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Disposition of diphenyl sulphoxide in rat

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1. Radiolabelled diphenyl sulphoxide (U-14C- or 35S-) was administered by gavage (1.0 mmol/kg body weight) to the adult male Wistar rat following an overnight fast.

2. For both labelled forms faeces was the major route of excretion of radioactivity (50%) with substantial amounts still being voided during the third and fourth days (13%). Urinary elimination (42%) was similar during the first (20%) and second (17%) days and a small amount of radioactivity (7%) was found within the carcass after 4 days.

3. Plasma data showed a peak concentration at 40 min (t_{max}), a distribution half-life of 2 h ($t_{\frac{1}{2}}\alpha$) and an elimination half-life of 22.5 h ($t_{\frac{1}{2}}\beta$). Biliary studies revealed that 16% of the dose traversed the bile duct during the first day with nearly half of this being excreted in the first 8 h.

4. From urinary data, metabolism occurred via ring hydroxylation with subsequent conjugate formation. Oxidation of the sulphur to form the sulphone also took place. No evidence for sulphoxide reduction, cleavage of the ring structures or exclusion of the sulphur was obtained.

Introduction

Diphenyl sulphoxide (1,1´-sulphinylbisbenzene) is a simple aromatic sulphoxide that finds use, together with its parent sulphide, as an intermediate in chemical synthesis especially in the plastics industry. The sulphoxide has both defoliant (Goodhue and Tissot 1953) and fungicidal properties (Siegler and Childs 1947, Esrolko 1949) but has negligible insecticidal activity when compared with the sulphide (Beran *et al*. 1951). It has also been investigated, along with other sulphoxides (Lavine 1935), as a tumour growth suppressant, but was without effect (Hammett 1942). Subcutaneous administration of diphenyl sulphoxide to mouse has led to neurotoxic effects including limb paralysis, convulsion and respiratory suppression (Hammett 1942). The present investigations have been undertaken as, to date, little is known concerning the fate of diphenyl sulphoxide within living systems.

Materials and methods

Chemicals

Diphenyl sulphide, diphenyl sulphoxide, diphenyl sulphone, benzene, sulphur, thionyl chloride, hydrogen peroxide and anhydrous aluminium chloride were obtained from Aldrich Chemical Co. Ltd (Dorset, UK). All other chemicals were of analytical grade and readily available within the laboratory.

[U-14C]-diphenyl sulphoxide was prepared by the thionation of [U-14C]-benzene (Sigma Chemical Co, St Louis, MO, USA). Small amounts (*c*.0.2 g) of anhydrous aluminium chloride were stirred into an ice-cooled mixture of [U-14C]-benzene (10 g, 128 mmol; 1 mCi) and thionyl chloride (3.8 g, 32 mmol) until the evolution of hydrogen chloride ceased (*c*.6 g). The mixture was then refluxed for 30 min after the addition of extra benzene (4 g, 51.2 mmol). On cooling, the mixture was poured into excess ice-water

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and the precipitated yellow oil washed several times with water. Excess benzene was removed by distillation and the residue recrystallized from petroleum ether (b.p. 60–80 °C) (Colby and McLoughlin 1887, Shriner *et al*. 1930).

The white crystalline substance (m.p. 69–70 °C uncorr.; lit. 70–1 °C, Colby and McLoughlin 1887, Shriner *et al*. 1930) gave a chemical analysis in agreement with the theoretical value (expected for $C_{12}H_{10}SO$; C 71.3, H 5.0; found C 71.1, H 5.2), UV peaks (ethanol) at λ_{max} 233 ($\log_e$ 4.14) and λ_{max} 265 ($\log_e$3.31), a strong IR band at *c*.1040 cm^{-1} (KBr) or *c*.1050 cm^{-1} (in CCl_4) (S-O stretching) (Barnard *et al*. 1949, Cymerman and Willis 1951) and a top mass ion at *m/z* 202 (mol. wt. 202) (Bowie *et al*. 1966). A specific activity of 15.6 mCi/mol (radiochemical yield 40%) was obtained with chemical and radiochemical purities > 99% as determined by thin-layer (tlc) and capillary gas-liquid chromatography (see below). The compound was highly soluble in most organic solvents (except cold hexane, heptane and petroleum ether) with a partition coefficient (log*P*, octanol/water) of 1.37.

[35S]-diphenyl sulphoxide was prepared in a similar manner via [35S]-diphenyl sulphide. Finely powdered anhydrous aluminium chloride (4.2 g) was dispersed in dry benzene (10 g, 128 mmol; excess) with constant stirring and [35S]-sulphur (1 g, 31.3 mmol; Amersham International, Amersham, UK) added slowly while the mixture was refluxed under a stream of nitrogen for 4 h . The radioactive hydrogen sulphide evolved was removed by passing the gas, amid the nitrogen stream, through acidified potassium dichromate solution (turns orange to green) which was constantly renewed. Dilute hydrochloric acid (8 ml, 1.5 M) was added to the cold refluxed mixture and the separated benzene layer washed with water and dried over calcium chloride before being removed by distillation. Anhydrous ethanol (16 ml) was added to the residue and the filtered liquid distilled under reduced pressure; the diphenyl sulphide coming over at 115 °C (3 mmHg) (1.1 g, 5.9 mmol; yield 19% relative to sulphur) (Böeseken 1905, Dougherty and Hammond 1935). Hydrogen peroxide (1 ml, 30% by wt) was slowly stirred into an ice-cold solution of [35S]-diphenyl sulphide (1 g, 5.3 mmol) in acetone (10 ml) and left for 24 h at room temperature. Following removal of the acetone under reduced pressure, the aqueous residue was extracted with chloroform (3× 5 ml) which was subsequently dried over anhydrous magnesium sulphate. Removal of the chloroform and repeated recrystallization from petroleum ether (b.p. 60–80 °C)/chloroform (95/5 v/v) afforded white crystals of diphenyl sulphoxide (0.67 g, 3.3 mmol, 62% yield,; 10.5% overall yield relative to sulphur; specific activity 31.9 mCi/mol, chemical and radiochemical purities 99% +), m.p. 69–70 °C and physical characteristics as previously reported (Hinsberg 1910, Barnard *et al*. 1949).

Animal dosing

Radioactive diphenyl sulphoxide (both U-14C- and 35S-) was suspended in an emulsion of corn oil and water (3/1 v/v) and administered by gavage (1.0 mmol/kg body weight) to adult male rats (Wistar strain, 250 g; National Institute of Medical Research, London, UK) following an overnight fast. The absolute dosage was determined gravimetrically.

Radioactive balance study

Following dosing with either [U-14C]-diphenyl sulphoxide or [35S]-diphenyl sulphoxide, animals were housed in separate glass metabolism cages ('Metabowls', Jencons Ltd, UK) for 4 days with free access to food ('Lab Sure' rat pellets; K. K. Greef Ltd, Croydon, UK) and water. Urine and faeces were collected separately each day over solid carbon dioxide. During the [U-14C]-diphenyl sulphoxide experiments, expired air was drawn through a series of Dreschel bottles containing ethanolamine in methoxyethanol (2/1 v/v) to trap $14CO_2$ and the solutions counted for radioactivity after 96 h as described below. At the end of the study animals were killed by cervical dislocation and stored frozen (– 20 °C). Cages were thoroughly cleansed with ethanol (50–100 ml) and the washings counted for radioactivity.

Tissue distribution and plasma levels

At known time intervals following the oral administration of [U-14C]-diphenyl sulphoxide, animals were killed by cervical dislocation, exsanguinated, and the required organs and tissues removed and weighed before being stored frozen (– 20 °C). In another series of studies, heparinized blood samples were removed at various times from the tail veins of previously dosed animals, centrifuged and the liberated plasma counted for radioactivity as described below.

Biliary secretion studies

Rats were anaesthetized with sodium pentobarbitone (50 mg/kg body weight, i.p.; 'Sagatal', Rhône Poulenc, Dagenham, UK) and the common bile duct cannulated via a ventral mid-line incision just caudal to the xiphoid cartilage. Bile was collected at regular intervals for up to 8 h; staggering the time

interval between [U-14C]-diphenyl sulphoxide dosing and cannulation permitted a complete 0–24 h profile to be obtained. The volume of bile produced was replaced with an equivalent volume of isotonic saline via a tail vein cannula which also enabled augmentation of anaesthesia ensuring that all animals remained unconscious throughout the study.

Quantification of radioactivity

Aliquots (0.2–1.0 ml) of urine, bile, plasma, CO_2 and SO_2 trapping fluids, cage washings and bands of silica gel removed from tlc plates, were added directly to vials containing scintillation fluid (10 ml, 'Ecoscint'; National Diagnostics Ltd, Atlanta, GA, USA) and counted by liquid scintillation spectrometry using a Packard Tri-Carb 4640 scintillation counter (Canberra-Packard Instruments Ltd, Pangbourne, UK) with external standards being used for quench correction.

Faecal samples were lyophilized, ground to a fine powder, and triplicate weighed samples (50–100 mg) combusted in oxygen (Harvey Biological Material Oxidiser, Harvey Instrument Corp., NJ, USA). Any $14CO_2$ or $35SO_2$ being produced was trapped in an alkaline diphenylethylamine-containing scintillation cocktail (15 ml) (Peterson *et al*. 1969) and counted as previously described.

Frozen carcasses were cut into small cubes with a bone-saw and dissolved in aqueous potassium hydroxide (10 M, 1 litre) at 18 °C for 7–10 days. The resultant liquid was homogenized, filtered through glass wool and aliquots (1 ml) in scintillation vials decolourized with hydrogen peroxide (30% v/v; 2 ml), methanol (2 ml) being added to prevent effervescence. After decolourization, the contents of the vials were mixed thoroughly with distilled water (5 ml), followed by scintillation fluid (10 ml) and the vials counted for radioactivity as described above. Stomach and intestines (both with contents) were digested in aqueous potassium hydroxide as above whereas organs and tissues were homogenized in water. Triplicate aliquots (1 ml) were then decolourized and counted for radioactivity as described above.

Chromatography

Tlc was performed on silica gel 60WF254s plates (0.2 mm thick, 20×20 cm, aluminium backed; Merck, Darmstadt, Germany) and developed in toluene/acetone (39/1, v/v; solvent 1) or toluene/ethyl acetate (1/1, v/v; solvent 2). Compounds were located under UV irradiation (254 nm) and the naphthoresorcinol reagent used to visualize glucuronides (Elliott *et al*. 1959).

Gas chromatography-mass spectrometry (gc-ms) was carried out on a Hewlett Packard 5890 II series gas chromatograph connected to a HP5971 mass selective detector operated in the electron impact mode controlled by HPG 1034C software from the MS Chemstation (Hewlett Packard, Cheshire, UK). The fused-silica capillary column (30 m×0.25 mm i.d.) was coated (film thickness 0.25 μm) with cross-linked phenyl-methyl silicone (5%) with a helium gas flow of 1 ml/min. The column oven was initially held at 70 °C for 2 min, then raised at 20 °C/min until 290 °C was reached which was maintained for a further 2 min. The injection port was held at 250 °C. The gc-ms interface temperature, the ionization energy and the ion source temperature of the mass spectrometer were 280 °C, 70eV and 185 °C, respectively.

Identification and quantification of metabolites

Aliquots (10–50μl) of neat radioactive urine were examined, either spotted or streaked, by tlc. Reference compounds dissolved in control urine were co-chromatographed to provide provisional identification. Consecutive bands of silica gel (0.3 cm) were removed from the origin to solvent front of dried developed tlc plates, added to vials containing scintillation fluid and counted as described above to provide quantification.

Pooled chloroform extracts (5×3 ml) of urine aliquots (1 ml) were dried (anhyd. $CaCl_2$), concentrated under nitrogen and examined by gc-ms. Additionally, urine (1 ml) was incubated with β-glucuronidase (1000 units, *E. coli* type IX; Sigma Chemical Co., Dorset, UK) in phosphate buffer (1 ml; 0.1 M, pH 6.8) for 18 h at 37 °C. Control samples contained no enzyme. Both hydrolysed and control incubates were lyophilized and the residues extracted with methanol (3×5 ml). Following centrifugation, the separated methanol supernatant was reduced in volume under a dry nitrogen stream and examined by gc-ms. In further studies, the lyophilized residues were vigorously shaken with methanol (2 ml) and kept sealed in the dark for 24 h at 4 °C. Excess diazomethane in diethyl ether (generated from KOH on *N*-methyl-*N*-nitroso-*p*-toluenesulphonamide, 'Diazald') was then added until the solution remained yellow and the mixture left for a further 24 h at room temperature. Following reduction to dryness under a nitrogen stream, the residue was extracted with methanol (3×5 ml), centrifuged, and the separated supernatant reduced in volume under nitrogen before examination by gc-ms.

Potential degradation of [35S]-diphenyl sulphoxide to inorganic sulphate was determined by a decrease in radioactivity of a centrifuged urine sample to which fine powdered barium chloride had been added until no further precipitation occurred. If a decrease in radioactivity was observed, the precipitate itself was isolated, washed with water then methanol, and subjected to paper chromatography (Whatman No 1; solvent isobutyric acid/1 M ammonia solution (5 : 3 v/v)) together with standard [35S]-sodium sulphate (Amersham).

Spectrometric methods

UV spectra were obtained for compounds dissolved in ethanol (1 mg/ml) in quartz cuvettes (1 cm) using a Shimadzu MPS-200 UV spectrophotometer with Shimadzu PR-3 graphic printer and computer facilities (V. A. Howe & Co., Ltd, London, UK). Infrared spectra were obtained via potassium bromide discs or in CCl_4 solution employing a Varian Fourier Transform I.R. (Varian Associates, Surrey, UK). Electron impact mass spectrometry was undertaken using a Hewlett Packard 5971 series mass selective detector attached to a capillary gas chromatograph as detailed above.

Results

Distribution and elimination studies

Virtually identical balance study profiles were obtained with both positions of radiolabel (table 1). Half of the administered radioactivity was recovered from the faeces during the 4 days of the study, most being voided during the first 2 days (35–38%) suggesting that a proportion of the dose may have passed through the gut unabsorbed. However, 16.4± 1.1% of the administered radioactivity traversed the bile duct in the first 0–24 h, with nearly half of this (7.5± 0.7%) being excreted in the first 8 h; the greatest flow rate (1.35% dose/h) occurring 1–1.5 h after dosing. This implies that enterohepatic cycling contributed to the retention of the radioactivity in the animal. This is also seen within the urinary collections where virtually the same amounts of radioactivity were voided during the first and second days ([U-14C]—19.3± 4.6%, 16.8± 2.2%; [35S]—20.4± 8.2%, 17.6± 4.8%, respectively; not significantly different, 25% $> p >$ 10%, Student's *t*-test), a total of 41–43% being collected in the urine over 4 days of the study (table 1).

Plasma level data approximated a two-compartment oral (three-compartment) model and displayed a peak concentration of 0.16% dose/ml (c_{max}) at 40 min (t_{max}) post-dosing. Assuming equal distribution and about 8 ml plasma in a 250 g rat (Creskoff *et al.* 1942), this accounts for 1.28% dose within the plasma at 40 min post-dosing. The fraction of the dose which was absorbed was taken up quickly ($t_{\frac{1}{2}}ab$ about 3 h) and rapidly distributed around the body ($t_{\frac{1}{2}}\alpha$ 2 h) but then slowly eliminated ($t_{\frac{1}{2}}\beta$ 22.5 h).

Results from tissue distribution studies were unremarkable. Small amounts of the dose were found in the liver (*c.*4%; 0.4%/g) and kidney (*c.*1.4%; 0.7%/g) during the first 6 h, presumably reflecting excretion by these organs. These amounts decreased slowly over the next 66 h. The majority of radioactivity was detected within the gastrointestinal tract, appearing to move slowly from the stomach to the intestines, although 3% of the dose still remained within the stomach after 24 h (table 2). Between 5 and 11% of the dose was found in the carcasses after 4 days. No radioactivity was detected in expired air (< 0.1% dose).

Metabolite identification and quantification

The failure to detect exhaled $14CO_2$ or any radiolabelled sulphate in the urine suggested little or no degradation of the diphenyl sulphoxide molecule during its passage through the rat. Excreta from the [35S]-diphenyl sulphoxide studies were not examined further.

Analysis by tlc of 0–48 h urine samples collected from the [U-14C]-diphenyl sulphoxide balance studies showed the presence of three radioactive areas. One co-chromatographed with authentic diphenyl sulphoxide (R_f 0.17 solvent 1; R_f 0.43 solvent 2) accounting for 2.8± 1.1% of the administered dose and another with

Table 1. Excretion of radioactivity from the adult male Wistar rat dosed orally with radiolabelled diphenyl sulphoxide (DPSO) (1.0 mmol/kg body weight).

	Percentage administered radioactivity excreted	
	[U-14C]-DPSO (n = 12)	[35S]-DPSO (n = 4)
Urine		
Day 1	19.3± 4.6	20.4± 8.2
Day 2	16.8± 2.2	17.6± 4.8
Day 3	3.8± 2.4	4.3± 2.1
Day 4	1.1± 0.5	0.8± 0.5
Total	41.0± 6.1	43.1± 8.3
Faeces		
Days 1 and 2	37.8± 14.6	35.2± 12.2
Days 3 and 4	12.4± 6.6	14.4± 5.6
Total	50.2± 12.5	49.6± 10.1
Carcass	6.8± 2.1	5.8± 3.1
Cage washings	0.5± 0.2	0.6± 0.2
Total	98.5± 5.5	99.1± 5.1

Values quoted are mean± SD.
No radioactivity (less than 0.1% dose) was detected in the expired air.

Table 2. Amounts of radioactivity remaining within organs of the male Wistar rat at various time intervals following oral administration of [U-14C]-diphenyl sulphoxide (1.0 mmol/kg body weight).

Time after oral dose (h)	Percentage of radioactive dose remaining in entire organ or structure					
	Stomach	Intestine	Liver	Kidney	Heart	Lung
1.0	72.0± 11.7	8.6± 2.6	4.3± 0.2	1.5± 0.2	0.3± 0.1	0.3± 0.1
3.0	57.1± 5.0	29.3± 3.3	4.4± 0.8	1.4± 0.1	0.2± 0.1	0.4± 0.2
6.0	28.2± 5.1	24.0± 1.8	3.5± 0.3	1.2± 0.2	0.2± 0.0	0.4± 0.1
9.0	19.8± 5.9	32.9± 6.3	2.7± 0.2	1.0± 0.1	0.2± 0.1	0.3± 0.2
12.0	16.1± 7.6	39.6± 2.0	2.6± 0.3	0.9± 0.3	0.2± 0.1	0.2± 0.0
18.0	8.1± 4.8	26.5± 3.2	2.4± 0.1	0.8± 0.3	0.1± 0.0	0.1± 0.0
24.0	3.2± 1.1	19.0± 6.2	1.7± 0.4	0.9± 0.2	0.1± 0.1	0.2± 0.1
36.0	2.3± 0.4	12.4± 1.5	1.2± 0.2	0.8± 0.1	0.2± 0.1	0.2± 0.0
48.0	2.0± 0.8	7.5± 1.0	1.2± 0.1	0.4± 0.2	0.2± 0.0	0.3± 0.1
60.0	2.2± 0.5	5.6± 0.2	0.8± 0.1	0.5± 0.1	0.1± 0.1	0.2± 0.0
72.0	1.2± 0.1	6.6± 1.2	1.0± 0.1	0.7± 0.1	0.2± 0.0	0.3± 0.0

Values are quoted as the mean± 1 SD (n = 3).
Values obtained for the adrenals and spleen were always 0.1% of the dose or below except for the spleen at 1 and 3 h which gave values of 0.2% dose.

authentic diphenyl sulphone (R_f 0.33; 0.54) accounting for 6.8± 2.4% dose. However, the majority of the radioactivity remained on the origin in both solvent systems and gave a positive reaction with naphthoresorcinol indicative of glucuronic acid conjugates although confusion with endogenous glucuronides was probable. No evidence for diphenyl sulphide (R_f 0.63; 0.61) was obtained.

Chloroform extracts of 0–48 h urine examined by gc-ms confirmed the presence of diphenyl sulphoxide (R_t 10.5 min) (M+, m/z 202) and diphenyl sulphone (R_t 10.9 min) (M+, m/z 218) and the absence of the sulphide (R_t 8.8 min). In addition, two peaks (R_t 12.8 and 13.0 min) were present which had virtually identical mass spectra (M+, m/z 234) and could be interpreted as ring-hydroxylated diphenyl

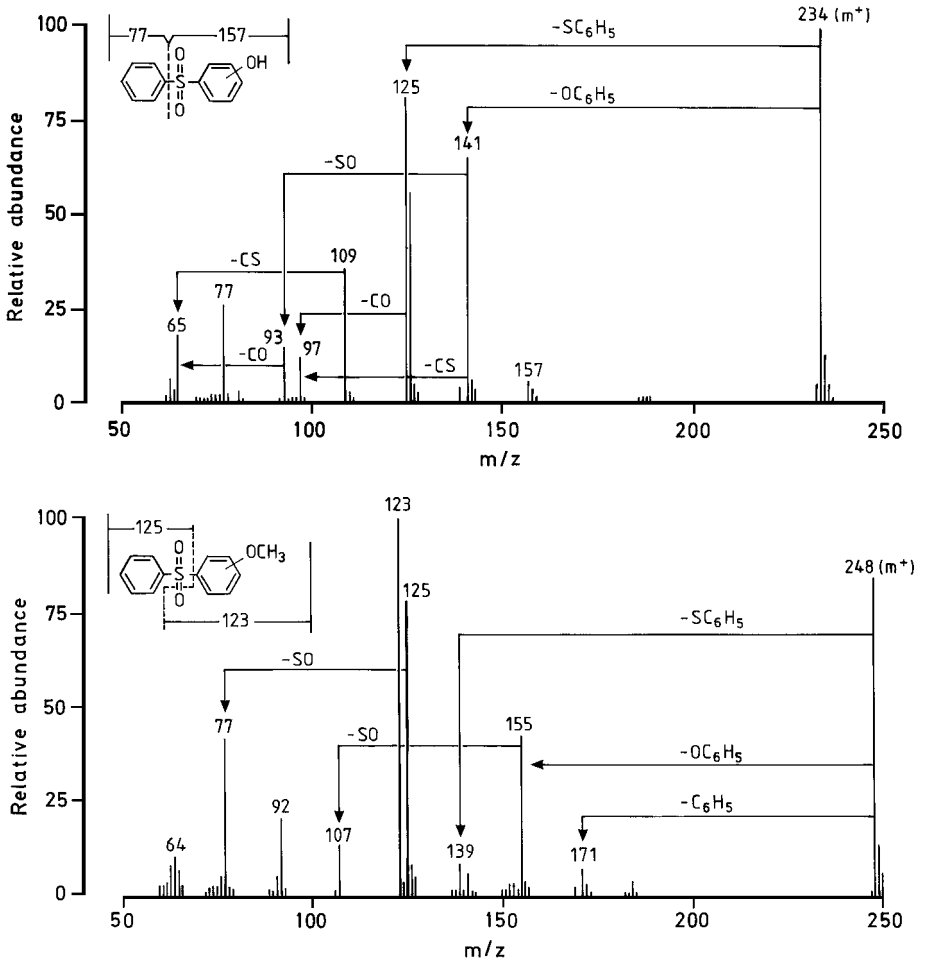

Figure 1. Electron impact mass spectra of ring-hydroxylated metabolite(s) of diphenyl sulphoxide (oxidized to the sulphone) (1a) and these metabolite(s) following methylation with diazomethane (1b). Interpretation of the fragmentation patterns is hampered by the formation of C-C and C-S bonds within the spectrometer and the exclusion of C-S, C-O and S-O fragments (Bowie *et al.* 1966).

sulphone (or the dihydroxylated sulphoxide) (figure 1). The areas of these latter peaks nearly doubled after β-glucuronidase treatment, suggesting that 58% of the hydroxylated metabolites were present in the free form and 42% as glucuronic acid conjugates (presumably both on the tlc origin). Methylation of the hydrolysed urine effectively abolished the peaks at R_t 12.8 and 13.0 min and gave two new peaks at R_t 12.0 and 12.4 min.

Mass spectral examination of these new peaks provided fragment ions which were 14 mass units higher (one methyl group) than previously observed (M+, m/z 248), permitting interpretation as ring mono-methoxylated diphenyl sulphone (figure 1). No further investigations as to the absolute position of the ring hydroxyl groups were pursued. No examination of faecal or bile radioactivity was undertaken.

Discussion

No overt toxicity was observed following oral diphenyl sulphoxide administration at 1.0 mmol(202 mg)/kg body weight during this study. However, previous studies have shown that the same dosage was fatal when administered i.p. to rat (unpublished data from our laboratory) or s.c. to mouse (Hammett 1942). Diphenyl sulphoxide appears to be poorly absorbed from the gastrointestinal tract with 50% of the dose eliminated via the faeces. However, biliary secretion (following absorption) undoubtedly contributes to this faecal excretion and enterohepatic recycling may prolong its retention within the animal. The lipophilic nature of diphenyl sulphoxide may facilitate its entry into the lymphatic system and also its retention within adipose cells (Barton *et al*. 1997), this latter phenomenon being previously observed for *p,p´*-dichlorodiphenyl sulphone which is concentrated in fatty tissues (Mathews *et al*. 1996).

Although only urinary metabolites were investigated, no indication of molecular degradation was observed and all metabolites contained the intact diphenyl structure. The sulphoxide moiety was further oxidized to the sulphone but there was no evidence for sulphoxide reduction. Previous studies employing perfused guinea-pig liver have shown that diphenyl sulphoxide was further oxidized to the sulphone presumably via cytochrome(s) P450 activity rather than FAD-monooxygenase (Yoshihara and Tatsumi 1990). However, under conditions of hypoxia, and in the presence of an electron donor (2-hydroxypyrimidine, benzaldehyde) for aldehyde oxidase, diphenyl sulphide was produced (Yoshihara and Tatsumi 1990). Similar enzymic conclusions have been drawn for the reduction of the sulphoxide within rat and rabbit liver cystosols. However, diphenyl sulphoxide reduction via rat and rabbit renal cystosols and via the cytosolic fraction of *Escherichia coli* was thought to be mediated by a different, thioredoxin-dependent, system (Lee and Renwick 1995a, b). Such interconversions are as expected, with the sulphoxide moiety being intermediary in a redox sequence (Mitchell and Nickson 1993). In addition to sulphone production, aromatic ring hydroxylation and the subsequent formation of glucuronic acid conjugates appeared to be the major route of metabolism for diphenyl sulphoxide. Previous studies have shown that *p,p´*-dichlorodiphenyl sulphone was excreted as a phenolic metabolite and its glucuronide both in the urine and faeces of the orally dosed rat (Mathews *et al*. 1996), and presumably the majority of radioactivity found within the faeces during the present studies may have been of a similar nature.

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