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Bioavailability and Pharmacokinetics of Natural Volatile Terpenes in Animals and Humans

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Abstract: **Herbal medicinal products containing natural volatiles are used in the treatment of gastrointestinal diseases, pain, colds and bronchitis. Many pharmacological studies report a wide variety of *in vitro* effects, with anti-inflammatory and antimicrobial activities investigated most frequently. In comparison, relatively few studies on the bioavailability and pharmacokinetics have been carried out. Thus, the relevance of the *in vitro* activity to the therapeutic effects found in individual studies or documented in textbooks of phytotherapy is still not established. Further studies with essential oils and their single compounds providing supporting evidence of efficacy and demonstrating systemic availability are necessary. Such data could also be important in the context of safety.**

Key words: **Terpenes, volatiles, herbal medicinal products, bioavailability, pharmacokinetics.**

Introduction

Essential oils are mixtures of lipophilic, liquid, volatile, and often terpenoid compounds present in higher plants. More than 3000 compounds have been described so far (1). The clinical efficacy of volatiles is particularly well-established for chronic pulmonary obstruction and acute bronchitis. For these indications clinical trials have been carried out with products containing 1,8-cineole (2), (3), (4), standardised myrtol (1,8-cineole, α-pinene, limonene) (5) and thyme extract (6), (7). Further clinical studies have been carried out with peppermint oil for the treatment of irritable bowel syndrome (8), (9), non-ulcer dyspepsia (10), and tension-type headache (11). Numerous essential oils and their components have shown antimicrobial or antimycotic activity in *in vitro* studies (12), (13), (14), (15), (16).

Various other *in vitro* activities of volatile oils or compounds have been reported. However, the clinical relevance of these activities depends on the systemic availability of these compounds in the respective target organs. Thus, investigation of absorption, distribution and metabolism is necessary to link *in vitro* with *in vivo* data. They may also be important in context of the safety of herbal medicinal products containing natural volatiles. However, pharmacokinetics of volatile natural compounds have not yet been investigated satisfactorily. For several monoterpenoid and phenylpropanoid compounds there is a large amount of experimental data, but crucially – especially with respect to humans – pharmacokinetic data are lacking.

This article reviews the data currently available on the systemic fate of natural volatile terpenes and phenylpropanes. Unfortunately, methodological details, particularly concerning validation of the analytical methods used have not been published for most of the studies. Therefore the results of those studies discussed here have to be considered cautiously, since the assays used might not match the requirements for analytical validation.

Absorption and Systemic Availability

Dermal absorption

Preparations of eucalyptus oil or mountain pine oil containing α- and β-pinene, camphor, 3-carene, and limonene have been used in most studies investigating the dermal absorption of essential oil compounds. These monoterpenoid compounds are readily absorbed after dermal application due to their lipophilic character. In order to avoid pulmonary absorption of evaporating compounds, an external air supply was provided for subjects in all studies. The skin did not represent a barrier to the diffusion of essential oil compounds (17), (18). Application by ointment to humans (19) and by bath to mice (18) resulted in a fast increase of plasma levels of the respective compounds. Maximum plasma levels were reached within 10 min of application (Fig. **1**). It was shown that the extent of absorption depended on the size of treated skin area (18), skin properties, concentrations of the administered compounds and on time of exposure (17). However, the latter results were obtained in a study performed with only one subject and thus allow only limited conclusions (For details see Tables **1** and **2**).

Absorption after oral administration

Only a few studies have addressed the absorption of volatile compounds after ingestion. From three studies with radio-labeled ^{14}C-citral and [1-^{14}C]-*trans*-anethole administered to

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rats, the rate of absorption was estimated from the recovery of ^{14}C in faeces or urine, respectively (20), (21), (22). The authors found 91 – 95 % of the original activity in faeces and urine and determined seven metabolites by HPLC.

In a pharmacokinetic study with human volunteers, enteric coated capsules containing a defined mixture of limonene, 1,8-cineole and α-pinene, were investigated (41). As capsules were administered uncrushed and crushed (as a surrogate of a liquid application), some information on the absorption of 1,8-cineole, which was the only compound detected in sufficient quantities in the plasma of all patients, can be extracted from this study. Patients who took the compounds as a capsule showed rather similar AUC values of 1,8-cineole to those who took the crushed capsules. The difference of the AUC of both administration modes was smaller than 7 %. As expected, the C_{max} of crushed capsules was more than 25 % higher, and t_{max} was 0.75 h compared to 2.5 h with uncrushed capsules. These data suggest that the upper part of the gastrointestinal tract has no significant role with respect to the absorption of 1,8-cineole, but additional data would be helpful for a better understanding of absorption of ingested volatile compounds. Data derived from radio-labeled compounds as published in (20) are not sufficient, as long as no information on the identity of the carrier after metabolization is provided.

Pulmonary absorption

The volatile monoterpenes are particularly suitable for inhalation as used in the treatment of respiratory tract infections. Following inhalation, the compounds may be absorbed by the lung, and systemic availability is possible. In particular, for α-pinene, camphor and menthol this absorption route has been confirmed experimentally. The reported range was 54 – 76 % of the dose supplied with inhaled air (Fig. **2**) (23), (24), (25). However, estimation of the amount absorbed by calculating the difference between inhaled and exhaled air does not account for various uncertainties, (e.g., regarding mucosal drug deposition and metabolism) and does not consider distribution into other compartments. These concerns were supported by a study that measured blood levels, since only 4 – 6 % of the amount assumed to be absorbed was actually found in the blood (24). Various factors seem to influence the extent of absorption during inhalation. Römmelt et al. (1988) demonstrated that pulmonary absorption depended on the kind of compound and the breathing mechanics of the subjects (23). Furthermore, it was evident that the release of compounds from water into the headspace depended on water temperature. At 80 °C, 12 % camphor but only 5 % menthol could be detected in the headspace within 15 minutes. (For details see Table **2.)**

Metabolism and Pharmacokinetics

Metabolism

The metabolic fate of essential oil components depends on their individual chemical structure, and hence generalisation is not possible. Metabolites resulting from both phase-I and phase-II reactions have been reported.

Oxidation products of thymol and carvacrol were determined after oral administration of the genuine compounds to rats (Fig. **3**) (26). As phase-II metabolites, glucuronides or sulfates were detected in rats, rabbits, and humans (26), (27). Unchanged compounds could be detected only in small amounts in 24 h urine (27).

Several studies investigated the metabolic fate of *t*-anethole in humans and rodents (28), (29), (30). Determination of urinary metabolites suggested that in humans ^{14}C-*trans*-anethole was completely metabolised by oxidative *O*-demethylation and various oxidative alterations of the side chain (30). Metabolites were excreted both unconjugated and conjugated to either glycine or glucuronic acid (29). No unchanged *t*-anethole was detected in urine. The pattern of metabolites in human urine differed only quantitatively from that seen in rodent urine. A dose-dependent variation in urinary metabolites was evident for *t*-anethole in rodents as well as ^{14}C-eugenol in rats (31). For *t*-anethole the formation of phase-I metabolites was dose-dependent (28), whereas ^{14}C-eugenol showed dose-dependent variations of conjugates.

Metabolites of menthol and peppermint oil were investigated in several human studies (32), (33), (34). After oral application of L-(–)-menthol or peppermint oil, respectively, 35 – 50 % of the original menthol content was excreted renally as menthol glucuronide. Only one study conducted by Bell et al. examined the free fraction of menthol and the glucuronide (34). No unchanged menthol and only traces of the sulphate conjugate were detected. There was a significant interindividual variation in the quantities excreted, which is likely to be due to differences in absorption and dietary habits (33). After administration of peppermint oil to ileostomy patients elimination of menthol glucuronide was less than after administration to healthy subjects. This indicated that absorption of menthol mainly took place in the small intestine (32).

Other components of peppermint oil like menthone or menthyl acetate were not assayed in these studies. However, these components could easily be metabolised to menthol and excreted as menthol glucuronide as well.

The metabolic fate of menthol was studied in detail in rats after oral administration (35), (36). Oxidation patterns were similar to thymol. Menthol was conjugated at the 3-hydroxy

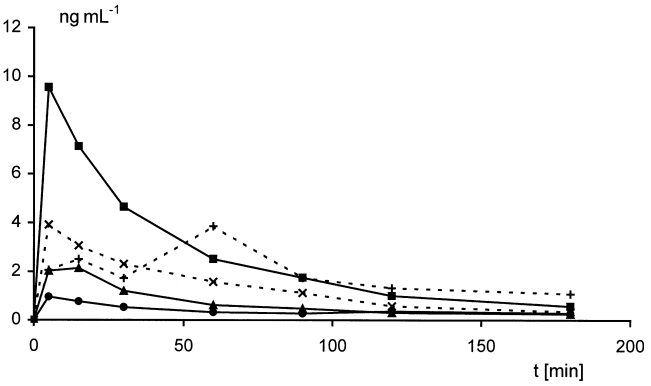

Fig. 1 Plasma levels of –■– α-pinene, –●– camphor, –▲– β-pinene, –×– 3-carene and –+– limonene after dermal application of 2 g Pinimenthol-S-ointment® at 400 cm^2 area in 12 human subjects (19).

Table 1 Pharmacokinetic data in animals and humans.

Compound(s)	ozothin[1]	(+)-α-pinene, (–)-α-pinene		1,8-cineole										men- thol	cam- phor
subject	humans	humans		humans	mice	humans				humans				humans	
n	9 and 27	8 ♂		6 ♂, 6 ♀	5	20 ♂, aged 19–42				2 ♀		2 ♂		10 ♂	
application	intravenous	2 h inhalation, light physical exercise in exposure chamber		dermal ointment, area: 400 cm²	1 h inhalation	oral				20 min inhalation				10 min inhalation from ointment in water	
					rosemary oil	capsule		capsule crushed						(500 mL; 80 °C)	
						cross over									
dose	10 mL	450 mg · m⁻³; 225 mg · m⁻³ 450 mg · m⁻³; (–)-α-pinene		2 g	0.5 mL	120 mg²	300 mg³	120 mg²	300 mg³	4 g				5 g ointment	
	α-phase	α-phase	β-phase		α-phase	β-phase				α-phase		β-phase			
distribution	n = 9														
and elimination	3–4	450 mg · m⁻³; 225 mg · m⁻³		26	6	45	92	221	100	206	2.0	4.8	31	33	35.5	39.9
half life (min)		4.8	38													
		450 mg · m⁻³ (–)-α-pinene														
		5.6	40													
volume of distribution (L · kg⁻¹)				8425												
t_{max} (min)		120		6.3			138	154	42	65	19		14		15	
c_{max} (ng · mL⁻¹)				7.4			72	168	108	205	868		1135		459	
clearance		Cl_{4h} (l · h⁻¹ · kg⁻¹)		14186 (L · h⁻¹)												
		1.4	1.3	1.4												
		Cl_{21h} (l · h⁻¹ · kg⁻¹)														
		1.1	1.1	1.2												
reference	(37)	(38)		(19)	(40)	(41)					(39)				(23)	

[1] Ozothin: myrtenal, myrtenol, pinocarveol, verbenon, terpinhydrate.

[2] Myrtol: 8 mg α-pinene, 30 mg limonene, 30 mg 1,8-cineole.

[3] Myrtol: 20 mg α-pinene, 75 mg limonene, 75 mg 1,8-cineole.

Table 2 Absorption in animals and humans.

Compound (s)	camphor, menthol, α-pinene, isobornyl acetate, limonene radiolabeled, main compounds from Pinimenthol®-bath		α-pinene, camphor, β-pinene, limonene	^{14}C-citral	^{14}C-*t*-anethole radiolabeled	α-pinene	limonene	camphor	borneol	menthol	camphor	(+)-α-pinene	(–)-α-pinene
subject	mice		human	rats	rats and mice	human, aged 20 – 30				humans aged 20 – 45		humans aged average 31	
n	3 ♂	3 ♂	1	>3	6 ♂, 6♀	6 ♂ and ♀				10 ♂		2 ♂	
application	solution shaved skin areas		solution intravenous	oral and intravenous	oral	inhalation				10 min inhalation from water (500 mL; 80 °C)		inhalation during light physical exercise	
	3 cm^2	1.5, 3, 6 cm^3											
dose	20 mL bathing concentrate containing one of the labeled compounds in 100 L water		450 L water, 30 min, 150 mL mountain pine oil i.v.: 50 mg · kg^{-1}	oral: 5, 50, 500 mg · kg^{-1} i.v.: 50 mg · kg^{-1}	250 mg·kg^{-1}	10 % aqueous solution of the compound concerned				5 g ointment		2 h exposure 450 mg · m^{-3}	225 10 450
absorption	no compound absorbed preferably	amount absorbed depended on treated skin area	depending on exposition time, covered skin area, concentration	91 – 95 % absorption	>95 % recovery of radioactivity in 24 h urine	61 %	66 %	54 %	58 %	76 %	67 %	58 %	60 % 40 % 58 %
						percentage found in blood unmetabolised							
						5.6 %	4.6 %	7.5 %	6.5 %				
reference	(18)		(17)	(21)	(22)	(24)				(23)		(25)	

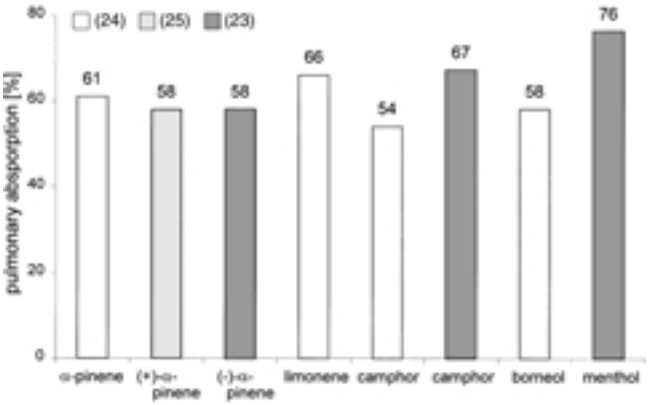

Fig. 2 Pulmonary absorption after inhalation

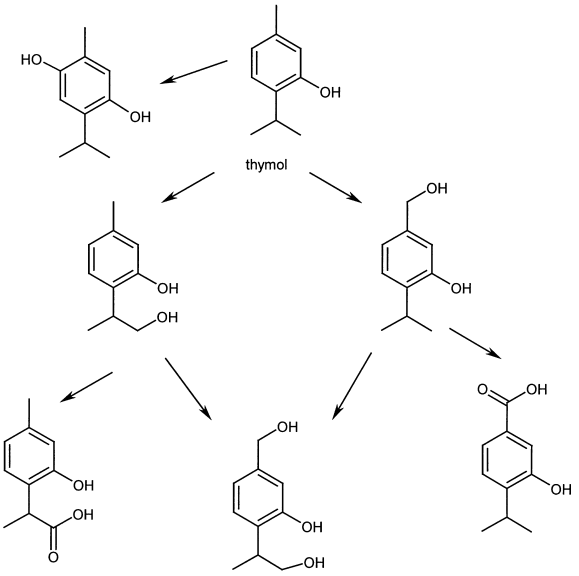

Fig. 3 Urinary phase-I metabolites and suggested metabolic routes of thymol in rats (26).

group (35). Its glucuronide was also the main urinary metabolite in rats (60%) (36). Therefore menthol glucuronide seemed to be the main urinary metabolite in both humans and rats.

Investigations of citral (isomeric mixture of neral and geranial) revealed complete and stereoselective metabolism in rats (21). In addition to the liver, other organs were involved in the metabolism. Comparing the routes of excretion after oral, intravenous, and dermal application, a cutaneous first pass effect was suggested (20). (For details see Table **3**.)

Pharmacokinetic data

In general, most essential oils and their components studied so far have been pharmacokinetically characterised by an elimination profile that is at least biphasic (37), (38), (39), (40). This suggests that these compounds are distributed from the blood into other tissues. Due to high clearance and short elimination half lives, accumulation was improbable.

The best approach to obtain basic pharmacokinetic parameters of a compound (half life, volume of distribution, and clearance) is to follow the change in plasma concentrations over time after intravenous injection. However, there was only one study carried out by Kleinschmidt et al. (1985), measuring plasma levels after intravenous injection of a bronchosecretolytic mixture of terpenes (ozothin®) in humans. Plasma concentrations were calculated as total terpene concentrations. The short half life in the α-phase of 3 to 4 min indicated fast distribution of terpenes into tissues. Elimination half life of the β-phase was 60 – 65 min due to metabolism and excretion (37).

Pharmacokinetic parameters of α-pinene were also determined after dermal application and inhalation (19), (38). The half life in the α-phase was very short, about 5 min, followed by longer half lives in the β-phase ranging from 26 to 38 min. In the latter study (38) a third γ-phase was determined with an elimination half life of 695 min. The other study (19) showed a rise of α-pinene concentrations in a few subjects six to ten hours after dermal application; however, considerable variations due to the methodological difficulties of measuring low concentrations prevented the authors from including these data into a pharmacokinetic calculation. This highlights the importance of long sampling periods combined with sensitive methods for the determination of pharmacokinetic data. The high volume of distribution of α-pinene suggested a disposition in compartments, which was likely due to the high affinity to lipophilic structures. Despite a high volume of distribution, the high clearance indicated fast elimination of α-pinene. Thus, there should be no accumulation even during long-term administration. In this study high standard deviation values were conspicuous. This might be explained by the heterogenous sample, including both men and women.

After inhalation of 1,8-cineole a substantial difference of elimination half lives in male and female subjects was found (39). Whereas pulmonary absorption and t_{max} were within a similar range, elimination half lives were at least twice as long in female subjects. Therefore it was assumed that subcutaneous fat was an important factor influencing the elimination of 1,8-cineole.

The bioavailability of 1,8-cineole and the pharmacokinetics of myrtol (a mixture of 30 mg 1,8-cineole, 30 mg limonene and 8 mg α-pinene), were evaluated after oral administration of crushed and uncrushed capsules (41). Comparison of the cineole plasma levels yielded a relative bioavailability of approximately 100% for the uncrushed capsules. As expected, plasma levels remained elevated for a longer time after administration of uncrushed capsules. Limonene and α-pinene could be detected in only a few subjects.

Elimination data for menthol and camphor after inhalation were fitted to a two-compartment model (23). Elimination half lives were 35.5 and 39.9 min for menthol or camphor, respectively, indicating that there should be no accumulation even during long-term application. (For details see Table **4**).

Excretion

Elimination of essential oil compounds was monitored in urine, faeces or expired air. The major part of the compounds

Table 3 Metabolism in animals and humans.

Compound	thymol			carvacrol	citral	*t*-anethole				eugenol	menthol					
subject	human	rabbit	rats	rats	rats	human	rats mice	rats/mice	rats	human	human aged 17 – 37		ileostomy patients		human	rats
n	2	3	♂	♂	>3	2♂	♀ ♂	♀ ♂	♀	20	4♂, 2♀		3♂, 3♀		4♂	♂
application	oral	oral	stomach-tube	stomach-tube	oral, intravenous, dermal	oral	oral/ intraperitoneal	oral/ intravenous	stomach tube	oral	oral				oral enteric	oral
											capsules	coated capsules	capsules	coated capsules	coated capsules	
dose	0.6 g	0.5 g · kg^{-1}	1 mmol · kg^{-1}	1 mmol · kg^{-1}	500 mg · kg^{-1}, 5, 50 mg · kg^{-1}	1 mg	50 mg · kg^{-1}	0.05, 5, 50, 1500 mg · kg^{-1}	0.05 – 1000 mg · kg^{-1}	500 mg menthol	0.4 mL peppermint oil = 95 mg menthol				180 mg peppermint oil	menthol 0.1 – 1.0; (19)	0.5 mg · kg^{-1} (39)
pase-I metabolism																
urine	thymohydroquinone		see Fig. **3**	oxidation pattern like thymol	reduction and hydroxylation of 2,3-double bond; oxidation of anhyde	4-MBA[2] 3 %	ω-oxidation; cinnamate derivatives;	dose dependent variation	reduction of double bond							oxidation on the methine and methyl sites
	oxidised products				function; stereoselective at C8		oxidation of olefinic double bond									traces of unchanged menthol
phase-II metabolism								calculated as percentage of original menthol content								
glucuronide	+	+[1]	+		+	+	+		at high doses	prior to and after GS-assay[4]:	after GS-assay[4]:				after GS-assay[4]:	after GS-assay[4]:
										50 % within 24 h traces	35 % within 24 h	40 % within 24 h	17 % within 24 h	29 % within 24 h	40 % within 14 h	60 %
sulphate	+	+[1]	+						at low doses							
further conjugates					cutaneous first pass metabolism	glycine conjugates 4-MHA[3] 56 %	glycine conjugates, glutathione conjugates									
number of metabolites			6	7	7		11	10								14
reference	(27)			(26)	(20), (21)	(30)	(29)	(28)	(31)	(34)	(32)				(33)	(35), (36)

[1] indirect analysis. [2] 4-MBA: 4-methoxybenzoic acid. [3] 4-MHA: 4-methoxyhippuric acid. [4] GS-assay: glucuronidase and sulfatase assay.

Table 4 Elimination in rodents.

Compound		menthol		linalool	citral			*t*-anethole				eugenol
subject		rats	bile duct cannulated rats	rats	rats			rats		mice		rats
n		3 ♂		2 ♂	>3 ♂			♀		♂		♀
application		oral gavage		stomach tube	oral	intravenous	dermal	oral/intravenous				stomach tube
dose		500 mg · kg^{-1}		500 mg · kg^{-1}	5, 50, 500 mg · kg^{-1}	5 mg · kg^{-1}	5 – 50 mg · kg^{-1}	0.05 mg · kg^{-1}	1500 mg · kg^{-1}	0.05 mg · kg^{-1}	1500 mg · kg^{-1}	0.05 – 1000 mg · kg^{-1}
route of elimination	urinary	19% preferred on 2nd day	7%		51%	58%	8.5%	56%	32%	72%	35%	75 – 80%
	pulmonary			23% CO_2	17% CO_2	8% CO_2	3.3% CO_2	28%	57%	20%	67%	
					unmetabolised							
					0.48%	0.33%	2.84%					
	faecal	26%	67% biliary		12%	7%	3.48%	1%		1%		10%
		preferred on 1st day										
enterohepatic circulation		+		+								
end of detection		48 h		72 h	72 h			72 h				24 h
reference		(35)		(42)	(20)			(28)				(31)

and their metabolites was eliminated by the kidneys and the lung. A minor part was eliminated via the faeces. Only traces of the compounds were eliminated unmetabolised in urine or faeces. Enterohepatic circulation was evident for menthol and linalool delaying their excretion (35), (42).

Pulmonary elimination

Due to their volatility, essential oil compounds or their metabolites are likely to be exhaled. However, only 1.5 – 5% of intravenously injected monoterpenes were eliminated unchanged by the lung. 75 – 95% of this fraction were exhaled within the first 10 – 40 min (17), (37). The amount of terpenes exhaled decreased with increasing boiling point of the respective compounds (17). The major part of the compounds was assumed to be metabolised and exhaled as CO_2 or renally eliminated as terpene conjugates (17), (25), (37). (For details see Table **5**.)

Balance of elimination

Complete elimination and excretion of the applied dose was only tracked for citral and *t*-anethole (20), (28). For these compounds urinary excretion was found to be the main route of elimination accounting for over 50% of the dose (20), (28), followed by pulmonary elimination. Faecal excretion was assumed to be a minor route of elimination.

After oral administration of *t*-anethole in humans, metabolites were mainly excreted renally (approximately 60%) (30), (43). A smaller fraction was metabolised and eliminated via the lung as $^{14}CO_2$. Cumulative excretion curves indicated that elimination was completed within 24 hours. Increasing doses had no influence on the excretion pattern of *t*-anethole (Fig. **4**) (43).

In contrast to these observations, elimination patterns of *t*-anethole in rats and α-pinene in humans were dose dependent (28), (25). At low doses, most of the applied ^{14}C-labeled *t*-anethole was eliminated through the lung as $^{14}CO_2$, indicating that oxidative *O*-demethylation predominated in rats after application of low doses (Fig. **5**). In contrast to *t*-anethole, the percentage of α-pinene excreted renally (main metabolites: verbenol) increased with decreasing exposure levels (25). These findings might be the result of saturation of particular metabolic enzymes after administration of high doses.

Changes in excretion profiles according to the kind of application were observed for citral. The differences after oral and intravenous application were interpreted as a consequence of

Table 5 Elimination in humans.

Compound		α-pinene	camphor	β-pinene	limonene	ozothin	^{14}C-*t*-anethole				(+)-α-pinene			(+)-α-pinene
subject		human				human	human		human		human			
n		1 ♂				27 and 9 ♂	3 ♂		5 ♂		2 ♂			
application		intravenous or bathing				intravenous	oral		oral		inhalation 2 h exposure			
dose		intravenous: 0.6 $\mu g \cdot kg^{-1}$				10 mL	1 mg	1 mg	50 mg	250	450 mg $\cdot m^{-3}$	225 mg $\cdot m^{-3}$	10 mg $\cdot m^{-3}$	450 mg $\cdot m^{-3}$
		bathing: 150 mL mountain pine oil in 450 L water												
route of elimination	urinary						60%	60.1%	68.6%	53.9%	1.7% verbenols	2% verbenols	3.8% verbenols	1.5% verbenols
											0.001% unchanged in urine			
	pulmonary	intravenous				5–7%	20%	13.5%	17%	13.8%	7.7%	not determined	not determined	7.7%
		5% unmet.[1]	3% unmet.[1]	3.6% unmet.[1]	1.4% unmet.[1]	unmet.[1]	as CO_2							
		75% eliminated after:												
		10–15 min bathing	10–15 min	10–15 min	20–30 min									
		2 h after bathing: % of the maximum value detectable:												
		60%	60%	60%	40%									
end of detection		after 45 min: 90% eliminated				60 min	8 h		48 h		2 h			
reference		(17)				(37)	(30)		(43)		(25)			

[1] unmetabolised.

decomposition by intestinal bacteria or first pass metabolism following gastrointestinal absorption. Overall transport or metabolism of citral revealed not to be dose dependent in the dose range studied (20). (For details see Table **4** and **5**).

Analytical Methodology of Essential Oil Compounds in Biological Matrices

In most studies presented here, the lack of documentation of the assay methodology applied is a striking and crucial fact. Appropriate sensitive and selective detection methods are a major prerequisite for the analysis of volatile compounds and their metabolites in biological matrices. Accuracy, precision and specificity have to be evaluated by appropriate validation assays.

Due to their volatile character essential oil compounds are accessible to gas chromatographic analysis (GC). Combined with appropriate detection GC provides a sensitive analytical method with limits of detection in the lower ng/mL range. The most selective detection system available today is GC/MS or GC/MS/MS using selected ion monitoring or selected reaction monitoring experiments. However, it has not yet been used for quantitative analysis in pharmacokinetic studies of essential oil components. For quantification in biological matrices at trace levels by GC/MS or GC/MS/MS, standards labeled with stable isotopes are needed.

Urinary metabolites have often been detected by GC analysis after enzymatic hydrolysis of phase II metabolites such as glucuronides and/or sulfates. In most studies the initially non-conjugated free compounds were not determined. This might be the reason for varying results in different studies with similar designs. For sample preparation compounds and the corresponding phase-I metabolites were either extracted from urine or plasma with lipophilic solvents (ethyl acetate, ether), separated by solid-phase extraction or analysed directly via headspace analysis. Lower limits of detection might have been achieved by using other extracting solvents (pentane, hexane, isopropanol) or adsorbents (e.g., ethyl-vinyl-benzene polymer). However, comparative evaluation of the different methods used can hardly be performed since method validation was rarely reported.

Adsorbents were also used to trap and determine pulmonarily eliminated compounds in two studies. A different method was applied by Levin (25). Infrared spectroscopy was used for the determination of pinene concentrations in an exposure chamber and in the exhaled air of the subjects. This method was the only one published which allowed simultaneous and on-line monitoring of exposure concentrations and pulmonary elimination. The limit of detection was not presented.

Determinations of compounds, which become non-volatile by phase-I and phase-II metabolism, require other analytical methods, such as HPLC. In order to achieve sufficient sensitivity, liquid scintillation detection of labeled compounds was used in these studies. However, radioactive labeling is not always the ideal method, because such data do not provide any information on the identity of the analyte. Additionally, exhaled $^{14}CO_2$ was trapped in alkaline solutions or charcoal, a method that carries the risk of underestimation.

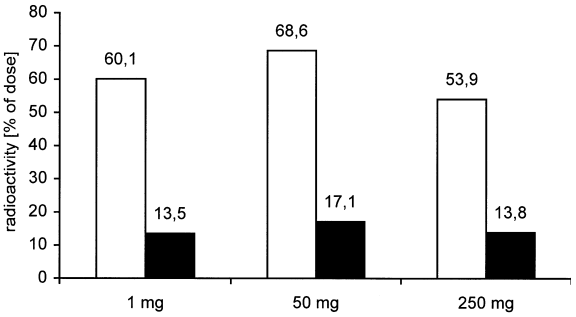

Fig. 4 Urinary (□) and pulmonary ($^{14}CO_2$) (■) elimination after application of different doses of ^{14}C-*t*-anethole to humans (30).

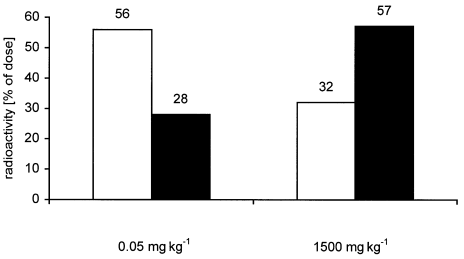

Fig. 5 Urinary (□) and pulmonary ($^{14}CO_2$) (■) elimination after application of different doses of ^{14}C-*t*-anethole to rats (28).

Nowadays multi-channel electrochemical detection offers a new, very sensitive detection method with liquid chromatographic analysis which may become significant with regard to non-volatile metabolites in the future.

The published data can only be evaluated with regard to the applied analytical methodology, but satisfactory analytical parameters of the assays used were only reported in a few studies, for example by Kaffenberger et al. and Zimmermann et al. (33), (41). In most other reports the different results obtained in similar studies therefore cannot be put down to differences in analytical methods, although this might have had a major impact on the outcome of the investigations. For all methods used, it is a minimum requirement in method validation to assess and document the precision of retention times, linearity over the desired concentration range, limit of quantification, and analyte recovery at different concentrations.

Conclusions

Despite the number of studies which provide a large quantity of data for several volatile compounds, there is a lack of good quality pharmacokinetic data in humans.

The few existing pharmacokinetic studies of essential oils after intravenous administration suggest that essential oil components are quickly eliminated in humans with an elimination half life of about one hour. The volume of distribution is considered to be high. Regarding these findings together with the high clearance of essential oil compounds, accumulation is unlikely.

Most studies suggest that essential oil compounds are quickly absorbed after oral, pulmonary, or dermal administration. Only a small fraction is eliminated unchanged by the lungs, whereas the major portion is metabolised and either eliminated by the kidneys in the form of phase-II conjugates – mainly glucuronides, or exhaled as CO_2.

Studies on the pharmacokinetics and bioavailability of essential oils and their compounds require a highly sensitive and specific assay methodology. Differing or even contradictory results from various studies might be caused by differences in the applied analytical methods. This, however, could not be critically evaluated since analytical details were not published in most of the studies. As the assays used might not match the requirements for analytical validation, the results of the studies reviewed have to be considered cautiously.

The data available so far express a great demand for further pharmacokinetic studies in humans. Reliable pharmacokinetic data in humans would be an important key to the question whether a volatile compound or its metabolites may have a potential effect on certain diseases which is therapeutically relevant. Detailed information about absorption, metabolism, distribution and elimination may also be important in the context of safety evaluations of herbal medicinal products. Some basic and principle pharmacokinetic parameters of isolated compounds are reported but they are not linked or compared to respective data in complex mixed essential oils.

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