THE FATE OF DIPHENYL SULPHIDE, DIPHENYL SULPHOXIDE AND DIPHENYL SULPHONE IN THE RAT

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SUMMARY

Radiolabelled [UL-^{14}C]-diphenyl sulphide, [UL-^{14}C]-diphenyl sulphoxide and [UL-^{14}C]-diphenyl sulphone were administered by gavage (1.0 mmol/kg body weight) to adult male Wistar rats following an overnight fast. For all compounds, faeces were the major route of excretion of radioactivity (50%). Urinary elimination (40%) was similar during the first (19%) and second (16%) days and a small amount of radioactivity (6%) was found within the carcass after four days. From urinary and faecal data, metabolism occurred via ring hydroxylation with subsequent conjugate formation. Oxidation of the sulphur to form the sulphoxide and sulphone also took place; a small amount of sulphoxide reduction was apparent but no sulphone reduction was found. No evidence for exclusion of the sulphur was obtained, and it appeared unlikely that extensive cleavage of the ring structures occurred.

KEY WORDS

diphenyl sulphide, diphenyl sulphoxide, diphenyl sulphone, rat, redox

INTRODUCTION

Diphenyl sulphide (phenyl sulphide; 1,1′-thiobisbenzene), diphenyl sulphoxide (1,1′-sulphinylbisbenzene) and diphenyl sulphone (sulphobenzide; 1,1′-sulphonylbisbenzene) are simple aromatic sulphur compounds which form a sequential sulphur redox-triad. Biologically, all three compounds possess fungicidal properties /1,2/ with the sulphide also being a potent insecticide /3/ and nematocide /4,5/ but not an anthelmintic /6/. The sulphide's inherent phytotoxicity, however, has limited its usefulness except, perhaps, as a weedkiller /7/ and, similarly, the sulphoxide has a defoliant action /8/. The addition of an oxygen to the sulphur moiety removes any insecticidal properties /3/, but the sulphone is highly ovicidal to the eggs of the red spider mite /9,10/. Interestingly, the sulphide has been shown to exhibit a weak oestrogenic activity /11/; the sulphoxide has been investigated as a tumour growth suppressant /12/ and is neurotoxic when subcutaneously administered /13/, whereas the sulphone displays a growth-retarding effect against both the tubercle bacillus /14/ and the poliomyelitis virus /15/.

No systematic metabolic investigation into these three compounds has been undertaken, although a few accounts have mentioned *in vitro* metabolism of diphenyl sulphoxide in the guinea-pig /16/. The present paper reports on the interrelated metabolic fates of diphenyl sulphide, diphenyl sulphoxide and diphenyl sulphone in the rat.

MATERIALS AND METHODS

Chemicals

Diphenyl sulphide, diphenyl sulphoxide, diphenyl sulphone, benzene, benzenesulphonyl chloride, sulphur chloride, thionyl chloride and anhydrous aluminium chloride were obtained from Aldrich Chemical Co. Ltd., Dorset, UK. Radioactive [UL-^{14}C]-benzene was purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were of analytical grade and readily available within the laboratory.

Radiolabelled compounds were synthesized in small quantities under a nitrogen atmosphere from [UL-^{14}C]-benzene by variations of

the Friedel-Crafts reaction in the presence of the Lewis acid catalyst, anhydrous aluminium chloride.

For [UL-^{14}C]-diphenyl sulphide, finely powdered anhydrous aluminium chloride (5.4 g; 40.5 mmol) was dispersed in dry [UL-^{14}C]-benzene (Sigma Chemical Co, St. Louis, MO, USA; 10 g, 128 mmol; 1 mCi) and the mixture cooled in ice whilst sulphur chloride (4.7 g, 34.8 mmol) in benzene (4.5 g; 57.6 mmol) was added slowly with constant stirring under a gentle stream of nitrogen. Stirring continued at room temperature for two hours followed by heating to 30°C for a further hour until the evolution of hydrogen chloride had ceased. The reaction mixture (the yellow viscous aluminium chloride complex may remain behind) was then poured onto cracked ice (about 10-15 g), the benzene layer separated and the benzene removed by distillation, leaving a dark-coloured oil which, on cooling to 0°C, was filtered to remove precipitated sulphur. The remaining oil was dissolved in anhydrous methanol (10 ml) and cooled to 0°C whilst stirring for four hours, after which any precipitated sulphur was removed by filtration. This process may need to be repeated until no further sulphur precipitates. Following the removal of methanol by gentle heating on a water-bath, the residue was distilled under reduced pressure. The yellow liquid distilling at 155-170°C (18 mm Hg) was decolourised by stirring with zinc dust (1 g) in aqueous sodium hydroxide (40% w/v) on a boiling water bath for one hour. Subsequent washing with water (2 x 10 ml) and drying over anhydrous sodium sulphate yielded, on further distillation at reduced pressure, a fraction of colourless diphenyl sulphide, b.p. 162-163°C (18 mm Hg) (2.6 g, 14.0 mmol; yield 40.0% relative to S_2Cl_2, 15.0% relative to total benzene; radiochemical yield 19.2%, being 4.2% greater than that calculated for benzene incorporation, perhaps suggesting a non-linear reaction) /17-21/.

[UL-^{14}C]-Diphenyl sulphoxide was prepared by thionation with thionyl chloride. Small amounts (~0.2 g) of anhydrous aluminium chloride were stirred into an ice-cooled mixture of [UL-^{14}C]-benzene (10 g, 128 mmol; 1 mCi) and thionyl chloride (3.8 g, 32 mmol) under a gentle stream of nitrogen until the evolution of hydrogen chloride ceased (~6 g). The mixture was then reluxed for 30 min after the addition of extra benzene (4 g, 51.2 mmol). On cooling, the mixture was poured into excess ice-water and the precipitated yellow oil washed several times with water. Benzene was removed by distillation

and the residue recrystallized from petroleum ether (b.p. 60-80°C) to afford white crystals of diphenyl sulphoxide, m.p. 69-70°C (5.2 g, 25.6 mmol; yield 80% relative to $SOCl_2$, 28.6% relative to total benzene; radiochemical yield 40%) /22,23/.

Synthesis of [UL-^{14}C]-diphenyl sulphone was afforded by a sulphonylation reaction. Dry [UL-^{14}C]-benzene (4 g, 51.2 mmol; 1 mCi) was added slowly with constant stirring under a gentle stream of nitrogen to a colourless solution of finely powdered aluminium chloride (6.0 g, 45.0 mmol) in benzenesulphonyl chloride (20.8 g, 117.8 mmol). Stirring continued at 25°C for two hours followed by a slight raising of the temperature to 30°C for a further hour. The reaction mixture was then added to an excess amount of aqueous sodium hydroxide (4 M) with rapid shaking and boiled for 40 min. After cooling, the solution was extracted with chloroform (3 x 50 ml). Each chloroform extract was washed with distilled water (50 ml) and the washings returned to the alkaline solution before the next extraction. The combined chloroform extracts were dried (anhydrous calcium chloride) and evaporated at reduced pressure, the residue being recrystallised from hot ethanol to give white crystals of diphenyl sulphone, m.p. 126-128°C (2.8 g, 12.8 mmol; yield 25% relative to benzene, 10.9% relative to benzenesulphonyl chloride; radiochemical yield 25%) /24,25/.

All products, following purification, were obtained in relatively low radiochemical yields (20-40%) but with high (>99%) chemical and radiochemical purities (t.l.c. and capillary g.c., see below) and possessed characteristics (Table 1) in agreement with those cited in the literature.

Animal dosing

Radioactive [UL-^{14}C]-diphenyl sulphide, [UL-^{14}C]-diphenyl sulphoxide and [UL-^{14}C]-diphenyl sulphone suspended in an emulsion of corn oil and water (3/1 v/v) were 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 (nominally, [UL-^{14}C]-diphenyl sulphide 3.43 μCi/250 g rat, [UL-^{14}C]-diphenyl sulphoxide 3.90 μCi/250 g rat, [UL-^{14}C]-diphenyl sulphone 4.88 μCi/250 g rat).

TABLE 1

Physical characteristics of synthesized [UL-^{14}C]-diphenyl sulphide (DPS), [UL-^{14}C]-diphenyl sulphoxide (DPSO) and [UL-^{14}C]-diphenyl sulphone ($DPSO_2$)

	Diphenyl sulphide	**Diphenyl sulphoxide**	**Diphenyl sulphone**
Physical characteristic	colourless oil	white crystals	white crystals
Specific activity (mCi/mol)	13.7	15.6	19.5
Melting point (°C)	–	69-70	126-128 *
Boiling point (°C)	295-297	–	–
Empirical formula	$C_{12}H_{10}S$	$C_{12}H_{10}SO$	$C_{12}H_{10}SO_2$
Chemical analysis			
expected	C 77.4%; H 5.4%	C 71.3%; H 5.0%	C 66.0%; H 4.6%
found	C 77.1%; H 5.7%	C 71.1%; H 5.2%	C 65.8%; H 4.8%
U.V. peaks (ethanol)	250 (4.1)	233 (4.1)	235 (4.2)
λmax ($\log_\varepsilon$)	275 (3.8)	265 (3.3)	255-275 (3.1)
Mass spectra m/z (% abundance)			
molecular ion (M+)	186 (100)	202 (100)	218 (27)
other fragment ions	171 (4), 109 (5), 92 (11), 77 (11), 65 (5), 51 (12)	186 (20), 154 (67), 125 (13), 109 (86), 97 (37), 77 (55), 65 (34), 51 (50)	125 (100), 97 (13), 77 (35), 51 (20)

Measured values quoted above are in agreement with those given in the literature (melting and boiling points /22,26,27/, * also an α-allotropic form /28,29/; U.V. spectra /30,31/; mass spectra /32-35/).

Radioactive balance study

Following dose administration, animals were housed in separate glass metabolism cages ('Metabowls', Jencons Ltd., Herts, UK) for 4 days with free access to food ('Lab Sure' rat pellets; K.K. Greef Ltd., Croydon, UK) and water. Urine was collected separately each day over solid carbon dioxide. Faeces were pooled over the four days for each animal and examined as one sample. 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.

Quantification of radioactivity

Aliquots (0.2-1.0 ml) of urine, cage washings and bands of silica gel removed from t.l.c. 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, Berks, 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 Oxidizer, Harvey Instrument Corporation, New Jersey, USA). Any $^{14}CO_2$ being produced was trapped in an alkaline diphenylethylamine-containing scintillation cocktail (15 ml) /36/ and counted as previously described above.

Frozen carcasses were cut into small cubes with a bone-saw and dissolved in aqueous potassium hydroxide (10 M, 1 l) at 18°C for 7-10 days. The resultant liquid was homogenised, filtered through glass wool and neutralised (conc. HCl) aliquots (1 ml) in scintillation vials decolourised with hydrogen peroxide (30% v/v; 2 ml), methanol (2 ml) being added to prevent effervescence. After decolourisation, the contents of the vials were mixed thoroughly with distilled water, followed by scintillation fluid, and the vials counted for radioactivity as described above.

Chromatography

Thin-layer chromatography (t.l.c.) was performed on silica gel 60WF254s plates (0.2 mm thick, 20 x 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 visualised with acidified iodoplatinate /37/, the naphthoresorcinol reagent being used to detect glucuronides /38/.

Gas chromatography-mass spectrometry (g.c.-m.s.) 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 x 0.25 mm i.d.) was coated (film thickness 0.25 μm) with cross-linked phenyl-methyl silicone (5%) with a helium gas flow rate of 1 ml/min. The column oven was initially held at 70°C for 2 min, then raised at 20°C/min until a temperature of 290°C was reached which was maintained for a further 2 min. The injection port was held at 250°C. The g.c.-m.s. 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 to 50 μl) of neat radioactive urine and concentrated chloroform extracts of lyophilized faeces were examined, either spotted or streaked, by t.l.c. Reference compounds dissolved in either control urine or chloroform faecal extract 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 t.l.c. plates, added to vials containing scintillation fluid and counted as described above to provide quantification.

Pooled chloroform extracts (5 x 3 ml) of urine aliquots (1 ml) and lyophilized faeces (1 g) were dried (anhydrous $CaCl_2$), concentrated under nitrogen and examined by g.c.-m.s. In addition, urine (1 ml) and evaporated chloroform faecal extracts reconstituted in phosphate buffer (1 ml; 0.1 M, pH 6.8), were 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 x 5 ml). Following centrifugation, the separated methanol supernatants were reduced in volume under a dry nitrogen stream and examined by g.c.-m.s.

In further studies, the lyophilized residues from control and hydrolysed incubates as above 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*-toluenesulfonamide [Diazald®], Aldrich Chemical Co., Ltd) 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 residues were extracted with methanol (3 x 5 ml), centrifuged, and the separated supernatants reduced in volume under nitrogen before examination by g.c.-m.s.

Spectrometric methods

Ultraviolet 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 and Company, Ltd., London, 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

Radioactive balance studies

All compounds behaved in a similar manner and good overall balances were achieved, with about 92% of the dose being recovered in the four-day excreta and 5-7% being retained within the carcasses after this time. Virtually identical balance study profiles were obtained with the three compounds, the only difference which reached statistical significance (Student's *t*-test, $p<0.05$) being the smaller amount of radioactivity voided in the total (0-96 h) urine following diphenyl sulphide administration when compared to the sulphone (Table 2). Half of the administered radioactivity was recovered from

the faeces during the four days of the study which may suggest that a considerable proportion of the dose had passed through the gut unabsorbed, although potential contributions from biliary excretion cannot be ignored. Retention within the body may be reflected within the urinary collections in which virtually the same amounts of radioactivity were voided during the first and second days ([UL-^{14}C]-diphenyl sulphide - 16.9±5.5%, 13.1±3.2%; [UL-^{14}C]-diphenyl sulphoxide - 19.3±4.6%, 16.8±2.2%; [UL-^{14}C]-diphenyl sulphone - 21.4±3.7%, 17.8±1.7%, respectively; not significantly different, $P>0.05$, Student's *t*-test), with a total of 36-44% being collected in the urine over the four days of the study (Table 2).

TABLE 2

Excretion of radioactivity from adult male Wistar rats dosed orally with radiolabelled diphenyl sulphide (DPS), diphenyl sulphoxide (DPSO) or diphenyl sulphone ($DPSO_2$) (1.0 mmol/kg body weight)

	Percentage administered radioactivity excreted		
	[UL-^{14}C]-DPS (n = 4)	[UL-^{14}C]-DPSO (n = 12)	[UL-^{14}C]-$DPSO_2$ (n = 4)
Urine			
day 1	16.9 ± 5.5	19.3 ± 4.6	21.4 ± 3.7
day 2	13.1 ± 3.2	16.8 ± 2.2	17.8 ± 1.7
day 3	5.1 ± 1.8	3.8 ± 2.4	4.0 ± 2.0
day 4	1.0 ± 0.7	1.1 ± 0.5	0.5 ± 0.5
days 1-4	36.1 ± 7.1	41.0 ± 6.1	43.7 ± 1.4
Faeces			
days 1-4	53.4 ± 16.8	50.2 ± 12.5	48.0 ± 3.6
Carcass	5.6 ± 2.6	6.8 ± 2.1	6.9 ± 2.0
Cage washings	2.5 ± 0.4	0.5 ± 0.2	0.7 ± 0.3
Total	97.6 ± 6.5	98.5 ± 5.5	99.3 ± 1.4

Values quoted are means ± SD

Metabolite identification and quantification

Preliminary analysis by t.l.c. of pooled 0-48 h urine samples and chloroform extracts of faeces showed the presence of up to four radioactive areas. Three of these areas co-chromatographed with authentic diphenyl sulphide, diphenyl sulphoxide and diphenyl sulphone, and chloroform extraction of these radioactive t.l.c. areas permitted identity confirmation by subsequent g.c.-m.s. (Table 3).

However, with both urine and facal extracts from all three studies, the majority of the radioactivity remained on the origin in both t.l.c. solvent systems and gave a positive reaction with naphthoresorcinol, indicative of glucuronic acid conjugates, although overlaying with endogenous glucuronides was presumed. In addition to diphenyl sulphide, sulphoxide and sulphone, chloroform extracts of 0-48 h urine and lyophilized faeces examined by g.c.-m.s. showed the presence of two other peaks (R_t 12.8 and 13.0 min) which had essentially identical mass spectra (M+, m/z 234) and could be interpreted as ring-hydroxylated diphenyl sulphone (or the di-hydroxylated sulphoxide or even the tri-hydroxylated sulphide). These two peaks were also detectable in chloroform extracts of the t.l.c. origin, as above. The treatment of urine samples and faecal extracts with β-glucuronidase significantly increased these peak areas (2- to 4-fold) suggesting that the majority (50-75%) of these hydroxylated metabolites were initially present as glucuronic acid conjugates, presumably (together with the free phenols) on the t.l.c. plate origins. Methylation of the hydrolysed urine and faecal extracts 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 (Table 4). No further investigations as to the absolute position of the ring hydroxyl groups were pursued. However, chloroform extraction of faeces only removed 50% of the radioactivity associated with the lyophilized faecal powder. Attempts at exhaustive extraction with chloroform (10 x 5 ml) and the use of other solvents did not enhance the situation. This retained radioactivity may have represented polymeric material bound to faecal components and was not further investigated (Table 3).

TABLE 3

Chromatographic properties of parent compounds and quantification of metabolites in excreta following the oral dosing of adult male Wistar rats with [UL-^{14}C]-diphenyl sulphide (DPS), [UL-^{14}C]-diphenyl sulphoxide (DPSO) or [UL-^{14}C]-diphenyl sulphone ($DPSO_2$) (1.0 mmol/kg body weight)

Metabolite	t.l.c.		g.c.-m.s.		Percentage of administered dose *					
	solvent 1	solvent 2	R_t value	M+	DPS		DPSO		$DPSO_2$	
	R_f value	R_f value	(min)	(m/z)	urine	faeces**	urine	faeces**	urine	faeces**
M1 (DPS)	0.63	0.61	8.8	186	n.d.	8.9±2.4	n.d.	1.2±1.0	n.d.	n.d.
M2 (DPSO)	0.17	0.43	10.5	202	3.1±1.8	n.d.	2.8±1.1	2.4±1.2	n.d.	n.d.
M3 ($DPSO_2$)	0.33	0.54	10.9	218	4.2±1.9	n.d.	6.8±2.4	n.d.	6.8±2.3	12.2±4.8
M4/5	0.0	0.0	12.8/13.0	234	22.7±1.8	13.4±5.9	26.5±2.2	18.4±5.5	32.4±2.3	14.2±4.8
Retained in faeces*** (identity unknown)						31.1±6.8		28.2±5.8		21.6±5.2

n.d. =not detected (below 0.1% administered dose)

* Quantification of metabolites occurred via t.l.c. using neat urine (0-48 h) and chloroform extracts of lyophilized faeces (0-96 h).

** For faeces, values have been corrected for chloroform extraction efficiencies for DPS, DPSO and $DPSO_2$ (all >95%).

*** This amount of radioactivity was retained in the lyophilized faeces following chloroform extraction, its chemical identity is unknown.

TABLE 4

Molecular ions and diagnostic mass spectral fragmentation ions that correspond to the ring monohydroxylated diphenyl sulphone (metabolites M4 and M5) and their synthetic ring monomethoxylated derivatives

Fragment ion lost		Hydroxylated fragment ion remaining	Methoxylated fragment ion remaining
Molecular ion (M+)		m/z 234 (100) ($C_{12}H_9SO_2$•OH) $C_6H_5SO_2$•C_6H_4OH	m/z 248 (85) ($C_{12}H_9SO_2$•OCH_3) $C_6H_5SO_2$•$C_6H_4OCH_3$
C_6H_5	(m/z 77)	m/z 157 (6) -SO_2•C_6H_4OH	m/z 171 (7) -SO_2•$C_6H_4OCH_3$
C_6H_5, O	(m/z 93)	m/z 141 (65) -SO•C_6H_4OH	m/z 155 (42) -SO•$C_6H_4OCH_3$
C_6H_5, O_2	(m/z 109)	m/z 125 (81) -S•C_6H_4OH	m/z 139 (8) -S•$C_6H_4OCH_3$
C_6H_5, SO_2	(m/z 141)	m/z 93 (15) C_6H_4OH	m/z 107 (14) $C_6H_4OCH_3$

Figures in brackets indicate the relative percentage abundance of the ions.

DISCUSSION

No overt toxicity was observed in the present studies following the oral administration of diphenyl sulphide (186 mg/kg), sulphoxide (202 mg/kg) or sulphone (218 mg/kg) to male Wistar rats. However, this dose rate (202 mg/kg) of diphenyl sulphoxide has been shown to be fatal when administered subcutaneously to the mouse /13/. Oral LD_{50} values for rats have been quoted at 2.63 mmol (490 mg)/kg for diphenyl sulphide and 6.38 mmol (1390mg)/kg for diphenyl sulphone /4,39/.

No indication of molecular degradation was observed and all metabolites contained the intact diphenyl structure. The majority of the dose excreted in the urine and faeces appeared in the form of the ring hydroxylated sulphone (either free or as a glucuronic acid conjugate), and, by analogy with work on *p,p'*-dichlorodiphenyl sulphone, biliary excretion of this metabolite may contribute

considerably to faecal excretion /40/. Whether oxidation of the sulphur moiety occurred before or after ring hydroxylation (and conjugation) is uncertain, but no obvious evidence for a ring hydroxylated sulphide or sulphoxide was apparent. Nevertheless, the sulphur moiety appeared to be extensively oxidised during its passage through the rat body.

Employing the present data, and limited information within the literature regarding the metabolism of diphenyl sulphoxide /16,41,42/ and closely related structures such as *p,p'*-dichlorodiphenyl sulphone /40/ and phenylmethylsulphide /43/, it is possible to project a metabolic sequence for these compounds. Diphenyl sulphide is extensively oxidised to the sulphoxide and then the sulphone, presumably via cytochrome(s) P450 activity. Reduction of the sulphoxide to the sulphide does occur under conditions of hypoxia and in the presence of aldehyde oxidase and/or thioredoxin-dependent systems, and in the present study the sulphide was detected in the faeces after sulphoxide administration, presumably formed via microbial reduction. The sulphone appears to be resistant to reduction. Such redox interconversions are expected /44/. In addition, aromatic ring hydroxylation and subsequent formation of glucuronic acid conjugates appears to be the major route of metabolism for these compounds. Following the oral administration of biphenyl to rats, the major metabolite was conjugated 4-hydroxybiphenyl, with smaller amounts of other monohydroxy and dihydroxy derivatives /45-48/. The 4(para)-position appears the most favoured for the present ring hydroxylated diphenyl sulphone. However, when the 4-positions of the aromatic rings were occupied, as with *p,p'*-dichlorodiphenyl sulphone, the major metabolite was the 3-hydroxy derivative and its glucuronide /40/, indicating that this position may also be hydroxylated.

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