COMPARATIVE PHARMACOKINETIC AND DISPOSITION STUDIES OF [1-14C]-EICOSANYLCYCLOHEXANE, A SURROGATE MINERAL HYDROCARBON, IN FEMALE FISCHER-344 AND SPRAGUE-DAWLEY RATS

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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 characterized 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.

Mineral hydrocarbons (MHCs), as defined in recent health studies and regulatory reviews, are a class of highly refined petroleum products that include white mineral oils (liquid paraffins), petrolatums, and petroleum waxes. These products are complex mixtures, which consist of almost entirely of saturated hydrocarbons, predominately naphthenic and isoparaffinic hydrocarbons of carbon chains ranging from C18 to C32. 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 characterized 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.

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Abbreviations used are: MHC, mineral hydrocarbon; S-D, Sprague-Dawley; F-344, Fischer-344; EICO, [1-14C]-1-eicosanylcyclohexane; JVC, jugular vein cannula; MLN, mesenteric lymph node; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography with tandem mass spectrometry; AUC, area under the systemic blood concentration-time curve from zero to time infinity; APQI, atmospheric pressure chemical ionization; CID, collision-induced dissociation; Rf, retention time; ABT, 1-aminobenzotriazole.

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to more extensive accumulation/retention of MHCs in the livers of F-344 rats. Londoño et al. (1998) evaluated rat strain differences in the pharmacokinetics and disposition of octadecane (C18), a representative lower molecular weight hydrocarbon in white oils and waxes (Londoño 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 toluene, high-purity solvent (Belco, Waynesboro, PA); 1-eicosanylcyclohexane and food-grade ethylene glycol and 2-methoxyethyl ether (Mallinckrodt, Paris, KY); 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. At least three rats per strain were killed at 4, 8, 14, 26, 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 1 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 Katju.
The column effluent was monitored radiochemically with a β-Ram Flow-Through Monitor System (IN/US Systems, Inc., Tampa, FL). The retention time (Rf) of [14C]EICO was 195 min under these conditions.

For analysis of urinary metabolites associated with [14C]EICO, collected urine 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 delivery, and a tandem mass spectrometer (Finnigan MAT, San Jose, CA) equipped with a Hewlett Packard 1100 series LC/MS spectrometer. The column effluent was monitored by radiochemically with a β-Ram Flow-Through Monitor System (IN/US Systems, Inc., Tampa, FL). The retention time (Rf) of [14C]EICO was 195 min under these conditions.

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/e 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 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 24–48 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 $C_{\text{max}}$ of 110 ng/ml was obtained at 16 h ($T_{\text{max}}$). The blood concentration then declined in a log-linear manner until the end of the study at 96 h. The percentage of dose eliminated in the urine was dose-dependent 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.

Fig. 3. Cumulative recovery of $[^{14}$C]EICO eliminated in the feces as a function of time after a high dose of MHC (1.80 g/kg) to female F-344 (■) and S-D (○) rats (A), or a low dose of MHC (0.18 g/kg) to female F-344 (□) rats and S-D (○) rats (B).

Data expressed as mean percentage of administered radioactivity ± S.D. ($n = 3–23$ time point for the high-dose studies and $n = 6$ time point for the low-dose studies). The solid lines are the nonlinear regression fits of the data. * statistically different between F-344 and S-D rats at $P < 0.05$. See “Statistical Analysis” under Materials and Methods for further explanation.

Fig. 4. Cumulative recovery of total radioactivity excreted in the urine as a function of time after a high dose of MHC (1.80 g/kg) to female F-34 (■) and S-D (○) rats (A), or a low dose of MHC (0.18 g/kg) to female F-344 (□) rats and S-D (○) rats (B).

Data expressed as mean percentage of administered radioactivity ± S.D. ($n = 3–23$ time point for the high-dose studies and $n = 6$ time point for the low-dose studies). The solid lines are the nonlinear regression fits of the data. * statistically different between F-344 and S-D rats at $P < 0.05$. See “Statistical Analysis” under Materials and Methods for further explanation.
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 \( [^{14}C] \)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 \( [^{14}C] \)EICO compared with S-D rats regardless of the dose. Based on the 96-h data, no statistically significant differences in hepatic retention of \( [^{14}C] \)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 0.5% in S-D rats.

Determination of Urinary Metabolites. HPLC analysis of pooled urine collected from rats treated orally with the high dose of MHC containing \( [^{14}C] \)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 \( [^{14}C] \)EICO metabolites in the urine. Although the profiles of \( ^{14}C \)-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, \( R_t \) 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 (\([\text{C}_4\text{H}_8\text{O}_2\text{+H}]\))
This peak had a retention time identical to that of \([^{14}\text{C}]\text{EICO}\).
MLN samples regardless of dose (radiochromatograms not shown).
analysis in prepared plasma samples and solubilized fecal, liver, and
ine possible inherent rat strain differences in pharmacokinetic param-
be due, in part, to differences in the disposition and metabolism of
affin wax), as well as an increase in tissue weights and MHC tissue
57, and 43 (major signal)\], and 29) are characteristic ions of fatty
acids.
Urinary metabolite J (Rₑ of 150 min) was identified as 10-
cyclohexyldecanoic acid by LC-MS/MS (Fig. 8B). The resulting CID
production ion spectrum of the [M + H]⁺ ion at m/z 255 contained
signals at m/z 167, 87, 71, 57, 43, and 29. The product ion at m/z 167
was assigned to a structure resulting from the loss of C₆H₄O₂. The
prominent ion detected at m/z 87 resulted from the ionization of this
fragment (\([\text{C}_6\text{H}_4\text{O}_2\text{+H}]\)). Much like urinary metabolite K, characteristic
ions of fatty acids were detected at the lower end of the spectrum.

Determination of \([^{14}\text{C}]\) in Blood, Feces, Liver, and MLNs. Only
one major radioactive peak at Rₑ of 195 min was detected by HPLC
analysis in prepared plasma samples and solubilized fecal, liver, and
MLN samples regardless of dose (radiochromatograms not shown).
This peak had a retention time identical to that of \([^{14}\text{C}]\text{EICO}\).

Discussion
Previous 90-day feeding studies have shown that female F-344 rats
are more susceptible to the development of liver and MLN micro-
granulomas 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 micro-
granuloma, and inflammation of the mitral valve (only with para-
affin 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, \([^{14}\text{C}]\text{EICO}\), dissolved in MHC to exam-
ine possible inherent rat strain differences in pharmacokinetic param-
eters 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 \([^{14}\text{C}]\text{EICO}\) in F-344 rats because of greater absorption and/or
decreased ability to metabolize \([^{14}\text{C}]\text{EICO}\) and by extrapolation
MHC.
Albro and Fishbein (1970) reported that MHCs (and/or their me-
tabolites) 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
\([^{14}\text{C}]\text{EICO}\). 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/\([^{14}\text{C}]\text{EICO}\) absorption. As reviewed by Porter
and Charman (1997), the process of intestinal lymphatic drug trans-
port 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 concentra-
tion 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/\([^{14}\text{C}]\text{EICO}\) admin-
istered 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/\([^{14}\text{C}]\text{EICO}\) into the systemic circulation.
Compared with small molecular weight, lipophilic agents, the ab-
sorption of \([^{14}\text{C}]\text{EICO}\) 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 \([^{14}\text{C}]\text{EICO}\) (estimated logP of 12.9), as well as the
complex process of chylomicron formation before systemic absorp-
tion (Levy, 1992). Also, the more extensive, but slower absorption
of \([^{14}\text{C}]\text{EICO}\) in F-344 rats suggests a prolonged gastrointestinal transit
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 [14C]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 [14C]EICO, the urinary excretion rates were also

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**Fig. 8.** Representative APCI-MS/MS spectra of the major urinary metabolite 12-cyclohexyldodecanoic acid (HPLC peak K; R<sub>t</sub> of 163 min) (A) and the urinary metabolite 10-cyclohexyldecanoic acid (HPLC peak J; R<sub>t</sub> of 150 min) (B).
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 cleared [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 (Lesher, 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-tetramethylhexadecane) 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; Nicolaides and Kellum, 1966; Bartley et al., 1971; Frommer et al., 1972; Toftgard and Nilsen, 1982; Perdu-Durand and Tulliez, 1985). Results from preliminary in vivo studies using 1-aminobenzotrazole (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 to be established.

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References