

# Dermal absorption and disposition of musk ambrette, musk ketone and musk xylene in human subjects

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## Abstract

Musk ambrette, musk ketone and musk xylene have a long history of use as fragrance ingredients, although musk ambrette is no longer used in fragrances. As part of the review of the safety of these uses, it is important to consider the systemic exposure that results from these uses. Since the primary route of exposure to fragrances is on the skin, dermal doses of carbon-14 labelled musk ambrette, musk ketone and musk xylene were applied to the backs (100 cm<sup>2</sup>) of healthy human volunteers (two to three subjects) at a nominal dose level of 10–20 µg/cm<sup>2</sup> and excess material removed at 6 h. Means of 2.0% musk ambrette, 0.5% musk ketone and 0.3% musk xylene were absorbed based on the amounts excreted in urine and faeces during 5 days. Most of the material was excreted in the urine with less than 10% of the amount excreted being found in faeces. No radioactivity was detected in any plasma sample, consistent with low absorption, and no radioactivity was detected (< 0.02% dose) in skin strips taken at 120 h. Analysis of urine samples indicated that all three compounds were excreted mainly as single glucuronide conjugates. The aglycones were chromatographically different, but of similar polarity, to the major rat metabolites excreted in bile also as glucuronides. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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## 1. Introduction

Several nitroaromatics, known as nitromusks are or have been used as fragrance ingredients (Ford, 1998). Three of these, musk ambrette, musk ketone and musk xylene (Fig. 1) were at one

time the most important, although musk ambrette was withdrawn from use primarily due to its rare, but confirmed photosensitization effect in humans (Cronin, 1984). At high doses it also causes neurotoxic and reproductive effects in rats (Spencer et al., 1984). Musk ketone and musk xylene do not exhibit these toxic effects and continue to be used as fragrance ingredients. Musk xylene has been shown to cause an increase in hepatocellular adenomas and carcinomas in a 2-year dietary feeding

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study (Maekawa et al. 1990) although there is evidence that it is a non-genotoxic murine carcinogen. The toxicity profiles of these compounds have been established in rats and as part of this programme the absorption, distribution, metabolism and excretion of dermal doses have been investigated using radiolabelled compounds (Hawkins and Ford, 1999). Means of about 40, 31 and 19% of a single dose (0.5 mg/kg, 11 µg/cm) under occlusion for 6 h of musk ambrette, musk ketone and musk xylene, respectively, were absorbed. The data from these studies were used to obtain approval by an ethical review board for the simulated exposure studies in human volunteers, which are reported here. An evaluation of the systemic human exposure that may result from use of products containing these materials provides critical information for risk assessment, particularly since it is well known that dermal absorption is often considerably lower in humans compared to rats (Wester and Noonan, 1980). It has been reported (Ford, 1998) that the highest use levels of the nitromusks in cosmetic products are found in hydroalcoholic product such as perfumes and colognes. Since such uses result in the highest exposures and since it is assumed that dermal absorption and the resulting systemic exposures will be enhanced by ethanol (Hotchkiss, 1998), a study was designed to determine the systemic availability under conditions simulating

the use of such products, e.g. in ethanol on the skin with wash-off 6 h after exposure. The total maximum daily exposure is estimated to be about 10 mg/day therefore a single application of 1–2 mg to an area of about 100 cm used in these investigations was considered to simulate human exposure.

## 2. Materials and methods

### 2.1. Chemicals

Samples of the non-radiolabelled and carbon-14 uniformly ring-labelled musks were obtained as described by Hawkins and Ford (1999). A sample of *O*-desmethyl musk ambrette was synthesised at Huntingdon Research Centre by demethylation of <sup>14</sup>C-labelled musk ambrette using pyridine hydrochloride.

### 2.2. Subjects

A total of seven healthy adult male volunteers of similar build were enrolled for the studies, which were conducted on three separate occasions. Three subjects participated in the study on musk ambrette, two subjects were treated with musk ketone and another two were treated with musk xylene. The subjects were in the age range 18–50 years and were within ±10% of normal weight for age and build. Within 7 days of commencement of the study, each subject was submitted to a suitable physical examination at which medical history was obtained and blood and urine samples were taken for laboratory analysis. The study was subject to review and approval by the Ethics Committee of the Institute of Clinical Pharmacology, Dublin, Ireland, where it was conducted. Within 7 days of the end of the study, a physical examination and blood and urine analysis was carried out which showed the test compound had no detectable effect on the subjects' health. For 12 h before dosing and during the 5-day experimental period the subjects were confined to the hospital clinical pharmacology unit.

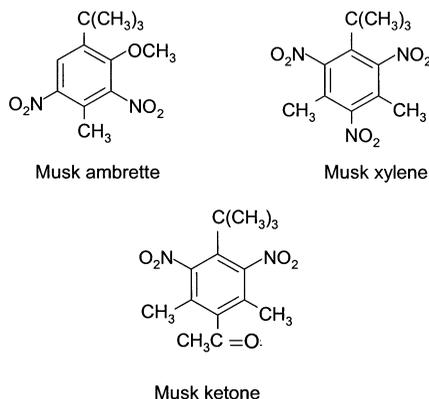


Fig. 1. Musk ambrette, musk ketone and musk xylene.

### 2.3. Test compound application and sampling

The carbon-14 labelled test compounds were dissolved in ethanol containing the minimum amount of phenylethyl alcohol (approximately 1%) to give a concentration of 1 mg/ml. The specific activities of the compounds were: musk ambrette 45  $\mu\text{Ci}/\text{mg}$ , musk ketone 20.8  $\mu\text{Ci}/\text{mg}$  and musk xylene 50.4  $\mu\text{Ci}/\text{mg}$ . Each subject fasted for 12 h before dose administration at which time 1 ml (2 ml for musk ketone) of the solution was applied evenly to an area of 100  $\text{cm}^2$  on the unshaven skin of the upper left quadrant of the chest and the ethanol allowed to evaporate for 30 min. The nominal dose levels were 1 mg (0.014 mg/kg) for musk ambrette and musk xylene and 2 mg (0.028 mg/kg) for musk ketone. The treated area was covered with a protective gauze held in position with adhesive tape. At 6 h after administration, the dressing was removed and the treated skin wiped with cotton wool swabs using phenylethyl alcohol/ethanol (1.99%, v/v). Blood samples (10 ml) were withdrawn into heparinised tubes at pre-dose and at 0.25, 0.5 and 0.75, 1, 2, 4, 6, 10, 12, 16, 24, 36, 48, 72, 96 and 120 h after dosing. A portion (1 ml) was retained and plasma separated from the remainder. Urine was collected at 0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–24, 24–48, 48–72, 72–96 and 96–120 h. Faeces were collected separately at 24-h intervals and all samples stored at  $-20\text{ }^\circ\text{C}$ . At 120 h a part (approximately 12.5  $\text{cm}^2$ ) of the treated skin was stripped with 10 successive applications of transparent adhesive tape and the strips retained for measurement of radioactivity.

### 2.4. Sample analysis

Faeces were extracted once by homogenisation in methanol. After centrifugation, samples of the separated extract and residue were measured for radioactivity. The gauze, cotton wool swabs and adhesive tape strips were separately extracted with acetone in a Soxhlet apparatus for 3 h. The extraction was repeated and radioactivity measured in both extracts. Samples of urine (4 ml) and plasma (0.5 ml) were mixed with MI-31 scintillator (Packard Instrument Company, Caver-

sham, UK). Samples of extracted faeces and cotton wool were combusted in oxygen using an Automatic Sample Oxidiser (Model 306, Mk2, Tri-Carb<sup>®</sup>, Packard Instrument Company, Caversham, UK). The combusted products in oxygen were absorbed into CarboSorb<sup>™</sup> and mixed with Permafluor<sup>®</sup>-V scintillator system. Radioactivity was measured with a Philips Liquid Scintillation analyser (Philips N.V., Eindhoven, Holland). Radioactivity in amounts less than twice background was considered to be below the limit of accurate measurement.

### 2.5. Chromatographic analysis

Urine samples were extracted with ethyl acetate before and after incubation with  $\beta$ -glucuronidase. For enzyme incubation, aliquots of urine were mixed with equal volumes of 0.1 M sodium acetate buffer (pH 5),  $\beta$ -glucuronidase (Type H1, Sigma) added and incubated for 16 h at  $37\text{ }^\circ\text{C}$ . Extracts were analysed using thin layer chromatography on pre-layered Kieselgel F<sub>254</sub> plates (Merck, Darmstadt, Germany) of layer thickness 0.25 mm using solvent systems hexane/ethyl acetate (6:4, v/v) and ethyl acetate/acetone (1:1, v/v). Radioactive components on thin-layer plates were detected either by opposition autoradiography using Singul XIRP X-ray film (Blisshen, London, UK) or with a Berthold Mark II radiochromatogram scanner (Model LB 2722). Chromatograms of human urine metabolites were compared with those for rat bile metabolites (Hawkins and Ford, 1999).

## 3. Results

The systemic availability as determined based on the excretion of radioactivity in urine and faeces was very low for all three compounds (Table 1). Most of the applied material was recovered from the surface of the skin as well as from the protective gauze at 6 h. Due to the rather low recoveries in two subjects treated with musk ambrette, a third subject was included. The urinary excretion ranged from 1.2 to 3.2% of the applied dose while faecal excretion was much lower at

Table 1

Disposition of a topical dose<sup>a</sup> of <sup>14</sup>C-labelled musk ambrette <sup>14</sup>C-labelled musk ketone or <sup>14</sup>C-labelled musk xylene following application to the backs of male human volunteers

	Musk ambrette			Musk ketone		Musk xylene	
	Subject 1	Subject 2	Subject 3	Subject 1	Subject 2	Subject 1	Subject 2
Cotton wool washes at 6 h	39.3	44.5	66.6	66.8	75.7	33.2	60.8
Protective gauze dressing	33.7	34.9	24.2	19.3	10.8	56.8	33.7
Urine (0–120 h)	1.16	1.51	3.18	0.49	0.34	0.20	0.33
Faeces (0–120 h)	0.05	0.06	0.02	0.02	0.09	<0.1	<0.1
Total recovery	74.2	81.0	94.0	86.6	86.9	90.2	94.8

Results expressed as percent applied dose.

<sup>a</sup>Doses were applied to a skin area of 100 cm<sup>2</sup> at a level of 10 µg/cm<sup>2</sup> for musk ambrette and musk xylene and 20 µg/cm<sup>2</sup> for musk ketone.

0.02–0.06%. For musk ketone results were very similar for the two subjects (Table 1) with 0.34 and 0.49% of the dose in urine and 0.09 and 0.02% in faeces, respectively. Subjects treated with musk xylene excreted 0.2 and 0.3% of the dose in urine with none detected in faeces (<0.1%) (Table 1). The maximum rate of excretion occurred during days 2–3 for all three compounds. During day 5, only 0.1% (musk ambrette), 0.02% (musk ketone) and 0.03% (musk xylene) of the doses were excreted.

No radioactivity was detected in any plasma or sample for the three materials with limits of detection of 0.0001% (10<sup>-4</sup>) dose/ml (musk ambrette) 0.00002% (2 × 10<sup>-5</sup>) dose/ml (musk xylene) and 0.0004% (4 × 10<sup>-5</sup>) dose/ml (musk ketone), which is consistent with the low absorption. Similarly, no radioactivity was detected in any blood samples at a limit of detection of 0.0001% dose/ml indicating that there was no material selectively bound to blood cells. Additionally, no radioactivity was detected (<0.02% dose) in the skin strips taken at 120 h.

Although concentrations of radioactivity in human urine samples were very low, some information on the nature of metabolites was obtained by analysis of solvent extracts of urine. In all cases, the amounts of radioactivity extracted were greatly enhanced after treatment with β-glucuronidase. About 90% was extracted for musk ambrette and for musk xylene samples and 60% for musk ketone. The parent compounds were

well separated from metabolites in the chromatographic systems developed and none were detected in any urine extracts. The musk ambrette sample extracts contained one major metabolite, which corresponded to a minor deconjugated rat bile metabolite, but not to the major rat metabolite, which was previously identified as a hydroxymethyl analogue (Hawkins and Ford, 1999). The metabolite also did not correspond to an authentic sample of the desmethyl analogue, another potential metabolite. Similarly, musk xylene human urine extracts contained a single major component, which did not correspond to the rat bile metabolite but was chromatographically similar. Musk ketone human samples contained one major component and a chromatographically similar minor component, which corresponded to a major rat bile metabolite.

#### 4. Discussion

The results of these investigations highlight the potential value of controlled human volunteer studies in safety evaluation and risk assessment. Studies in rats have shown that at 5 days after administration of dermal doses only 0.4–2.1% of applied material was still in the carcass and tissues (Hawkins and Ford, 1999). Therefore, it is reasonable to conclude that the amount of material measured in excreta provides a good index of the extent of absorption by humans under the condi-

tions of simulated exposure especially since metabolism and excretion is less complicated than in rats. The technical difficulties associated with this type of study particularly when a large amount of the dose is not absorbed means that recoveries of around 90% can be considered to be effectively quantitative. This was achieved in most subjects and while recoveries were rather low in the first two subjects for musk ambrette this was much greater in a subsequent third subject with the major difference being in the amount recovered from the treated skin.

For these compounds, human studies are particularly pertinent since there are key differences in the disposition of dermal doses between humans and rats, the main species used for toxicological evaluation. Firstly the dermal absorption was 20- to 60-fold lower in humans compared to rats, resulting in a correspondingly lower systemic exposure for equivalent doses. Interestingly, although absorption was very low in humans, the ranking order was the same as for the rat, namely musk ambrette > musk ketone/musk xylene. In contrast to the rat, urinary excretion was the predominant route of excretion of absorbed material. This could be predicted since biliary excretion is usually the main contributing reason for extensive faecal excretion in the rat. The higher molecular weight threshold for active biliary excretion in humans (> 500) compared to the rat (> 250) results in many cases in greater urinary excretion of metabolites in the former.

The primary metabolism of all three compounds is probably similar for human and rat in that phase I oxidation is involved but with different hydroxylated metabolites being formed. For both musk ambrette and musk xylene there is good evidence that the major initial Phase I metabolism involves oxidation of the arylmethyl to a benzylic alcohol. For all three compounds, the major human metabolites were different from those in the rat but the polarity indicated they could be monohydroxylated analogues. A common structural feature of these compounds is the *t*-butyl group and one possibility could be methyl hydroxylation of this function. Metabolites resulting from hydroxylation of the arylmethyl and aryl-*t*-butyl groups have been reported for musk xylene after oral doses to rats

(Minegishi et al., 1991). Hydroxylation of an aryl-*t*-butyl is a well-known pathway, one example being the antihistamine drug, terfenadine, where a corresponding hydroxy compound is a major metabolite in humans (Yun et al., 1993). It therefore seems highly probable that the major human urinary metabolites for all three nitromusks are formed by this pathway, these metabolites being conjugated with glucuronic acid and excreted in urine. The glucuronides of the hydroxylated rat metabolites are however eliminated in bile and a consequence is that they are available for further metabolism, including reduction of the nitro groups to potentially toxic arylamines, which can be reabsorbed. Arylamines have been reported as important metabolites of musk xylene excreted in both faeces and urine (Minegishi et al., 1991).

In conclusion, these studies have provided valuable information on dermal absorption and metabolism of nitroaromatics in humans, which can be applied directly to the assessment of risk resulting from exposure to products containing nitromusks.

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