however, F-344 rats fed relatively high levels of some mineral oils and waxes developed inflammatory changes in tissues, principally in the liver and mesenteric lymph nodes (MLNs) (Baldwin et al., 1992; Smith et al., 1996). Female F-344 rats had a slightly greater response than males.

When several types of highly refined food-grade white oils and waxes were tested in 90-day feeding studies in F-344 rats (Smith et al., 1996), the higher molecular weight products (microcrystalline waxes and higher viscosity oils) were without biological effects, while paraffin waxes and low- to mid-viscosity oils produced biological effects that were inversely related to molecular weight, viscosity and melting point. Additionally, the type of crude oil the MHC was derived from and the refining method did not appear to affect the response.

Of the MHCs tested in the F-344 rat, low melting point paraffin wax (LMPW) produced the most liver and MLN histopathological changes associated with inflammatory macrophages. Food-grade paraffin waxes are saturated solid hydrocarbons made up mainly of linear alkanes with varying proportions of branched alkanes and smaller amounts of cyclo-alkanes. LMPWs which have melting point ranges of about 45–60 °C are derived from lower boiling base oil distillates and have viscosities ranging from 3 to 6 centiStokes (cSt; mm^2/s) at 100 °C. In addition to the liver and MLN effects seen with other low-viscosity MHCs, inflammatory changes in the cardiac mitral valve were also observed in F-344 rats fed LMPW (Smith et al., 1996; Scotter et al., 2003).

In a direct comparative study where F-344 rats and a common S–D-derived strain were both fed a low viscosity, 15 cSt, paraffinic white mineral oil for 90 days, the S–D rats showed only a slight increase in inflammatory cells in the liver at the highest dose with no formation of microgranulomas. In contrast, the F-344 rats had hematological and clinical chemistry changes, increased liver, MLN and splenic weights, and microgranulomas in the liver and MLNs (Firriolo et al., 1995). The MHC concentrations determined by chemical analysis in the MLNs from both rat strains were similar but the F-344 rats had a much greater liver burden of MHC than the S–D rats. The absence of significant findings in other species and in other rat strains suggests that the F-344 rat may be particularly sensitive to these lower viscosity/molecular weight MHCs.

The objective of the present study was to further investigate possible mechanisms for the different biological response to lower viscosity or lighter MHCs between F-344 and S–D rats, a representative less sensitive strain. LMPW was selected as the test material because of its greater potential to cause liver and MLN microgranulomas compared to other MHC products. Additional objectives of this study were to characterize the cellular responses in the liver and MLNs using immunohistochemical techniques and electron microscopy (EM) and to determine the concentration and size range of hydrocarbons found in target tissues.

2. Materials and methods

2.1. Test animals and maintenance

Female F-344 rats (CDF® CF-344/CRLBR) and female S–D-derived rats (CRL:CD) were obtained at approximately 4 weeks of age from Charles River Laboratories, Raleigh, NC, and acclimated for 14 days before use. For the first week of acclimation, two rats of the same strain were housed in the same cage; thereafter, the rats were individually housed. The animal rooms were maintained at 20–23 °C and 34–76% relative humidity with a 12:12 h light/dark cycle. A certified rodent diet (CRD) in meal form (No. 5002, PMI Feeds Inc., St. Louis, MO, USA) and tap water were available *ad libitum*. This study was conducted under US federal guidelines for the care and use of laboratory animals in a facility fully accredited by the American Association for Accreditation of Laboratory Animal Care.

2.2. Test material

The test LMPW (CAS number 8002-74-2) was a pelleted white crystalline solid refined by hydrogenation from crude oil and had the following characteristics: viscosity 3.35 cSt at 100 °C; melting point 55 °C; oil content 0.1%; sulfur content < 5 ppm; carbon number of *n*-alkanes C_{19}–C_{42}. The pelleted test material was stored in a closed container at ambient laboratory temperature.

2.3. Test diets

The test material was incorporated into the diet at two concentrations, 0.2 or 2.0% (w/w). The LMPW pellets were first converted to a fine powder by generating a fine spray of molten LMPW with an atomizing nozzle operated with heated nitrogen and then cooling the sprayed material (Walters et al., 1994). The LMPW powder was stored frozen (−20 °C) until used to prepare the test diets. Fresh batches of test diet were prepared weekly and stored in sealed containers under refrigeration until use. Dietary concentration, homogeneity and stability were verified using the hydrocarbon analysis methodology described below. Dietary concentration was determined at study weeks 1, 2, 3, 4, 9 and 13; all samples were 90–101% of the nominal concentration. In the homogeneity determinations, the mean recoveries ± standard deviation (SD) at the 0.2% and 2.0% treatment levels were 98.5 ± 3.85% and 99.1 ± 4.74%, respectively.

The ability of the rats to differentiate between the powdered test material and CRD and thus selectively consume CRD was assessed by determining the test material concentration of samples taken from the feeders of five rats per strain from the high-dose groups at the end of a 7-day feeding period. No increase in test material concentration was observed in the samples, confirming that the rats had not selectively consumed CRD.

2.4. Study design

Groups of F-344 or S–D rats were fed LMPW at dietary concentrations of 0.2% or 2% *ad libitum* for up to 90 days (Table 1). Control rats received CRD *ad libitum* for the same periods. Ten control and high-dose rats of each strain were euthanized and

Table 1
Experimental design of 90-day feeding study with low melting point paraffin wax in female Fischer-344 (F-344) and Sprague–Dawley (S–D) rats.

Dose group	Rat strain	Dietary concentration (% w/w)	Mean dose (mg/kg/day)	Number of rats evaluated at specified interval					
				Subchronic study animals			Only used for analyses of tissues for mineral hydrocarbon		
				30 days	60 days	90 days	30 days	60 days	90 days
I	F-344	0	0	10	10	10			5
IV	S–D		0	10	10	10			5
II	F-344	0.2	157			10			5
V	S–D		160			10			5
III	F-344	2	1609	10	10	10	5	5	5
VI	S–D		1644	10	10	10	5	5	5