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Metabolism of inhaled methylethylketone in rats

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Abstract

1. Methylethylketone (MEK) is widely used in industry, often in combination with other compounds. Although nontoxic, it can make other chemicals harmful. This study investigates the fate of MEK in rat blood, brain and urine as well as its hepatic metabolism following inhalation over 1 month (at 20, 200 or 1400 ppm).
2. MEK did not significantly accumulate in the organism: blood concentrations were similar after 6-hour or 1-month inhalation periods, and brain concentrations only increased slightly after 1 month's exposure. Urinary excretion, based on the major metabolites, 2,3-butanediols ($\pm$ and meso forms), accounted for less than 2.4% of the amount inhaled. 2-butanol, 3-hydroxy-2-butanone and MEK itself were only detectable in urine in the highest concentration conditions investigated, when metabolic saturation occurred.
3. Although MEK exposure did not alter the total cytochrome P450 concentration, it induced activation of both CYP1A2 and CYP2E1 enzymes. In addition, the liver glutathione concentration (reduced and oxidised forms) decreased, as did glutathione S-transferase (GST) activity (at exposure levels over 200 ppm).
4. These metabolic data could be useful for pharmacokinetic model development and/or verification and suggest the ability of MEK to influence the metabolism (and potentiate the toxicity) of other substances.

Keywords: Methylethylketone, 2-butanone, metabolism, inhalation, rat, 2,3-butanediol

Introduction

Methylethylketone (MEK) (or 2-butanone) (CAS n° 78-93-3) is commonly used as a solvent in the manufacture of adhesives and coatings (especially vinyl, nitrocellulose and acrylic), dewaxing agents of mineral oils, and extraction agents for foodstuffs, and also as a synthesis intermediate (INRS, 2009). In these contexts, it is often associated with other substances (Yoshikawa *et al.*, 1995). MEK exposure alone shows low acute and subchronic toxicity but, when combined with other substances, it can make the other chemicals harmful (Yang, 1986; ATSDR, 1992; INRS, 2009). Indeed, it has been demonstrated that MEK potentiates the hepatotoxic effects of carbon tetrachloride (Dietz and Traiger, 1979), and the neurotoxicity of ethanol, n-hexane, ethyl-n-butyl ketone (EnBK) and methyl-n-butyl ketone (MnBK) (O'Donoghue *et al.*, 1984; Yang, 1986; Noraberg and Arlien-Soborg, 2000).

Recently, it has been demonstrated that the presence of MEK, at concentrations consistent with those found in industrial environments, can modify the metabolism of toluene, an aromatic solvent known to be neurotoxic and ototoxic (ATSDR, 2015). The interaction between the two solvents was notably linked to an increase in the blood toluene concentration in rats co-exposed to the solvents by inhalation (Cosnier *et al.*, 2014). However, the nature of this interaction and its impact on MEK metabolism or urinary elimination have yet to be fully elucidated. Occupational exposure to MEK is generally bio-monitored by measuring the MEK concentration in exposed workers' blood (at the end of a work shift), or in the urine (for high exposures) (Miyasaka *et al.*, 1982; Ong *et al.*, 1991; Sia *et al.*, 1991; Kawai *et al.*, 2003).

Before assessing the impact of the interaction between toluene and MEK on short and medium-term excretions of various biomarkers, thorough investigation on the metabolism of MEK was necessary.

Previous investigations established that MEK can be absorbed through the skin and by inhalation. In rodents, MEK is reduced by alcohol dehydrogenase to 2-butanol (2BuOH) and

oxidised to 3-hydroxy-2-butanone (OHMEK), presumably by cytochrome P450-dependent monooxygenases. OHMEK is then reduced to 2,3-butanediol (23BD). These metabolites can be excreted in the urine, as O-glucuronides or O-sulfates, or may enter the intermediary metabolism to be eliminated as CO₂ or incorporated into tissues (ATSDR, 1992) (Figure 1). These pathways were described based on observations made after i.p. injection of guinea pigs with MEK (DiVincenzo *et al.*, 1976) and its oral administration in rats (Dietz *et al.*, 1981). To the best of our knowledge, no study has investigated the excretion of MEK metabolites in animals after inhalation exposure. In humans, few investigations have studied 2-butanone metabolism. However, OHMEK and 23BD have been detected in human urine after inhalation exposure (Perbellini *et al.*, 1984; Liira *et al.*, 1988b; Liira *et al.*, 1990a).

To complete what is known about MEK metabolism, this study investigated the fate of MEK in rats after inhalation exposure for 1 month by analysing blood, brain and urine samples. To simultaneously determine the concentrations of urinary metabolites of MEK (MEK, 2BuOH, OHMEK and 23BD), a specific method had to be developed. The hepatic metabolism of MEK by measuring cytochrome P450 (CYP) concentrations, CYP1A2, CYP2E1 and glutathione S-transferase (GST) activities, as well as the GSH/GSSG balance was also investigated. Among the various cytochromes, a special attention has been brought to CYP2E1 and CYP1A2 since they are mainly involved in the metabolism of carbon tetrachloride, ethanol, hexane and aromatics; solvents which could be found in mixtures with MEK.

Materials and methods

Animals

4-week-old male Brown Norway rats were purchased from Janvier Labs (Le Genest St Isle, St Berthevin, 53941, France) and weighed between 70 and 90 g on arrival at the animal facility. Before exposure, all animals were acclimatised for at least 2 months with a 12:12 h day/night cycle in polycarbonate cages containing irradiated cellulose BCell8 bedding (ANIBED, Route de Lude, 72510 Pontvallain. A04 irradiated pelleted food (SAFE, Augy, France) and tap water were available *ad libitum*. The temperature in the animal facility was 22 ± 2 °C and the relative humidity was $55 \pm 10\%$. Animal experiments were performed in line with ethical regulations on animal experimentation (European Directive 2010/63/EU) in a French Ministry of Agriculture-accredited animal facility (Accreditation D54-547-10). The present study was approved by the local ethics committee and was registered as CELMEA-2012-0029.

Test material

Methylethylketone puriss. p.a., ACS reagent, $\geq 99.5\%$ (GC) (Fluka ref # 04380) was purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). All other chemicals used were of analytical grade.

Inhalation exposure

Groups of rats (~ 250 g at the beginning of the exposure) were exposed to MEK vapours using a whole-body exposure system. Animals were exposed to 20, 200 or 1400 ppm for 6 hours/day (from 09:00 am to 03:00 pm), 5 days/week for 4 weeks in 200 L glass/stainless steel inhalation chambers. The 20, 200 and 1400 ppm MEK concentrations were chosen because they correspond to 1/10 and to the French 8-hour occupational exposure limit and to

the maximum level of exposure detected in French industries between 2000 and 2010 (based on the INRS Solvex database), respectively. Unexposed rats (controls) were maintained in similar chambers, but exposed to clean air only. During the 6-hour exposure period, the rats did not have access to food or water. All the exposure parameters: temperature (22 ± 2 °C), humidity ($55 \pm 10\%$), air flow in the inhalation chambers, test substance concentrations, etc. were determined in accordance with OECD guideline No. 412 to test chemical toxicity (Subacute Inhalation Toxicity: 28-Day Study. Details of how **test substance** vapours were generated and concentrations controlled can be found in Cosnier *et al.* (2014).

For each exposure condition (MEK20, MEK200 and MEK1400), 3 groups of rats were investigated. Urine was collected from the first group exposed to MEK ($n = 12$); blood was collected and organs were removed (in particular liver, brain and kidneys) from the second group exposed to MEK ($n = 2 \times 8$); blood and organs were also collected from the third group, control animals ($n = 2 \times 8$).

Urine collection

Five time-points were considered for urine collection: the first sample was taken 3 days before the start of inhalation exposure (control time = d0) and subsequent samples were taken after the first (d1), the fifth (d5), the tenth (d10) and the twentieth (d20) days of exposure. Animals were placed in individual metabolic-type stainless steel cages with free access to food and water from 3:00 pm to 9:00 am the following day. During this 18-h period, urine was collected and refrigerated (by precaution) by a cooling system surrounding the collection tubes. Urine samples were frozen immediately after collection and stored at -20 °C. They were thawed just before analysis.

Blood and organ collection

Blood samples were collected from eight rats each from the second and third groups, at d1 and d5, and from the remaining eight rats per group at d10 and d20. Rats were removed one by one from the inhalation chamber using an airlock while atmospheric concentrations were maintained constant. Blood collection was performed and completed on one rat before taking another animal from the chamber. Briefly, blood was collected from the tail vein of rats anaesthetised with isoflurane; sampling was completed within 5 min (3.5 min in mean). Blood samples (≈ 0.8 mL) were dispensed into 2-mL heparinised (30 μ L/150 UI) vials and frozen at - 20 °C.

At d5 and d20, once blood collection had been completed, rats were sacrificed following a deep anaesthesia induced by *i.p.* injection of a ketamine/xylazine mixture (45mg/kg and 5 mg/kg, respectively). Anaesthetised animals were then intracardially perfused with heparinised physiological saline, exsanguinated and dissected to retrieve organs, including the liver. Organs were frozen in liquid nitrogen immediately after collection prior to storage at - 80 °C.

Urinary analysis

Creatinine concentrations in urine were determined by the Jaffe method using a creatinine test kit (Randox Laboratories, Roissy en France, France). For the purposes of this study, creatinine results were primarily used to identify or explain possible extreme values of MEK metabolites concentrations.

Urinary metabolites of MEK, whether in free or glucuroconjugated forms, were determined by a method specifically developed for this study. The method developed was based on the

works of Kezic *et al.* (Kezic and Monster, 1988; Kezic *et al.*, 1989) and can simultaneously measure the five metabolites: MEK, 2BuOH, OH-MEK, $\pm$ 23BD and meso23BD.

Sample preparation

Frozen urine samples were allowed to thaw and equilibrate to room temperature. Samples were agitated and 300- μ L aliquots were transferred into 2-mL Eppendorf Safe-Lock microcentrifuge tubes. Two internal standards: 1-butanol (50 μ L, 0.54 g/L) and 1,2-butanediol (12BD) (50 μ L, 0.06 g/L) were added, and the total volume was adjusted to 500 μ L with ultrapure water. Derivation of 12BD and 23BDs in samples was performed in a few seconds by adding a solution of 4-bromophenylboronic acid in dichloromethane (DCM) (3.2 g/L). 900 μ L of DCM was then added. The mixture was vortex-mixed for 5 sec and centrifuged at 10 000 rpm for 3 min before collecting the organic layer. The sample was then ready for GC-MS/MS analysis. All samples were analyzed in duplicate.

To determine total metabolites (free plus conjugated), an enzymatic hydrolysis step was added prior to the derivation step. For this, β -Glucuronidase from *Helix pomatia* (type HP-2, aqueous solution, $\geq 100\,000$ units/mL) (30 μ L) and phosphate buffer pH = 4.7 (170 μ L, solution of Na₂HPO₄/NaH₂PO₄, 0.18/13.7 g/L) were added to defrosted urine samples. The mixtures were incubated at 37 °C for 18 hours.

Gas chromatography

Gas chromatography (GC) was performed on a 450-GC system (Bruker, Wissembourg, France) equipped with a CP-8400 autosampler (Bruker). Compounds were separated on an Rxi-17 MS (Restek, France) capillary column (30 m length $\times$ 0.25 mm I.D. $\times$ 0.25 μ m film thickness) using helium as the carrier gas at a constant flow of 1.0 mL/min. The sample (1 μ L) was injected in splitless mode (injector type 1177). Injection temperature was 300 °C.

The split delay and split ratio were set at 0.2 min and 1/30, respectively. The oven temperature was held at 40 °C for 3.0 min, then increased to 300 °C at a rate of 20 °C/min (total time: 16 min). The transfer line was maintained at 250 °C throughout.

Tandem mass spectrometry

GC-MS/MS analyses were performed on a 320 triple quadrupole mass spectrometer (Bruker, Wissembourg, France) in electronic impact (EI) mode. The ion source temperature was set to 300 °C with ionization energy at 70 eV. Mass spectra were acquired in selected reaction monitoring (SRM) mode using argon 99.9999% (Air Liquide) as collision gas at a pressure of 2.0 mtorr in the collision cell. The source was flushed with helium gas for the first 1.6 min of the injection. To ensure selectivity, two SRM ion transitions were chosen per compound, when possible based on their abundance. Selecting two transitions maximised the signal-to-noise ratio in matrix extracts. One transition was used as quantifier and the second as a qualifier. Dwell time and collision energy were set at 0.1 s and 10 eV, respectively, for all transitions.

Details regarding which SRM transitions were used for each compound are provided in Table 1.

The main performance characteristics of the method (validated linearity ranges, limits of detection (LOD), limits of detection (LOQ), within-run and between-run precisions and recoveries) are summarised in Table 2.

Blood and brain analysis

MEK and 2BuOH concentrations in blood and brain were determined using a similar protocol involving liquid/liquid extraction of the tissues (800 µL of blood or half a brain) in DCM (500

or 750 μL for blood or brain samples, respectively) using n-propylacetate as an internal standard (5 μL , 1.5 mg/L in DCM). For brain samples, a homogenate was first prepared by grinding the brain in tubes containing ceramic beads (Fast Prep instrument, MP Biomedicals, with Lysing Matrix D tubes). After 30 min agitation, samples were centrifuged at 1250 rpm, 4 $^{\circ}\text{C}$ for 10 min. The lower DCM layer contained the MEK and the 2BuOH. 0.3 mL of this layer was transferred to a vial containing a glass insert. The sample (1 μL) was then ready for GC-MS analysis using the chromatographic conditions described in Cosnier *et al.* (2014).

In these conditions, the LODs (and LOQs) for MEK were 0.018 (and 0.054) $\mu\text{g/g}$ in blood and 0.060 (and 0.180) $\mu\text{g/g}$ in brain. For 2BuOH, the LOD (and LOQ) was 0.024 (and 0.072) $\mu\text{g/g}$ in blood (this compound was not investigated in brain).

Liver assays

Biochemical assays were carried out on liver homogenates (reduced (GSH) and oxidised glutathione (GSSG)), liver microsomes (total cytochrome P450 (CYP) level and CYP1A2 and CYP2E1 activities), and cytosolic extracts (activity of GSTs).

GSH was measured by a colorimetric method using 5,5'-dithiobis 2-nitrobenzoic acid. GSH was blocked with 2-vinylpyridine, then its oxidised form (GSSG) could be measured following reduction by glutathione reductase (Griffith, 1980).

The total cytochrome P450 level was determined by the Omura and Sato (1964) method; this method is based on the absorption difference at 450 nm between the reduced form of the pigment and the carbon monoxide-conjugated form in the presence of dithionite.

CYP2E1 activity was determined according to the method described by Koop (1986), based on the (colorimetric) measurement at 456 nm of 4-nitrocatechol obtained from p-nitrophenol after oxidation (hydroxylation) by CYP2E1. CYP1A2 activity was measured in a similar manner by monitoring the O-deethylation of ethoxyresorufin.

GST was measured according to the method developed by Habig *et al.* (1974) using 1-chloro-2,4 dinitrobenzene as substrate.

For assays of GSH, GSSG, CYP or GST, the amounts measured were normalised based on the total protein content measured in the different samples by the Lowry assay (Lowry *et al.* (1951)). Except for GSH and GSSG, all the assays discussed above were carried out on a fully automated random access bench-top clinical chemistry analyzer (Randox RX-Daytona).

Statistical analysis

All statistical analyses were performed using Statgraphics Centurion XVI Software (Version 16.2.04) (StatPoint Technologies, Inc.). The threshold for statistical significance was set at 5%. For the analysis of results, different statistical procedures were implemented. For blood and urine data, analyses were performed using a general linear model including fixed effects (the MEK exposure level and/or the collection day), and a random effect (subject) to take the non-independent nature of the data into account. The interaction between the two fixed effects was also tested. Post-hoc tests were performed using the Bonferroni method. Atmospheric, blood and urinary concentrations are presented in the tables in the form arithmetic mean $\pm$ standard deviation (unless indicated otherwise). Analyses of variance of two factors (group and day) were used to analyse brain and liver data (group = exposed or control, day = d5 or d20).

Results

Animal and organ weights

Exposure to MEK did not affect weight-gain in animals (~ 340 g at the end of the exposures); in addition, the weights of the brains, lungs and kidneys of animals exposed by inhalation were no different from control animals (data not shown). However, the relative weights of livers (as a ratio of body weight) were significantly lower in exposed animals compared to controls. This difference was stable ($-10.5 \pm 2.8\%$) and did not depend on the atmospheric concentration of MEK nor the day of sacrifice (d5 or d20).

Blood and brain concentrations

The blood concentration for MEK (MEK-B) increased with its atmospheric concentration (MEK-A), from ~ 0.20 µg/g for MEK20 to ~ 162 µg/g for MEK1400 (Table 3). Except for MEK1400 between d1 and d20, no significant day-effect was identified based on paired t-test and Bonferroni correction. MEK-B was thus quite stable during the inhalation period. Classical linear ($y = mx + c$) and multiplicative ($\log y = m \log x + c$) models failed to describe the relation between MEK-B and MEK-A.

In contrast to MEK, its metabolite 2BuOH was only quantifiable in blood with exposure to MEK1400 (Table 3). When detected, the 2BuOH concentration (~ 2.4 µg/g) was 68 times lower than the MEK concentration.

The MEK brain concentration (MEK-C) was found to increase proportionally to MEK-B (Table 3), but was quantifiable only for MEK200 at d5 and for MEK1400 at d5 and d20. The concentrations at these time-points were between 6 and 3 times lower than those measured in the blood. However, it should be noted that brain levels of MEK were significantly higher at d20, suggesting accumulation over time.

Metabolite assay in urine

The LODs of the analytical method for a 300- μ L aliquot of urine were 0.70, 0.26, 0.50, 0.11 and 0.29 mg/L for 2BuOH, MEK, OHMEK, $\pm$ 23BD and meso23BD, respectively (Table 2). These limits were lower than those reported by Kezic *et al.* (Kezic and Monster, 1988; Kezic *et al.*, 1989); expressed in terms of minimum detectable quantities (taking into account the volume of urine used), the method developed for this study presented a 3.5-, 8-, 6-, 15- and 6-fold gain in sensitivity for 2BuOH, MEK, OHMEK, $\pm$ 23BD and meso23BD, respectively. Compared to methods described in the literature to assay MEK, OHMEK or 23BDs individually (Van Doorn *et al.*, 1989; Otsuka and Ohmori, 1992, 1994), in the worst case the current method was 5, 12 or 13 times less sensitive, respectively. This loss in sensitivity could be related to the fact that these individual assays used larger volumes of urine and more sophisticated techniques: headspace, micro-solid phase extraction, oxidation and derivatisation steps or used a specific detector (Miyasaka *et al.*, 1982; Gori *et al.*, 1995; Chou *et al.*, 1999; Antunes and Linden, 2008; Pacenti *et al.*, 2010).

In rats exposed to MEK20 and MEK200, free 2BuOH, MEK and OHMEK were not present in measurable amounts in their urine; in contrast, exposure to MEK1400 triggered a marked increase in the amount of these free metabolites (Table 4). For MEK itself, the increase was spectacular: lower than 0.26 mg/L at MEK200 to 31.9 mg/L (on average) at MEK1400.

23BDs were detectable from endogenous sources in urine from unexposed animals (Table 4). At MEK20, the levels excreted by exposed animals were not statistically different from those excreted by controls, however, at MEK200 and at MEK1400 the difference was significant. No day-effect was observed with MEK200 in terms of excretion, which remained stable over time; however, excretion of 23BDs after exposure to MEK1400 gradually increased between d1 and d20. 23BDs were excreted with a mean distribution ratio of 1:3 between $\pm$ 23BD and

meso23BD forms (1:5 for endogenous excretion). At 1400 ppm, combined excretion for the two forms was 8 to 22 times higher (depending on the day) than MEK excretion (31.6 mg/L); OHMEK (~ 3.1 mg/L) and the 2BuOH (~ 1.5 mg/L) followed in order of abundance (Table 4).

Assays of the total (free + conjugated) metabolite concentrations (at d0, d1 and d5) after hydrolysis of urine revealed that the proportion of free form was metabolite-dependent. Indeed, most OHMEK (~ 78%) and half the 2BuOH were conjugated; in contrast, more than 85% of urinary MEK was unconjugated. With 23BDs, the endogenous fractions (measured at d0) increased when urine was hydrolysed and were not homogenous (between animals in the MEK20, MEK200 and MEK1400 groups). This effect complicated the analysis of results. The proportion of conjugated forms of $\pm$ 23BD related to exposure appeared to decrease with the exposure level. Thus, at MEK20, conjugated $\pm$ 23BD was a major form, whereas at MEK1400, it was negligible compared to the free form (Figure 2). For meso23BD, this phenomenon was even more pronounced: more than half of the total amount excreted was in the free form at MEK20 and MEK200, and more than 90% at MEK1400.

Liver assays

No statistically significant difference in the total CYP concentration between exposed and controls groups was found for any MEK concentration, or day considered (520 pmol/mg protein on average). In contrast, CYP1A2 and CYP2E1 activities measured in the liver of exposed animals were almost always higher than those measured in control livers (16 pmol/min/mg protein for γ CYP1A2 and 230 pmol/min/mg protein for γ CYP2E1) (Figure 3). For both CYP isoforms, a dose/effect relationship was observed; the day-effect was however not statistically significant.

In addition, GST activity was altered only with MEK200 and MEK1400 (data not shown), where the concentrations observed in exposed animals were less than those of controls. Finally, with regard to the GSH concentrations, controls always had a higher concentration than treated animals. This phenomenon was less pronounced with GSSG.

Discussion

A limited number of studies describing the uptake, distribution, metabolism, and elimination of MEK in experimental animals and humans have been published. This study is the first to explore the absorption and metabolism of MEK in rats following inhalation exposure through the measurement of some hepatic parameters and its elimination in the urine.

As previously shown by Liira *et al.* (1991), after a 600-ppm inhalation for 6 hours over 1 day or for 6-10 hours/day over 8 days, MEK does not appear to accumulate in the blood based on the duration of exposure. Thus, blood concentrations were similar after 6-hour or 1-month inhalation periods. In addition, blood concentrations measured at 200 ppm ($\sim 1.8 \mu\text{g/g}$) or 1400 ppm ($\sim 160 \mu\text{g/g}$) were consistent (not equals but same order of magnitude) with those found in rats exposed to 600 ppm ($\sim 1090 \mu\text{mol/L} = 78 \mu\text{g/g}$) or in humans exposed for 4 hours at the same concentration ($3.5 - 7.2 \mu\text{g/mL} \approx 3.3 - 6.8 \mu\text{g/g}$) (Liira *et al.*, 1988b). It should be mentioned that in our case the blood concentrations measured are not at “steady-state” but at the very beginning of the declining phase (around 3.5 min after the end of exposure) knowing the fact that half-life of MEK in blood is 30 min (Liira *et al.*, 1988b).

As expected from solubility data for MEK in various tissues (Perbellini *et al.*, 1984; Thrall *et al.*, 2002), Liira *et al.* (1991) reported a constant MEK concentration in perirenal fat ($\sim 0.70 \mu\text{mol/g} = 50 \mu\text{g/g}$). In the present investigation, a similar concentration was found in brain after 20 days' exposure at 1400 ppm. However, we did observe a slight increase in brain

concentrations between d5 and d20 at MEK200 and MEK1400; thus, a slight accumulation in this organ over time cannot be excluded.

The strong increase in MEK blood concentration between MEK200 and MEK1400 was probably the result of metabolic saturation, as reflected by the appearance of 2BuOH in urine after exposure to MEK1400. Indeed, using F344 male rats exposed to 100-2000 ppm MEK in a closed, recirculating gas uptake system, Thrall *et al.* (2002) demonstrated that MEK is metabolised by a process which is both a saturable and first-order. Using physiologically relevant pharmacokinetic model simulations (PBPK) for 8-hour exposures, Liira *et al.* (1990a) estimated that this metabolic saturation would occur in humans at 100 ppm at rest and at 50 ppm during exercise.

As in humans (Liira *et al.*, 1988b), 23BD is the major metabolite in rat urine following inhalation. It exists under two forms: $\pm$ 23BD and meso23BD (with a mean distribution ratio of 1:3). 23BD are also produced endogenously and excreted in urine. This endogenous production is related to pyruvate (a product of glycolysis) metabolism in mammals (Montgomery *et al.*, 1990). Other metabolites: 2BuOH and OHMEK, but also MEK itself, were only detectable in urine at the highest concentration investigated (1400 ppm = 7 $\times$ 8h-OEL) when metabolic saturation occurred; therefore, these metabolites should not be present except in accidental conditions. This represents a difference between humans and rats, because MEK and OHMEK have been detected in urine from humans exposed to normal occupational levels (Miyasaka *et al.*, 1982; Perbellini *et al.*, 1984; Liira *et al.*, 1988a; Ong *et al.*, 1991; Sia *et al.*, 1991). Another difference between human and rat metabolism and excretion lies in the fact that the glucuronide or sulfate form of these metabolites does not appear to be a major metabolic pathway in rats.

If we consider the rat minute ventilation equal to 110 mL/min (Strohl *et al.*, 1997), the amount of MEK inhaled during a 6-h exposure would correspond to 330 and 2307 μ mol for MEK200 and MEK1400 exposure, respectively. The corresponding amounts of MEK and urinary metabolites excreted (free or total) during the 18-h post-exposure period (considering an average diuresis of 7.5 mL) were less than 1.5 and 56 μ mol, respectively. Thus, the urinary excretion measured accounted for less than 0.45% (at MEK200) and 2.4% (at MEK1400) of the total amount inhaled, or 0.9% and 4.8% of the absorbed MEK if pulmonary uptake was equivalent to that measured in humans (~50%) (Liira *et al.*, 1988a; Liira *et al.*, 1988b; Liira *et al.*, 1990b). These data are consistent with data from human studies (Perbellini *et al.*, 1984; Liira *et al.*, 1988b; Liira *et al.*, 1990b) and are clearly different from those obtained in rats after oral administration, where about 30% of the dose administered was converted to 2,3-butanediol, 4% was converted to 2-butanol, and 4% was converted to 3-hydroxy-2-butanone (Dietz *et al.*, 1981).

In contrast to what was observed by Toftgard *et al.* (1981) and Cavender *et al.* (1983) after exposure of rats to 800-ppm MEK for 5 weeks and 5000 ppm for 12 weeks, respectively, in our hands, a slight decrease in absolute and relative liver weight was observed in exposed animals. This decrease in liver weight was associated with a slight induction of the enzymatic activity of the two CYP isoforms investigated (CYP1A2 and CYP2E1). However, MEK exposure (even at 1400 ppm) was not found to alter the total cytochrome P450 concentration. Until now, no changes had been observed in CYP isozyme profiles after MEK inhalation (Toftgard *et al.* 1981), and only few studies had shown that MEK could induce microsomal liver enzymes in rats after acute oral exposure (Robertson *et al.*, 1989; Raunio *et al.*, 1990; ATSDR, 1992) or after acute inhalation of high MEK concentrations ($\geq$ 1500 ppm) (Brondeau *et al.*, 1989).

In addition to these enzymatic effects, MEK exposure induced a decrease in the liver glutathione concentration (both reduced and oxidised forms) as well as in GST activity (at exposure levels over 200 ppm). For Liira *et al.* (1991), this decrease could be caused by production of thioethers. However, our preliminary results have found no evidence for the formation of mercapturic acid.

These metabolic data suggest that MEK could influence the metabolism (and potentiate the toxicity) of other substances, such as, for example, aromatic hydrocarbons which are primarily metabolised through oxidation by CYP1A2 and CYP2E1, but can also be eliminated via the glutathione pathway (Liira *et al.*, 1988a; Raunio *et al.*, 1990; Liira *et al.*, 1991; Ueki *et al.*, 1995; Cosnier *et al.*, 2014). This work provides the foundation for future studies related to interactions with MEK. Another benefit of this study is to provide blood and urine data that could be useful for pharmacokinetic model development/and or verification.

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Declaration of interest

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Figure 1. MEK metabolism, adapted from DiVincenzo *et al.* (1976) and Dietz *et al.* (1981)

Figure 2. Urinary 23BD concentrations (free and total in mg/L) – bars represent the difference between exposed and control rats

Figure 3. Enzymatic activity of two CYP isoforms - bars represent the ratio between exposed and control rats

Table 1. Retention times and transitions used in selected reaction monitoring mode for the compounds studied and internal standards.

Compound	Derived compound	RT (min)	MS segment (min)	Precursor ion (m/z)	Product ion (m/z)	Q/q *
2BuOH	No	2.12	1.6 – 3.5	59	41	Q
MEK	No	2.32	1.6 – 3.5	72	43	Q
				72	57	q
1BuOH	No	2.70	1.6 – 3.5	56	41	Q
OHMEK	No	4.04	3.6 – 6.9	88	45	Q
				88	43	q
±23BD	Yes	11.54	7.0-15.0	256	241	Q
				256	184	q
m23BD	Yes	12.02	7.0-15.0	256	241	Q
				256	184	q
12BD	Yes	12.22	7.0-15.0	256	227	Q

* Q : quantifier ion; q : qualifier ion

Table 2. Performance parameters for the analytical method developed to simultaneously measure urinary metabolites

	Linearity range* (mg/L)	LOD (mg/L)	Within-run precision (%RSD)	Between-run precision (%RSD)	Recovery (%)
2BuOH	2.1 - 30	0.70	≤ 12.0	≤ 12.6	87 ± 18
MEK	0.8 - 50	0.26	≤ 12.1	≤ 15.8	95 ± 12
OHMEK	1.5 - 25	0.50	≤ 11.5	≤ 15.6	87 ± 13
±23BD	0.3 - 120	0.11	≤ 3.3	≤ 12.3	66 ± 11
m23BD	0.9 - 500	0.29	≤ 4.6	≤ 9.2	79 ± 9

* The lower limit corresponds to the limit of quantification (LOQ) = 3 × LOD

Table 3. Atmospheric (MEK-A), blood (MEK-B and 2BuOH-B) and brain (MEK-C) concentrations in $\mu\text{g/g}$ (mean $\pm$ standard deviation or median*)

	Day	MEK 20	MEK 200	MEK 1400
MEK-A [#]	d1	18.3 $\pm$ 2.1	212 $\pm$ 20	1454 $\pm$ 34
	d5	21.3 $\pm$ 0.8	207 $\pm$ 5	1389 $\pm$ 22
	d10	21.9 $\pm$ 0.4	209 $\pm$ 3	1439 $\pm$ 20
	d20	21.7 $\pm$ 1.1	195 $\pm$ 4	1413 $\pm$ 13
MEK-B	d1	0.186 $\pm$ 0.043	1.86 $\pm$ 0.96	181 $\pm$ 34
	d5	0.208 $\pm$ 0.050	1.65 $\pm$ 0.78	163 $\pm$ 26
	d10	0.211 $\pm$ 0.062	2.11 $\pm$ 0.73	163 $\pm$ 14
	d20	0.203 $\pm$ 0.064	1.48 $\pm$ 0.41	142 $\pm$ 15
	Mean	0.202 $\pm$ 0.053	1.79 $\pm$ 0.79	162 $\pm$ 26
2BuOH-B	d1	/	[0.052]	2.86 $\pm$ 0.57
	d5	/	[0.026]	2.36 $\pm$ 0.34
	d10	/	[0.051]	2.13 $\pm$ 0.25
	d20	/	[0.032]	2.17 $\pm$ 0.26
	Mean	/	[0.035]	2.38 $\pm$ 0.47
MEK-C	d5	[0.122]	[0.304]	33.1 $\pm$ 17.1
	d20	[0.158]	0.66 $\pm$ 0.32	54.3 $\pm$ 13.9

[#] based on three measurements during the 6-h exposure

* medians are given in brackets when multiple measurements were between the limits of detection and the limits of quantification ($=3 \times \text{LODs}$) = 0.054, 0.072 and 0.18 $\mu\text{g/g}$ for MEK-B, 2BuOH-B and MEK-C, respectively.

Table 4. Concentrations of urinary metabolites (free or total) in mg/L (mean $\pm$ standard deviation or median*)

	Day	2BuOH	MEK	OHMEK	$\pm$ 23BD	meso23BD
Free	MEK20	d0			[0.25]	1.75 $\pm$ 0.37
		d1			0.59 $\pm$ 0.17	2.52 $\pm$ 0.47
		d5			[0.35]	1.89 $\pm$ 0.37
		d10			[0.34]	1.81 $\pm$ 0.27
		d20			0.42 $\pm$ 0.10	2.44 $\pm$ 0.63
	MEK200	d0			[0.24]	1.83 $\pm$ 0.66
		d1			2.04 $\pm$ 0.97	7.24 $\pm$ 2.55
		d5			2.43 $\pm$ 0.49	8.26 $\pm$ 2.68
		d10			2.48 $\pm$ 0.71	7.30 $\pm$ 3.48
		d20			2.05 $\pm$ 0.40	6.47 $\pm$ 2.91
	MEK1400	d0			[0.21]	1.42 $\pm$ 0.33
		d1	[1.69]	34.7 $\pm$ 15.8	[1.85]	59.0 $\pm$ 22.4
		d5	[1.41]	33.6 $\pm$ 8.37	2.76 $\pm$ 0.85	93.2 $\pm$ 29.6
		d10	[1.34]	30.7 $\pm$ 6.80	4.30 $\pm$ 1.38	146.5 $\pm$ 43.8
		d20	[1.07]	27.7 $\pm$ 14.2	3.25 $\pm$ 0.88	153.1 $\pm$ 32.7
Total	MEK20	d0			1.62 $\pm$ 0.81	1.55 $\pm$ 0.64
		d1			3.46 $\pm$ 1.36	2.76 $\pm$ 0.76
		d5			3.34 $\pm$ 0.73	1.84 $\pm$ 0.42
	MEK200	d0		[0.75]	7.43 $\pm$ 3.06	6.37 $\pm$ 1.90
		d1		[0.97]	12.4 $\pm$ 5.5	16.6 $\pm$ 5.2
		d5		[1.17]	11.2 $\pm$ 4.0	16.2 $\pm$ 2.4
	MEK1400	d0		[1.16]	12.1 $\pm$ 1.97	8.37 $\pm$ 2.80
		d1	[3.15]	41.0 $\pm$ 20.2	7.91 $\pm$ 5.00	87.7 $\pm$ 30.4
		d5	[3.53]	37.7 $\pm$ 9.4	12.4 $\pm$ 8.30	170.2 $\pm$ 58.1
						378.3 $\pm$ 124.3

*medians are given in brackets when multiple measurements were between the limits of detection and the limits of quantification ($= 3 \times \text{LODs}$) = 2.1, 0.8, 1.5, 0.3 and 0.9 mg/L for 2BuOH, MEK, OHMEK, $\pm$ 23BD and meso23BD, respectively.