URINARY METABOLISM OF ORALLY ADMINISTERED ORTHO-PHENYL PHENOL IN DOGS AND CATS

by

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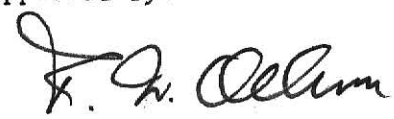

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INTRODUCTION

Ortho-phenyl phenol (OPP) has a broad bacteriological spectrum and is used as a germicide, household disinfectant, and fungicide. It is found in dishwashing formulations and is used to control mold on citrus fruits, in fungistatic dips and waxes for coating vegetables, and as a paper-liner coating to reduce rotting of stored food.

Although this compound has a low oral toxicity, species and age differences may be seen in both the toxicity and kinetics of OPP. In particular, this compound is much more toxic to cats than to other animals, and it is thought that toxicity is due to a defect in the cats ability to biotransform OPP to lesser-toxic metabolites.

Since little work had been done on the comparative metabolism of chronically administered OPP, the present study investigated the urinary metabolites of OPP when this compound was given repeatedly to both young and adult dogs and cats.

URINARY METABOLISM OF ORALLY ADMINISTERED ORTHO-PHENYL PHENOL IN DOGS AND CATS

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SUMMARY

The urinary metabolites from repeated oral doses of 3.7 mg ortho-phenyl phenol (OPP) to mature and immature dogs and cats were studied. At both age levels, dogs excreted significantly more OPP as sulfate and glucuronide than did cats. Puppies produced four times the level of glucuronides than mature dogs. No such age differences were seen with glucuronide formation by cats, nor were there any age differences in either group of animals for sulfate formation. Some sex differences were observed in conjugation of OPP in cats and dogs. The dominant urinary excretion product of oral OPP administration was the unchanged OPP.

INTRODUCTION

Ortho-phenyl phenol (OPP) has a broad bactericidal spectrum and is used as a germicide, household disinfectant and fungicide. It is found in dish-washing formulations and is used to control molding of citrus fruits, in fungistatic waxes for coating vegetables, and as a paper-liner coating to reduce the rotting of stored food. OPP administration has been reported the

method of choice for treatment of ulcerative stomatitis (one of the most common infectious diseases in snakes) in Crotalid snakes [1]. This compound is also used as an intermediate in the dye industry, as well as a preservative of water-oil emulsions encountered in the rubber, textile and metal-working industries. The highly soluble sodium salt of OPP is used for protecting water soluble paints from decomposing prior to use and is used as a preservative for proteins and other types of decomposable adhesives [2].

Ortho-phenyl phenol has a very low oral toxicity. Macintosh [3] reported the mean oral lethal dose of OPP dissolved in nut oil to be 0.5 g/kg for cats and 3 g/kg for rats. This same study reported that feeding this compound at levels of 2, 20, and 200 mg/kg in rats for 32 days did not cause any ill effects or changes in hemoglobin or white blood cell count. In a two-year study by Hodge et. al. [2], rats fed a diet of 0.02 or 0.2% OPP showed no adverse chronic effects, although rats fed a diet of 2% OPP had slight growth retardation, dilation of kidney tubules, and OPP residues in kidney tissues. In part of the same study, no adverse effects could be seen in dogs fed 0.02, 0.2, and 0.5 g OPP/kg/day for one year.

Work by Oehme [4] and Oehme and Smith [5] showed that although dogs survived a dose of 1 or 3 g OPP/kg, cats succumbed to the lower dose in 15 hrs and the higher dose within 6 hr. Dogs required about three times the OPP dosage as cats to develop similar signs. Rachofsky [6] found that plasma levels of OPP peaked within 2 hrs after oral low doses were given to adult and immature dogs. Adult and immature cats required 12 and 36 hrs (respectively) to achieve peak plasma concentrations. These results suggest that the cat has difficulty transporting OPP across the gastrointestinal wall. It has recently been proposed [7,8,9] that phenol is transported across the intestinal lumen after first becoming conjugated by the mucosal cells to

either phenyl-glucuronide or phenyl-sulfate. Since the cat has a defect in its ability to form glucuronides to any extent [10], and since the process of sulfate conjugation is easily saturated [11], these two observations may explain the longer times required for peak plasma concentrations of OPP in the cat.

Since little work has been done on the comparative metabolism of chronically administered OPP, this current study will investigate the urinary metabolites of OPP given repeatedly to both young and adult dogs and cats.

MATERIALS AND METHODS

Animals

Each of the four groups of animals were composed of six animals, three of each sex. Adult beagle-type dogs (greater than six-months old) and domestic short haired cats (greater than six-months old) were used in the mature groups, while the immature groups were comprised of recently weaned puppies and domestic short haired kittens. It was previously determined stoichiometrically [6] that the actual amount of OPP in a four-second burst of aerosol disinfectant was 3.7 mg. A single dose for the study contained 3.7 mg of chemically-pure OPP plus a tracer quantity of UL-^{14}C- OPP in a geletin capsule. Regardless of weight, animals received a single dose every other day for 25 doses. Urine samples were collected weekly and frozen for subsequent analysis of metabolites.

Chromatography

Samples (25-75 µl) of freeze-concentrated urine were spotted on 5 cm wide strips of Whatman No. 1 paper, and chromatographed with n-propanol - ammonia

(7:3 by vol.) using the descending technique. The strips were scanned with a radiochromatogram scanner (Packard Model 7201). To confirm the presence of a glucuronide or sulfate metabolite, 200 µl of urine was incubated in a shaker bath at 37° with 300 µl of the appropriate enzyme in 0.1 M acetate buffer, pH 4.5. β-glucuronidase (40,000 units, No. G-0251, Sigma Chem. Co.) was used for hydrolysis of glucuronides, while sulfates were hydrolyzed using β-glucuronidase with sulfatase activity (6,000 units, No. G-0751, Sigma Chem. Co.). After incubation, urine samples were centrifuged and the supernatent chromatographed as above. Glucuronide metabolites were identified by comparing the radiochromatograms of the treated and untreated urine, noting the loss or decrease of the glucuronide peak and subsequent increase in area of the free OPP peak. Sulfates were analyzed by comparison of the two enzyme-treated groups and noting the loss or decrease in peaks by the use of β-glucuronidase with sulfatase activity, but not with β-glucuronidase activity alone (Fig 1). The OPP peak was verified by chromatography of pure OPP (Eastman Co.) using various solvent systems and color reactions [9].

Statistical Analysis

The data was analyzed using Students t-test at a significance level of $p<0.05$. In the case of unequal sample size, t was computed from the weighted average of the sample variance [12].

RESULTS

Results are shown in Table I. Only parent OPP and glucuronide and sulfate conjugation products were detected in any of the samples.

Canine OPP Metabolism

Immature and mature dogs excreted about the same percentage (approx. 7%) of o-phenyl phenol sulfate; however there was an age difference in degree of glucuronide conjugation. While mature dogs produced only about 5.2% glucuronides, the puppies were excreting four times that value (21.1%).

There were no significant differences in metabolites between male and female puppies, although in adult dogs the females metabolized more OPP to OPP sulfate ($p<0.05$) than did the male dogs (7.6% in females compared to 4.9% in the males).

The low specific activity of many of the urine samples prevented accurate analysis of the conjugation pattern as the OPP accumulated in the body (Table II).

Feline OPP Metabolism

OPP was metabolized to its glucuronide conjugate to about the same extent in both mature and immature cats, but the mature female cats metabolized more OPP to its glucuronide ($p<0.05$) than did the mature males. There were no sex or age differences in conjugation to sulfates by the kittens and cats.

There were no significant changes in the OPP conjugation pattern in kittens as the doses accumulated in the body (Table II). The same held true for cats after their third week of dosing; however no glucuronides could be detected in the urine before the third week of OPP dosing.

DISCUSSION

Recent work by Cassidy and Houston [13] has shown that phenol will

undergo a large first-pass effect when administered orally, and that the major conjugative contribution is due to intestinal mucosal enzymes rather than the hepatic enzymes, which seem to have a minimal role in detoxication of orally ingested phenol. The intestinal mucosa is an important site of glucuronide formation. Glucuronyl transferase activity is present throughout most of the gastrointestinal tract, its activity being highest in the microsomal fraction of the mucosal cells [14].

Although intestinal glucuronyl transferase seems to resemble that of the liver and kidney, the development of these enzymes is very different [15]. Activity in the liver and kidney is low or absent in early fetal life and develops to maximal capacity within a few weeks after birth [16], while activity of gastrointestinal glucuronyl transferase is at maximal activity before birth and declines postnatally [14]. The puppies in the present experiment probably had a much higher glucuronyl transferase activity in the intestinal mucosa, where most of the orally ingested phenol is conjugated, than did the mature dogs. This may explain why the recently-weaned puppies formed four times the amount of urinary OPP-glucuronides than the mature dogs (Table I).

Neither age group of the cats were able to glucuronidate OPP very well. This observation was not surprising, since cats are poor at forming glucuronides with most compounds due to a deficiency in the enzyme UDP-glucuronyl transferase [9,10,17,18]. The cat is not lacking in UDP-glucuronic acid [18], nor does it have a different tissue distribution of β-glucuronidase than other animals [10]. Although this enzyme deficiency exists, the cat has been shown to glucuronidate phenol in trace amounts [9,18,19], and may even form glucuronides of some selected compounds with ease [17,18].

As in the case of glucuronide conjugation, cats conjugated less OPP with sulfate than did dogs. With the exception of a sex difference in the mature

dogs, there were no differences in sulfate conjugation in either species group due to age or sex.

It was expected that cats would have higher urinary levels of OPP sulfate than were observed if they were to follow the same urinary pattern as has been shown to occur with the excretion of phenol [9,20]. Several factors may account for the limited sulfate conjugation. Sulfate conjugation is a readily saturable process [11,21,22], although the levels of OPP given were probably below the level needed for saturation. The degree of conjugation may be strongly influenced by the chemical group in the ortho position of phenol. It has been demonstrated in the rabbit [23] that acidic groups ortho to the hydroxyl of phenol tend to prevent sulfation of the OH group, while basic groups tend to increase the degree of conjugation. Neutral groups, such as phenyl, in the ortho position have little effect. Sulfate bonds might be broken by circulating sulfatases, which are believed to have a role in controlled synthesis and degradation of arylsulfates [24]. Decreased activity in intestinal gut flora may result in a decreased level of sulfation. In the rat, which normally excretes phenol as the glucuronide and sulfate, the germ free state of the digestive tract results in loss of the sulfation route of detoxication [25]. Dosage of the phenolic substance has been shown to influence the conjugation pattern in the cat and other species, with generally an inverse relationship between the formation of phenolic sulfates and dose level [11,22,23,26]. The possibility of autocatalytic degradation must also be considered, since sulfate conjugates are very labile [27,28]. Radiolabelled compounds may be unstable due to the energy produced by nuclear disintegration, resulting in breakdown during storage. In general, ^{14}C compounds stored as aqueous solutions are more stable at temperatures less than -20° C than at 0° C or room temperature; however this is not a universal effect [29].

The differences in glucuronide conjugation observed between male and

female adult cats could be due to the effects of sex hormones on UDP-glucuronyl transferase activity or on β-glucuronidase levels [18,28,30]. Androgens have increased kidney levels of β-glucuronidase in rats [30], but work by Musa et. al. [31] failed to show a significant increase in the levels of this enzyme in cats receiving androgens. A high β-glucuronidase activity could produce the low levels of glucuronides excreted in the male cats.

It has been proposed [32] that steroid sulfatases may be responsible for a fluctuating relationship between free steroid and steroid sulfate levels in circulation. These sulfatases, and perhaps less specific sulfatases, may have different activities in the two sexes and may account for the higher concentration of OPP sulfate found in the adult female dog compared to the adult male dog.

Other metabolites, namely monophenyl phosphate [33] and quinol [11,22], have been reported to be formed in vivo by cats. Neither of these were observed in the present study, nor was catechol, a metabolic product formed in small amounts by phenol detoxication in rabbits [23,24]. Even small amounts of catechol or quinol will cause darkening of the urine due to oxidation yielding dark polymerization products [11,34]. The lack of dark samples in the present study supports that these metabolites were not present in greater than trace amounts. The alkaline pH of cat urine would further enhance the oxidation process.

Sulfate conjugation in the intestinal mucosa would not likely be saturated at the low dose of OPP given, and this may explain the absence of quinol metabolites, since less free OPP would be available for oxidation. Indeed, in the cat, greater amounts of quinol conjugate are formed after an intraperitoneal injection of phenol than after an oral dose [9]. It has been postulated [22] that quinol formation is an obligatory step in phenolic poisoning

in the cat, and that the characteristic clinical signs result after further oxidation of quinol to the corresponding quinone. Quinones inhibit mitochondrial respiration by acting as ubiquinone analogues [22]. The present study supports the above hypothesis, since the administration of a phenolic compound (OPP) in doses low enough not to produce clinical signs resulted in sulfate and glucuronic acid metabolites, but no quinol metabolites were apparently formed.

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Figure 1. Chromatogram of a single urine sample showing the use of β-glucuronidase and sulfatase to identify OPP-glucuronide (peak C), OPP-sulfate (peak B) and free OPP (peak A). Chromatogram I is the untreated urine; II shows the results of treatment with β-glucuronidase; III is the result of treatment with β-glucuronidase with sulfatase activity.

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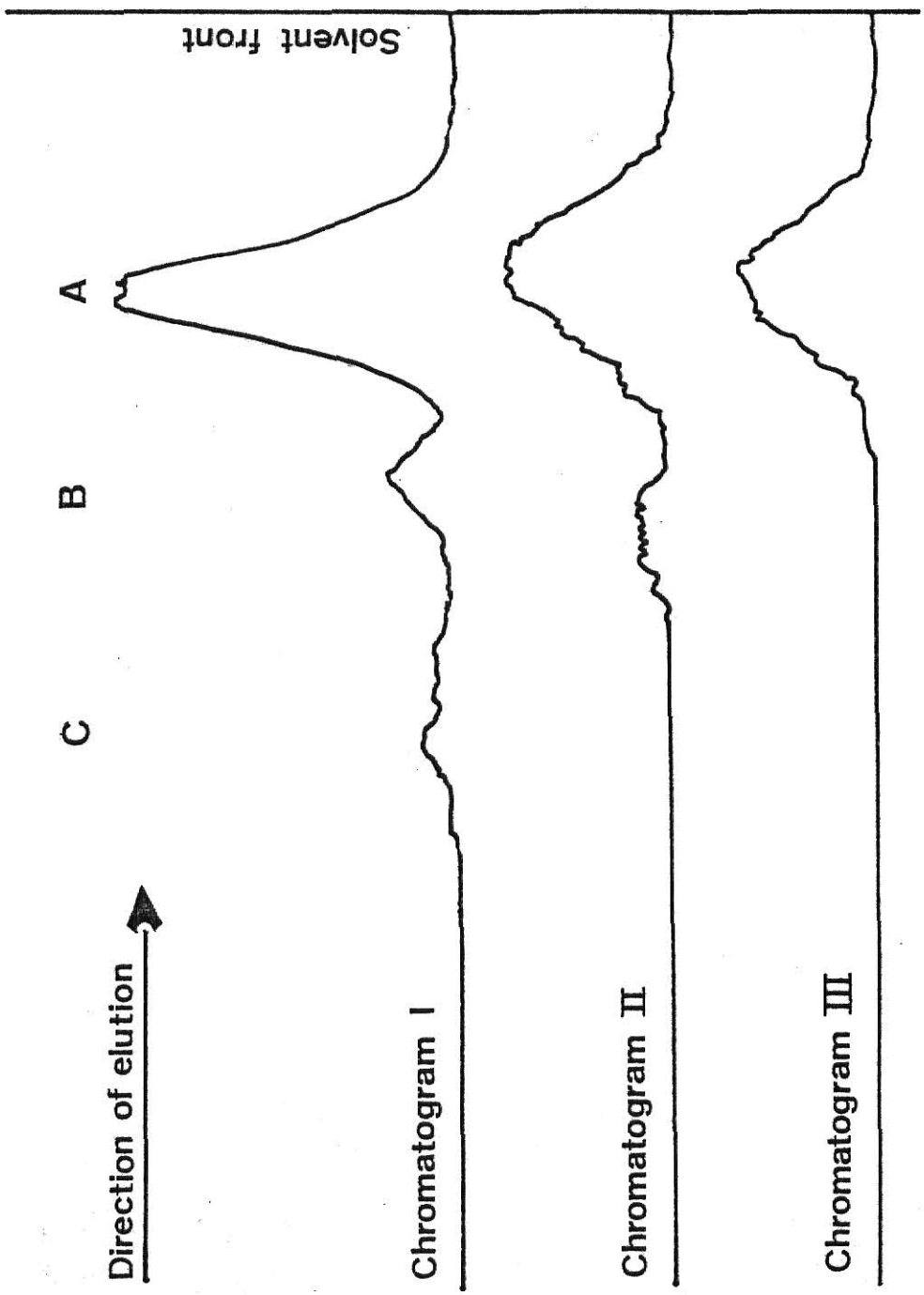
Solvent front
A
B
C
Direction of elution
Chromatogram I
Chromatogram II
Chromatogram III

Table I

URINARY EXCRETION PRODUCTS OF REPEATED ORAL DOSES OF 3.7 mg o-PHENYL PHENOL (OPP) IN PUPPIES, DOGS, KITTENS AND CATS

Values represent mean percentage of the amount in urine ± standard error of mean.
Significant differences within groups ($p \leq 0.05$) represented by *

		OPP-glucuronide	OPP-sulfate	free OPP
Puppies	female n=12	22.5 ± 3.7	8.7 ± 2.9	68.1 ± 3.5
	male n=7	18.7 ± 6.5	7.5 ± 2.1	73.7 ± 8.5
	mean	* 21.1 ± 3.2	8.3 ± 2.0	72.8 ± 3.6
Dogs	female n=14	5.5 ± 0.7	* 7.6 ± 0.9	86.5 ± 1.4
	male n=18	4.9 ± 1.1	* 4.9 ± 0.7	90.0 ± 1.1
	mean	* 5.2 ± 0.7	6.1 ± 0.6	88.4 ± 0.9
Kittens	female n=7	0.14 ± 0.14	1.6 ± 0.6	98.3 ± 0.5
	male n=22	1.22 ± 0.46	3.8 ± 0.9	94.9 ± 1.1
	mean	0.96 ± 0.36	3.3 ± 0.7	95.7 ± 0.9
Cats	female n=22	* 1.18 ± 0.37	2.6 ± 0.5	96.0 ± 0.8
	male n=18	* 0.26 ± 0.18	2.2 ± 0.6	97.4 ± 0.7
	mean	0.76 ± 0.23	2.4 ± 0.4	96.6 ± 0.6

Table II

WEEKLY URINARY PRODUCTS OF o-PHENYL PHENOL (OPP) IN PUPPIES, DOGS, KITTENS AND CATS RECEIVING 25 ALTERNATING DAY DOSES OF 3.7 mg OPP ORALLY

Values are mean percentage of the product in urine ± standard error of mean.

	Urinary Product	Week of Dosage						
		1	2	3	4	5	6	7
Puppies	Glucuronide	...	...	24.5 ± 3.1	...	4.2 ± 4.2	...	...
	Sulfate	...	...	9.3 ± 2.2	...	4.7 ± 4.7	...	...
	Free OPP	...	...	65.6 ± 3.2	...	91.1 ± 8.9	...	...
Dogs	Glucuronide	...	...	4.9 ± 0.8	...	4.2 ± 0.9	6.0 ± 1.4	...
	Sulfate	...	...	7.9 ± 0.8	...	5.0 ± 1.2	5.2 ± 1.0	...
	Free OPP	...	...	86.9 ± 1.5	...	90.4 ± 2.1	88.3 ± 1.4	...
Kittens	Glucuronide	0.8 ± 0.6	0.6 ± 0.3	1.5 ± 1.1	0.9 ± 0.8	...	0.4 ± 0.3	...
	Sulfate	3.0 ± 0.9	1.6 ± 0.6	4.0 ± 1.4	2.5 ± 0.8	...	3.1 ± 1.0	...
	Free OPP	96.1 ± 1.5	97.6 ± 0.6	94.4 ± 2.4	96.6 ± 1.6	...	96.5 ± 0.9	...
Cats	Glucuronide	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.6	0.4 ± 0.3	0.0 ± 0.0	1.7 ± 0.9	1.1 ± 0.6
	Sulfate	4.0 ± 1.0	2.2 ± 0.8	2.2 ± 1.0	0.8 ± 0.3	1.8 ± 0.6	2.2 ± 0.7	2.0 ± 0.8
	Free OPP	96.0 ± 1.0	97.8 ± 0.6	96.5 ± 1.3	98.8 ± 0.5	98.2 ± 0.6	95.3 ± 2.2	96.9 ± 1.6

APPENDIX I

A Review of Commonly Used Chromatography Techniques for Phenolic Compounds

A REVIEW OF COMMONLY USED CHROMATOGRAPHY TECHNIQUES FOR PHENOLIC COMPOUNDS

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Phenol is used in the manufacture of a large variety of aromatic compounds, including fertilizers, coke, asbestos goods, paints, wood preservatives, pharmaceutical preparations and perfumes. Phenolic solutions are general disinfectants and used as topical anesthetics for pruritic lesions. About one-fourth of the pesticides on the market posess a substituted phenol moiety which can often be easily cleaved from the molecule. These pesticides include herbicides and fungicides, in addition to carbamate and organophosphorous insecticides. Other phenolics, such as o-phenyl phenol, are employed as general disinfectants, and are used in the citrus fruit industry to prevent mold growth and to aid in the proper storage of food. This industry, as well as several others, is responsible for the introduction of a wide variety of phenols into the environment. The major source of pollution by phenolics occurs in the waste effluent of coking plants, brown coal distillery plants, and the pulp and paper industry. Because of the toxicity and ubiquitous nature of these compounds, it is apparent that analytical procedures are needed to detect and monitor the level of various phenolic substances in a variety of environmental and biological samples. Since these samples often contain a mixture of organic compounds, of which phenols might be only a minor component, various chromatographic methods offer specific advantages and

effectiveness for the separation, identification, and quantitation of phenols in the presence of other possibly interfering compounds. This paper will review the techniques commonly used for the chromatography of simple phenols.

GAS CHROMATOGRAPHY

Quantitative gas chromatographic (GC) analysis of phenolic mixtures may be difficult to achieve because of the high polarity and low vapor pressure of most phenolics. Compounds with similar vapor pressure and polarity may have identical retention times on one or even two columns, so it is imperative to employ several types of columns when separating a variety of phenols. The chromatography of acidic phenols must usually take place at temperatures less than 125°, because the commonly employed liquid phases are destroyed by these compounds at high temperatures (1). In the separation of alkylphenols, structural information from the chromatograms may be difficult to analyze because of the simultaneous influences of vapor pressure and polar interactions (2). Despite the difficulties of phenol detection and resolution, GC is a widely used method for separating these compounds. It offers speed, sensitivity, resolution, and the ability to be coupled with mass spectroscopy for peak confirmation.

A flame ionization detector (FID) is usually used in phenolic analysis by GC. The phenols may be analyzed intact, or they may be modified to form their methyl or trimethylsilyl (TMS) derivatives. The derivatization procedure causes a transformation of the non-volatile and thermally unstable phenols into more volatile compounds, which can then be analyzed at temperatures low enough to prevent thermal decomposition. TMS ethers are of value in resolving mixtures of hydroxy compounds that are difficult to separate, and comparison

of retention times of the phenol and its derivatives can yield valuable structural information (3).

Two other detectors used in the analysis of phenols are the electron capture detector (ECD) and the flame photometric detector, both of which detect a derivative of phenol rather than the phenol itself. Reaction of some phenols with bromine or pentafluorobenzoyl chloride produces a derivative that is easily detected by ECD at levels much lower than as detectable by the FID (4). A problem with analysis using this method is the relative lack of specificity (5). Where sensitivity is not as important as selectivity, the phenolic substances may be derivatized to their phenyl-diethyl-phosphate esters and subsequently analyzed using flame photometric detection. Although only one-fifth as sensitive as the ECD, it provides high selectivity and a stable baseline (5).

Examples of some of the types of stationary phases, supports, and column temperatures used in GC are given in Table 1.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography (HPLC) is often used in preferance to GC to separate the less volatile phenols because no derivatization step is necessary. The avoidance of high temperatures prevents thermal degradation, and there are no limits on molecular size capable of being detected. Most of the analyses employ the use of an ultraviolet (UV) detector set at 254 nm. Fluorescence detection may provide high sensitivity to phenol derivatives, such as dansyl chloride derivatives of some hydroxyaromatics (6). Phenols from the HPLC column effluent may be reacted with cerium(IV) sulfate, forming fluorescent cerium(III), which is carried in the combined effluent to the

detector (7). A third type of detector used to detect phenols, including o- and p-amino phenol and o-phenyl phenol, is the amperometric detector. This detector has a lower limit of detectability than the UV detector, as well as good specificity and low cost. The sensitivity of this detector varies, depending on the compound to be analyzed and the condition of the working electrode. The amperometric method is 100 and 1000 times more sensitive to o- and p-amino phenol (respectively) than the UV detector, but is only slightly more sensitive to o-phenyl phenol (8,9). It is useful to make a new standard curve each day when using this detector, because the slope of the curve often decreases due to deterioration of the working electrode (8).

Reversed-phase columns are commonly used to chromatograph phenols (Table 2), although normal phases are also useful in separation and identification. Work by Callmer et. al. (10) and Schabrom et. al. (2) showed the retention times of various alkylphenols, napthols and phenylphenols were related to their structural features. In normal phase chromatography, the numbers of ortho substituents, aromatic rings, and aliphatic carbons affect retention. But in the reversed-phase system, retention time is affected by the numbers of aliphatic carbons and aromatic double bonds (2,11). A general scheme for separating mixtures of phenols (2) includes separation of phenolic compounds with more than one aromatic ring from phenol and alkylphenols by use of a normal phase column with a $Si(CH_2)_3NH_2$ or $Si(CH_2)_3CN$ functional group. Fractions from this column are then introduced to a reversed-phase column, which separates the phenols by aliphatic carbon number (assuming all compounds have the same number of aromatic double bonds).

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) is a popular method of chromatographing phenols because it is fast, inexpensive, and provides good resolution. Multiple elimination TLC, in combination with ultraviolet or fluorometric analysis, will theoretically allow separation and quantitative determination of almost all naturally occuring phenols (12). TLC is faster than paper chromatography and is usually superior in separating ability; however, it is time consuming compared to GC and HPLC, and the phenols may be readily oxidized on the thin layer plates or not as easily moved (12).

Most TLC of phenols is performed on silica gel plates, although thin layers of cation and anion exchangers, polyamide, and aluminum oxide have also been used (13,14). A wide variety of solvent systems are used in TLC of phenols. Solvents employed include benzene and diisopropyl ether, as well as mixtures of benzene-dioxane-acetic acid, benzene-methanol, and chloroform-acetic acid. For a list of R_f values of the major phenols chromatographed by several solvent systems, as well as detection reagents used, references (14) and (15) are recommended.

Several methods for the detection of phenols on TLC plates are known, most which involve the use of spray reagents. These reagents include Gibbs and iron(III) chloride-hexacyanoferrate(III) reagent, and diazotized benzidine, sulphanilamide, sulphanilic acid or p-nitroaniline. The diazotized reagents must be prepared at a low temperature and are stable for only 2-3 hrs. The disadvantages of the iron(III) chloride-hexacyanoferrate(III) reagent are that it cannot detect monohydric phenols and that a black background color rapidly develops on the plates (16). Sodium nitrite may be used as a spray reagent

for detecting various phenols if the silica gel G layer had been impregnated with anilinium chloride when the plate was prepared (16). Phenolic spots appear instantly in various colors and are stable for several days.

RADIOCHROMATOGRAPHY

Radiochromatograms of thin layer chromatograms or paper chromatograms are often used for the determination of phenolic metabolites. The radioactive areas of the chromatograms may be determined and quantitated by exposure to photographic paper (autoradiography), scanning of the chromatogram with an ionization detector (radiochromatogram scanning), or by liquid scintillation counting. Autoradiography gives a good visual impression of where the radiolabelled substances and their metabolites are located on the chromatogram. Long exposure times may be needed for weak beta emitters like tritium, and this may cause contamination or fogging of the film. This problem may be avoided by spraying the chromatogram with a fluorescent compound.

The spots may be quantitated from the film by densitometry, which is not very accurate, or the corresponding area may be cut out from the paper (or scraped from the TLC) and subjected to liquid scintillation counting. In radiochromatogram scanning, the chromatogram is either held stationary and scanned by a moving ionization counter, or it is moved on a bed or rollers under a stationary counter. The advantages of this method over scintillation counting is that it is quick, the chromatogram is retained for further study, and interpretation of partially separated peaks is possible (17). A disadvantage is that there is a very low count rate due to the small area being analyzed at any given time. This leads to poor statistics.

PAPER CHROMATOGRAPHY

Paper chromatography (PC) is used for the separation of all types of phenolic compounds, from the simple phenols to the more complex flavanoids. It is a very popular method for separating the naturally-occuring polyphenols because they have a solubility range that provides good separation and because they can often be observed without the use of spray reagents (18). Common solvent systems include 1-butanol-ethanol-water, ethyl acetate-acetic acid-water, and 1-propanol-ammonia in various proportions. For a list of R_f values of the major phenols chromatographed in several solvent systems, and detection reagents, the reader is referred to reference (14).

Solvent systems and detection reagents for the more complex flavanoids are described by Harborne (18). The reagents used will depend on the class of phenols to be detected and will allow the chromatographer to distinguish between certain groups of phenols on the basis of whether or not a color reaction had taken place. Two popular sprays are ferric chloride-ferricyanide, which responds to all phenols by turning blue, and diazotized p-nitroaniline, which responds to most phenols by turning yellow, brown or red. Some techniques involve the use of pre-treated chromatography paper, eiliminating the need for spray reagents. For example, during chromatography on molybdate-treated paper, ortho hydroxy-phenols react with the paper and are identified by intense orange-brown spots (19).

PAPER ELECTROPHORESIS

Paper electrophoresis has no general advantage over paper chromatography (18). The phenolic substances to be separated must be either ionized or complexed with a metal ion to become mobile in the electrical field. Non-complexing electrolytes are used to separate nonpolar phenols from phenols having charged functional groups. For example, in phosphate buffer (pH 7.2) or acetate buffer (pH 5.2), phenolic acids and amines will move in the electrical field, but phenol will remain immobile. Catechol and its derivatives may be complexed with metal ions to increase their mobility, while related monophenols fail to so complex. This provides a system for separating catechols from many other phenols.

COLUMN CHROMATOGRAPHY

Column chromatography is often used to separate naturally-occuring phenols and as a separation technique in large scale separations, such as in the preliminary purification of the phenolic fractions in botanical sources. Various column packings have been used to separate phenols, including Sephadex, polyamide, cellulose, ion exchange resins, and silica gel. The compounds are usually assayed by their absorption at a particular wavelength, but refractive index detectors may also be used.

Sephadex, a cross-linked dextran gel, has a tendancy to adsorb phenol and other aromatic compounds. This phenomenon allows separation of phenols due to

their differing degrees of bonding to the gel matrix rather than on the basis of molecular size (20). Through the use of these adsorptive properties, the application of several aqueous solvents allows the separation of a wide range of phenols and flavonoids. The elution volume is a reflection of the number and position of the hydroxyl groups, as well as the presence of other groups. Work by Brook (21) has shown a large variation in the elution coefficient K_D with temperature, especially when working with halogen-substituted phenols. As temperature was increased, the phenolics were eluted off the column with lower elution volumes (i.e., smaller K_D value). He suggested that mixtures of phenols might be separated on Sephadex columns by the use of temperature programming.

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Table 1. Column Supports and Oven Temperatures Used in the Gas Chromatography of Simple Phenols

Phenol class	Derivative	Column supports	Column temp(°C)	Ref.
Mixtures of phenols	none	Modified and unmodified Tenax GC	250	22
	none	Col 1: 40% rubidium benzenesulphonate + 2% Carbowax 20M + 2% Ascorbic acid on Chromosorb P (60-80 mesh)	150	23
		Col 2: 12% Apiezon L + 0.5% Carbowax 20M + 1% Ascorbic acid on Chromosorb W (60-80 mesh acid washed)	135	23
	none	0.3% SP-1000 and 0.3% Phosphoric acid on Carbowax A	165-195	1
	trimethylsil	5% Cyanosilicone GE XE-60 on DMCS Chromasorb G (80-100 mesh)	75, 150 or 200	3
	phenyl diethyl phosphate esters;FPD[a]	3% OV-1 on Chromasorb W (100-120 mesh)	190	5
	trimethylsil	1.5% SE-30 + 1.5% SE-52 on Chromasorb W AW DMCS (80-100 mesh)	80-300	12

Table 1. (cont'd)

Phenol class	Derivative	Column supports	Column temp(°C)	Ref.
Alkyl phenols	trimethylsil	5% [1,2,3-tris(cyanoethoxy)propane] on Chromasorb G HP	160	10
	none	5% polyethylene glycol-20M on Chromasorb W (AW-DMCS) (60-80 mesh)	200	11
	none	30% Silicone KF-96 on Chromasorb W (AW) (60-80 mesh)	240	11
Phenyl phenols	none	30% Silicone KF-96 on Chromasorb W (AW) (60-80 mesh)	240	11
	penta fluorobenzoyl ester; ECD[b]	5% DEGS on Gas Chrom Q	190	4
	none	OV-101, OV-1, or OV-17	50-270	24
	none	QF1 on Chromasorb W (AW-DMCS) (80-100 mesh)	180	25
Alkyl catechols	none	1.5% Silicone OV-1 on Chromasorb W (AW-DMCS) (80-100 mesh)	170	11
Phenolic Acids	trimethylsil	3% SE-30 on Chromasorb W (AW-DMCS) (60-80 mesh)	175	18
	trimethylsil	3% OV-1 or 3% UCW-98 on Chromasorb WHP (100-120 mesh)	175	18

[a]FPD Flame photometric detection; [b]ECD Electron capture detection; All others Flame ionization detection

Table 2. Column Packings and Mobile Phases Commonly Used in the High Performance Liquid Chromatography of Simple Phenols

Phenol class	Column packing	Mobile phase	Detection	Ref.
Mixtures of phenols	HC-ODS	Acetonitrile-water (2:8)	Fluorescence	26
	µBondapack C_{18}, µPorasil	Methanol-water(6:4) or (8:2)	UV-254 & 280	27
	µBondapack C_{18}, Corasil	gradient- acetonitrile/water	UV-254	7
	µBondapack C_{18}, Porasil	gradient- methanol/ 0.1 N Potassium dihydrogen phosphate	UV-350	28
Alkyl phenols	TCEP, Porasil	Isooctane	UV	10
	Fractonitrile Vl, Porasil	Isooctane	UV	10
	Cyano Sil-X-1	Isooctane + 0.5% 2-propanol	UV	10
	µBondapack C_{18}, µPorasil	Ethanol-water (6:4)	UV	10
	µBondapack CN, normal phase	n-heptane	UV-254	2
	µBondapack NH_2, normal phase	n-heptane-isopropyl alcohol (99:1)	UV-254	2
	µPorasil, normal phase	n-heptane-chloroform (9:1)	UV-254	2

Table 2. (Cont'd)

Phenol class	Column packing	Mobile phase	Detection	Ref.
	μBondapack C_{18}, rev. phase	methanol-water (65:35)	UV-254	2
	μBondapack CN, rev. phase	methanol-water (50:50)	UV-254	2
Phenyl phenols	μBondapack NH_2	gradient- ethanol/isooctane-acetonitrile-isoamyl alcohol	UV-254	29
	μBondapack C_{18}, Corasil	Ethanol-water (4:6)	UV-254 or amperometric	8
	μBondapack C_{18}, Porasil	Methanol-water (6:4) or (8:2)	UV-254 & 280	27
	small particle (7-18μ) silica gel	Hexane-chloroform (9:1) or (7:3)	UV-254 or fluorometric	6
Catechols	Partisil-1025 ODS	various phosphate solutions	UV-254	30
Phenolic acids	μBondapack C_{18}	water-acetic acid (95:5), water-acetic acid-methanol (90:5:5) and other eluants	UV-254 & 280	31

Note: In the case of gradient elution, the compound preceeding the slash is being increased in concentration

APPENDIX II

Individual Animal Data on the Urinary Metabolism of Orally Administered Ortho-Phenyl Phenol in Dogs and Cats

Animal # P-1, Species Canine, Age group Immature, Sex F

Date of Initial Dose 7/11, Date of Final Dose 8/28

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
8/3	0.647	3.0	0.740	4.4	0.853	92.6
	0.648	29.6	0.770	12.1	0.885	54.8
	0.694	10.2	0.775	6.1	0.879	83.8

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # P-2 , Species Canine , Age group Immature , Sex M

Date of Initial Dose 7/11 , Date of Final Dose 7/28

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP

activity of all samples from this animal were too low to determine accurate percentages of conjugation products.

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # P-3, Species Canine, Age group Immature, Sex M

Date of Initial Dose 7/11, Date of Final Dose 8/28

Sample date	Glucuronide[a] Rf	% OPP± glucuronide[b]	Sulfate[c] Rf	% OPP± sulfate[b]	OPP[d] Rf	% Free OPP
8/3	0.596	28.3	0.711	10.6	0.838	61.1
	0.679	29.6	0.770	12.1	0.885	58.4
	0.645	33.8	0.760	13.0	0.878	53.1
	0.691	37.5	0.765	10.8	0.890	51.6

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # P-4, Species Canine, Age group Immature, Sex F

Date of Initial Dose 7/11, Date of Final Dose 8/28

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
8/3	0.602	27.9	0.773	2.24	0.871	69.8
	0.587	28.7	0.715	1.6	0.835	69.7
	0.627	2.2	0.701	39.8	0.872	58.0
	0.674	34.2	0.786	5.4	0.886	60.4
	0.704	33.4	0.791	6.6	0.881	59.9
	0.721	27.0	0.808	4.1	0.893	63.4
	0.698	36.9	0.789	5.4	0.879	57.7
	0.696	28.0	0.781	7.6	0.872	64.4
8/17	0.647	8.4	0.778	9.4	0.888	82.2

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # P-5, Species Canine, Age group Immature, Sex M

Date of Initial Dose 7/11, Date of Final Dose 8/28

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
8/3	0.613	2.0	0.698	6.3	0.825	91.6
8/17	nd	...	nd	...	0.838	100
8/31	nd	...	nd	...	0.856	100

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # P-6, Species Canine, Age group Immature, Sex F

Date of Initial Dose 7/11, Date of Final Dose 8/28

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP

activity of all samples from this animal were too low to determine accurate percentages of conjugation products.

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # D-1, Species Canine, Age group Mature, Sex M

Date of Initial Dose 5/28, Date of Final Dose 7/15

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
7/14	0.643	4.7	0.714	4.5	0.886	90.1
	0.684	16.4	0.751	2.2	0.855	81.3
	0.684	11.6	0.750	1.0	0.854	87.7
	nd	...	0.666	3.7	0.892	92.6
	nd	...	0.714	12.7	0.922	87.2
	0.573	2.9	0.626	3.5	0.816	93.5
	0.586	2.4	0.722	3.1	0.834	94.6

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # D-2, Species Canine, Age group Mature, Sex F

Date of Initial Dose 5/28, Date of Final Dose 7/15

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
6/22	0.611	3.8	0.766	12.7	0.903	83.5
	0.573	4.8	0.737	8.6	0.851	86.6
	0.626	4.9	0.728	9.1	0.863	83.8
	0.651	8.3	0.772	10.8	0.856	80.8
7/6	0.588	7.0	0.754	10.4	0.845	82.6
	0.594	3.6	0.704	7.8	0.844	86.0
	0.709	3.3	0.772	2.9	0.878	93.8
	0.613	7.1	0.745	6.5	0.840	86.3
	0.642	2.7	0.758	2.5	0.858	94.8
	0.655	5.6	0.768	5.1	0.861	89.3
7/14	0.636	5.3	0.734	8.8	0.838	85.7
	0.687	12.6	0.766	11.5	0.852	75.8

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # D-3, Species Canine, Age group Mature, Sex F

Date of Initial Dose 6/3, Date of Final Dose 7/21

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
	activity of all samples from this animal were too low to determine accurate percentages of conjugation products.					

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # D-4 , Species Canine , Age group Mature , Sex F

Date of Initial Dose 5/28 , Date of Final Dose 7/15

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
6/22	0.531	8.9	0.696	7.9	0.803	83.2
	0.519	4.4	0.680	5.4	0.756	90.2
	0.503	7.3	0.691	9.3	0.819	83.3
	0.716	3.2	0.823	4.7	0.907	92.0
	0.568	0.7	0.737	3.8	0.852	95.5
	0.574	2.9	0.731	7.1	0.849	90.0
7/6	nd	...	nd	...	0.837	100
7/14	0.626	3.5	0.689	6.8	0.886	89.7
	0.592	6.8	0.730	2.3	0.813	89.7
	0.605	9.7	0.729	3.1	0.835	87.3
	0.554	2.1	0.632	6.6	0.835	91.2

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # D-5 , Species Canine , Age group Mature , Sex F

Date of Initial Dose 5/26 , Date of Final Dose 7/13

Sample date	Glucuronide[a] Rf	% OPP± glucuronide	Sulfate[c] Rf	% OPP± sulfate	OPP[d] Rf	% Free OPP

activity of all samples from this animal were too low to determine accurate percentages of conjugation products.

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # D-6, Species Canine, Age group Mature, Sex F

Date of Initial Dose 5/26, Date of Final Dose 7/13

Sample date	Glucuronide[a] Rf	% OPP± glucuronide[b]	Sulfate[c] Rf	% OPP± sulfate[b]	OPP[d] Rf	% Free OPP
6/8	0.630	4.1	0.740	7.0	0.836	88.9
6/24	0.651	4.4	0.777	2.6	0.859	93.1

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # K-1, Species Feline, Age group Immature, Sex F

Date of Initial Dose 7/13, Date of Final Dose 8/30

Sample date	Glucuronide[a] Rf	% OPP± glucuronide	Sulfate[c] Rf	% OPP± sulfate	OPP[d] Rf	% Free OPP
7/21	nd	...	0.696	1.7	0.895	98.3
7/30	nd	...	0.658	4.6	0.865	95.4
8/7	nd	...	0.601	0.9	0.897	99.1
8/15	nd	...	0.683	1.9	0.879	98.1
8/22	nd	...	nd	...	0.900	100
8/29	nd	...	0.684	2.1	0.898	97.9

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # K-2, Species Feline, Age group Immature, Sex M

Date of Initial Dose 7/13, Date of Final Dose 8/30

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
7/21	0.581	1.7	0.685	4.3	0.892	94.0
7/30	nd	...	0.695	0.9	0.892	99.1
8/7	nd	...	0.708	2.4	0.895	97.6
8/15	nd	...	0.635	1.3	0.877	98.7
8/29	nd	...	0.611	6.6	0.882	93.4
	0.402	1.6	0.641	1.7	0.872	96.7

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # K-3 , Species Feline , Age group Immature , Sex F

Date of Initial Dose 5/24 , Date of Final Dose 7/11

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
		activity of all samples from this animal were too low to determine accurate percentages of conjugation products.				

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # K-4 , Species Feline , Age group Immature , Sex M

Date of Initial Dose 5/22 , Date of Final Dose 7/9

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
6/7	0.408	2.1	0.644	1.7	0.881	96.2
	0.449	1.3	0.616	2.7	0.824	96.0
6/8	nd	...	0.643	1.7	0.861	97.7
6/15	nd	...	0.702	4.3	0.896	95.7
6/22	nd	...	0.657	2.8	0.905	97.2
7/6	nd	...	0.681	2.0	0.907	98.0
7/13	nd	...	0.595	17.8	0.883	82.1
	0.620	3.8	0.674	4.6	0.861	91.6

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # K-5, Species Feline, Age group Immature, Sex F

Date of Initial Dose 5/24, Date of Final Dose 7/11

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
6/8	0.262	1.0	nd	...	0.898	99.0

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # K-6, Species Feline Age group Immature, Sex M

Date of Initial Dose 5/22, Date of Final Dose 7/9

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
6/8	nd	...	nd	...	0.861	100
6/15	0.432	8.3	0.709	12.7	0.903	79.1
	0.357	2.6	0.748	1.6	0.882	95.8
	nd	...	0.640	4.0	0.880	96.0
	nd	...	0.586	2.1	0.823	97.9
6/22	nd	...	0.681	2.5	0.878	97.5
	nd	...	nd	...	0.892	100
	0.541	5.5	0.686	6.3	0.887	88.2

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # A-1, Species Feline, Age group Mature, Sex M

Date of Initial Dose 4/26, Date of Final Dose 6/13

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
5/9	nd	...	nd	...	0.710	100
5/16	0.455	1.8	0.596	5.3	0.879	92.9
5/30	nd	...	0.596	3.6	0.849	96.4
6/15	nd	...	0.627	1.3	0.869	98.7
6/24	nd	...	0.621	6.6	0.836	93.4

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # A-2, Species Feline, Age group Mature, Sex M

Date of Initial Dose 4/26, Date of Final Dose 6/13

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
5/9	nd	...	0.610	2.8	0.884	97.2
5/16	nd	...	nd	...	0.923	100
5/23	nd	...	nd	...	0.847	100
5/30	nd	...	0.607	2.0	0.846	98.0
6/7	nd	...	nd	...	0.954	100
6/14	nd	...	nd	...	0.682	100

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # A-3, Species Feline, Age group Mature, Sex M

Date of Initial Dose 6/5, Date of Final Dose 7/23

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
6/7	0.147	2.8	0.678	8.1	0.877	89.0
6/14	nd	...	0.703	5.0	0.953	95.0
	nd	...	0.651	1.8	0.860	97.0
6/23	nd	...	0.671	2.2	0.890	97.8
6/30	nd	...	0.644	1.4	0.875	98.6
7/30	nd	...	nd	...	0.909	100

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # A-4, Species Feline, Age group Mature, Sex F

Date of Initial Dose 6/3, Date of Final Dose 7/21

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
6/7	nd	...	0.605	4.9	0.886	95.1
6/15	nd	...	nd	...	0.873	100
7/7	nd	...	nd	...	0.896	100
7/14	nd	...	nd	...	0.891	100
7/22	0.345	2.6	0.651	4.8	0.893	92.6
7/30	0.518	3.8	0.687	7.1	0.881	89.0

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # A-5, Species Feline, Age group Mature, Sex F

Date of Initial Dose 6/3, Date of Final Dose 7/21

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
6/7	nd	...	0.710	1.3	0.976	98.7
6/14	nd	...	0.654	1.4	0.884	98.6
6/23	0.337	3.1	0.710	1.5	0.875	95.4
7/14	0.545	1.0	0.649	3.0	0.875	96.0
7/24	0.446	1.9	0.752	1.8	0.875	96.3

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Animal # A-6, Species Feline, Age group Mature, Sex F

Date of Initial Dose 6/17, Date of Final Dose 8/4

Sample date	Glucuronide[a] Rf	% OPP[b] glucuronide	Sulfate[c] Rf	% OPP[b] sulfate	OPP[d] Rf	% Free OPP
6/23	nd	...	0.641	1.6	0.883	98.4
6/30	nd	...	0.601	1.5	0.883	98.5
7/7	0.560	3.4 L	0.672	3.6 L	0.877	93.0
7/14	0.420	1.1	0.664	1.0	0.881	97.9
7/22	nd	...	0.715	1.5	0.929	98.5
7/30	0.311	5.8	0.657	5.4	0.858	83.9
	0.405	3.2	0.656	2.5	0.876	94.2
	nd	...	0.636	1.0	0.833	99.0
	nd	...	0.626	1.5	0.823	98.5

L -- indicates low activity

[a] Confirmation of glucuronide by use of β-glucuronidase

[b] Peak area determined by a disc integrator and percentage of total peak area computed.

[c] Confirmation of sulfate by use of β-glucuronidase with sulfatase activity.

[d] Confirmation by chromatography of pure OPP and development with color reagents.

nd indicates no peak observed

Weighted Means of Individual Animal Data

Group	Glucuronide Rf	% OPP-glucuonide	Sulfate Rf	% OPP-sulfate	OPP Rf	% free OPP
Immature Dogs	0.657	21.1	0.759	8.3	0.868	72.8
Mature Dogs	0.615	5.2	0.729	6.1	0.851	88.4
Immature Cats	0.450	0.96	0.662	3.3	0.881	95.7
Mature Cats	0.408	0.76	0.653	2.4	0.873	96.6

URINARY METABOLISM OF ORALLY ADMINISTERED ORTHO-PHENYL PHENOL IN DOGS AND CATS

by

MICHAEL CHRIS SAVIDES

B.A., Rutgers University, 1977

AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Ortho-phenyl phenol (OPP) has a broad bactericidal spectrum and is used as a germicide, household disinfectant, and fungicide. It is found in dish-washing formulations and is used to control mold on citrus fruits, in fungi-static dips and waxes for coating vegetables, and as a paper-liner coating to reduce rotting of stored food. Although this compound has a low oral toxicity, species and age differences may be seen in both the toxicity and kinetics of OPP. In particular, this compound is much more toxic to cats than to other animals, and it is thought that toxicity is due to a defect in the cats ability to biotransform OPP to lesser-toxic metabolites. Since little work had been done on the comparative metabolism of chronically administered OPP, the present study investigated the urinary metabolites of OPP when this compound was given repeatedly to both young and adult dogs and cats.

The animals received oral doses of 3.7 mg OPP every other day for a total of 25 doses. At both age levels, dogs excreted significantly more OPP as sulfate and glucuronide than did cats. Puppies produced four times the level of glucuronides that mature dogs did. No such age differences were seen with glucuronide formation by cats. There were no age differences in either group of animals for sulfate formation, but mature dogs excreted larger amounts of free phenol than did the puppies. Some sex differences were observed in conjugation of OPP in cats and dogs. The dominant urinary excretion product of oral OPP administration was the unchanged OPP.