THE *IN VIVO* ABSORPTION, DISTRIBUTION AND EXCRETION OF ^{14}C-METHYLEUGENOL IN MALE F-344 RATS AFTER TOPICAL APPLICATION

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ABSTRACT

Methyleugenol, an essential oil, is used as a flavoring agent for foods and as a fragrance material. Because of its use in fragrances, it is important to understand the extent of systemic exposure following dermal application. In this study aluminum skin traps containing activated charcoal were affixed to the skin. The dose of C-ME (5 mg/kg body weight, 10 µCi/animal) was applied to 8.4 cm of skin under the charcoal trap. Dosing vehicles were 100 µl of 100% ethanol (EtOH) or a solution of 25:75 diethyl phthalate (DEP):ethanol. After application, the animals were placed into metabolism cages for collection of urine and feces. At 144 hr after dosing the animals were euthanized and blood and major tissues harvested. Urine, cage rinse samples, the skin rinse at the treatment site and the rinse of the trap were analyzed directly by scintillation counting. Radioactivity adsorbed to the activated charcoal was desorbed and measured by scintillation counting. Furthermore the homogenized tissue and fecal samples, the excised skin site and the charcoal were analyzed for radioactivity by tissue oxidation. Absorbed compound was considered that excreted in the urine, feces, recovered from the cage rinse and found in tissues and in the skin at the application site. When applied in 100% ethanol, ~20% of the applied dose was absorbed; when applied in DEP-EtOH more than 40% was absorbed. Urine was the major route of elimination (60-90%) with the remaining 10 to 30% appearing in the feces. The radioactivity excreted in the urine was considerably higher and peaked 24 hr later (around 48 hr) using the DEP-EtOH vehicle. These data suggest better and slower absorption through the skin with the DEP:EtOH vehicle.

INTRODUCTION

Methyleugenol (ME) is an alkenylbenzene compound found in many essential oils and is incorporated into cosmetics, soaps, fragrances, and herbal products. It is not currently added to cosmetic products directly as an ingredient, it enters cosmetics through its use as a fragrance raw material. Human exposure to this compound is limited to low levels, but its use in many products makes it likely that exposure is relatively constant. Methyleugenol is of toxicological concern because it has been reported to be a rodent carcinogen when administered at high daily doses by gavage [1].

EXPERIMENTAL PROCEDURES

- Aluminum skin traps containing activated charcoal were affixed to the skin [2]
- The dosing solution was applied to the surface of the skin through a plastic tubing inserted through the charcoal
- After dosing the animals were placed in Nalgene metabolism cages for collection of urine and feces during the experiment
- At the end of 144 hr blood and major tissues (liver, kidneys, lungs, skin at the application and at control site) were harvested
- Urine, cage rinse, skin rinse and charcoal elute samples were analyzed directly by LSC
- Tissues, feces and charcoal samples were combusted in the sample oxidizer

REFERENCES

[1] National Toxicology Programme (NTP) (1998) NTP Technical Report on the Toxicology and Carcinogenesis Studies of Methyleugenol in F344/N rats and B6C3F1 mice. NTP TR 491. NIH Publication No. 98-3950, Research Triangle Park.

[2] Winter, S. M. and Sipes, I. G.: *Fd. Chem. Toxic.* **31 (9)**, 615-621 (1993)

[3] Burkey, J: Ph.D. Dissertation, The University of Arizona, College of Pharmacy (1999)

RESULTS

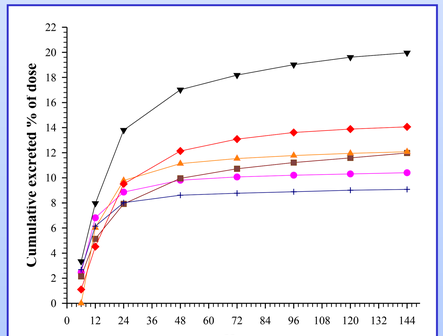


Figure 1. Cumulative excretion of C-ME equivalents in urine by individual rats

DOSING VEHICLE: 100% ETHANOL

	Mean	SD
Absorbed through the skin *(urine, cage rinse, feces, application site and tissues)*	22.9	5.5
Not absorbed *(skin rinse, trap wash, charcoal elution and burn)*	61.4	13.1
Not recovered	15.7	10.9

	Mean	SD
Urine	12.9	3.8
Cage rinse	2.4	0.8
Feces	4.2	1.2
Skin rinse	11.9	13.7
Charcoal elution	24.2	7.9
Charcoal burn	19.3	7.2
Trap wash	9.2	4.4
Application site	1.1	0.7
Other tissues	0.3	0.2
Total recovery	**84.3**	**10.9**

- **17-31% of the applied dose absorbed**
- **66.8% of the absorbed dose excreted in the urine**
- **18.6% of the absorbed dose excreted in the feces**
- **<1% of the applied dose was found in selected tissues**

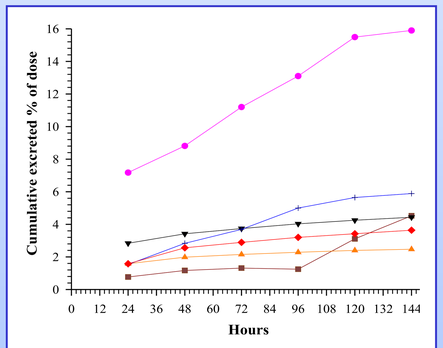
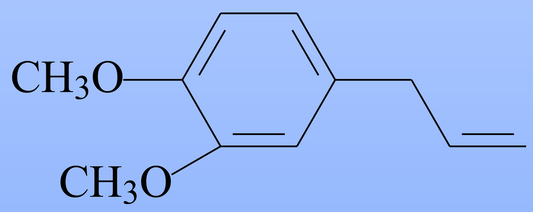


Figure 2. Cumulative excretion of C-ME equivalents in feces by individual rats



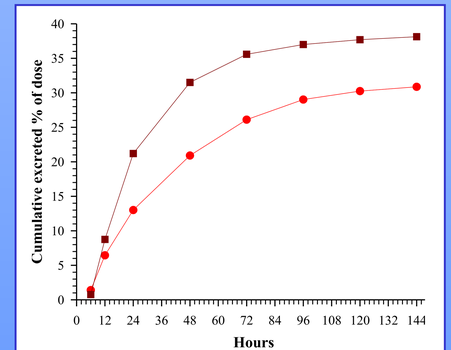


Figure 3. Cumulative excretion of C-ME equivalents in urine by individual rats

DOSING VEHICLE: 25% DEP - 75% EtOH

	Mean	SD
Absorbed through the skin *(urine, cage rinse, feces, application site and tissues)*	46.5	3.9
Not absorbed *(skin rinse, trap wash, charcoal elution and burn)*	33.5	8.6
Not recovered	20.0	4.7

	Mean	SD
Urine	34.5	5.1
Cage rinse	5.5	1.5
Feces	6.1	0.1
Skin rinse	0.1	0.1
Charcoal elution	15.9	3.1
Charcoal burn	14.0	2.5
Trap wash	3.5	3.1
Application site	0.2	0.2
Other tissues	0.2	0.1
Total recovery	**80.0**	**4.7**

- **44-50% of the applied dose absorbed**
- **86% of the absorbed dose excreted in the urine**
- **13.1% of the absorbed dose excreted in the feces**
- **<1% of the applied dose was found in selected tissues**

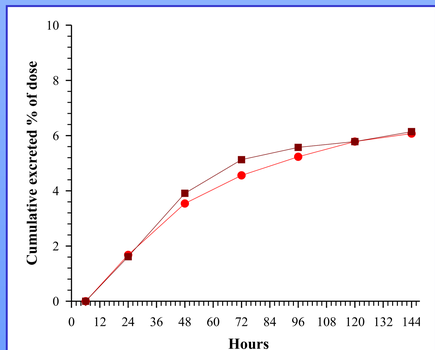


Figure 4. Cumulative excretion of C-ME equivalents in feces by individual rats

CONCLUSION

The urinary and fecal excretion of C-ME was studied after dermal application to male Fischer-344 rats. The dose was applied either in 100% ethanol or a solution of 25% diethyl phthalate – 75% ethanol.

The absorbed C-ME was associated with urine, cage rinse, feces, application site and tissues. The non-absorbed C-ME was associated with the activated charcoal, skin trap, and skin at the site of the application.

The majority of the absorbed radiolabel was eliminated in the urine and feces. This pattern of elimination is similar to that observed after oral or *iv* administration of C-ME [3]

As assessed by the excretion of C-ME equivalents, the dosing vehicle alters dermal absorption (Figure 5-6).

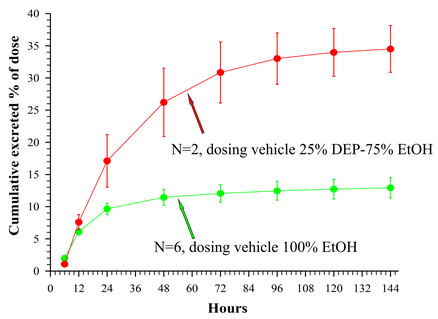


Figure 5. Comparison of the average cumulative excretion of ^{14}C-ME equivalents in urine

When the dosing vehicle was DEP-EtOH:

- **more C-ME was absorbed through the skin**
- **more C-ME equivalents were excreted in urine and feces**
- **less C-ME remained on the skin surface**

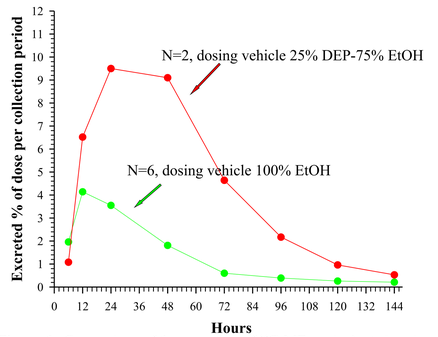


Figure 6. Comparison of the excretion of ^{14}C-ME equivalents in urine: % eliminated per collection period

When the dosing vehicle was DEP-EtOH:

- **the maximum level of radioactivity in urine and feces occurred around 48 hr instead of 24 hr**