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Disposition and metabolism of dipropyl disulphide *in vivo* in rat

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Abstract

The metabolism of dipropyl disulphide (DPDS), a sulphur compound from onion, was investigated *in vivo* in the rat. A single dose (200 mg kg^{-1}) was administered by gastric intubation and the time courses of DPDS and its metabolites were followed over 48 h by gas chromatography coupled with mass spectrometry in the stomach, intestine, liver, and blood. DPDS was detected in the stomach where it was transformed into propyl mercaptan, whereas the liver contained only traces of DPDS and none at all in the other examined organs. The metabolites methylpropyl sulphide, methylpropyl sulphoxide (MPSO), and methylpropyl sulphone ($MPSO_2$) were sequentially formed in the liver. The route of elimination from the liver seemed to be mainly via the blood. The bile also participated in the excretory process, but only for $MPSO_2$. The pharmacokinetic parameters were determined for all of the above compounds. Whereas the bioavailability of DPDS was very low (0.008 h mM), the areas under the curve were higher for the *S*-oxidized metabolites MPSO and $MPSO_2$, i.e. 9.64 and 24.15 h mM, respectively. The half-lives for DPDS and its metabolites varied between 2.0 and 8.25 h, except for $MPSO_2$, which had a half-life of 29.6 h. $MPSO_2$ was the most abundant and persistent of these metabolites.

Keywords: *Allium, onion, metabolism, dipropyl disulphide, mercaptan, sulphoxide, sulphone*

Introduction

Plants of *Allium* species such as onion and garlic are among the oldest of all cultivated plants, have fascinated man since the earliest times, and have been used as foodstuffs and as drugs in folk medicine (Rivlin 2001). More recently it is becoming increasingly evident

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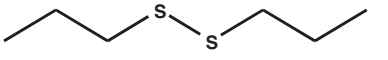
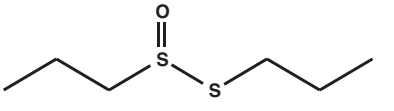
that they have beneficial effects on cardiovascular diseases and some cancers (Le Bon & Siess 2000; Bianchini & Vainio 2001; Rahman 2001) and most of their properties have been attributed to specific sulphur compounds present in high levels in these vegetables (Lanzotti 2006). Sulphur compounds from garlic and onion have been shown to have an impact on many processes involved in carcinogenesis as well as atherogenesis and anti-inflammatory, antithrombic, hypotensive, hypolipidemic, antibacterial, antiviral, and antifungal properties have been demonstrated (Augusti 1996; Bianchini & Vainio 2001; Rahman 2001; Griffiths et al. 2002). Most experimental studies have focused on allyl sulphides, mainly derived from garlic, whereas much less is known about alkyl sulphides, which are present in other *Allium* species such as onion (Block et al. 1992) and leek (Nielsen & Poll 2004).

Dipropyl disulphide (DPDS) (Figure 1) is the major polysulphide produced among a wide range of sulphur compounds resulting from a series of chemical reactions. This chain of reactions is initiated by the action of allinase on *S*-alk(en)yl-cysteine sulphoxides, the flavour

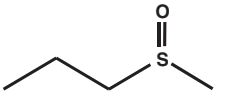


DPDS : dipropyl disulphide

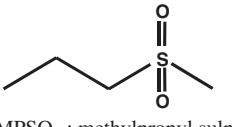


DPDSO : dipropyl thiosulfinate

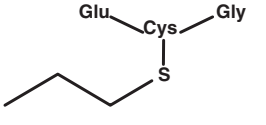
MPS : methylpropyl sulphide



MPSO : methylpropyl sulphoxide



$MPSO_2$: methylpropyl sulphone



PGS : propyl glutathione sulphide

PM : propyl mercaptan

Figure 1. Molecular structures of sulphur compounds.

precursors, after disruption or crushing of an onion bulb (Lancaster & Boland 1990). Up to now little is known about the biotransformation of allyl and alkyl sulphides in animals and man. The metabolism of monosulphides such as diallyl sulphide and dipropyl sulphide has been studied in rats, where oxidation of the sulphur atom results in the production of sulphoxides and sulphones (Brady et al. 1991; Chen et al. 1994; Nickson & Mitchell 1994). Metabolism of disulphides, such as diallyl disulphide (DADS) and DPDS, have been investigated more recently. *In vitro*, these compounds have also been reported to be metabolized by sulphur oxidation. In the presence of rat liver subcellular fractions, the metabolism of DPDS occurs by the oxidation of one atom of sulphur to give the corresponding thiosulphinate DPDSO or by conjugation with glutathione mediated by glutathione *S*-transferase to yield propylglutathione sulphide (PGS) and propyl mercaptan (PM) (Figure 1) (Teyssier & Siess 2000). With DADS, similar metabolites have been observed in human and rat using subcellular fractions (Teyssier et al. 1999; Germain et al. 2003). *Ex vivo*, in an isolated perfused rat liver, DPDS is transformed into PM, methylpropyl sulphide (MPS), methylpropyl sulphoxide (MPSO), and methylpropyl sulphone ($MPSO_2$) (Figure 1) (Teyssier & Siess 2000). The latter authors hypothesized that the methylation of PM to MPS and its further oxidation to $MPSO_2$ occurred. *Ex vivo*, DADS is transformed into allyl mercaptan, allylmethyl sulphide, allylmethyl sulphoxide, and allylmethyl sulphone (Egen-Schwind et al. 1992; Germain et al. 2003), whereas in primary rat hepatocytes the products of DADS metabolism are allyl mercaptan and allylmethyl sulphide (Sheen et al. 1999). Germain et al. (2002) have studied the *in vivo* metabolism of diallyl disulphide (DADS) and have also demonstrated extensive catabolism. They identified allyl mercaptan, allylmethyl sulphide, allylmethyl sulphoxide and allylmethyl sulphone as metabolites.

The *in vitro* metabolism of DPDS is well established in rat (Teyssier & Siess 2000), but has to be confirmed *in vivo* before postulating any role of DPDS on health and disease. The present study was undertaken to investigate the *in vivo* fate of DPDS and its metabolites in rats after a single oral administration of 200 mg of DPDS kg^{-1} body weight. We compared the metabolites detected with those observed *in vitro* and *ex vivo*. For this purpose we analysed sulphur compound distribution in the stomach, intestine, plasma, and liver tissues by a quantitative and sensitive gas chromatography coupled with mass spectrometry (GC-MS) method which has already been validated (Martin-Lagos et al. 1995; Germain et al. 2002), and we additionally determined their pharmacokinetic parameters.

Materials and methods

Chemicals

DPDS (purity 98%), PM, MPS, sodium metaperiodate, *p*-cymene and nonane were purchased from Sigma Aldrich Chemical (L'Isle D'Abeau, France). All other chemicals and reagents were of the highest commercial quality available. Dichloromethane was distilled before use for solvent extractions.

Synthesis of MPSO and $MPSO_2$

MPSO and $MPSO_2$ were obtained by oxidation of MPS with sodium metaperiodate in a methanolic solution thawed on ice overnight as already described (Furniss et al. 1989;

Teyssier & Siess 2000). After extraction, the compounds were injected into gas chromatography coupled with mass spectrometry (GC-MS) in order to assess their structure by comparison with a library of spectra. The reaction was complete but gave a mixture of MPSO (69.77%) and of $MPSO_2$ (30.23%) as estimated by GC-MS.

Drug administration

Male 6-week-old Wistar rats ($n = 24$, average weight 263 ± 19 g) were purchased from Janvier (Le Genest, Saint Isle, France). Rats were housed in individual stainless-wire cages. They were fed *ad libitum* with a semi-synthetic diet consisting of (on a dry matter basis) 18% casein, 43% starch, 21% saccharose, 5% corn oil, 2% cellulose, 5% mineral mixture, and 1% vitamin mixture for 1 week before the beginning of the experiment. Water was added to the diet in the ratio 50 g water/100 g dry matter. The liquid diet was prepared daily. Drinking water was provided *ad libitum*. The animals were fasted for 18 h before and 5 h after drug administration. DPDS, dissolved in corn oil, was administered by gastric intubation at a dose of 200 mg kg^{-1} body weight. This dose was selected because it is equivalent to dose consumed per rat and per day in previous experiments showing an inhibiting effect of DPDS on the genotoxicity of carcinogens (Guyonnet et al. 2001). Three animals were included as negative controls in order to check that no sulphur compounds were detectable in their tissues. After oral administration, animals were maintained separately in metabolic cages. In each experiment, three animals were killed by exsanguination under anaesthesia (isoflurane 2.5% in oxygen) at 1, 2, 4, 8, 24, 36, and 48 h after oral dosing. Blood samples were withdrawn into tubes containing EDTA. Livers, stomachs, and intestines (40 cm long from the stomach) were removed, weighed, and stored at -20°C for a few days until further analysis. Blood was immediately centrifuged and plasma was stored at -20°C for a few days before analysis. Subsequently, the tissues were thawed on ice and were extracted as previously described (Germain et al. 2003). Briefly, stomachs, intestines and livers were homogenized in distilled water. *p*-Cymene in ethanol solution (0.17 mg ml^{-1}) was added as an internal standard. After trichloroacetic acid protein precipitation, the homogenate was centrifuged and the supernatant extracted three times with dichloromethane. The same protocol without homogenization was used for the plasma samples. The samples were then concentrated by evaporation under a nitrogen stream to a final volume of 500 µl. To this volume, 100 µl of nonane dissolved in dichloromethane (0.147 mg ml^{-1}) was added in order to standardize the sample concentration and an aliquot of 1 µl was injected into the gas chromatograph under the conditions described below.

GC-MS conditions for organosulphur compounds analysis

GC-MS was carried out using a 5973N Mass Selective Detector coupled to a 6890 gas chromatograph (Agilent Technologies, Massy, France) equipped with a DB^{TM}1701 capillary column (30 m × 0.32 mm i.d., 1 µm film thickness) (J&W Scientific, Folsom, CA, USA). Samples were injected in splitless mode into an injection port maintained at 200°C. Helium was the carrier gas with a 35 cm s^{-1} constant velocity. The oven was programmed from 35°C (2 min) to 220°C at a linear rate of 5°C min^{-1}. Mass spectra were obtained in a positive-ion mode with an electronic impact of 70 eV. Each sample was analysed twice. First acquisition, GC-MS was used in scan mode from m/z 29 to 300 amu in order to ascertain the identification of compounds by comparison with standards based

on their retention time and their spectra. Second acquisition, GC-MS was used in selected ion-monitoring (SIM) mode. The quantification of PM, MPS, MPSO, $MPSO_2$ and DPDS was achieved by monitoring the ions at m/z 76, 90, 106, 81 and 150, respectively. The purity was continuously checked by the ratio between the quantifying ion and another one, specific for each compound.

Quantitative determination of the organosulphur compounds

For each sulphur compound the sensitivity and linearity of the GC-MS detector used in the SIM mode was calculated from a series of standard solutions dissolved in dichloromethane. The concentration of each sulphur compound in the biological samples (600 µl) was calculated according to the following equation:

$$\text{Concentration (mM)} = \frac{\text{area sulphur compound}}{\text{area } p\text{-cymene} \times \text{area nonane}} \times \frac{1}{\text{response coeffecient}} \times \frac{1}{\text{yield of extraction}}$$

The response coefficient (a) was calculated from the curve:

$$y = ax + b,$$

where y is the adjusted area (with the areas of p-cymene and nonane) of the quantified ion; x is the concentration of the compound (mM) in the vial; and b is the detection limit. The extraction yield was determined for each sulphur compound in each analysed tissue and corresponded to the percentage of extracted compound from the tissue. From the concentration of sulphur compounds, the quantity (µmol) of sulphur compounds per organ was calculated. The limit of detection varied depending on the sulphur compound from 0.33 nmol ($MPSO_2$) to 3.42 nmol (PM). Results are presented as means of three rats.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined from plasma concentrations using a non-compartmental method and were calculated using Kinetica® software (InnaPhase, Champs sur Marne, France). The area under the plasma concentration–time curve (AUC) was computed using the trapezoidal rule, when $C_n > C_n - 1$. The elimination half-life ($t_{1/2}$) was calculated as:

$$t_{1/2} = \frac{\ln 2}{L_z},$$

where L_z is the elimination rate constant. The plasma clearance (Cl_p) was calculated as:

$$Cl_p = \frac{\text{dose}}{\text{AUC}}.$$

T_{max} corresponded to the time at which the maximum concentration was reached (C_{max}).

Results

Identification of the detected products

Figure 2 represents a typical GC-MS chromatogram (total ion current) observed in the liver 4 h after DPDS administration. When DPDS was administered to rats, this molecule

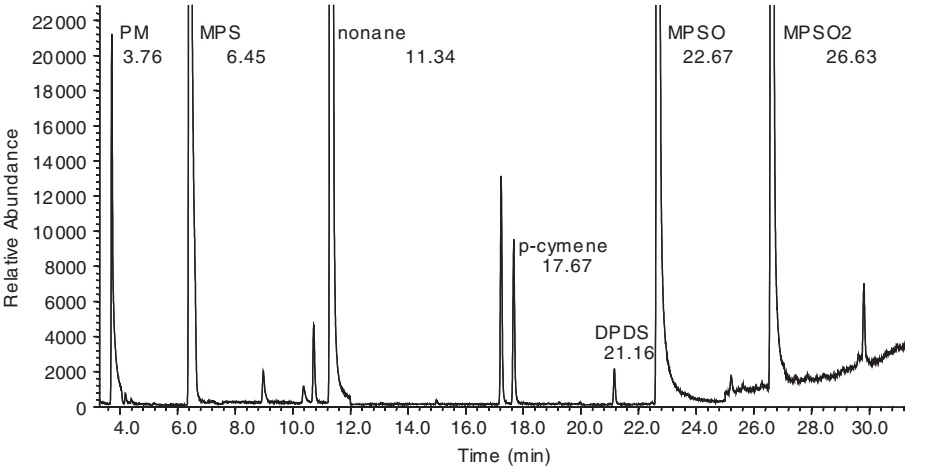


Figure 2. Total ionic current chromatogram of extracted liver at 4 h obtained on GC-MS. Numbers under the compound names are retention times.

was detected by GC-MS in all extracted tissues (stomach, intestine, liver and plasma). PM, MPS, MPSO, and $MPSO_2$ were additional metabolites and were identified by comparing their retention times and fragmentation spectra with those of standards and/or those derived from a spectra library (Wiley library). Figure 3 presents, for each organ, the quantities of these sulphur compounds as a function of time.

Profile of DPDS in the organs examined

One hour after administration, DPDS was mainly detected in the stomach (Figure 3A). The absorption of DPDS is probably almost complete in this organ, as the DPDS quantity in the intestine is quite negligible (less than 0.01 µmol in the intestine compared with more than 50 µmol in the stomach at 2 h). For these two organs, the amount of DPDS declined rapidly between 4 and 8 h after administration.

Profile of DPDS metabolites in the organs examined

In each organ, the metabolites were detected with various rates of appearance and different concentration–time profiles. In the stomach, DPDS and PM were detected very early (from the first hour after administration). The DPDS concentration–time profile was similar to that of PM. However, the concentrations of DPDS were higher than the concentrations of PM (Figure 3A). In the liver, MPS appeared first and transiently and then followed by the appearance of MPSO, and $MPSO_2$. Whereas PM, MPS and MPSO were detected 1 h after administration of DPDS, $MPSO_2$ appeared only at 2 h and its concentration gradually increased until 24 h and then gradually decreased. At 48 h its concentration was comparable to that observed at 8 h. The slow appearance of $MPSO_2$ in the liver suggested a slow biotransformation from its precursor MPSO. $MPSO_2$ also demonstrated a transient high concentration in the liver. In plasma (Figure 3D), PM reached a maximal concentration very early whereas the maximal concentration of MPSO was at 8 h, and that of $MPSO_2$ was delayed to 24 h. In the stomach and in

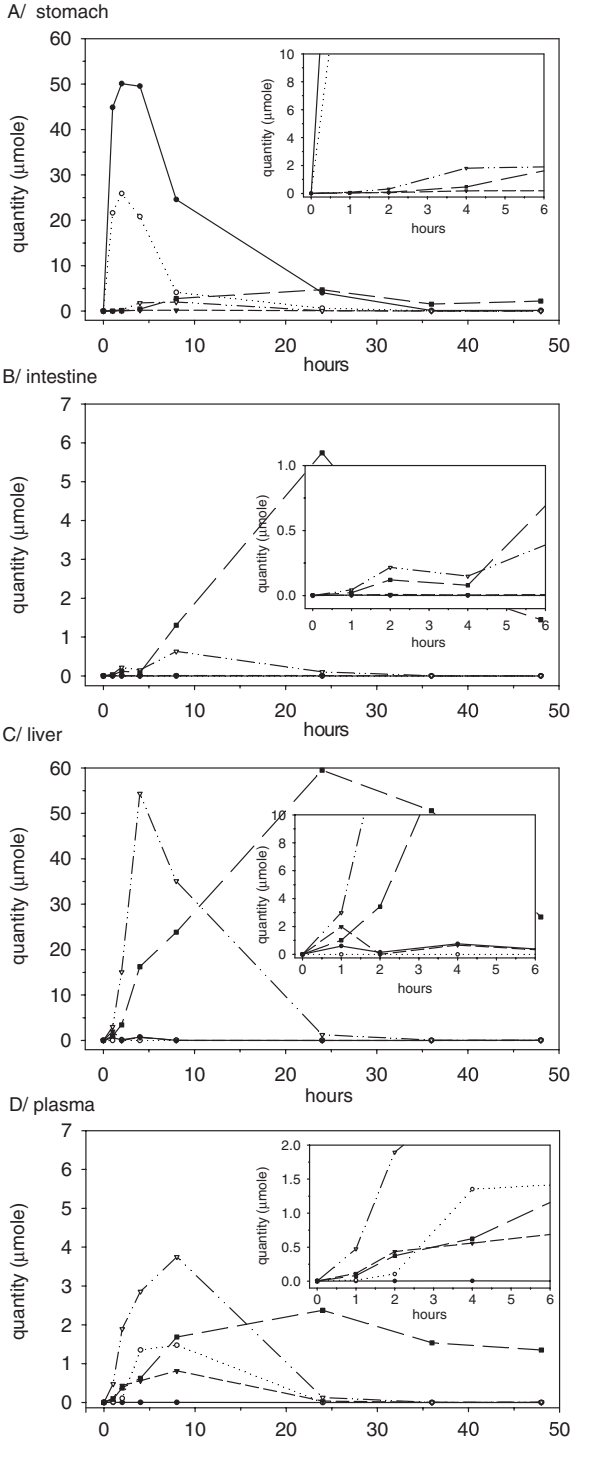


Figure 3. Quantity of volatile metabolites during the 48-h period in the stomach, intestine, liver, and plasma after one oral administration of 200 mg kg^{-1} of DPDS to male rats. —●— DPDS, ·····○····· PM, — –▼– - MPS, · · –▽· · – MPSO, — ■— - $MPSO_2$, Results are the mean of three rats.

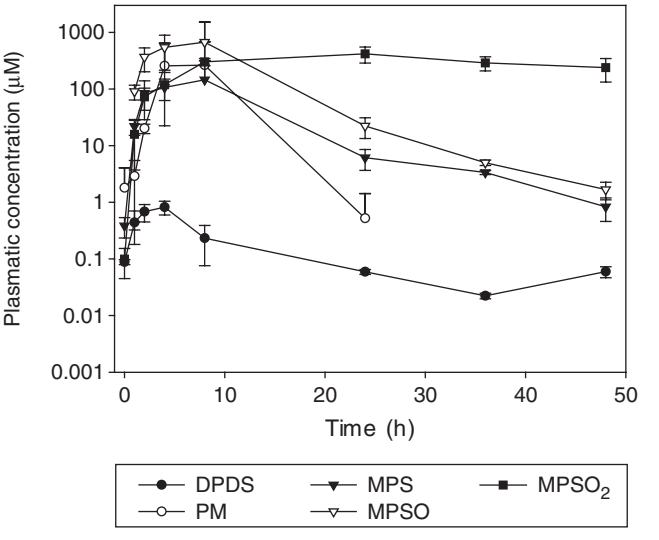


Figure 4. Plasma concentration–time curve of metabolites of DPDS after one oral administration to male rats. Data are means of three rats ± standard deviation.

the intestine, the maximal concentrations were at 8 h for MPSO and at 24 h for $MPSO_2$. The presence of these metabolites in the digestive tract at this time was probably caused by the introduction of these compounds at this time rather than an *in situ* metabolic transformation of DPDS or PM. The quantity of $MPSO_2$ in stomach and intestine represented 7.9 and 9.6%, respectively, of that of the liver.

Pharmacokinetic parameters

The plasma concentration–time curves of DPDS and its metabolites after oral administration to rats are shown in Figure 4 and the pharmacokinetic parameters are summarized in Table I. All parameters were determined only for DPDS as the plasmatic clearance could only be determined for the administered compound. The $t_{1/2}$ (between 2 and 8 h) were quite similar for all metabolites, except $MPSO_2$, whose $t_{1/2}$ was 30 h. The bioavailability, measured by the AUC of each compound, was very low for DPDS (0.008 h mM), because it was immediately transformed after administration. The AUC values were higher for MPSO and $MPSO_2$ with values of 9.64 and 24.15 h mM, respectively. The T_{max} values for the majority of these sulphur compounds were short (between 4 and 8 h) indicating the non-persistence of these compounds in the plasma, whereas the T_{max} for $MPSO_2$ was delayed to 24 h.

Discussion

The present study was carried out in order to investigate the *in vivo* biotransformation of DPDS after a single oral administration to rats. PM, MPS, MPSO, and $MPSO_2$ were identified as DPDS metabolites in the stomach, intestine, liver, and plasma. Based upon their plasma concentrations, their pharmacokinetic parameters were determined. These compounds seemed to be rapidly metabolized or eliminated within a few hours, with a

Table I. Pharmacokinetics parameters of dipropyl disulphide (DPDS) and its metabolites after a single oral administration of DPDS in the rat.

	Dipropyl disulphide (DPDS)	Propyl mercaptan (PM)	Methylpropyl sulphide (MPS)	Methylpropyl sulphoxide (MPSO)	Methylpropyl sulphone ($MPSO_2$)
$t_{1/2}$ (h)	8.25	2.08	6.30	5.85	29.57
C_{max} (mM)	0.001	0.263	0.145	0.670	0.419
T_{max} (h)	4	8	8	8	24
AUC_{total} (h mM)	0.008	3.44	2.07	9.64	24.15
Cl_p ($l\,h^{-1}$)	39.57	n.c.	n.c.	n.c.	n.c.

n.c., not calculated.
Parameters were calculated from three rats.

rate of biotransformation in rats similar to other sulphur compounds originating from *Allium*. The $t_{1/2}$'s of vinyldithiins, from garlic, were about 5 h. They were detected in many tissues over a period of 24 h, but the maximal serum concentration was observed within the 30 first min after ingestion (Egen-Schwind et al. 1992). After DADS administration to rats, the $t_{1/2}$'s of the sulphur metabolites were reported to be between 4.0 and 8.5 h with a T_{max} of 24 or 48 h (Germain et al. 2002).

The uptake of DPDS in the liver was observed only during the first hours after dosing. This level did not exceed 1% of the DPDS level in the stomach. The fact that DPDS was hardly detected outside the digestive tract could be linked to its metabolism. Indeed, the $t_{1/2}$ of DPDS was about 8 h. PM was detected in the stomach at 1 h post-dosing. As DPDS was administered by gastric intubation, PM could result only from a transformation of DPDS in this organ. Glutathione *S*-transferases have previously been identified as catalysing the formation *in vitro* of PM from DPDS (Teyssier & Siess 2000). The presence of these enzymes in the stomach (Pinkus et al. 1977) could explain the formation of PM. The AUC_{tot} and C_{max} of DPDS (0.008 h mM and 0.001 mM, respectively) are lower than the corresponding pharmacokinetic parameters of PM (3.44 h mM and 0.263 mM, respectively). These parameters, in association with the persistence of DPDS and PM in the stomach, indicate that there is a low absorption of DPDS in blood, but a rapid one of PM. In stomach, the concentration of DPDS was higher than the concentration of its metabolite PM and the decrease in PM was not compensated for by the appearance of a new metabolite in the stomach. This suggested that (1) DPDS was almost totally transformed into PM and (2) that PM, once formed, was directly and rapidly absorbed though the stomach wall.

The present data show that DPDS is transformed into PM, MPS, MPSO, and $MPSO_2$. Among these metabolites, MPSO and especially $MPSO_2$ are the major and more persistent volatile metabolites found in all tissues examined. $MPSO_2$ appears to be the ultimate metabolite since no new metabolite was detected when its level decreased. We have previously proposed a scheme for the *in vitro* metabolism of DPDS based on subcellular fractions and isolated perfused liver experiments (Teyssier & Siess 2000) and the pharmacokinetics described in the present study validate this hypothesis. PM is the first detectable metabolite of DPDS which is sequentially transformed into PM, then MPS, then MPSO, and finally to $MPSO_2$. This agrees with the chemical stability of the hydrophilic sulphone, which is therefore readily detected in the urine. This conclusion is supported by the work of Nickson et al. (1995), who only detected dipropyl sulphone in the urine of rats after oral ingestion of dipropyl sulphoxide. In the case of administration of DADS to rats, the ultimate metabolite was also a sulphone detected in the urine (Germain et al. 2002).

In agreement with the literature, the present study emphasizes that the liver plays a major role in the *in vivo* metabolism of DPDS. All metabolites of DPDS, excepted PM, first appeared in this latter organ. Moreover, they have been previously identified in rat liver perfused with DPDS (Teyssier & Siess 2000), therefore they are probably formed in this organ. The slow elimination of $MPSO_2$ ($t_{1/2} = 29.57$ h) and the level of this molecule in the liver suggests a role for this organ in the storage of sulphur compounds. Compounds containing a sulphoxide or sulphone moiety may interact strongly with their surrounding environment (via hydrogen bond formation, etc.) and thereby delay their excretion (Mitchell 1988). At the same experimental time, the *S*-oxidized metabolites in the stomach and the intestine appeared in the absence of potential substrate in these organs and after their formation in the liver (Figure 3A and B). This indicated elimination from the liver of a fraction of MPSO and $MPSO_2$ by the bile. In fact, elimination was higher via the plasma for MPSO, and via the intestine for $MPSO_2$ (70.8% of the elimination). With our experimental protocol, it was not possible to identify an enterohepatic cycle, but the latter might explain the persistence of $MPSO_2$ in the body. Nickson & Mitchell (1996) demonstrate the existence of such a cycle for the dipropyl sulphone, a very similar sulphur compound. Dipropyl sulphone is below the generally assumed minimum threshold molecular weight required for a notable biliary excretion. The hypothesis of the authors was the chemical properties of the S-O bond which could end at molecular stacking increasing the apparent molecular weight. Even if one could explain this by a phenomenon of molecular stacking, the biliary excretion of $MPSO_2$, we cannot explain why this is not also the case for MPSO which is eliminated at a rate of 85.6% by the plasma.

This study has clearly established the presence of volatile metabolites in several organs after DPDS ingestion in the rat. The formation of other metabolites cannot be ruled out, which could not be detected using the analysis method. In particular, it has been reported that several glutathione conjugates are formed *in vivo* from diallyl sulphide (Jin & Baillie 1997) and *in vitro* from DADS and DPDS (Teyssier & Siess 2000; Germain et al. 2003). This conjugation could also happen during the *in vivo* DPDS metabolic process. Hence, if such conjugates actually exist, it would be of major interest to compare their levels with those of MPSO and $MPSO_2$.

In conclusion, it has been confirmed that the metabolites PM and MPS and $MPSO_2$ already observed *in vitro* are also *in vivo* metabolites of DPDS and MPSO has been identified as a new and previously unreported metabolite of DPDS in rat, which has not been observed in any previous study. DPDS has been shown to have anticarcinogenic, antimutagenic, and hypolipidemic effects (Wattenberg et al. 1989; Liu & Yeh 2000; Guyonnet et al. 2001). Nevertheless, due to the rapid metabolism described herein, DPDS seems to have no systemic bioavailability after oral administration. Taking into account the time of exposure and quantity of the different metabolites of DPDS, the *S*-oxidized compounds are certainly involved in the beneficial effects ascribed to onion. Further studies are needed to assess the biological effects of these two metabolites of DPDS. Finally, as DPDS and its metabolites are probably biologically active component of *Allium* species, it would be of great interest to explore the bioavailability and metabolism of such compounds in man.

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