Abstract: White oils or waxes [mineral hydrocarbons (MHCs)] with substantial levels of saturated hydrocarbons in the range of C18 to C32 have produced hepatic microgranulomas and lymph node microgranulomas (also referred to as histiocytosis) after repeated administration to female Fischer-344 (F-344) rats. Female Sprague-Dawley (S-D) rats are less sensitive to these MHC-induced hepatic and lymph node effects. Studies reported herein characterize the pharmacokinetics and disposition of a representative C-26 MHC, [1-14C]1-eicosanylcyclohexane ([14C]EICO), in these two rat strains. Female F-344 and S-D rats were administered by oral gavage either a high (1.80 g/kg) or a low (0.18 g/kg) dose of MHC in olive oil (1:4, v/v) containing [14C]EICO as a tracer. Blood, urine, feces, liver, and mesenteric lymph nodes (MLNs) were analyzed for [14C]EICO and 14C-metabolites. After the high dose, F-344 rats had a higher blood Cmax of [14C]EICO, a longer time to Cmax, and a greater area under the systemic blood concentration-time curve from zero to time infinity compared with S-D rats. After the low dose, F-344 rats displayed a unique triphasic blood concentration-time profile, meaning two distinct Cmax values were observed. Fecal excretion was the major route of [14C]EICO elimination for both rat strains (70–92% of the dose). S-D rats eliminated the majority of [14C]EICO metabolites recovered in the urine by 16 h (8–17% of the dose), whereas F-344 rats did not excrete the same amount until 72 to 96 h. Beyond 24 h, a greater level of [14C]EICO was recovered in livers of F-344 rats; at 96 h, 3 and 0.1% of the dose was retained in livers of F-344 and S-D rats, respectively. The major urinary metabolites of EICO in both rat strains were identified as 12-cyclohexyldecanoic acid and 10-cyclohexyldecanoic acid. Based on the pharmacokinetic parameters and disposition profiles, the data indicate inherent strain differences in the total systemic exposure, rate of metabolism, and hepatic and lymph node retention of [14C]EICO, which may be associated with the different strain sensitivities to the formation of liver granulomas and MLN histiocytosis.
to more extensive accumulation/retention of MHCs in the livers of F-344 rats.

Lonardo et al. (1998) evaluated rat strain differences in the pharmacokinetics and disposition of n-octadecane (C18), a representative lower molecular weight hydrocarbon in white oils and waxes (Lonardo et al., 1998). Female S-D rats, after a single oral gavage dose of [14C]octadecane (2 g/kg), exhaled greater amounts of 14CO2 and had lower levels of radioactivity in liver 48 h postexposure than did similarly treated F-344 rats. These data suggested that S-D rats may be more efficient at metabolizing and clearing MHCs compared with F-344 rats after a single oral gavage dose. Thus, strain differences in the absorption, distribution, metabolism, and/or elimination of MHCs may play an important role in the observed differential toxicological responses observed in the MHC feeding studies.

Additional pharmacokinetic and disposition studies were conducted to test this hypothesis further. These studies were designed to better define inherent strain differences in the pharmacokinetics, disposition, and metabolism of MHCs. [1-14C]-Eicosanylcyclohexane (14C[EICO], illustrated in Fig. 1, was chosen as the surrogate MHC in these studies because it contains 26 carbons, the peak or average carbon number in many of the complex mixtures of MHCs (Concawe, 1984). 14C[EICO] was labeled with 14C in the cyclohexane ring, which minimizes the loss of 14C signal due to exhalation of 14CO2 and 14C-organics after metabolism.

Materials and Methods

Chemicals. The following chemicals were purchased from the vendors indicated: [1-14C]-eicosanylcyclohexane, specific activity 17 mCi/mmol (99.9% pure; Moravek Biochemicals, Brea, CA); 1-eicosanylcyclohexane and food-grade white mineral oil with a viscosity 15 centistokes at 40°C, an average molecular weight 350, and specific gravity approximately 0.85 g/ml (American Petroleum Institute, Washington, DC); olive oil, reagent grade, specific gravity approximately 0.91 g/ml (ICN Pharmaceuticals Biochemicals Division, Aurora, OH); ethylene glycol and 2-methoxyethyl ether (Mallinckrodt, Paris, KY); Carbosorb E and Flo-Scint III (Packard Instrument Company, Inc., Meriden, CT); tissue solubilizer TS-2 (Research Products International Corp., Mount Prospect, IL); toluene, high-purity solvent (Burdick and Jackson, Inc., Muskegon, MI); and Universeal Cocktail (ICN Pharmaceuticals, Irvine, CA).

Animal Studies. Animals. Female F-344 and S-D rats and male S-D rats in a weight range of 175 to 199 g were purchased from Hilltop Laboratory Animals, Inc. (Scottsdale, PA). Upon arrival, the animals were acclimated for 5 to 7 days in a temperature-controlled room (20–22°C) with a 12:12-h light/dark cycle before any treatment. Food (Teklad 4% Mouse-Rat Diet; Harlan Teklad, Madison, WI) and water were provided ad libitum. Some of these animals were fitted with an indwelling jugular vein cannula (JVC).

Dosing Solutions. Radiolabeled [14C]EICO, a representative MHC with 26 carbons (Fig. 1), was incorporated as a tracer in the dosing solutions to characterize the pharmacokinetics and disposition of MHCs. The dosing solutions were prepared in a stepwise procedure. Approximately 400 μl (400 μCi) of [14C]EICO in hexane was added to 1.6 ml of white oil and warmed slightly to remove the hexane volume and facilitate dissolution. Once dissolved into solution, 6.4 ml of olive oil was added to the white oil/[14C]EICO mixture (1.6 ml) without further warming and thoroughly mixed. The resulting solution (8.0 ml) was allowed to cool to room temperature before dosing. In the high-dose study, each rat was administered a single oral gavage dose (dosing volume 2 ml/kg) of MHC in olive oil (1:4, v/v) containing [14C]EICO (2.14 mg/kg; 100 μCi/kg). Using the densities of 0.85 g/ml (white oil) and 0.91 g/ml (olive oil), the dose of MHC was 340 mg/kg and that of olive oil was 1.46 g/kg. This represents a total oil dose (MHC + olive oil + [14C]EICO) of 1.80 g/kg.

In the low-dose study, each rat received 0.2 ml/kg of the dosing solution described above. Therefore, the dose of [14CEICO] was 0.21 mg/kg (10 μCi/kg), the dose of MHC was 34 mg/kg, and that of olive oil was 0.15 g/kg. This represents a total oil dose of approximately 0.18 g/kg.

Routes of Elimination Study. All rats were fasted for 18 h before oral administration of MHCs, but continued to receive water during this time. After dosing (1.80 g/kg; 100 μCi/kg), rats were immediately placed individually into sealed glass metabolism cages maintained with a constant inflow of ambient air. Two hours after dosing, food was reintroduced ad libitum. Urine, feces, and exhaled organics and CO2 were collected at 8, 16, 24, 48, 72, and 96 h. Total airflow through each cage was passed through a series of traps containing either 2-methoxyethyl ether for collection of expired 14C-organics or Carbosorb E and ethylene glycol (2:1, v/v) for collection of expired 14CO2. All trapping solvents were collected and changed at selected times and measured for total radioactivity by direct liquid scintillation counting (LS5000TD liquid scintillation counter; Beckman Coulter, Inc., Fullerton, CA). Rats were killed at 96 h by inhalation of carbon dioxide. Blood was immediately collected from the inferior vena cava into a heparinized syringe, and liver, mesenteric lymph nodes (MLNs), kidneys, lung, heart, spleen, and subcutaneous fat were excised. All samples were weighed and stored immediately at −80°C until analyzed. Direct liquid scintillation counting was used to determine total radioactivity associated with urine and exhaled products. Samples of homogenized feces and selected tissues were oxidized to 14CO2 (Oxidizer 306; Packard Instrument Company, Inc., Downers Grove, IL) (Winter et al., 1992). Each oxidized product was collected into scintillation fluid and then counted directly for total radioactivity by direct liquid scintillation counting. A body composition estimate of 9% of body weight for subcutaneous fat was used (Mathews and Anderson, 1975).

Oral Pharmacokinetic and Disposition Studies. Rats with indwelling JVCs were orally administered either a high dose (1.80 g/kg) or a low dose (0.18 g/kg) of MHC, olive oil, and [14C]EICO tracer as described above. In another study, eicosanylcyclohexane was substituted for MHC. Female rats of both strains were administered a single high oral dose of eicosanylcyclohexane (340 mg/kg + 2.14 mg/kg [14C]EICO) in olive oil (total oil dose 1.80 g/kg). After administration, rats were immediately placed individually into Nalgene metabolism cages to allow collection of urine and feces at selected times. Two hours after dosing, food was reintroduced ad libitum. Serial blood samples (200 μl) were collected through the JVC at selected times (0.5–96 h), and an equal volume of saline was injected to replace the blood volume. Blood samples were measured directly for 14C equivalents by direct liquid scintillation counting (3 × 50 μl of whole blood). At least three rats per strain were killed at 4, 8, 16, 24, 48, 72, and 96 h by carbon dioxide asphyxiation in the high-dose study, whereas only six rats per strain were killed at 96 h in the low-dose study. At these times, blood, liver, and MLNs were collected. Direct liquid scintillation counting was used to determine the total 14C associated with urine. Homogenized fecal and selected tissue samples were prepared for radioactive counting by the addition of 1 ml of the tissue solubilizer TS-2. Once the samples were completely solubilized, glacial acetic acid was added to eliminate chemiluminescence and, together with scintillation cocktail, made possible accurate liquid scintillation counting for total 14C.

Metabolite Identification Study. Male S-D rats were orally administered a high dose (1.80 g/kg) of eicosanylcyclohexane (unlabeled material), olive oil, and the [14C]EICO tracer. After administration, rats were immediately placed individually into Nalgene metabolism cages to allow collection of urine at 4, 8, 16, 24, 48, 72, and 96 h. Urine samples were analyzed by high-performance liquid chromatography (HPLC) to determine the metabolite profile and by liquid chromatography with tandem mass spectrometry (LC-MS/MS) to identify the major urinary metabolites.

Data Analysis. The blood concentration-time data after a single oral gavage dose of MHC, olive oil, and [14C]EICO to female JVC F-344 and S-D rats were analyzed by compartmental methods using nonlinear regression analysis (WinNonlin; Scientific Consulting, Inc., 1995). F-344 rats administered the low MHC dose exhibited an unexpected and unique systemic blood concentration-time curve at early time points (0.5 to 24 h). Therefore, these data were analyzed using noncompartmental methods as described by Rowland and...
HPLC method for $^{14}$C-EICO and $^{14}$C-metabolites at varying times. Whole blood samples were collected from each rat and centrifuged to separate the plasma. Plasma was then removed and concentrated by vacuum centrifugation to approximately 200 µl. Solubilized fecal, liver, and MLN samples that were counted for total radioactivity (described above) were also analyzed by HPLC. All samples were centrifuged to remove any precipitate before being injected (100 µl) onto a Luna C$_{18}$ analytical column (250 × 4.6 mm; Phenomenex, Torrance, CA) and eluted with a mobile phase of water, methanol, and toluene; a flow rate of 1 ml/min; and a total run time of 210 min. The mobile phase gradient was run from 100% water to 100% methanol over a 177.5-min time period. After 5 min of 100% methanol, the gradient then proceeded to 100% toluene, which was held for 5 min. The column was brought back to initial conditions over 25 min. The HPLC system used throughout was composed of a SP8800 ternary HPLC pump and SP8775 autosampler (Spectra Physics, San Jose, CA). The column effluent was monitored radiochemically with a β-Ram Flow-Through Monitor System (IN/US Systems, Inc., Tampa, FL). The retention time ($R_T$) of $^{14}$C-EICO was 195 min under these conditions.

For analysis of urinary metabolites associated with $^{14}$C-EICO, collected urinary samples at each time point were pooled, diluted (1:1, v/v) with distilled water, thoroughly mixed, and centrifuged at low speed to remove any precipitate. Prepared urine samples (100 µl) were analyzed using the same HPLC system, column, and gradient conditions as described above.

**Isolation of Urinary Metabolites.** The two major urinary metabolites, labeled as peaks J and K, were isolated using solid phase extraction. Approximately 400 µl of urine was loaded onto a hydrophilic/lipophilic balance-solid phase extraction cartridge (3 ml; Waters, Milford, MA) and washed with ~1 ml of water. After washing, the metabolites were eluted with 1 ml of methanol. The methanol extract was analyzed by HPLC (same conditions described above) to confirm that only the two urinary metabolites were extracted and analyzed by LC-MS/MS to identify the urinary metabolites.

**Metabolite Identification Using Mass Spectrometry and MS/MS Analysis.** LC-MS was carried out using a Finnigan TSQ 7000 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) equipped with a Hewlett Packard HPLC system (Series 1050) consisting of an HPLC pump, automatic solvent degasser, and an atmospheric pressure chemical ionization (APCI) ion source. Extracted urinary metabolites were injected onto a Luna C$_{18}$ analytical column (250 × 4.6 mm; Phenomenex) and eluted with a mobile phase of water and methanol, a flow rate of 0.5 ml/min, and a total run time of 45 min. The HPLC mobile phase gradient was run from 90% water and 10% methanol containing 0.1% trifluoroacetic acid for 5 min and then to 100% methanol over 15 min. These conditions were held for 20 min before being brought back to initial conditions over 5 min.

Protonated ions were formed using positive APCI. The optimum positive ion APCI conditions included a nitrogen nebulizer pressure of 70 psi, a vaporizer temperature of 400°C, a capillary temperature of 250°C, a corona current of 4.0 µA, and a fragmentor voltage of 100 V. Ions with $m/z$ values corresponding to putative metabolites were subjected to collision-induced dissociation (CID) with argon gas within a tandem mass spectrometer and the subsequent product ion signal masses analyzed to produce a product ion mass spectrum. Logical fragmentation patterns observed in the resulting MS/MS spectrum provided further evidence as to metabolite identity.

**Statistical Analysis.** Statistical evaluations between F-344 and S-D rats were conducted using parametric and nonparametric procedures at the 5% level of significance. For the parametric procedures, a one-way analysis of variance program to assess significance was used (SigmaStat, 1994). If sig-
significant differences among the means were indicated, Student-Newman-Keuls method was used. For nonparametric procedures, the Kruskal-Wallis one-way analysis of variance on ranks was applied (Holland and Wolf, 1973). Statistical analyses of the fecal and urinary excretion data (Figs. 3 and 4, respectively) between the two rat strains were based on the individual amounts of $^{14}$C equivalents recovered from blood of rats after either a high oral dose (1.80 g/kg) or a low dose (0.18 g/kg) of MHC in olive oil. After administration of $[^{14}$C]EICO from blood of rats after either a high oral dose (1.80 g/kg) or a low dose (0.18 g/kg) of MHC to female F-344 (closed circle) and S-D (triangle) rats, the solid lines are the nonlinear regression fits of the data. Significant differences among the means were indicated, Student-Newman-Keuls method was used. For nonparametric procedures, the Kruskal-Wallis one-way analysis of variance on ranks was applied (Holland and Wolf, 1973). Statistical analyses of the fecal and urinary excretion data (Figs. 3 and 4, respectively) between the two rat strains were based on the individual amounts of $^{14}$C equivalents recovered during the sample collection periods (0–16, 16–24, 24–48, 48–72, and 72–96 h) and not based on the cumulative percentage of dose values graphically displayed in the figures. For example, the amount of $^{14}$C equivalents recovered in the fecal samples of F-344 rats during the 24- to 48-h collection period was significantly greater compared with the amount recovered from S-D rats, even though the cumulative data points overlap at 48 h in Fig. 3.

Results

Blood Kinetics. Fig. 2 displays the concentration-time data of $[^{14}$C]EICO from blood of rats after either a high oral dose (1.80 g/kg) or a low dose (0.18 g/kg) of MHC in olive oil. After administration of the high dose to F-344 rats, the $C_{max}$ of 110 ng/ml was obtained at 16 h ($T_{max}$). The blood concentration then declined in a log-linear manner until the end of the study at 96 h. In contrast, the $C_{max}$ in S-D rats was nearly 3-fold less (41 ng/ml) and occurred at an earlier $T_{max}$ of 6 h. After the low dose, S-D rats had even lower $C_{max}$ and $T_{max}$ values (27 ng/ml and 5 h, respectively), whereas F-344 rats displayed a triphasic blood concentration-time profile. An initial peak concentration of 44 ng/ml was reached at 2 h, which has been labeled the first phase in the triphasic curve. The concentration in blood then decreased until 6 h but then increased again to 34 ng/ml at 16 h (second and third phases, respectively). After this time, the blood concentration of $[^{14}$C]EICO declined log linearly until the end of the study at 96 h. After peak concentrations were achieved, a relatively slow elimination of $[^{14}$C]EICO from blood was apparent in both rat strains at the two doses. Pharmacokinetic parameters are presented in Table 1.

Excretion and Distribution Data. Fecal excretion of total radioactivity was the major route of elimination for both rat strains in the high- and low-dose studies (Fig. 3). After the high dose, 92% (F-344) and 88% (S-D) of the administered radioactivity was recovered in the feces by 96 h, whereas 76% (F-344) and 70% (S-D) was recovered after the low dose. Although comparable amounts of $[^{14}$C]EICO were excreted in the feces by 96 h within each study, fecal elimination was more rapid in S-D rats. At 16 h the majority of the administered radioactivity was present in the intestinal contents of F-344 rats (data not shown), whereas the bulk of $[^{14}$C]EICO had been excreted in the feces by S-D rats. At 16 h the majority of the administered radioactivity was present in the intestinal contents of F-344 rats (data not shown), whereas the bulk of $[^{14}$C]EICO had been excreted in the feces by S-D rats.

The percentage of dose eliminated in the urine was dose-dependent in both rat strains. At 96 h, 11% of the high dose and 22% of the low dose were excreted in the urine of F-344 rats (Fig. 4). By 96 h, 11 and 27% of the dose was recovered in the urine of S-D rats after the high dose and low dose, respectively. S-D rats excreted nearly all of the radioactivity recovered in the urine by 16 h, regardless of the dose (8% of the high dose and 17% of the low dose). On the other hand,
F-344 rats maintained relatively linear urinary elimination rates throughout the 96-h studies. Total recovery in urine and feces ranged from 97 to 103%. No radioactivity was associated with expired carbon dioxide or organic vapors (data not shown).

Livers of F-344 rats retained more of the dose of [14C]EICO than those of S-D rats (Fig. 5A). Maximum hepatic concentrations in F-344 rats occurred at 24 and 48 h (5 and 4% of the dose, respectively). At 96 h, 3% of the dose was still present in the liver. In S-D rats, maximum hepatic concentrations of 2% occurred at 16 and 24 h; however, by 96 h only 0.1% remained in livers of S-D rats. Liver samples were only collected at 96 h after the low dose of MHC (Fig. 5B). At that time, nearly 3% of the administered radioactivity was recovered in the livers of F-344 rats, compared with 0.5% in S-D rats. Thus, livers of F-344 rats retained more of the absorbed [14C]EICO compared with S-D rats regardless of the dose. Based on the 96-h data, no statistically significant differences in hepatic retention of [14C]EICO were seen between the two doses within each rat strain.

In the other target tissue analyzed, MLN of F-344 rats retained approximately 0.01% of the administered radioactivity at 96 h, compared with 0.003% in S-D rats after the high dose of MHC (Fig. 5C). At 96 h after administration of the low dose, 0.02 and 0.009% of the dose was present in F-344 and S-D rats, respectively (Fig. 5D).

When the retention of [14C]EICO at 96 h was compared as concentration (percentage of administered radioactivity per gram of tissue, or %/g) (Fig. 6), the concentration of [14C]EICO in each respective strain was similar in both liver and MLNs.

Less than 0.9% of the administered radioactivity was associated with the kidney (0.2%), lung (0.1%), heart (0.03%), spleen (0.04%), and subcutaneous fat (0.5%) at 96 h postadministration of MHCs to female F-344 rats.

When the large dose of EICO only (no added MHCs) was administered to female F-344 or S-D rats, the pharmacokinetic characteristics of [14C]EICO were similar to those obtained for this dose of [14C]EICO in the presence of added MHCs. For example, AUC (nanogram/hour per milliliter), Cmax (nanograms per milliliter), and Tmax (hours) values were 9500 and 2000; 132 and 49; and 19 and 6 for F-344 and S-D rats, respectively. The rates and routes of elimination and the liver retention of [14C]EICO (at 96 h) were also the same as those reported earlier for the high-dose MHC study.

**Determination of Urinary Metabolites.** HPLC analysis of pooled urine collected from rats treated orally with the high dose of MHC containing [14C]EICO revealed that only metabolites of EICO were excreted. A large number of metabolites were present, as evidenced by the HPLC radiochromatograms (Fig. 7). Interesting strain differences were observed with respect to the elimination of [14C]EICO metabolites in the urine. Although the profiles of 14C-containing peaks were the same for both rat strains, S-D rats eliminated the great bulk of these metabolites within the first 16 h. Only minute amounts were present in urine collected at 24 or 48 h, and none were present at 72 or 96 h. On the other hand, F-344 rats excreted metabolites much more slowly. Similar amounts of each metabolite appeared in the urine at all times.

The major urinary metabolite (peak K, Rr of 163 min) in both rat strains was identified as 12-cyclohexyldodecanoic acid (Fig. 8A). The protonated molecule ([M + H]+, m/z 283) of this metabolite was subjected to CID within a tandem mass spectrometer. The resulting product ion spectrum contained signals at m/z 265, 71, 57, 43, and 29.
was assigned to a structure resulting from the loss of C4H8O2. The fragment ([C4H8O2-H]+)
This peak had a retention time identical to that of [14C]EICO.
MLN samples regardless of dose (radiochromatograms not shown).
analysis in prepared plasma samples and solubilized fecal, liver, and
ingo possible inherent rat strain differences in pharmacokinetic param-
be due, in part, to differences in the disposition and metabolism of
strain-dependent responses to MHC is unknown, but the effects may
comments and disposition profiles after oral administration of a single dose. The data obtained demonstrate strain differences in pharmacokinetic and disposition profiles for this representative MHC. In general, the results show higher systemic exposure and enhanced hepatic retention of [14C]EICO in F-344 rats because of greater absorption and/or decreased ability to metabolize [14C]EICO and by extrapolation MHC.
Albro and Fishbein (1970) reported that MHCs (and/or their meta-
are mainly absorbed within the small intestine and are subsequently transported via the lymphatic and portal systems into the systemic circulation. Although not directly assessed, one or both of these processes may be more efficient in F-344 rats compared with S-D rats, thus resulting in the greater systemic blood exposure of [14C]EICO. Possible strain differences in the absorption processes may have been observed in the low dose studies. After administration of the low dose of MHC, a unique triphasic blood concentration-time profile (two distinct Cmax values) was seen in F-344 rats, but not in S-D rats. All F-344 rats (n = 6) displayed this type of profile, whereas all six S-D rats did not. The two Cmax values may be the result of two separate routes of MHC/[14C]EICO absorption. As reviewed by Porter and Charman (1997), the process of intestinal lymphatic drug transport often continues over time periods longer than typically observed for drug absorption via the portal vein. Based on this information, the initial rise in blood concentration (first phase) may be absorption via the hepatic portal system, and the second increase in blood concentration may be the result of absorption through the lymphatic system. This two-phase absorption profile was not detected in F-344 rats after the high dose, where the larger amount of MHC/[14C]EICO administered may have masked these processes. Interestingly, this type of profile was not detected in S-D rats regardless of the dose. This suggests that differences could exist between the two rat strains in the uptake of MHC/[14C]EICO into the systemic circulation.
Compared with small molecular weight, lipophilic agents, the ab-
sorption of [14C]EICO into the systemic blood circulation was a slow process. After administration of the high dose of MHC, Tmax did not occur until 6 and 16 h after oral dosing in the S-D and F-344 rats, respectively. This slow absorption process may relate to the extremely lipophilic nature of [14C]EICO (estimated logP of 12.9), as well as the complex process of chylomicron formation before systemic absorption (Levy, 1992). Also, the more extensive, but slower absorption of [14C]EICO in F-344 rats suggests a prolonged gastrointestinal transit.

Discussion

Previous 90-day feeding studies have shown that female F-344 rats are more susceptible to the development of liver and MLN microgranulomas after exposure to white mineral hydrocarbons and waxes than other species and/or rat strains (Firrillo et al., 1995; Smith et al., 1995; Miller et al., 1996). The pathological responses observed in F-344 rats include hepatic granulomas, mesenteric lymph node microgranuloma, and inflammation of the mitral valve (only with paraffin wax), as well as an increase in tissue weights and MHC tissue concentrations. The mechanism(s) for the reported sex, species, and strain-dependent responses to MHC is unknown, but the effects may be due, in part, to differences in the disposition and metabolism of MHC. To test this hypothesis further, studies were performed using a representative tracer MHC, [14C]EICO, dissolved in MHC to examine possible inherent rat strain differences in pharmacokinetic param-
time compared with S-D rats, which would allow more contact time for increased MHC absorption. However, when gastrointestinal transit times of female F-344 and S-D rats were experimentally determined by two different methods, no differences were observed (data not shown). The delay in fecal elimination of $[^{14}\text{C}]$EICO observed in F-344 rats most likely relates to the large variations in the amount of feces eliminated between individual F-344 rats. In general, the less stool that was eliminated at these early times (16–24 h), the less radioactivity that was recovered. S-D rats showed considerably less animal-to-animal variation at these times.

Along with strain differences in systemic exposure and fecal excretion profiles of $[^{14}\text{C}]$EICO, the urinary excretion rates were also
distinctly different. S-D rats excreted the majority of the absorbed 14C equivalents by 16 h, a rate significantly faster than that observed for F-344 rats. The F-344 rats eliminated 14C-metabolites of [14C]EICO at a relatively constant rate through 96 h. The time-dependent rate of urinary excretion is indicative of a depot effect, where a rate-limiting process is involved before urinary excretion. This rate-limiting step in F-344 female rats could include tissue retention of [14C]EICO and/or impaired capacity to biotransform [14C]EICO into excretable metabolites.

Previous studies have shown that F-344 rats retain greater amounts of MHC in target tissues compared with other rat strains and species after 60- and 90-day dietary exposure (2% in diet) to MHCs (Firriolo et al., 1995; Hoglen et al., 1998). This observation is consistent with the data presented herein. Although peak concentrations of [14C]EICO in livers of both rat strains were reached at the same time after administration, S-D rats clear [14C]EICO more efficiently from livers compared with F-344 rats. As a result, higher hepatic levels of [14C]EICO are maintained in F-344 rats.

The greater AUC, the relatively long terminal t1/2 value, the time-dependent urinary excretion profile, and the observed hepatic retention of [14C]EICO may all result from a reduced capacity of F-344 rats to metabolize [14C]EICO. Initial metabolic steps include oxidative metabolism of the saturated hydrocarbon in the liver (and intestine) to the corresponding alcohols and fatty acids. Subsequently, the oxidized metabolites can undergo the same metabolic fate as endogenous fatty acids and saturated fats (Lesiter, 1979; Le Bon et al., 1988; Barrowman et al., 1989). This metabolic process of saturated hydrocarbons was further confirmed in these studies by the identification of 12-cyclohexyldecanoic acid and 10-cyclohexyldecanoic acid, C18- and C16-carboxylic acids, respectively, in the urine of rats after [14C]EICO administration. The initial oxidative step of the saturated hydrocarbon chain could be the rate-limiting step in female F-344 rats, as described for the metabolism of phytane (2,6,10,14-tetramethylpentadecane) before urinary elimination by rats (Albro and Thomas, 1974). Evidence for this is that only parent [14C]EICO is retained by the liver of F-344 rats. Therefore, the retention of parent [14C]EICO occurs because of the lack of hepatic clearance due to impaired metabolism.

The initial oxidative step of [14C]EICO, and most likely of other MHCs, involves cytochrome P450 enzymes (McCarty, 1964; Nicolas and Kellum, 1966; Bartley et al., 1971; Frommer et al., 1972; Rowland and Tozer, 1995). Results from preliminary in vivo studies using 1-aminobenzotriazole (ABT) to inhibit P450 enzymes support this view. The hepatic retention of [14C]EICO was increased 3- to 4-fold in ABT-pretreated F-344 rats compared with non-ABT-pretreated rats at 96 h after [14C]EICO dosing (data not shown).

In summary, the results reported herein demonstrate that inherent differences in the pharmacokinetic and disposition of [14C]EICO are present between female F-344 and S-D rats. F-344 rats are systemically exposed to higher blood concentrations of [14C]EICO; excrete metabolites of [14C]EICO in urine in a slower, time-dependent manner; and retain a significant amount of [14C]EICO in livers at 96 h. Based on the hepatic retention of parent [14C]EICO in F-344 rats, a difference in the ability of the livers to metabolize [14C]EICO most likely explains the pharmacokinetic/dispositional differences observed between the two rat strains. This lack of metabolism and retention of MHC by livers of F-344 female rats relates to the development of hepatic granulomas after repeated dosing being established.

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