

Sensitization Potential of Vetiveryl Acetate Evaluated Using the Local Lymph Node Assay

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ABSTRACT

Vetiveryl acetate is a fragrance material prepared by the acetylation of vetiver oil. Three possible production processes yield complex mixtures that vary in composition based on the geographic and botanic origins of the starting material. These production processes result in four distinct commercial qualities of vetiveryl acetate that are used in perfumery. A sample of each commercial quality was tested in the Local Lymph Node Assay (LLNA) to characterize its sensitization potential and to compare the potency of each. The LLNAs were conducted according to the methods described in OECD Guideline 429. Groups of five CBA/J female mice were utilized throughout the study. Each material was tested at five dose levels ranging from 1% to 25% w/v in 1:3 ethanol:diethyl phthalate. The Stimulation Index (SI) values were calculated for each dose level based on pooled lymph nodes, and an SI of 3 or more was considered a positive response. Linear interpolation of the dose response data from each LLNA was used to derive an estimated concentration (EC3) required to elicit an SI value of 3. The EC3 value was then taken as a measure of relative potency and used to compare the sensitization potential between each quality. All four samples of vetiveryl acetate can be regarded as potential sensitizers because the tests resulted in SI values greater than 3. EC3 values ranged from 9.3% to 13.3% (2317 µg/cm² to 3325 µg/cm²). The study demonstrated that there was little difference in the sensitization potential between each quality, and the materials are considered weak dermal sensitizers. The data from this study will be useful in guiding the direction of future human studies to confirm a No Expected Sensitization Induction Level (NESIL) and provide information to a Quantitative Risk Assessment (QRA) for these materials.

INTRODUCTION

Vetiveryl acetate is produced by the acetylation of vetiver oil (*Vetiveria zizanioides* Stapf) or the parent alcohol, vetiverol. More than 10 metric tons are used per year in the fragrance industry (IFRA, 2004). Four of several production qualities were chosen for testing based on the characterization most representative of the available qualities and the highest volumes of use in commerce. Groups of 5 mice per dose per material were tested by LLNA to determine whether vetiveryl acetate would induce a hypersensitivity response as measured by the proliferation of lymphocytes in the draining lymph nodes.

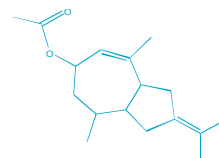


Table 1: Predominant Component of Test Material

METHODS AND MATERIALS

The 4 tested qualities were produced by acetylation, purification and rectification of vetiver oil from Brazil, China, Haiti, India, Indonesia, France, Madagascar and Venezuela. The individual components, terpenes/sesquiterpenes, ketones and acetates, were distributed differently in each type of vetiveryl acetate (see table 2).

Table 2: Sum of Constituents in 4 Tested Qualities

Sample	Terpenes/ Sesquiterpenes	Ketones	Acetates
Quality 1	20	15	54
Quality 2	5	15	69
Quality 3	1	6	87
Quality 4	15	17	54

The LLNAs were conducted in accordance with OECD Guidelines 429 and OPPTS Guidelines 870.2600. For 3 days, groups of 5 female CBA/J mice received daily topical applications on the dorsal surface of both ears with 1%, 2.5%, 5%, 10% or 25% (w/v) of vetiveryl acetate in 1:3 ethanol:diethyl phthalate. Control groups were treated with the vehicle alone. On day 6, the mice were intravenously injected in the tail vein with 20 µCi of radiolabeled ³H-thymidine in sterile saline. The mice were killed by CO₂ asphyxiation 5 hours later, and the draining auricular lymph nodes were removed. A single cell suspension was prepared by pooling the lymph nodes from each group. After washing twice with phosphate buffered saline, cells were precipitated overnight at 2-8 °C with 5% trichloroacetic acid. A β-scintillation counter was used to count the pellets, and increases in ³H-thymidine incorporation relative to control groups were then derived and recorded as stimulation indices for each group.

ANALYSIS

To measure the sensitization potential of each quality of vetiveryl acetate, the EC3 value was derived from the dose-response data by linear interpolation. Using two data points on the dose-response curve immediately above and below the SI value of 3, the EC3 value was calculated using the following equation presented by Basketter et al., (1999):

$$EC3 = c + [(3-d)/(b-d)](a-c)$$

Data points lying directly above and below the SI value of 3 on the dose-response curve have the coordinates (a,b) and (c,d), respectively. The concentrations of test material, given as % w/v, lie on the x-axis, and the SI values lie on the y-axis.

RESULTS AND DISCUSSION

Vetiveryl acetate did not appear to cause any overt toxicity, but the lymph nodes of the groups treated with the 25% doses were enlarged. No erythema or edema was noted during the study. All 4 qualities tested induced 3-fold or greater increases in proliferative lymphocyte activity relative to controls. The SI values were greater than 3, and the EC3 values ranged from 9.3%-13.3% (2317 µg/cm² to 3325 µg/cm²) (see table 3). Based on the potency classifications recommended by ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) in Technical Report Number 87, these materials are considered weak to moderate dermal sensitizers. As shown by the similar EC3 values in table 3, there were no significant differences between the sensitization potentials of the four qualities.

Table 3: Vetiveryl Acetate Local Lymph Node Assay Results

SAMPLE	CONCENTRATIONS (%)	DPM/LYMPH NODE	STIMULATION INDEX	EC3
Quality 1	1:3 DEP/EtOH Control	1578	-	13.1% (3277 µg/cm ²)
	1.0	1346	0.85	
	2.5	1469	0.93	
	5.0	2391	1.52	
	10.0	1767	1.12	
Quality 2	25.0	16064	10.18	13.3% (3325 µg/cm ²)
	1:3 DEP/EtOH Control	1481	-	
	1.0	1702	1.15	
	2.5	2209	1.49	
	5.0	3390	2.29	
Quality 3	10.0	3310	2.23	9.3% (2317 µg/cm ²)
	25.0	8493	5.73	
	1:3 DEP/EtOH Control	812	-	
	1.0	1358	1.67	
	2.5	1543	1.90	
Quality 4	5.0	2053	2.53	10.9% (2722 µg/cm ²)
	10.0	2703	3.33	
	25.0	8196	10.09	
	1:3 DEP/EtOH Control	1302	-	
	1.0	2072	1.59	
Quality 4	2.5	1151	0.88	
	5.0	2606	2.00	
	10.0	2835	2.18	
	25.0	20747	15.93	

REFERENCES

- Basketter, D.A., Lea, L.J., Dickens, A., Briggs, D., Pate, I., Dearman, R. and Kimber, I. (1999) A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. *Journal of Applied Toxicology*, 19, 261-266
- European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Technical Report 87. Contact sensitization: classification according to potency, Brussels, 2003
- Environmental Protection Agency (EPA). 2003. Health effects test guidelines. OPPTS 870.2600 Skin Sensitization. Washington, D.C.: GPO
- International Fragrance Association (IFRA), 2004. Use Level Survey, December 2004
- Organization for Economic Co-Operation and Development (OECD), 2002. Guideline reference 429. Paris, France:OECD