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Evaluation of the interindividual human variation in bioactivation of methyleugenol using physiologically based kinetic modeling and Monte Carlo simulations

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ABSTRACT

The present study aims at predicting the level of formation of the ultimate carcinogenic metabolite of methyleugenol, 1′-sulfooxymethyleugenol, in the human population by taking variability in key bioactivation and detoxification reactions into account using Monte Carlo simulations. Depending on the metabolic route, variation was simulated based on kinetic constants obtained from incubations with a range of individual human liver fractions or by combining kinetic constants obtained for specific isoenzymes with literature reported human variation in the activity of these enzymes. The results of the study indicate that formation of 1′-sulfooxymethyleugenol is predominantly affected by variation in i) P450 1A2-catalyzed bioactivation of methyleugenol to 1′-hydroxymethyleugenol, ii) P450 2B6-catalyzed epoxidation of methyleugenol, iii) the apparent kinetic constants for oxidation of 1′-hydroxymethyleugenol, and iv) the apparent kinetic constants for sulfation of 1′-hydroxymethyleugenol. Based on the Monte Carlo simulations a so-called chemical-specific adjustment factor (CSAF) for intraspecies variation could be derived by dividing different percentiles by the 50th percentile of the predicted population distribution for 1′-sulfooxymethyleugenol formation. The obtained CSAF value at the 90th percentile was 3.2, indicating that the default uncertainty factor of 3.16 for human variability in kinetics may adequately cover the variation within 90% of the population. Covering 99% of the population requires a larger uncertainty factor of 6.4. In conclusion, the results showed that adequate predictions on interindividual human variation can be made with Monte Carlo-based PBK modeling. For methyleugenol this variation was observed to be in line with the default variation generally assumed in risk assessment.

Introduction

Methyleugenol (3,4-dimethoxyallylbenzene) is a member of a family of chemicals known as alkoxy allylbenzenes which includes also compounds such as safrole and estragole (Robison and Barr, 2006). Methyleugenol enters the diet via different food sources including herbs and spices (e.g. basil, sweet bay, cloves and lemon grass) which are consumed at low levels in the human diet (Gardner et al., 1997) or foods flavored with these herbs or their essential oils. Methyleugenol was selected to be studied by the National Toxicology Program (NTP) because of its broad use and its structural resemblance to the other carcinogenic alkoxy allylbenzenes including safrole and estragole. Based on the results of the National Toxicology Program (NTP) study, methyleugenol was observed to have a carcinogenic effect in both F344 rats and B6C3F1 mice (NTP, 2000). The Expert Panel of the Flavor and Extracts Manufacturers Association (FEMA) classified methyleugenol as GRAS (generally recognized as safe) at the proposed levels of flavor use in 1965 (Hall and Oser, 1965). In 2001, the FEMA Panel reassessed the available data for methyleugenol and confirmed that there is no considerable cancer risk resulting from consumption of methyleugenol as flavouring substance and affirmed the GRAS status of methyleugenol as a flavoring substance given the low levels of exposure (Smith et al., 2002). In 2001, the Scientific Committee on Food (SCF) of the European Union published a scientific opinion on methyleugenol in which it was concluded that methyleugenol is genotoxic and carcinogenic and that reductions in exposure and restrictions in use levels are indicated (SCF, 2001). The average daily intake of methyleugenol was estimated by the SCF to be 13 mg/day, corresponding to 0.217 mg/kg bw/day for a 60 kg person (SCF, 2001). The FEMA, using a different methodology, estimated the average daily intake of methyleugenol from flavor use to be less than 0.01 mg/kg bw/day

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(Smith et al., 2002). The estimation of the SCF was based on theoretical maximum use levels of methyleugenol in various food categories and consumption data for these food categories, whereas the intake estimation of the FEMA was based on production volume data for flavor use (SCF, 2001; Smith et al., 2002).

Methyleugenol is unreactive by itself and requires metabolic activation to produce electrophilic metabolites that act as the DNA reactive intermediates (Gardner et al., 1997; Miele et al., 2001). Fig. 1 displays the metabolic pathways of methyleugenol. Important metabolic pathways include *O*-demethylation of the methoxy moieties on the benzene ring, and 2′,3′-epoxidation and 1′-hydroxylation of the allylic side chain (NTP, 2000; Solheim and Scheline, 1976). *O*-demethylation of the methoxy substituents of methyleugenol yields the corresponding phenolic derivatives, which may be excreted as sulfate or glucuronic acid conjugate (Smith et al., 2002). Epoxidation of the side chain yields a 2′,3′-epoxide. This epoxide is detoxified by epoxide hydrolase to form the dihydrodiol or via glutathione conjugation (Luo and Guenthner, 1995). Hydroxylation at the 1′-position of methyleugenol is considered to represent the bioactivation pathway producing the proximate carcinogenic metabolite 1′-hydroxymethyleugenol (Drinkwater et al., 1976; Miller et al., 1983). In a next step 1′-hydroxymethyleugenol can be sulfonated by sulfotransferases to form 1′-sulfooxymethyleugenol which readily undergoes desulfonation to a putative reactive carbocation intermediate that can form DNA or protein adducts (Miller et al., 1983).

Genotype- and lifestyle-based factors can influence the activity of the enzymes involved in the bioactivation and detoxification of methyleugenol, which could in theory lead to large variability in the

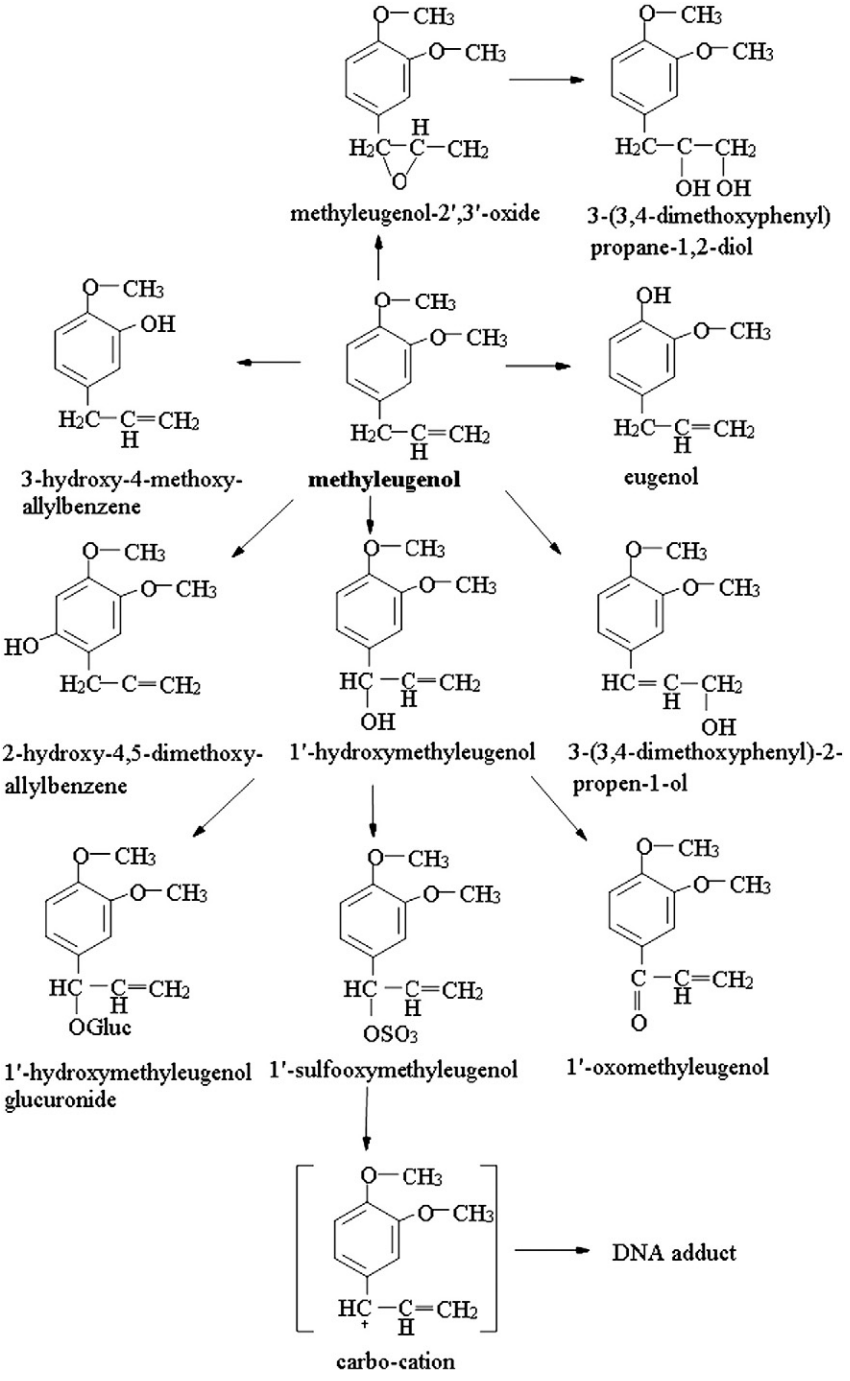

Fig. 1. Suggested metabolic pathways of methyleugenol.