Title page.

Pharmacokinetic and metabolism of apigenin in female and male rats after a single oral administration.

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Running title page.

Running title
Pharmacokinetics of apigenin in rats

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4 figures
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186 words in the abstract
716 words in the introduction
1275 words in the discussion

Abbreviations.
BW: body weight ; CYP : cytochrome P450 monooxygenase ; Equiv.: equivalent ; GA: glucurononoconjugate of apigenin ; HPLC : high performance liquid chromatography ; LC-MS: liquid chromatography-mass spectrometry ; SA: sulfoconjugate of apigenin.
Abstract.

The metabolism of apigenin, a weak estrogenic flavonoid phytochemical, was investigated in the rat. After a single oral administration of radiolabelled apigenin, 51.0% of radioactivity were recovered in urines, 12.0% in faeces, 1.2% in the blood, 0.4% in the kidneys, 9.4% in the intestine, 1.2% in the liver and 24.8% in the rest of the body within 10 days. Sex differences appear with regard to the nature of compounds eliminated via the urinary route: immature male and female rats like mature female rats excreted a higher percentage of mono-glucuronoconjugate of apigenin than mono-sulfoconjugate of apigenin (10.0-31.6% vs 2.0-3.6% respectively). Mature male rats excreted the same compounds in an inverse ratio (4.9% and 13.9% respectively). Radioactivity appeared in the blood only 24 hours after oral administration. Blood kinetics showed a high elimination half-time (91.8 hours), a distribution volume of 259 ml and a plasmatic clearance of 1.95 ml/h. All of the parameters calculated from these experiments suggested a slow metabolism of apigenin, with a slow absorption and a slow elimination phase. Thus a possible accumulation of this flavonoid in the body can be hypothesised.
Introduction.

Flavonoids are polyphenolic compounds that occur ubiquitously in plants and that are present in variable amounts in the diet. Beneficial health properties of flavonoids have been reported and much attention has been paid to their antioxidant activities that affect oxygen free radicals and lipid peroxidation, both of which are involved in many diseases (Di Carlo et al., 1999). Cancer prevention by flavonoids has also been observed (Le Marchand, 2002). Several mechanisms such as modulation of the xenobiotic metabolizing enzymes involved in the bioactivation of carcinogens, or apoptosis induction could explain such an effect (Wang et al., 1999, Middleton et al., 2000).

Some of them have additional properties: several isoflavones including genistein are well known as potent phytoestrogens and could act on hormonal cancers, on coronary heart diseases, strokes, osteoporosis and menopausal symptoms. Apigenin (figure 1) is a flavone present in the western diet: in aromatic plants (camomilla, rosemary, parsley) but also in celery, apple, honey, fennel and wheat germ (Hertog et al., 1992, Karakaya and Nehir EL, 1999). Even though apigenin is less active than its homologous isoflavone (genistein), its estrogenic properties have already been described. In vitro, apigenin has estrogenic activity on growth of transfected cells that are estrogen-dependent and has additive effects with 17-β estradiol (Le Bail et al., 1998). In vivo, apigenin decreases the endogenous estrogen receptor levels in mice uterus (Breinholt et al., 2000), enhances the estrogenicity of low doses of estradiol in immature rats (Stroheker et al., 2003) and has a protective effect on skin tumorogenesis, an hormonal dependent cancer (Birt et al., 2001). Anti-fertility properties have also been observed: apigenin is an active constituent of S. Orobancheoides, a medicinal plant used for its contraceptive properties (Hiremath et al., 2000). Many mechanisms of its action have been proposed to explain these (anti-) estrogenic and (anti-) carcinogenic properties: interaction with estrogen receptors (ERs) (Miksicek, 1995, Le Bail et al., 1998), modulation

Even though the biological and health properties have been extensively studied, our knowledge concerning its metabolism and its pharmacokinetic needs to be extended. In vivo metabolism studies published in 1972 showed that maximal excretion of apigenin and its metabolites occurred between 48 and 72 h following oral administration and that derivatives were all conjugated compounds (primary metabolites were not detected) (Griffiths and Smith, 1972). More recently, apigenin elimination in human urine after parsley consumption has been studied (Nielsen et al., 1999). Apigenin appeared in urine after 24 h, the fraction of apigenin intake excreted being 0.58%. In vitro studies have already shown that apigenin undergoes transformation into luteolin and conjugated derivatives (glucurono- and sulfono-conjugates). Recently, using rat hepatic microsomes we have identified two new hydroxylated derivatives, namely scutellarein and iso-scutellarein. More, phase II apigenin biotransformation leads to the formation of three mono-glucuronated and one mono-sulfated compounds. Its major phase I metabolite, luteolin, can also be conjugated to four different mono-glucuronated, two sulfated and one methylated compounds (namely diosmetin). Despite the diversity of possible phase I and II metabolites that can be formed, only phase II derivatives were recovered from a perfused rat liver ex vivo system and one metabolite, mono-glucuronated and mono-sulfated were detected only in these conditions (Gradolatto et al., 2004). But this work needs to be completed with the in vivo apigenin metabolism study, in order to provide new elements concerning the bioavailability and kinetical parameters of this compound.

Thus, the aim of the present work was to investigate in vivo apigenin metabolism in rats. Intragastric route was chosen because this kind of compounds are provided by food
consumption and must be metabolized and pass through the intestinal barrier to join systemic circulation. Firstly, the elimination of radioactivity was measured in urine and faeces after a single oral administration of [\(^3\)H]-apigenin to male and female Wistar rats. Potential storage tissues were also analysed (blood, livers, kidneys, intestines, and body residues) and specific time-point samples were further analysed to investigate potential sex differences. Secondly, blood kinetics were investigated in order to determine kinetic parameters. Finally, a bile excretion experiment was carried out to check whether, like other flavonoids, apigenin metabolism can go under an enterohepatic pathway.
Materials and methods.

Chemicals. Apigenin (CAS number: 520-36-5), luteolin (CAS number: 491-70-3) and diosmetin (CAS number: 520-34-3) were purchased from Extrasynthèse (Genay, France, purity > 90%). $[^3]$H-apigenin (specific activity: 15-20 Ci/mmol, radiochemical purity above 95%) was provided by Sibtech, Inc. (Newington, United states). Tritiation is randomly distributed on the phenolic skeleton. Sulfatase (type VI) and $\beta$-glucuronidase (type H-3AF) were purchased from Sigma-Aldrich Chimie (Saint-Quentin Fallavier, France). Soluene-350 and the scintillation fluid Ultima Gold were purchased from Packard (Rungis, France). Isofluran was provided by Baxter (Maurepas, France). All other chemicals were provided by standard commercial sources and were of the highest quality available.

Animals and diets. Mature and immature SPF Wistar rats were obtained from Janvier (Le Genest Saint-Isle, France). They were housed in wire metabolic cages in a room maintained at 22°C with a 12 h light/dark period. The animals were fed ad libitum on a purified semi-liquid phytoestrogen-free diet (synthesised at Jouy-en-Josas, INRA, France). They were maintained in accordance with the French Ministry of Agriculture guidelines for care and use of laboratory animals. Animals were fasted for 16 hours before and 2 hours following drug administration; water was provided ad libitum.

Apigenin elimination. Ten female and ten male rats (8 weeks old) weighing 200 ± 30 g received, via intragastric route, one single dose of 10 mg unlabelled apigenin coupled with 10 $\mu$Ci of $[^3]$H-apigenin b.w. (specific activity of the administered dose: 1 $\mu$Ci/mg) dissolved in corn oil (dosing volume: 5 ml / kg b.w.). One male and one female control rat received only corn oil. The same experiment was repeated with male and female immature animals (3 weeks old) (n=6) for the first 24 hours of experiment (55.6 ± 1.0 g). Urine and faeces were collected daily for ten days in pre-cooled flasks. Volume of urines samples was measured and adjusted to pH4 with HCl. Urine samples corresponding to the
maximum elimination of radioactivity were analysed using LC-MS technique. Faeces were directly frozen after weighing and stored at –80°C for further analysis.

At the end of sample collection, aorta blood collection was carried out under isofluran anaesthesia. The blood was immediately treated for radioactivity counting. The livers, kidneys and intestines were frozen at –80°C for further analysis. The rest of the body was crushed with a mixer and frozen at –80°C for further analysis.

**Blood kinetics.** Four female rats (8 weeks old) weighing 225 ± 25 g received one single dose of 10 mg coupled with 10 µCi of apigenin / kg b.w. dissolved in corn oil (dosing volume: 5 ml / kg b.w.). One female control rat received only corn oil.

Blood was withdrawn under heparin from the orbital vein at 8, 24, 48, 72, 120, 170, 216, 242, 264, 288 and 360 hours after intragastric administration. At the end of the experiment carbon dioxide was used for euthanasia of the animals.

Data were analysed using non-compartmental methods with Kinetica software (InnaPhase, Champs sur Marne, France). The AUC was computed using the Log Linear Method, trapezoidal when Cn > Cn-1. The elimination half-life (t1/2) was calculated as t1/2=ln2/Lz where Lz is the elimination rate constant. The plasma clearance (Clp) was calculated as Clp=dose/AUC.

**Bile collection.** The bile duct of four female rats (8 weeks old) weighing 253 ± 4 g were canulated and the bile was collected with a system allowing the animals to move freely (Harvard Apparatus, Les Ulis, France). After 16 hours of fasting, the animals received one single dose of 10 mg coupled with 10 µCi of apigenin / kg BW dissolved in corn oil via the intragastric route (dosing volume: 5 ml / kg b.w.). One control female rat received only corn oil.
Bile was collected with an auto-sampler hour by hour during 48 hours. At the end of the experiment carbon dioxide was used for euthanasia of the animals.

**Radioactivity measurement.** Samples from the control rats were counted to determine the background count rate for each matrix. The measurements were made by a model TRI CARB 2000 (United technologies Packard, France) liquid scintillation counter. Counts per min were converted to disintegrations per min based on a quench curve stored in the instrument’s microprocessor. The yield of counting was determined by a standard curve. Quenching was corrected by external standardisation. The yield of counting was of 91.57 ± 0.73% for blood, 100.94 ± 3.20% for carcasses, 91.41 ± 1.66% for faeces, 103.37 ± 2.54% for intestine, 119.38 ± 14.73% for kidneys, 95.18 ± 0.84% for livers and 88.12 ± 2.85 for urine.

Aliquots of urine (1 ml) and bile (100 µl) were directly counted in 10 and 5 ml of scintillation fluid respectively. Aliquots of blood (150 µl) were digested by soluene-350 in presence of isopropanol and bleached by H₂O₂ 30% for 1 hour at 50°C before the addition of 10 ml of scintillation fluid for counting.

The kidneys, livers and intestines were homogenised with ultrapure water (1g:1ml) by ultraturax. The faeces were first hydrated with ultrapure water (1g:4ml) for 2 hours before homogenisation with ultraturax. Aliquots (400 µl) were digested by soluene-350 in the presence of isopropanol and bleached by H₂O₂ 30% for 2 hours at 50°C before the addition of 10 ml of scintillation fluid for counting.

**LC-MS analysis.** Analyses of 24 hours urine samples were performed after HPLC separation as previously described (Gradolatto 2004). The interface was realised between a Water Alliance system (Milford, MA, USA) including a model 2690 separation module a 996 photodiode array detector and a single quadrapole Waters LCZ Platform equipped with an
ESI source operated in a negative ionization mode. The following conditions as describe elsewhere (Gradolatto, 2004) were used: ESI capillary voltage: 3.50 kV; temperature desolvation: 150°C; desolvation gas: 400 l/h; fragmentor voltage: 20 V.

**Statistical analysis.** Data from the apigenin elimination experiment (% of cumulative and final radioactivity counting), and data from urine and blood LC-MS analysis (% of recovered compounds) were submitted to an ANOVA (p≤0.05). Data from the LC-MS urine analysis of mature and immature male and female rats were submitted to the Kruskal-Wallis test (α≤0.05). Calculations were made with Stat Box Pro software.
Results.

Apigenin elimination. At the end of the experiment, 51% of radioactivity was recovered in urine, 12% in faeces, 1.18% in blood, 0.32% in kidneys, 9.41% in intestines and 1.18% in livers with a significant difference between males and females (average values from male and female data, details are given in table 1). Approximately a quarter of the recovered radioactivity (24.8%) is still present in the rest of the body after 10 day post-ingestion.

A single oral dose of [3H]-apigenin administered to male and female rats lead to a major elimination of radioactivity via urine pathway and to a lesser extent via faeces (51% and 12% of the ingested dose at the end of the experiment). This excretion mainly occured during the first 24 hours post-ingestion. No significant difference between male and female rats was observed when considering radioactivity recovery (table 2). LC-MS analysis of 24 hour urine samples of mature and immature animals revealed that both rat groups excreted a high proportion of unchanged apigenin (64.8-87.8%; m/z 269). In contrast, the proportion of conjugated derivatives highlighted a strong difference related to sex and maturity of animals (figure 2). Analysis of urine samples collected from immature males and females revealed the presence of two compounds eluted at 25 and 30 minutes (m/z of 445 and 349 respectively). These products correspond to glucuronated and sulfated derivatives of apigenin (10.0 vs 31.6% for female and male glucurono-conjugates and 2.2 vs 3.6% for female and male sulfoconjugates respectively). In mature female samples these metabolites were in the same proportion as those observed for immature animals (21.1 and 2.0% for glucurono and sulfo derivatives respectively). In contrast, LC-MS analysis of mature male samples revealed the presence of the same compounds in an inverse proportion: 4.9 vs 13.9% for glucuronated and sulfated derivatives respectively.
**Blood kinetics.** Preliminary experiments were first realised to determine which time point could be analysed during this kinetic.

During the first 8 hours following apigenin administration, no radioactivity was detected. It appears at 9 hours post-administration time and increased to reach a maximum at 24 hours post ingestion time. Then it slowly decreased in a monophasic manner, suggesting a slow distribution phase (figure 3). The calculated pharmacokinetic parameters showed that blood radioactivity decreased with a high elimination half-life of 91.8 ± 5.6 hours and with a \( T_{\text{max}} \) occurring at 24 hours. The volume of distribution was quit high (259.1 ± 25.8 ml) and plasmatic clearance was about 2 ml/h.

**Bile excretion.** Radioactivity in bile samples appeared during the second hour after \([^{3}\text{H}]-\)apigenin ingestion and increased during the third hour to a dose equivalent to 0.59 ± 0.2 mg apigenin/ml (figure 4). Radioactivity decreased during the fourth hour and increased once again during the fifth and sixth hours to a dose equivalent to 0.35 ± 0.03 apigenin/ml. Then it slowly decreased and baseline (0.12 equiv mg apigenin/ml ± 0.05) was recovered approximately 15 hours after ingestion of \([^{3}\text{H}]-\)apigenin.
Discussion.

To our knowledge, no information concerning the pharmaco-kinetics of apigenin in rat were available. Our data show that apigenin excretion occurred mainly via the urinary pathway, with 16% of ingested radioactivity recovered from male urine samples within the first 24 hours, and 18% for female. After this time point, radioactivity excretion decreased and 40% was recovered within 5 days. Because glucurono-conjugates of apigenin (GA) only represent a part of this percentage (figure 2), we can assume that within 5 days, the proportion of GA excreted is less than 40% of the ingested dose. Griffiths et al (1972) observed GA excretion after administration of one single dose of apigenin to rats. But in this previous work, GA excretion was not maximal after 24 hours and increased until the second day post-ingestion and finally 75% of GA was recovered within 5 days. The differences observed between these two studies could be explained by the initial dose administration since a 100 mg / kg b.w. dose was administered by Griffiths, i.e. ten fold higher than the one delivered in our study. Even though the kinetics of elimination are not exactly the same, both works show that urinary excretion of apigenin is an important route.

These results are very different from those observed in human volunteers after parsley ingestion: within 24 hours following ingestion, only 0.58% of apigenin was recovered in urine samples (Nielsen and Dragsted, 1998). Moreover, even though the authors highlighted the importance of individual differences in apigenin absorption, they could not detect any differences between male and female subjects after the enzymatic digestion of derivatives. This was also the case in our study concerning radioactivity recovery alone. Nevertheless LC-MS analysis quickly invalidated this observation, by pointing out sex maturity different evolution resulting in different proportions of metabolism products. The application of a detection method allowing estimation of metabolites proportion (Basly et al., 2003) showed that the ratio of glucuronated (GA) to sulfated (SA) metabolites of apigenin in immature rats
(male and female) and mature female was the inverse ratio in mature male rats (10-31% : 2.0-3.6% in immature rats and mature female rats, vs 4.9%: 13.9% in mature males for GA and SA respectively). To our knowledge this is the first time that such difference has been observed and this could be explained by differences in enzyme activities related to sex maturation of animals. It is well-known that phase I enzyme metabolism and more especially CYP which are growth hormone gender regulated (Shapiro et al., 1995 and Pampori et al., 2001). This is also the case for some phase II metabolism enzymes (Mugford and Kedderis, 1998). Some UDP-glucuronosyl and sulfo-transferases can be regulated via endogenous steroid hormones and are consequently sex and age regulated, thus explaining discrepancies between metabolites obtained from immature and mature males and females (Mugford and Kedderis, 1998). Moreover, some aryl sulfotransferase activities in mature male rat livers are 2-3 fold times greater than those in mature female livers, thus explaining why SA are predominant in urine samples of mature males (Mulder, 1986).

Apigenin kinetics appear to differ from others flavonoids like quercetin. When administered to rats, only 20% of this flavonol was absorbed through the intestinal barrier, 30% was excreted in expired CO₂ and 30% was recovered unchanged in the faeces (Ueno et al., 1983). In the present work 63% of radioactivity was excreted via the urinary and faecal route (only 12% for the latter) suggesting a high absorption of apigenin. After ten days, 24.8% of radioactivity are still present in the rest of the body suggesting an accumulation of this compound in the body and slow elimination.

More, it could be noted that none of the phase I derivative of apigenin was recovered at any time of the experiment. Previous works have showed that in vitro apigenin phase I metabolism (hepatic rat microsomes) mainly led to the formation of luteolin while only phase II conjugates were recovered in ex vivo experiments (Gradolatto et al., 2004, Nielsen and
Dragsted, 1998). Current work has confirmed the fact that phase I metabolites are compounds obtained only in vitro.

Previous data from literature concerning blood kinetics showed that, when diosmin was administered to human, its half-life ranged from 26 to 43 hours, with a peak occurring 2 hours after administration (Cova et al., 1992), whereas diosmetin administered per os to rats appeared in blood after 6 hours as unchanged and glucuronated compound (Boutin et al., 1993). Quercetin has been studied in different species and showed a half-time ranging from 0.7 to 2.4 hours, a distribution volume of 6.21 up to 92.61 liters and a total body clearance varying from 28.1 to 34.6 hour⁻¹ (Graefe et al., 1999). In pigs, after an oral dose of 50 mg/kg, only 17% of the quercetin administered was recovered in blood as free, conjugate and derivative products within 8 hours post-administration (Ader et al., 2000). In man, the peak in blood occurred more rapidly, approximately 2.9 hours after the flavonol administration (Hollman et al., 1995). In rat, flavonoids seemed to occur more rapidly. Luteolin, the in vitro phase I apigenin metabolite, given via gastric intubation appeared in plasma after 15 minutes (Shimoi et al., 1998). Apigenin given to rats via the intra-peritoneal pathway appeared in plasma 30 minutes after administration (Romanova et al., 2000).

Taken together, these results from literature suggest a relatively rapid absorption of flavonoids, the presence in blood occurring within a few minutes to a few hours and half-times not exceeding 48 hours. After 2 days, all of these compounds seem to be excreted from the body. In contrast, our results suggest a slow absorption of apigenin with the appearance of blood radioactivity 24 hours after ingestion, with no traces before this time point. In addition, the half-time of 91.8 hours and the fact that basal level is recovered 10 days after [³H]-apigenin treatment confirm the hypothesis of the slow absorption and excretion of apigenin.

In conclusion, i) urinary elimination seems to be the major elimination pathway of apigenin ii) absorption seems to be a slow phenomenon as tritium was detected only after 24 hours, iii)
48 hours after ingestion, blood radioactivity is still high and corresponds to about 60% of the peak previously observed and iii) the estimated distribution volume of radioactivity is 25 times higher than the theoretical blood volume (about 10 ml), suggesting accumulation in the tissues. Moreover, persistence of radioactivity in blood suggests an active enterohepatic pathway already described for others flavonoids (Hackett, 1986). These results corroborate the hypothesis of the slow metabolism and elimination of this compound.

Concerning biliary excretion it was shown that flavonoid administration ([14C]-quercetin or –catechin) leads to a peak of radioactivity in bile after 2 hours, containing the unchanged aglycone and its derivatives, with total recover of baseline 48 hours after administration (Shaw and Griffiths, 1980). In the case of genistein, 70 to 75% of radioactivity is recovered over a 4-hour period (Sfakianos et al., 1997). In contrast, our results show that peak radioactivity is not detected before 3 to 6 hours and that the basal level is recovered only 15 hours after administration. Consequently our results differ from other flavonoids and sustain the idea of slow apigenin pharmaco-kinetics. Therefore, biological effects of apigenin can be explained by its slow metabolism, its enterohepatic pathway leading to high persistence in blood and consequently accumulation in the body.

As apigenin is still present in the blood detected 9 days after the administration of one single dose, accumulation seems possible and raises the question of the eventual consequences of chronic exposure to this compound, which is present in the daily western diet and which may affect animal and human health.
Acknowledgements.

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References.


Footnotes.

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Legends for figures.

FIG. 1. Structure of apigenin.

FIG. 2. Percentage of compounds recovered from urine samples collected during the 24 hours following one single intragastric administration of 10 mg apigenin / kg BW to male and female, mature and immature rats. Values are mean ± standard error mean (n=4) and asterisks indicate significant difference from others animal groups (Kruskal-Wallis test, p<0.05).

FIG. 3. Milligrams equiv of apigenin per milliliter recovered in the whole blood of female rats following intra-gastric administration of 10 mg and 10 µCi of [3H]-apigenin / kg b.w..

FIG. 4. Milligram equiv apigenin per milliliters of ingested radioactivity recovered in the cumulative bile samples of female rats following intra-gastric administration of 10 mg and 10 µCi of [3H]-apigenin / kg b.w.. Mean ± standard error mean (n=4).
### TABLE 1

**Spread of the radioactivity after single oral administration of radiolabelled apigenin.**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>male</th>
<th>female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>50.58 ± 2.99</td>
<td>51.36 ± 3.34</td>
</tr>
<tr>
<td>Faeces</td>
<td>11.60 ± 2.44</td>
<td>12.91 ± 1.89</td>
</tr>
<tr>
<td>Blood</td>
<td>1.28 ± 0.14</td>
<td>1.09 ± 0.19</td>
</tr>
<tr>
<td>Liver *</td>
<td>1.35 ± 0.09</td>
<td>1.01 ± 0.09</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.25 ± 0.03</td>
<td>0.39 ± 0.07</td>
</tr>
<tr>
<td>Intestine</td>
<td>9.21 ± 0.43</td>
<td>9.61 ± 0.70</td>
</tr>
<tr>
<td>Rest of the body</td>
<td>25.74 ± 1.42</td>
<td>23.62 ± 1.35</td>
</tr>
</tbody>
</table>

Values are mean percents ± sem (n=10) of the recovered radioactivity after administration of one single dose of 10 mg unlabelled apigenin coupled with 10 µCi of \([^3]H\)-apigenin / kg b.w. (specific activity : 1 µCi/mg) to male and female mature Wistar rats. Data for urine and faeces correspond to the cumulative radioactivity detected for 10 days. Data for the other tissues correspond to the recovered radioactivity on the 10\(^{th}\) day. *: significant difference between sex (ANOVA, p<0.05).
TABLE 2

_Cumulative excretion (% of the recovered dose) of radioactivity in urine and faeces after single oral administration of [³H]-apigenin to male and female rats._

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Male urine</th>
<th>Male faeces</th>
<th>Female urine</th>
<th>Female faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>16.02 ± 0.69</td>
<td>7.05 ± 2.53</td>
<td>17.94 ± 1.37</td>
<td>6.22 ± 1.88</td>
</tr>
<tr>
<td>48</td>
<td>25.45 ± 0.95</td>
<td>10.10 ± 2.44</td>
<td>27.61 ± 1.50</td>
<td>9.24 ± 1.71</td>
</tr>
<tr>
<td>72</td>
<td>31.51 ± 1.48</td>
<td>10.54 ± 2.45</td>
<td>33.96 ± 2.20</td>
<td>10.18 ± 1.64</td>
</tr>
<tr>
<td>96</td>
<td>35.13 ± 1.86</td>
<td>10.82 ± 2.45</td>
<td>38.01 ± 2.42</td>
<td>11.68 ± 1.70</td>
</tr>
<tr>
<td>120</td>
<td>38.66 ± 2.16</td>
<td>11.03 ± 2.44</td>
<td>41.54 ± 2.55</td>
<td>11.92 ± 1.70</td>
</tr>
<tr>
<td>144</td>
<td>43.50 ± 2.46</td>
<td>11.17 ± 2.43</td>
<td>43.86 ± 2.43</td>
<td>12.07 ± 1.71</td>
</tr>
<tr>
<td>168</td>
<td>45.62 ± 2.62</td>
<td>11.26 ± 2.44</td>
<td>46.59 ± 2.84</td>
<td>12.41 ± 1.81</td>
</tr>
<tr>
<td>192</td>
<td>47.87 ± 2.81</td>
<td>11.38 ± 2.43</td>
<td>48.87 ± 3.10</td>
<td>12.67 ± 1.89</td>
</tr>
<tr>
<td>216</td>
<td>49.28 ± 2.92</td>
<td>11.51 ± 2.44</td>
<td>50.28 ± 3.21</td>
<td>12.81 ± 1.89</td>
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<td>240</td>
<td>50.58 ± 2.99</td>
<td>11.60 ± 2.44</td>
<td>51.36 ± 3.34</td>
<td>12.91 ± 1.89</td>
</tr>
</tbody>
</table>

Values are mean percents ± sem (n=10 rats) of the recovered radioactivity. Data of urine and faeces correspond to the cumulative radioactivity recovered each day for 10 days.
### TABLE 3

**Single dose pharmacokinetic parameters for total radioactivity in female rats (n=4).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>female rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (µg equiv./ml)</td>
<td>6.91 ± 0.54</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>24 ± 0.0</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (h.µg equiv./ml)</td>
<td>1038.0 ± 64.4</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>91.8 ± 5.6</td>
</tr>
<tr>
<td>$V_o$ (ml)</td>
<td>259.1 ± 25.8</td>
</tr>
<tr>
<td>$Cl_p$ (ml/h)</td>
<td>1.95 ± 0.11</td>
</tr>
</tbody>
</table>
Figure 1
Figure 2

This article has not been copyedited and formatted. The final version may differ from this version.
Figure 3

![Graph showing the relationship between post-dosing time (h) and mg equiv. apigenin/ml.](https://example.com/graph.png)
Figure 4

[Graph showing post-dosing time (h) vs. mg equiv. apigenin/ml]