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Influence of Lipophilicity on the Accumulation and Distribution of Halogenated Phenols and a Pyridinol as Metabolites of Pesticides in the Rat

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INFLUENCE OF LIPOPHILICITY ON THE ACCUMULATION AND DISTRIBUTION OF HALOGENATED PHENOLS AND A PYRIDINOL AS METABOLITES OF PESTICIDES IN THE RAT

by

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Assed Attumi

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ABSTRACT

Influence of Lipophilicity on the Accumulation and Distribution of Halogenated Phenols and a Pyridinol as Metabolites of Pesticides in the Rat

by

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Exposure to halogenated phenols and pyridinols is of increasing concern because of their wide use and distribution. This research was initiated to determine the distribution, accumulation, and depletion of a group of halogenated phenols and a pyridinol in selected tissues of male weanling rats at different time intervals following a single oral dose of 0.33 or 1.66 m moles per kg body weight. The halogenated phenols and pyridinol were distributed differently in every tissue sampled following their administration, even though the amount administered was the same in each case. The concentrations in tissue were found in the order: 2,4,5-trichlorophenol > 4-bromo-2,5-dichlorophenol > 4-iodo-2,5-dichlorophenol > 3,5,6-trichloro-2-pyridonol in kidney and fat, whereas the series 3,5,6-trichloro-2-pyridinol > 4-iodo-2,5-dichlorophenol > 4-bromo-2,5-dichlorophenol > 3,5,6-trichlorophenol occurred in liver. No

structurally significant series was observed for their concentrations in blood.

All halogenated phenols and pyridinol concentrations in tissues declined rapidly with time but not always in an apparently log linear fashion. Rates were greatest for clearance from blood. The highest concentration of halogenated phenols was in kidney among the tissues studied, whereas the highest concentration of halogenated pyridinol was in liver.

Relationships were found between the relative lipophilicity, as indicated by the chromatographic R_m value, and the concentrations of these compounds in tissues. The R_m (i.e., relative lipophilicity) was generally very well correlated with the log concentration of compounds in tissues observed 24 h after dosing. The correlation coefficients ranged between .517 and .995 among tissues. Correlations were positive between the R_m values and 24 h concentrations in adipose tissue, and kidney, but negative for the relationship between the R_m and 24 h concentrations in blood and liver.

(91 pages)

INTRODUCTION

The lower halogenated phenols have been in use as fungicides, herbicides, insecticides and precursors in the synthesis of other pesticides since the early 1930's. Production of these chlorinated phenols has at times been the cause of problems with regard to industrial hygiene but has otherwise not created notable environmental problems. Many organophosphate and organochlorine insecticides are well known man-made environmental pollutants that have been used for several decades in agriculture and industry. It is well known that organochlorine pesticides are soluble in fat and some are highly persistent in the tissue of animals; halogenated phenols may act similarly. On the other hand, these halogenophenols are major metabolites of several organophosphorus and a few organochlorine insecticides which are, themselves, readily biodegradable. Most literature reports indicated that the halogenated phenolic metabolites of these insecticides were found in urine, but only recently have these metabolites also been found in the animal tissues. As of this date, there have been few reports of studies with the lower halogenated phenols in animals and data on accumulation and depletion rates of halogenated phenols as residues in animal tissues are lacking. A similar lack exists for information about those halogenated pyridinols used in pesticide manufacture.

There is a general relationship between lipophilicity and many types of biological activity for related structures among classes

of foreign compounds and it has been found that lipophilicity is one of the most important factors in controlling the interaction of chemicals with biological systems. However, no work was found in the literature regarding the relationship between the structure of the halogenated phenols and pyridinols, their lipophilicity and their concentration in animal tissues. The purpose of this research was to identify the importance of relative lipophilicity as a characteristic influencing the biological distribution of these compounds. This study was planned to determine if rats do indeed accumulate halogenophenols and halogenopyridinols and, if so, in which major tissues. Knowledge of the tissue distribution of halogenated phenols and pyridinols accumulated by rats will be of value in elucidating sublethal physiological responses of these animals to low level exposures to agents yielding these compounds upon metabolism.

Two objectives in this study purused structural relationships among selected halogenated phenols and a pyridinol in comparison to: a) their distribution in blood, kidney, liver, and fat (adipose tissue); and b) their persistence in and depletion from these tissues with time.

REVIEW OF LITERATURE

Historical Background and Use of Halogenated Phenols

The first halogenated phenol synthesized was pentachlorophenol (Erdmann 1841; Laurent 1841). However, neither of those chemists determined the correct structure of the compound. Several different methods for synthesis were later tried until the preparation of pentachlorophenol by direct chlorination of phenol in the presence of a catalyst was reported by Merz and Weith (1872). Modern production of pentachlorophenol or lower chlorophenols may be performed in two ways (Melnikov 1971; Zollner et al. 1978): by direct chlorination of phenol, chlorophenols, or polychlorophenols with chlorine in the presence of catalysts at an elevated temperature or by alkaline hydrolysis of chlorobenzenes in mixtures of different solvents. Both methods may give rise to the formation of contaminants such as chlorinated dibenzodioxins, dibenzofurans and 2-phenoxyphenols (predioxins) (Ahlborg 1977).

In 1936, production of halogenated phenols was started on a commercial scale, particularly of pentachlorophenol which was used as a preservative for wood and wood products and an agent to control mold, mildew and termites on wood (Carswell and Nason 1938). 4-Bromo-2,5-dichlorophenol has also found use as a wood preservative (Imamura et al. 1978; Tilemans 1977). Pentachlorophenol has been used as a herbicide in pineapple and sugarcane fields (Bevenue and Beckman 1967). For many years, 2,4,5-trichlorophenol has been

used effectively as a fungicide in many major industries, including textile, leather, and glue manufacturing processes. More recently, pentachlorophenol, 4-bromo-2,5-dichlorophenol, and 4-iodo-2,5-dichlorophenol have found use as molluscicides (Roushdy et al. 1974; Yamamoto and Kasuga 1976, 1977; Imamura et al. 1977). More than that, halogenated phenols and halogenated pyridinols have found use as intermediates in the production of several important pesticides developed during the last two decades, such as Ronnel, leptophos, iodophos, and chlorpyrifos (McCollister et al. 1959). The annual use of pentachlorophenol in the U.S.A. is probably more than 20,000 tons and was estimated to be more than 24,000 tons in 1974 (Arsenault 1976).

Toxicity of Halogenated Phenols

Modern data on the toxicity of lower halogenated phenols seems to be absent although many studies have been conducted on the pentachlorophenol and tetrachlorophenols. Early studies on their toxicity were performed due to an interest in them as antiseptic compounds (Bechhold and Ehrlich 1906). The literature up to 1949 has been excellently reviewed by Von Oettingen (1949). The conclusions that can be drawn from the earlier work reviewed by Von Oettingen (1949) were confirmed in a study by Farquharson et al. (1958). The authors concluded that with increasing chlorination there is an increase in toxicity in which the convulsant action of phenol is replaced by the signs characteristic of poisoning by dinitrophenol, a well-known uncoupler of oxidative phosphorylation.

Based on determinations of the dissociation constants for the chlorinated phenols, they suggested that the higher chlorinated phenols interfere with oxidative phosphorylation. The convulsant action of the lower chlorinated phenols, on the other hand, was assumed to be associated with the undissociated molecule.

Although 2,4,5-trichlorophenol has been used in industry for many years, little toxicity data have been reported in the literature. Deichman and Mergard (1948) reported an acute oral LD50 in rats in the range of 0.62-0.825 g per kilogram body weight. Anderson et al. (1949) fed groups of cattle dosages of 0.8, 2.4, and 7.2 g per 100 lbs animal weight per day of zinc 2,4,5-trichlorophenate and 2,4,5-trichlorophenyl acetate in the diet for 78 and 154 days. They observed no adverse effects at any of the levels fed. For a 98-day feeding trial using rats and a dose range of .03-1.0 g/kg/day of 2,4,5-trichlorophenol. McCollister et al. (1961) found that rats maintained at the higher dose level showed diuria, but only mild reversible pathological changes were seen in the liver and kidneys. Those fed lower dose levels showed no evidence of adverse effects. Repeated oral feeding (20 doses in 28 days) by intubation to rabbits produced very slight pathological changes in liver and kidneys in those animals receiving 0.5 g/kg doses of 2,4,5-trichlorophenol. In a similar study, there was no evidence of adverse effect in rats at 0.3 and 1.0 g/kg. The only significant effect attributable to 2,4,5-trichlorophenol was a slight increase in average weight of the kidneys. No pathological changes were found upon microscopic examination. 2,4,5-trichlorophenol at a

dose rate as high as 400 mg/kg administered orally for 14 days in rats decreased microsomal NADPH-cytochrome C reductase activity and cytochrome P450 content (Carlson 1978). UDP-glucuronyltransferase was slightly inhibited in vitro and was not altered in vivo. The compound was not hepatotoxic as assessed by measurement of hepatic glucose-6-phosphatase and serum sorbitol dehydrogenase.

Toxicity data for 4-bromo-2,5-dichlorophenol, 4-iodo-2,5-dichlorophenol, and 3,5,6-trichloro-2-pyridinol are completely absent in reported literature.

Organophosphate Insecticides with Halogenated Phenolic Metabolites

Halogenated phenols and related compounds such as halopyridinols are mammalian metabolites of many insecticides, including Leptophos (phosvel) [O-(4-bromo 2,5-dichlorophenyl)-O-methyl phenyl phosphonothioate], chlorpyrifos (Dursban) [O,O-diethyl O-(3,5,6 trichloro-2-pyridyl) phosphorothioate], and Ronnel [O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate]. They are organophosphate insecticides that control a wide variety of insect pests including important forage insects (Collier and Dieter 1965; Gray 1965). Table 1 lists representative organophosphate and organochlorine insecticides that release halogenated phenols upon metabolism or degradation.

It was found that Dursban residues remained relatively stable when treated corn plants were chopped and ensiled for 30 days (Leuck et al. 1969). Other studies showed that Dursban and 3,5,6-trichloro-2-pyridinol residues were found in the soil and

Table 1. Representative Organophosphate and Organochlorine Insecticides with Phenolic Metabolites

Common Name	Chemical Name of Systematic Name	Phenolic Metabolite or Degradation Product
Dursban (chlorpyrifos)	0,0-diethyl-0-(3,5,6-trichloro-2-pyridyl) phosphorothioate	3,5,6-trichloro-2-pyridinol
Chloropyrifos-methyl (Dowcor 214)	0,0-dimethyl-0-(3,5,6-trichloro-2-pyridyl) phosphorothioate	3,5,6-trichloro-2-pyridinol
Leptophos (Phosvel)	0-(4-bromo-2,5 dichlorophenyl)-0-methyl-phenyl phosphorothioate	4-bromo-2,5-dichlorophenol
Ronnel	0,0-dimethyl-0-2,4,5-trichloro-phenyl phosphorothioate	2,4,5-trichlorophenol
Iodophos (Iodofenphos)	0-(2,5-dichloro-4-iodophenyl)-0,0-dimethyl phosphorothioate	4-iodo-2,5-dichlorophenol
Iodofenphos ethyl	0-(2,5-dichloro-4-iodophenyl)-0,0 diethyl phosphorothioate	4-iodo-2,5-dichlorophenol
Trichlor metafos-3	0-methyl-0-ethyl-0-(2,4,5-trichloro-phenyl) thiophosphate	2,4,5-trichlorophenol
	0,0-diethyl-0-2,5,6-trichloro-phenyl) thiophosphate	
Bromophos (Nexion)	0-(4-bromo-2,5-dichlorophenyl) 0,0-dimethyl phosphorothioate	4-bromo-2,5-dichlorophenol

Table 1. (Continued)

Common Name	Chemical Name or Systematic Name	Phenolic Metabolite or Degradation Product
Bromophos ethyl	O-(4-bromo-2,5-dichlorophenyl) O,O-diethyl phosphorothioate	4-bromo-2,5-dichlorophenol
Gardona (stirofos)	2-chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate	2,4,5-trichlorophenol
2,4,5-T	2,4,5 trichlorophenoxyacetic acid	2,4,5-trichlorophenol
Erbon	2-(2,4,5-trichlorophenoxy)ethyl 2,2-dichloropropionate	2,4,5-trichlorophenol
Silvex (2,4,5-TP)	2-(2,4,5-trichlorophenoxy) propionic acid	2,4,5-trichlorophenol
Lindane (γ-HCH)	γ-1,2,3,4,5,6-hexachlorocyclohexane	2,4,5-trichlorophenol
BHC	1,2,3,4,5,6-hexachlorocyclohexane	2,4,5-trichlorophenol
Haloprogin	2,4,5 trichlorophenyl-γ-iodopropargyl ether	2,4,5-trichlorophenol
Trichlorobenzene	1,2,4-trichlorobenzene	2,4,5-trichlorophenol

in plants (radishes and carrots) after one year from treated soil in field plots treated with Dursban at 3.4 kg AI/ha (Chapman and Harris 1980; Smith et al. 1967). Leuck et al. (1969) examined and reported the persistence of chlorpyrifos and its phenol in forage corn to be more persistent than those of most organophosphorus insecticides.

Cows fed a complete ratio containing Dursban residue for 2 weeks showed residues of Dursban and 3,5,6-trichloro-2-pyridinol in milk and cream samples (McKellar et al. 1976). Residues of chlorpyrifos and its metabolites 3,5,6-trichloro-2-pyridinol, were determined in fat, muscle, liver, and kidney tissues of swine at 1 week posttreatment with chlorpyrifos, but only in fat and liver tissue at 2 weeks posttreatment (Ivey and Palmer 1979). McKellar et al. (1972) found residues of chlorpyrifos predominantly in fat tissue and 3,5,6-trichloro-2-pyridinol in liver and kidney tissues of swine when the animals were fed chlorpyrifos in their diet for 30 days. The levels of the residues were small, ca. 0.05 ppm, even at the highest dietary concentration of 10 ppm. The residues declined rapidly to undetectable or very low levels within 7 days after withdrawal of the insecticide from the feed. Similar results were obtained when chickens were fed chlorpyrifos (Dishburger et al. 1972) and when turkeys were confined in pens on treated soil (Mann et al. 1973). Also, residues of 3,5,6-trichloro-2-pyridinol were found to be greater in liver and kidney than those found in fat when the cattle were fed chlorpyrifos in their diet for 30 days (Dishburger et al. 1977). Recently, it was

shown that the residues of 3,5,6-trichloro-2-pyridinol were found in liver, kidney and fat of cattle wearing chlorpyrifos-impregnated plastic ear bands (Ivey et al. 1978; Ivey 1979). Smith et al. (1967) reported that rats metabolized Dursban to 3,5,6-trichloro-2-pyridyl phosphate (75 to 80%) and 3,5,6-trichloro-2-pyridinol (15 to 20%). Bakke et al. (1976) reported that rats metabolized single oral doses of Dursban to at least six urinary metabolites. The urine contained about 90% of the dose. Three of these metabolites were identified as the glucuronide of 3,5,6-trichloro-2-pyridinol (80%), a glucoside of 3,5,6-trichloro-2-pyridinol (4%), and 3,5,6-trichloro-2-pyridinol (12%). Also, Bakke and Price (1976) showed that sheep and rats metabolized a single oral dose of chlorpyrifos methyl (the dimethyl analogue of Dursban) to three major metabolites that were excreted in the urine (70% of original dose). These were the glucuronide of 3,5,6-trichloro-2-pyridinol (68.6%), O-methyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate (17.8%), and 3,5,6-trichloro-2-pyridinol (13.8%). The latter two metabolites and the present compound were isolated from sheep feces. Sheep plasma contained the same metabolites that were found in sheep urine. However, in the same study it was found the sheep excreted a single oral dose of 3,5,6-trichloro-2-pyridinol unchanged in the feces and as a glucuronide in the urine. Only the bile, liver, kidney and gastrointestinal tract with its contents contained detectable residues of 3,5,6-trichloro-2-pyridinol.

Although leptophos administered orally to mice is rapidly metabolized and excreted as degradation products (Holmstead

et al. 1973), the major product was conjugated 4-bromo-2,5-dichlorophenol. Leptophos is relatively persistent in the envirionment in relation to other organophosphate insecticides (Leuck et al. 1969; Braun et al. 1975; Leuck et al. 1970). Leptophos fed to dairy cattle as residues on corn silage was observed to increase body gain and decrease milk production while not affecting feed intake. The cows ingested Leptophos residues averaging 0.41 to 1.71 mg/kg body weight and all secreted milk containing both Leptophos and its phenol. Also, Leptophos and its phenol were excreted in feces, but only phenol was excreted in the urine (Johnson et al. 1971). The chemical properties of Leptophos suggest that this pesticide may be deposited in the fat of animals (Davies et al. 1975; Freed et al. 1976). Recently, it was shown that Leptophos is rapidly absorbed by adipose tissue of hens after a single oral dose (Nabuhiro and Hideo 1978). Leptophos reduced the number and weight of eggs laid (Abou-Donia and Preissing 1976). Urinary excretion of 4-bromo-2, 5-dichlorophenol metabolite of Leptophos was found to be 12% of the total administered dose in rats (Bradway et al. 1977). Also 4-bromo-2,5-dichlorophenol was found as a metabolite of Leptophos in the white mouse urine, cotton plants and houseflies (Holmstead et al. 1973 and Lee and Fukuto 1976).

Ronnel, in addition to its use as an insecticide, has been shown to enhance feed efficiency and improve weight gain in beef when directly employed as a feed additive (Ramsey et al. 1975). Bradway et al. (1977) reported that rats excreted single oral doses of

Ronnel as 2,4,5-trichlorophenol (40.8 to 46.9% of the administered dose) in urine. It was found that Iodophenphos ethyl was inactive while its metabolite 2,5-dichloro-4-iodophenol was more active than 3,5,6-trichloro-2-pyridinol (Abo-Khatwa and Hollingworth 1974), to stimulate oxygen uptake both in the presence and absence of phosphate in vitro rat with liver mitochondria.

In studies with radioactive iodophenphos (14C) in rats, it was shown that elimination of iodophenphos equivalents in rat urine and feces accounted for 92% of the dose in 24 h after oral treatment. Greater than 98% of the total of the radioactivity in the urine was in the form of acidic metabolites (Frederick and Charles 1970). Recently, Ivey and Oehler (1976) studied the metabolism of iodofenphos which is used systemically to control a variety of ticks on livestock. They detected 4-iodo-2,5-dichlorophenol in tissues and urine of cattle fed iodofenphos.

4-Bromo-2,5-dichlorophenol was found to be the main metabolite of bromophos in tomato plants, reaching 13% of the total dose applied after 7 days (Stiasni et al. 1969). That metabolic pattern in plants is identical with that found in rats (Stiasni et al. 1967).

Organochlorine compounds (organochlorine insecticides and polychlorinated biphenyls) have become major environmental pollutants. They have been used for several decades in agriculture and industry. Their low degradation in nature, their accumulation in living beings, their biomagnification (increase of residue in the food chain) and their biological effects in natural exposure and animal experimentation may create environmental problems, in part

by increasing halogenated phenols in the environment. Chlorophenols are major metabolites of many organochlorine compounds. Some of these compounds were listed in Table 1.

Examples of biota contamination with chlorophenols following specific pesticide usage are numerous in the literature. For example, Fitzgerald et al. (1967) showed the degradation of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in wood plants led to 2,4,5-trichlorophenol. Also, 2,4,5-trichlorophenol was found in sunflower seedlings and strawberry leaves after treating with 2,4,5-T (Chkanikov et al. 1970). Recently, it has been found that 2,4,5-trichlorophenol is produced from 2,4,5-trichlorophenoxyacetic acid in soil and water (Rosenberg and Alexander 1980). Clark et al. (1975) reported tissue residues of 2,4,5-trichlorophenol in sheep and cattle given oral doses of the herbicides 2,4,5-T and silvex. Liver and kidney contained the highest levels and withdrawal of the animals from treatment for 1 week before killing resulted in significant reduction in tissue residue level. 2,4,5-trichlorophenol was found to be the principal metabolite of the 4-(2,4,5-trichlorophenoxy)-butyric acid herbicide following oral administration to rats (Boehme and Grunow 1974).

Erbon, 2-(2,4,5-trichlorophenoxy)-ethyl-2,2-dichloropropionate is a herbicide used extensively in noncrop areas to control weeds and grass. Erbon is more toxic than other members of the 2,4,5-T herbicide family. 2,4,5-trichlorophenol was found in blood, urine and feces shortly after administration of erbon given orally to sheep and the highest residue level of 2,4,5-trichlorophenol was found in the kidney, liver and fat (Wright et al. 1970).

In 1965 Grover and Sims showed that rats metabolized both lindane, γ-1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH) and (γ-PCCH) γ-2,3,4,5,6-pentachlorocyclohex-1-ene to 2,3,5- and 2,4,5-trichlorophenol which are excreted in the urine as free phenols, sulfates, and glucuronic acid conjugates (Chadwick and Freal 1972a and b; Freal and Chadwick 1973). Recently it was shown that lindane and hexachlorocyclohexene administered to rats produced 2,4,5-trichlorophenol as one of the many other chlorophenol metabolites both in vivo and in vitro (Chadwick et al. 1975; Tanaka et al. 1979a). Tanaka et al. (1979b) showed the metabolic fate of polychlorocyclohexenes [1,3,4,5,6-pentachlorocyclohexenes (PCCHE) and 1,2,3,4,5,6-hexachlorocyclohexene (HCCHE)] using microsomes from rat liver and housefly abdomen. They found that PCCHE and HCCHE were metabolized to 2,4,5-trichlorophenol as one of the many other chlorophenol metabolites.

Weikel and Bartek (1972) reported that, in the rat and rabbit, most of the administered dose of haloprogin, 2,4,5-trichlorophenyl-8-iodopropargyl ether, an antifungal agent, was excreted in urine as the major metabolic products 2,4,5-trichlorophenol and its sulfate conjugate. Also, 2,4,5 and 2,3,5-trichlorophenol were identified as major rabbit urinary metabolites of 1,2,4-trichlorobenzene (Kohli et al. 1976).

It is well known that organochlorine pesticides are soluble in fat and persistent for a long time in the tissue of animals. On the other hand, halogenophenols are major metabolites of many

organophosphorus and some organochlorine pesticides, which in general, are readily biodegradable as shown before. As of this date, there has been very little work reported in the literature about distribution of the halogenated phenols and pyridinols in the tissues. Most of the studies were limited to the determination of urinary excretion of some halogenated phenol metabolites of biodegradable pesticides.

Relation Between Chemical Structure and Biological Activity

The question of whether there would be some relationship between chemical structure and biological activity arose as early as the last century, and once it appeared possible to extend the range of drugs gradually by synthetic routes, many efforts were made to find an answer (Albert 1971).

There are numerous examples to substantiate the claim that studies on the relationship between the structure of a substance and its activity in biological systems have great practical value and have made significant contributions to the understanding of biological phenomena in general. The term structure relates to the physical characteristics, such as solubility, surface area, and molecular size, and the chemical factors of intramolecular electron distribution, pK, state of charge and stereochemistry. Any one, all, or any combination of these parameters might be influential in structure-activity considerations. Exploitations of

structure-activity relationship has at times led to the synthesis of valuable therapeutic agents.

The Importance of Partition Coefficient in Structure-Activity Relationship

The partition coefficient is one of the most important factors in controlling the interaction of chemicals with biological systems (Daniels et al. 1966).

Fundamental work was carried out by Richet (1893), who found that the toxic effects of ethers, alcohols, aldehydes and ketones are inversely proportional to their solubility in water; by Traube (1904), who established a linear relationship between surface tension and activity for a series of narcotics; and by Fuehner (1907), who noted the possibility of there being a quantitative relationship between a biological property, for example the narcotic activity, and the number of carbon atoms.

All of this research may be viewed in conjunction with the postulation of Crum-Brown and Fraser (1869) that the physiological action, ϕ, of a molecule is a function of its chemical constitution (C):

$$\phi = f(C) \qquad (1)$$

Meyer and Overton (1901) were studying independently the mode of action of the indifferent narcotics or physical toxicants which are quite diverse in structure and have depressant properties, especially in cells that are particularly rich in lipids, such as nerve cells.

From their studies it was discovered that most organic compounds foreign to the organism penetrate tissue cells as though the membranes were lipid in nature. They also found that the passage across these barrier systems and the subsequent narcotic action parallel the oil-water partition coefficients of the structures investigated (Rekker 1977).

While it has become apparent that biological activity can rarely be coupled to a single parameter but should rather be envisaged as the result of the interplay of various parameters, the partition coefficient did remain the major parameter in structure activity relationship studies. The period between the research of Meyer and Overton and the present situation is about half a century. For a review of the most important events during this period, reference is made to Purcell et al. (1973). Rekker (1977) outlined the following improved parameters that are meaningful for the understanding of biological action and the better approaches used in structure-activity studies:

(1) Hammett substituent constants

Hammett (1937) was among the first to offer a quantitative treatment of the effects of structure on chemical activity. His equation (2) makes it possible to calculate a rate or an equilibrium constant of a meta- or para-substituted derivative of C_6H_5-R with R as the reacting center:

$$\text{Log} \frac{K_s}{K_o} = \rho\sigma \qquad (2)$$

where, K_o represents the equilibrium constant of an unsubstituted structure and K_s that of a substituted derivative. The symbol σ is called the substituent constant, reflecting its ability to attract or repel electrons. Electron withdrawing groups have positive substituents, with respect to hydrogen. The symbol ρ is the rection constant in equation (1), and measures the sensitivity of the reaction to electronic effects on the m, p-substituents. When the log of the dissociation constants of various substituted benzoic acids is plotted as a function of the substituent constant of the same compounds, meta and para substituents fall on a straight line. Ortho-substituted compounds deviate because of steric interference, but when these steric interferences are removed by operating in the gas phase, the ortho substituents fall on the line. The Hammett treatment has been applied to many reactions and functional groups, and correlates well with a substantial amount of data (Jaffe 1953). Since the electronic effects of substitutions as conducted by the Hammett method proved to largely determine the partitioning behavior of a series of analogues, the biological processes highly dependent on distribution of the chemical, can often be correlated with the Hammett constant.

(2) Hansch approach to structure-activity relationship

After the Hammett σ-constant was found useful in evaluating the chemical constitution of a molecule upon which biological activity is highly dependent, Hansch et al. (1963, 1964 and 1965a and b) found an alternatively very useful structure-activity correlate, by use of π. This was defined as

$$\pi = \log \left(\frac{P_X}{P_H}\right) \qquad (3)$$

where P_X and P_H are the octanol-water partition coefficients of a parent compound H and its derivative X, differing by a substituent group. Thus the constant π indicates the change in the logarithm of the partition coefficient resulting from the introduction of the substituent group.

The π values for many substituent groups were directly measured by Hansch et al. (1964, 1965b) for a variety of drugs (over 200 aromatic compounds) and used in order to calculate the lipophilic character, as expressed by the partition coefficient function log P or by $\Sigma\pi$ of other compounds.

Hansch and Fujita (1964) offered statistical models to explain the relationship between structure and biological activity, and to reduce it to a series of linear regression equations. They take a logical approach to the penetration of a compound to the active site and the subsequent response evoked. It was assumed, as a first approximation, for many types of biologically active molecules there is one key rate-controlling reaction at the active site. This is formulated as in equation (4).

$$\text{Rate of biological responses} = \frac{d(\text{response})}{dt} = ACK_X \qquad (4)$$

A is the probability of a molecule reaching a site of action in a given time interval, C is the extracellular molar concentration of the compound being tested and K_X is a constant.

Thus, it was held that the biological response is a function of how much is applied, how successful the compound is in reaching the target, and how it reacts once it arrives. These factors are a direct result of the molecular structure. On the same studies, Hansch and Fujita (1964) developed five types of regression equations to fit various types of biologically active structural series. The simplest case was designated as Type I, which is given by the following equation:

$$\text{Log } \frac{1}{C} = a\,\pi + b \qquad (5)$$

where C is the concentration required for a definite effect-usually EC_{50}. The symbols a and b represent the slope and intercept, respectively. The activity of compounds in this type varies with lipophilicity. Examples of type II activity are given in equation (6), which is represented by the action of phenols on Salmonella typhosa and the carcinogenic activity of the dimethylaminoazobenzenes.

$$\text{Log } \frac{1}{C} = -a\,\pi^2 + b\,\pi + C \qquad (6)$$

The other types, represent more complex situations, including factors for electronic and steric effects.

(3) Boyce and Milborrow approach to structure and biological activity

Hansch et al. (1965) pointed out that the calculated partition coefficient log P or $\Sigma\pi$ cannot completely replace the experimental determination of partition coefficient because of

possible group interactions, and Bird and Marshall (1967) found some anomalies in the calculated $\Sigma\pi$ values of penicillins. On the other hand, in order to avoid the practical difficulties of the direct determination of a partition coefficient, particularly when the compound is highly insoluble in either of the solvent phases, Boyce and Milborrow (1965) suggested the use of the chromatographic R_m value as a simplified assessment of partition coefficient and its derivatives by exploiting the theoretical relationship between partition coefficient P and R_f value deduced by Martin (1949) for liquid-liquid partition chromatography:

$$P = K(1/R_f - 1) \tag{7}$$

where K is a constant for the system.

Bate-Smith and Westall (1950) introduced the term R_m, shown to be related to the partition coefficient and calculated from the formula:

$$R_m = \text{Log}\left(\frac{1}{R_f} - 1\right) \tag{8}$$

Thus, equation (9) can be used in place of equation (7)

$$\text{Log } P = \text{Log } K + R_m \tag{9}$$

The change in the value of R_m for a substituent (ΔR_m) is a free energy-based constant analogous to π used by Hansch. It is therefore possible in principle to correlate the penetration of substances with their R_m values. As a corallary of this, R_m and π usually have a linear correlation. One of the purposes of the present

work is to show that reversed phase thin layer chromatography is a suitable technique and simple and rapid method for the determination of lipophilicities for halogenated phenols and pyridinols. The lipophilic nature of a series of compounds has been described by Boyce and Milborrow using this approach (1965), and it has frequently been used since, for example, by Biagi et al. (1969, 1970). The chromatographic R_m value determination is more convenient than measuring partition coefficients (Dearden et al. 1974).

Studies of the Relationship Between Molecular Structure of Halogenophenols and Chromatographic Behavior

To date, there are very few papers in the literature dealing with the relationship between structure of halogenophenols and pyridinols and chromatographic behavior. Bark and Graham (1966a) chromatographed 60 halogenated and halogenoalkyl substituted phenols on alumina surfaces in eight eluent systems. They found the R_f values of the phenols decreased with an increase in the number of halogen atoms in the molecule thus indicating that the alumina surface acts as a proton donor towards the halogen atom as well as towards the phenolic group. It was seen that the R_f values of analogous halogenophenols increased with an increase in the size and corresponding decrease in the electronegativity of the halogen atom. In the same year, Bark and Graham (1966c) studied the chromatographic behavior of halogenated phenols, chromatographed in the reversed-phase thin layer chromatographic system ethyl oleate-aqueous ethanol. They suggested the mechanism of the

chromatographic process is dissolution of the phenol in the hydrophobic stationary phase, followed by the removal of the phenol from the interface as a result of solvation of the phenolic group by the polar mobile phase. They also found that the R_f values of halogenated phenols decreased with an increase in the number of halogen atoms in the molecule. This suggested that the effect of partial molar volumes of the halogen atoms is more important in governing the separation process than are electronic effects. It was shown that the number and nature of the substituents and their relations will have an effect in determining the chromatographic behavior of a compound (Bark and Graham 1966b). For the series of monohalogenated phenols, fluoro-, chloro-, bromo-, iodo-, Marcinkiewicz and Green (1963) found separations to occur according to the molar volumes of the halogen atoms. Bark and Graham also found the order of R_f values to be: fluoro < chloro < bromo < iodo, i.e., in order of the molar volumes of the halogen atoms. Bark and Graham (1967) reported that the migration of halogenated phenols was related to the site of solvation of the molecule by the mobile phase, the solvation site being the hydrophobic part of the molecule for the non-aqueous eluent and the phenolic group for the acqueous eluent. They suggested that the above mechanisms may be influenced by a number of factors such as electron withdrawal or donation by the substituent groups, steric effects and position of substituents in the molecule. All these were shown to affect the R_f values.

It is well known that atoms within groups in molecules tend to attract or release electrons to other regions of the molecule, depending on the electronegativity of the atom. This alters the intramolecular distribution of electrons, making some region of the molecule electron-deficient and another electron-rich. Examples of substituent groups with electron withdrawing capacity are nitro, quaternized nitrogen, cyano, carboxyl, and chlorine, whereas some groups that release electrons are hydroxyl, methoxyl, methyl, amine, and phenyl.

The distribution of a phenol between an aqueous and a lipoid solvent can be approximately calculated by use of the π constant for each substituent, as derived by Hansch (Fujita et al. 1964).

MATERIALS AND METHODS

Experimental Animals

Male weanling rats of the Sprague-Dawley strain, weighing 145 to 170 g, were purchased from Simonsen Laboratories, Inc. of Gilroy, California. They were individually housed in stainless steel cages with wire mesh bottoms. The rats were fed Purina Laboratory Chow in cubed form ad libitum with fresh water prior to and throughout the study. The cages were cleaned daily and the rats were observed for six days before dosing with the test chemicals.

Chemicals Tested

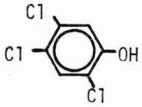
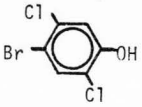
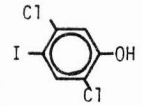
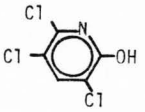
The compounds used in the studies are presented in Table 2, together with the data on source, purity and structural formula.

Experimental Design

Experimental animals were randomly assigned to treatments, and adjustments were made to equalize the body weight within ±2 g in each treatment, so that differences in group weights might not be a factor.

Seventy-two rats were allocated into five groups comprising 16, 16, 16, 16, and 8 rats, respectively. Rats in groups 2, 3, and 4 were divided into two equal subgroups to make eight rats for each treatment. The subgroups were used with administration of the higher dose level of 2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol and 3,5,6-trichloro-2-pyridinol. Each rat, except for those in

Table 2. Compounds Used in Investigation, Including Chemical Structure, Purity and Sources

Compound	Structure	Purity
2,4,5-trichlorophenol[a]		94%
4-bromo-2,5-dichlorophenol[b]		99.1%
4-iodo-2,5-dichlorophenol[c]		94%
3,5,6-trichloro-2-pyridinol[d]		99%

[a]Provided by Aldrich Chemical Company, Inc., Milwaukee, Wisconsin.

[b]Provided by Velsicol Chemical Company, Chicago, Illinois.

[c]Provided by Agricultural Division of CIBA-GEIGY Corporation, Greensboro, North Carolina.

[d]Provided by Dow Chemical Company, Midland, Michigan.

group 1 (controls) was treated with a single dose of a test chemical. Two rats from each treatment were killed at 1, 3, 6, and 12 day(s) after administration of the compounds, as were two control rats.

Dose Administration

The dose levels of all compounds were based on the LD50 of 2,4,5-trichlorophenol because the LD50 values of the other compounds were not available. Dose levels of 0.4 and 0.08 of the LD50 (1.66 m moles/kg and 0.33 m moles/kg respectively) were used for each compound under study (except 4-iodo-2,5-dichlorophenol which was tested only at the lower dosage). The lower dose 0.33 m moles/kg is equivalent to 65.6, 80.4, 95.9 and 65.9 mg/kg for 2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol, 4-iodo-2,5-dichlorophenol and 3,5,6-trichloro-2-pyridinol, respectively. The higher dose 1.66 m moles/kg is equivalent to 328, 401.9, and 329.5 mg/kg for 2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol and 3,5,6-trichloro-2-pyridinol, respectively. The compounds were administered by gavage as solution in corn oil.

All compounds dissolved in corn oil, except 3,4,6-trichloro-2-pyridinol which was dissolved in 1:9 v/v of ethanol and corn oil. The concentrations of the compounds in solution were adjusted so that each animal received 0.5 ml of the corn oil solution per 100 g of body weight. The control rats received an equal quantity of pure corn oil.

Animal Termination

Rats were anesthetized by placing them into a jar in which the

air was saturated with diethyl ether. At this time as much blood was removed as possible via the dorsal aorta. Animals were then sacrificed immediately and the liver, kidneys and the peri-renal plus sub-cutaneous pockets of adipose tissue were dissected free and cleaned of extraneous tissues. Except for blood, the sample tissues were weighed, placed in labeled vials and quickly frozen for storage until they were analyzed (within three months). Blood was analyzed within 24 h after being drawn.

Apparatus

A Micro-Tek 220 gas chromatograph (GC) equipped with ^{63}Ni electron capture detector (ECD) and a 6 ft x 1/4 in. glass column packed with 4% SE-30 + 6% QF-1 on 80-100 mesh chromosorb W (HP) was used. The column was operated under the following parameters: nitrogen carrier gas flow rate, 60 ml/min; column temperature, 170°C; inlet, 210°C; detector, 275°C.

Solvents and Reagents

All solvents were pesticide quality or nano-grade (Fisher and Baker). Deionized water was used throughout the procedures and was extracted with benzene. N-ethyl-N'-nitro-N-nitrosoquanidine (Aldrich) was used to prepare the ethylating reagent according to Stanley (1966). The silica gel (Woelm, active grade 1) was prepared according to Shafik et al. (1973).

Residue Analysis

Procedure for blood. Blood was analyzed for residues using modification of the methods in the Manual of Analytical Methods

(Thompson 1979). Extracting was by adding to 2 ml blood contained in a round-bottomed tube 1 ml of 4% trichloracetic acid, 2 ml of 2% Na_2SO_4, and 6 ml of hexane. The mixture was rotated in a Roto-rack at 50 rpm for 2 h, then centrifuged at 2000 rpm about 5 min to separate the layers. Five ml of the hexane extract was transferred to a 15 ml graduated centrifuge tube and the organic residue was extracted on a Roto-rack with 6 ml methylene chloride as before. Five ml of the CH_2Cl_2 was combined with the hexane extract. Ten drops of keeper solution (1% paraffin oil in iso-octane) were added to the combined extract solvent which was then concentrated to 0.5 ml by using a nitrogen evaporator (Meyer N-evaporator). One ml of benzene was added followed by freshly prepared diazoethane solution dropwise until a definite yellow color persisted. After allowing the mixture to stand 15 min, any excess reagent was removed by bubbling dry nitrogen through the solution. The derivatized sample was then "cleaned up" by use of the deactivated silica gel procedure of Shafik et al. (1973). The first fraction was collected, concentrated to an appropriate volume, and analyzed by EC-GC.

Procedure for liver and kidney. Four grams of liver or one kidney (0.7-1.0 g) was extracted by blending in a Lourdes homogenizer with 100 ml acetone and filtered through Whatman 40 filter paper into a 500-ml Erlenmeyer flask. The homogenizer and filter were washed with acetone. A Snyder column was attached to the flask and the extract was reduced by distillation to 25 ml and cooled to room temperature. An aliquot of the extract was taken for lipid analysis while the remaining extract was transferred to a 125 ml

separatory funnel and the sample extracted with benzene as described by Ivey et al. (1978). The benzene extracts containing 2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol or 4-iodo-2,5-dichlorophenol were transferred to the alumina column for cleanup as described by Bowman and Beroza (1969). In the case of 3,5,6-trichloro-2-pyridinol, the extract was cleaned up with the same method of Ivey et al. (1978). The eluate containing the halogenated phenols or the pyridinol was concentrated in a flash-evaporator under water pump vacuum to 50°C to near dryness and transferred to a 15 ml graduated centrifuge tube. Freshly prepared diazoethane reagent was added drop-wise until a persisting yellow color occurred, and the reaction mixture was then allowed to stand for 15 min. Excess reagent was removed by bubbling dry nitrogen through the solution. From this point on the silica gel column cleanup method of Shafik et al. (1973) was followed. The first fraction was collected, concentrated or diluted to an appropriate volume and analyzed by EC-GC.

Procedure for adipose tissue. An 8-10 g sample of fat was extracted by blending in a Waring blender with 75 ml acetone. The homogenate was transferred to a 600-ml beaker, heated on a hot plate to near boiling, and filtered through Whatman 40 filter paper into a 500-ml Erlenmeyer flask. The blender, beaker, and filter were washed thoroughly with acetone. The filtered mass was returned to the blender, blended with hexane, filtered into a 300-ml Erlenmeyer flask, and washed with additional hexane. A Snyder column was attached to the acetone flask and the extract was

reduced to ca 30 ml by distillation and cooled to room temperature. The column was removed, and the corresponding hexane extract was transferred to the flask containing acetone. The Snyder column was replaced, and the extract was again reduced to ca 75 ml (until the solvent vapors in the Snyder column reached 63-64°C, a temperature that indicates the absence of acetone). The extract was transferred to a 500 ml separatory funnel and partitioned 3 times with 50 ml portions of acetonitrile. The acetonitrile extracts were combined, reduced to ca 10 ml by distillation through a Snyder column, and cooled to room temperature; 30 ml of hexane was added through the column, and the column was removed. The solvent was evaporated to dryness and the residue was taken up in 5 ml of benzene. Derivation and cleanup were then carried out and the analytical procedure completed in an identical manner to the liver and kidney samples. Because residues were to be calculated on an extracted fat basis, the hexane solutions remaining after the acetonitrile extraction were transferred to a tared flask, the solvent was evaporated, and the fatty residue was weighed and recorded as sample weight.

Analytical Recovery Studies

Recovery studies were performed by adding known amounts of standards to each type of tissue sample (Table 2). After mixing, the fortified tissue samples were carried through the analytical procedure in an identical manner to unfortified samples. If the control samples contained any halogenated phenol or halogenated pyridinol, as found in the liver tissue for 2,4,5-trichlorphenol

and 3,5,6-trichloro-2-pyridinol, the amount was subtracted from the appropriate value obtained during recovery studies.

Procedure for Lipid

The procedure used for total lipid analysis was that described by Bragdon (1951), based on oxidation of the extracted lipid with dichromate and spectrophotometric measurement of the resulting chromate concentration. Calibration was made with stearic acid as a reference.

Reversed-Phase Thin Layer Chromatography

To estimate the relative solubility characteristics of the compounds tested for accumulation and distribution in the tissues, a reversed-phase thin layer chromatography procedure adapted from Biagi et al. (1969) was utilized. Chrom-Ar 500 (Mallinckrodt) cut in strips of 190 by 180 mm was impregnated with a 5% silicone oil (Dow Corning 200, 350 centistokes) in ether (w/v) solution for 2 min, and then air dried. A solvent feed line was drawn 15 mm from the bottom, and the origin at 30 mm. Solutions of halogenated phenols and pyridinols were spotted on the origin 10 mm apart (and beginning 25 mm from the edge to avoid edge effects). The mobile phase consisted of 37.5% of v/v aqueous ethanol, saturated with silicon oil. The spotted chromatograms were suspended in equilibrated chromatography chambers, allowed to equilibrate for 20 min, then the chromatogram was lowered into the mobile phase to the solvent feed line. When the solvent front had reached a line 100 mm from the origin, the chromatogram was removed and dried with the aid

of a forced-air heater. The halogenated phenols and pyridinols were detected as yellow spots on a purple background by spraying the eluted chromatograms with alkaline potassium permanganate (Bark and Graham 1966a). The yellow spots were marked at their centers and extremes. The R_f values were determined from the center of the spots. Each compound was chromatographed four times on four separate chromatograms and from these values the mean and standard error of the mean were computed. The R_m for each compound was calculated from the following relationship:

$$R_m = \text{Log}\left(\frac{1}{R_f} - 1\right) \qquad (8)$$

Equation (8) was introduced by Bate-Smith and Westall (1950).

RESULTS

Animal Studies

No adverse effects were observed in any of the rats treated with either the low dose level (.33 m moles/kg) or higher dose level (1.66 m moles/kg) of any compound used in these studies. Gross appearance and behavior, mortality, food consumption, weight gain, average body and organ:weight ratios were not affected by the chemicals. All animals were healthy and active in pre- and post-administration periods, no deaths were observed and no significant difference in the body weight was observed among those given the compounds and the controls. A decrease in body weight was noted one day after the administration among the animals exposed to the higher dose level of pyridinol; however, this was all regained by the third day after administration. The kidney and liver weight relative to the body weight was not altered by administered compounds, except a slight decrease in the liver was found in animals treated with a high dose of 2,4,5-trichlorophenol. These data are presented in Appendices A and B.

Gas Chromatographic Analysis of Halogenated Phenols and a Pyridinol in Tissues

The percent recovery, limit of detection in part per billion (ppb), and detector sensitivity in picogram (pg) for each chemical are shown in Table 3. These are based upon duplicate analyses of the fortified tissues using the methods described. The blood and

Table 3. Electron-Capture Detector Sensitivities, Limits of Detection and Percent Recovery of Halogenated Phenols and Pyridinol

Compound	Tissue	Detector Sensitivity pg	Limit of Detectability ppb	Recovery*
2,4,5-TCP	Blood	10.5	0.5	100.0
4-Br-2,5-DCP	Blood	9.0	0.1	88.6
4-I-2,5-DCP	Blood	11.7	0.1	83.9
3,5,6-TC-2-Pyridinol	Blood	11.3	0.1	89.6
2,4,5-TCP	Fat	12.0	9.0	80.7
4-Br-2,5-DCP	Fat	10	2.0	64.0
4-I-2,5-DCP	Fat	13	1.8	64.6
3,5,6-TC-2-Pyridinol	Fat	12.5	2.1	54.1
2,4,5-TCP	Kidney	15.0	12.6	104.4
4-Br-2,5-DCP	Kidney	11.5	9.5	69.0
4-I-2,5-DCP	Kidney	12.5	10.0	86.8
3,5,6-TC-2-Pyridinol	Kidney	18.0	9.5	69.0
2,4,5-TCP	Liver	12.0	9.7	66.5
4-Br-2,5-DCP	Liver	10.0	8.9	70.8
4-I-2,5-DCP	Liver	11.5	6.0	81.6
3,5,6-TC-2-Pyridinol	Liver	14.3	11.0	103.8

*Mean of duplicate analysis from adding 300 ng or 250 ng of each compound to the tissue.

fat control samples contained no halogenated phenols or pyridinols, but control kidneys and livers did contain a component which had a retention time identical to that of 2,4,5-trichlorphenol and 3,5,6-trichloro-2-pyridinol. Before the recovery data were calculated, the recorder response of this component was subtracted from the 2,4,5-trichlorophenol or 3,5,6-trichloro-2-pyridinol response obtained from the fortified samples. No other interfering component or halogenated phenol and pyridinol was detected in chromatograms of the control sample at a concentration exceeding its corresponding level of detectability. Figure 1 illustrates chromatograms of halogenated phenols and pyridinol of fortified and control kidney tissue samples. A small interference peak was present in kidney and liver treated samples analyzed for 2,4,5-trichlorphenol and 3,5,6-trichloro-2-pyridinol, just beyond the 2,4,5-trichlorphenol and 3,5,6-trichlor-2-pyridinol peak on the trailing edge. This interference was not identified and it did not affect the determination of compounds present because the determination was based on peak height.

Chromatographic R_m Value Determinations as an Index of Lipophilicity

In this study, R_m values were experimentally determined for 14 halogenated phenols and pyridinols as shown in Table 4. The common name, sources, melting points, purities, structural formulas and R_m values are presented in Table 4. Four of these compounds were then selected to be used through the animal study because they have been in use for several decades in agriculture and

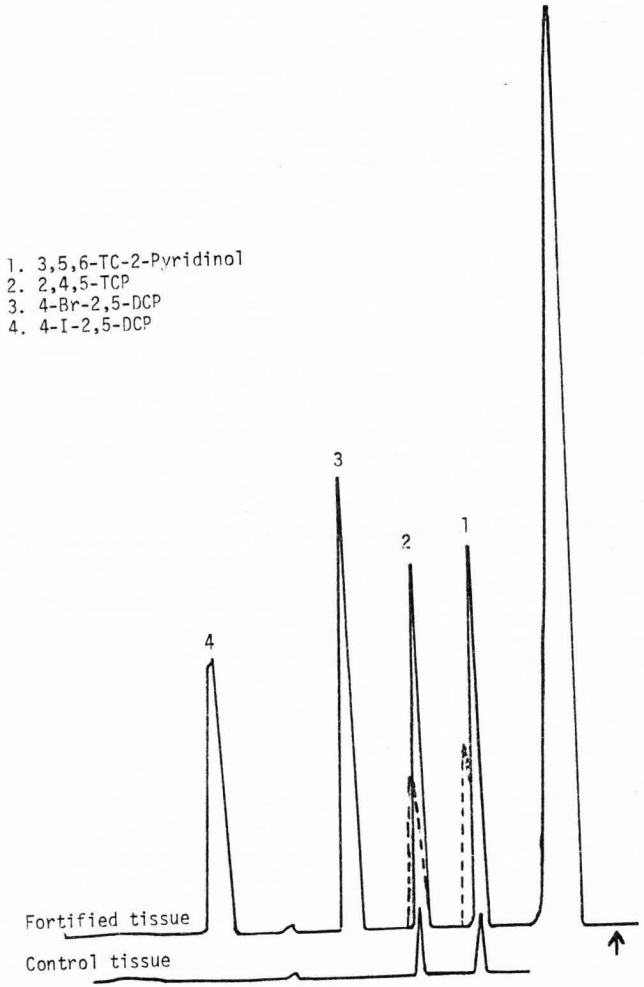


Figure 1. Gas chromatograms of halogenated phenols and pyridinol of fortified and control kidney tissues of rats.

Table 4. Common Name, Melting, Sources, Purities, Structure Formulas and Chromatographic R_m Values of Compounds Tested for Lipophilicity

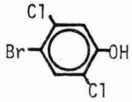
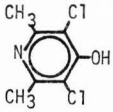
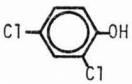
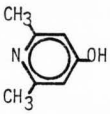
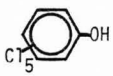
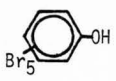
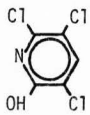
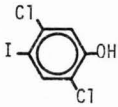
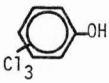
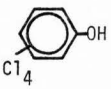
Common Name	Structural Formula	Melting Point	$R_m \pm S_{\bar{x}}$[g]
4-Bromo-2,5-dichloro-phenol[d] 99.1% used as received		71°C	-0.0045±.0003
3,5-Dichloro-2,6-dimethyl-4-pyridinol[c] 99%, used as received		320°C	1.234±.071
2,4-Dichlorophenol[c] 99.8% used as received		45°C	0.00
2,6-Dimethyl-4-pyridinol[c] analytical grade, used as received		--	0.00
Pentachlorophenol,[b] used as received		188-189°C	-.640±.032
Pentabromophenol[e] used as received		229°C	-.632±.057

Table 4. (Continued)

Common Name	Structural Formula	Melting Point	$R_m \pm S_x$[g]
3,5,6-Trichloro-2-pyridinol.[c] 99% used as received			-.908±.040
4-Iodo-2,5-dichlorophenol[a] technical grade, used as received		--	-.231±.045
Trichlorophenols[b]			
2,3,4		77-78°C	+.042±.043
2,3,5		58-59°C	-.084±.021
2,3,6		53-54°C	-.627±.064
2,4,5 - (95%)		64°C	+.035±.010
2,4,6		67°C	-.475±.032
3,4,5[c]		98°C	+.2926±.41
all recrystallized			
2,3,4,6-Tetrachlorophenol[b] technical grade used as received		--	-.720±.003

[a]Provided by Agricultural Division of CIBA-GEIGY Corporation, Greensboro, North Carolina.

[b]Purchased from Aldrich Chemical Company, Inc., Milwaukee, Wis.

[c]Provided by Dow Chemical Company, Midland, Michigan.

[d]Provided by Velsicol Chemical Company, Chicago, Illinois.

[e]Purchased from K. K. Lab, Inc., Planeview, New York.

[f]Melting values of compounds not reported were not determined.

[g]Mean of four chromatograms.

industry, and as of this date, there has been very little work reported in the literature relating to these compounds. The R_m was used in this study as the estimator of the relative lipophilicity of halogenated phenols and pyridinols.

Concentrations Based on the Tissue Weight

Halogenated phenols and the halogenated pyridinol were differentially distributed in every tissue sampled following the administrations. These data are presented in Appendix D. Figure 2 shows the corrected concentration of 2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol, 4-iodo-2,5-dichlorophenol and 3,5,6-trichloro-2-pyridinol, respectively in selected tissues of rats 24 h after single oral administration of 0.33 m moles/kg or 1.66 m moles/kg. All tissues showed different accumulation of halogenated phenols and pyridinol. The average 24 h concentrations of halogenated phenols and pyridinol in blood were 2 to 2.6 ppb in animals administered 0.33 m moles/kg body weight, whereas the average 24 h concentrations in animals dosed with 1.66 m moles/kg were 16.9 to 17.4 ppb. It seems there was no significant difference among the 24 h concentrations of each compound observed in blood, whereas significant differences among 24 h concentrations in fat, kidney, and liver were readily observed.

The 2,4,5-TCP mean concentration in fat 24 h after administration of 0.33 m moles/kg was found to be 247.1 ppb. This value was 1.7, 1.7, and 15.9 times greater than that found for 4-Br-2,5-DCP, 4-I-2,5-DCP, and 3,5,6-TC-2-Pyridinol, respectively, in the same

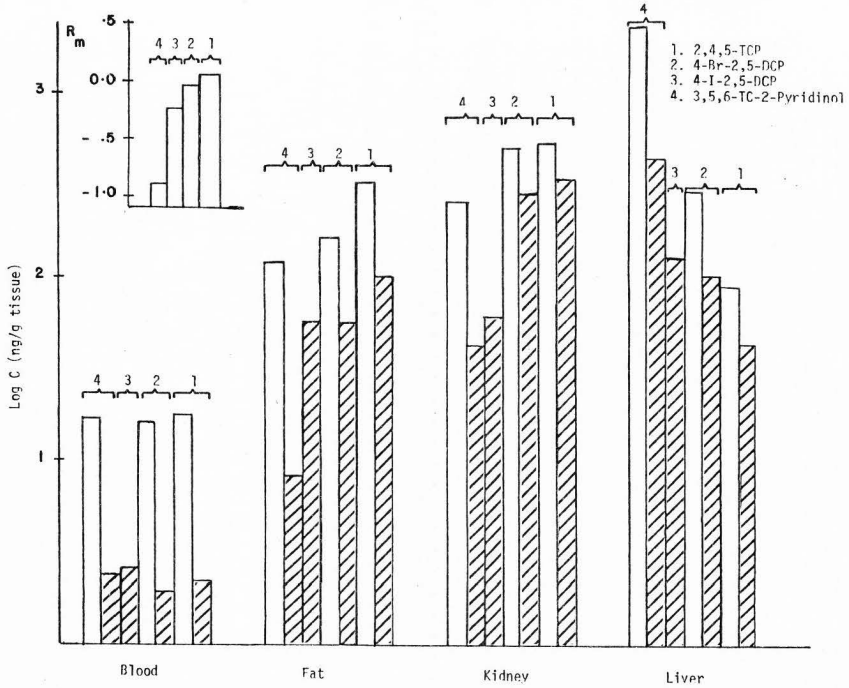


Figure 2. Comparison of lipophilicity of halogenated phenols and pyridinol with concentration in tissue. Tissue data taken after 24 h of a single orally administered dose of 0.33 m moles/kg (▨) or 1.66 m moles/kg (□), corrected for analytical recovery.

tissue. The 2,4,5-TCP mean 24 h concentration in animal fat dosed with 1.66 m moles/kg was about 3 and 2.7 times greater than that found for 4-Br-2,5-DCP and 3,5,6-TC-2-Pyridinol, respectively.

The average 24 h value of 2,4,5-TCP concentration in kidney-based on the tissue weight (Figure 2) was elevated 1.1, 6.1, and 7.5 times over the values for 4-Br-2,5-DCP, 4-I-2,5-DCP and 3,5,6-TC-2-Pyridinol, respectively in animals administered 0.33 m moles/kg. The kidney concentration of 2,4,5-TCP in animals administered 1.66 m moles/kg was 2.7 and 3.0 more than that of 4-Br-2,5-DCP and 3,5,6-TC-2-Pyridinol values, respectively.

The average value of liver 3,5,6-TC-2-Pyridinol concentration 24 h after administration was found to be more than 3.6, 4.5 and 9.8 greater than values that were found for the liver 4-Br-2,5-DCP, 4-I-2,5-DCP and 2,4,5-TCP, respectively, in animals given 0.33 m moles/kg. The liver 3,5,6-TC-2-Pyridinol in animals administered 1.66 m moles/kg was 7.7 and 26 times over the values found for liver 4-Br-2,5-DCP and 2,4,5-TCP, respectively.

Concentrations Based on the Lipid Weight

Figure 3 illustrates the concentration of halogenated phenols and pyridinol in the selected tissues calculated on a tissue lipid basis 24 h after single oral administrations of 0.33 m moles/kg and 1.66 m moles/kg. The average 24 h concentration of all compounds based on tissue lipid content, follow the same order and the same pattern as the 24 h concentration based on tissue weight. The only difference found was that the 24 h concentrations of all compounds were higher on a tissue lipid basis than that found on

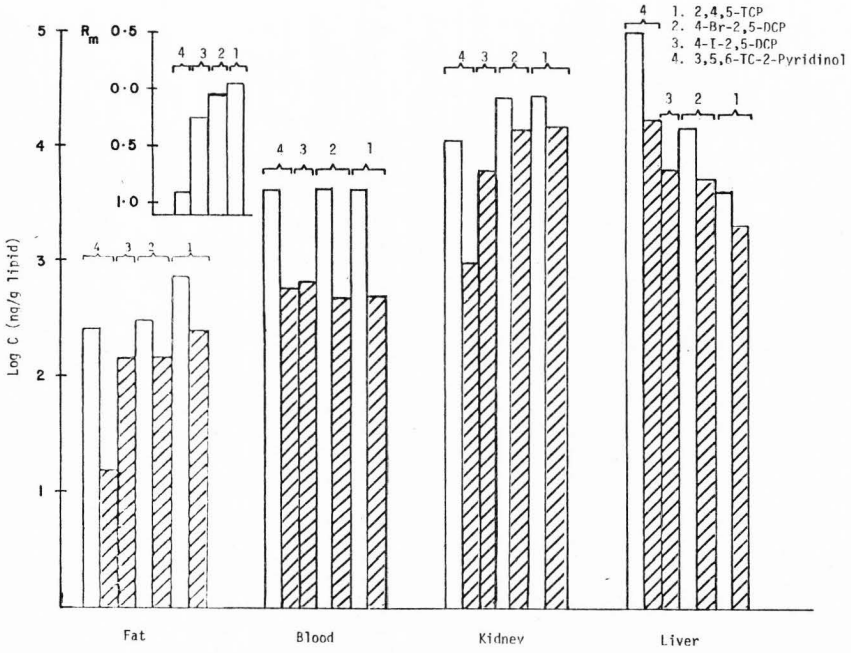


Figure 3. Comparison of lipophilicity of halogenated phenols and pyridinol with concentration in tissue calculated on tissue lipid basis. Data taken after 24 h of a single orally administered dose of 0.33 m moles/kg (▨) or 1.66 m moles/kg (□).

tissue weight basis, especially for 3,5,6-trichloro-2-pyridinol in liver. The concentrations of halogenated phenols and pyridinol on a tissue weight basis and on tissue lipid basis after administration 0.33 m moles/kg and 1.66 m moles/kg are presented in Appendix D.

Depletion of Halogenated Phenols and Pyridinol in Tissue

The halogenated phenols and halogenated pyridinol used in this study were distributed in every tissue sampled following their administrations. Figures 4, 5, 6 and 7 show the concentration of 2,4,5-TCP, 4-Br-2,5-DCP, 3,5,6-TC-2-Pyridinol and 4-I-2,5-DCP, respectively, in selected tissues of rats after a single oral administration of 0.33 m moles/kg or 1.66 m moles/kg at different time intervals. With the exception of liver, in the case of 3,5,6-TC-2-Pyridinol, and kidney in the case of 2,4,5-TCP, all residues in tissues declined rapidly with time but not always in an apparently log linear fashion. The rates of decline were different for the different tissues and for the different compounds. Generally, concentrations of all compounds at 24 h after treatment were depleted by 96% or reached undetectable limits on the sixth or the twelfth day for animals given either dose. The only exception found was the 2,4,5-TCP concentrations in blood and kidney which slowly depleted by 10% and 21% of the 24 h peak concentration, respectively, on the third day, and proceeded rapidly on the sixth day after treatment with 0.33 m moles/kg (Figure 4). On the other hand, the kidney 2,4,5-TCP 24 h concentration depleted by 44.5% on the third day, after which the rate of depletion was very slow; apparent persistence between the third and the sixth day

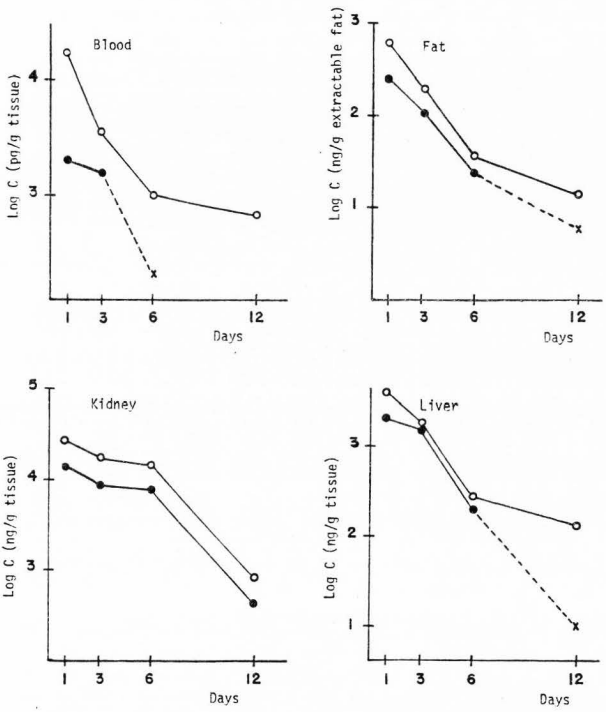


Figure 4. Depletion of 2,4,5-trichlorophenol in blood, fat, kidney, and liver tissues of rats following a single orally administered dose of 0.33 m moles/kg (●) or 1.66 m moles/kg (O) of 2,4,5-trichlorophenol. Each point represents the mean of data from two rats. The broken lines indicate the terminal depletion to the minimum limit of detection (x).

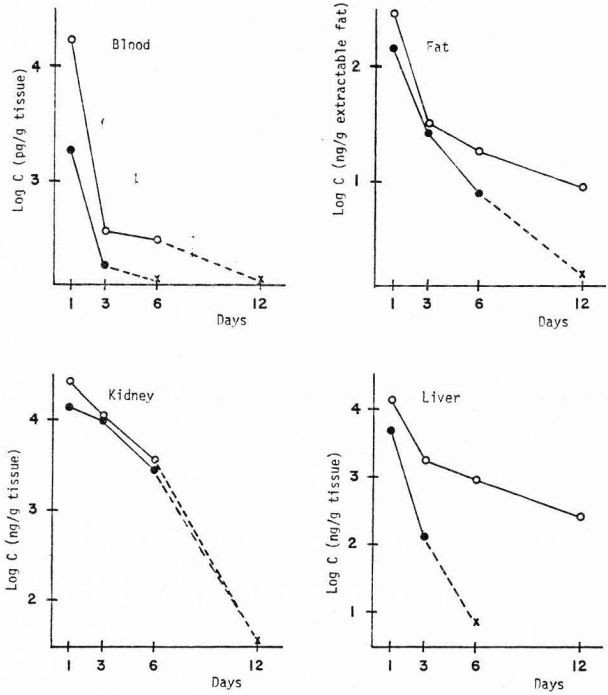


Figure 5. Depletion of 4-bromo-2,5-dichlorophenol in blood, fat, kidney and liver tissues of rats following a single orally administered dose of 0.33 m moles/kg (●) or 1.66 m moles/kg (O) of 4-bromo-2,5-dichlorophenol. Each point represents the mean of data for two rats. The broken lines indicate the terminal depletion to the minimum limit of detection (x).

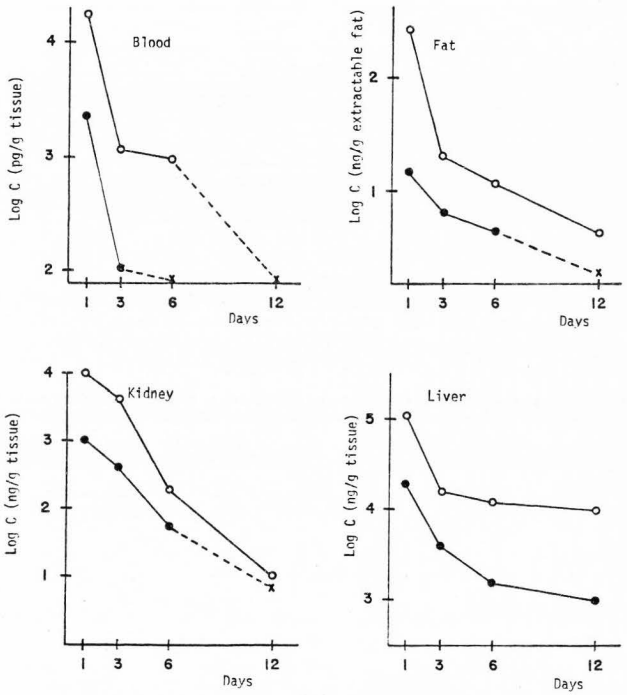


Figure 6. Depletion of 3,5,6-trichloro-2-pyridinol in blood, fat, kidney and liver tissues of rats following a single orally administered dose of 0.33 m moles/kg (●) or 1.66 m moles/kg (O) of 3,5,6-trichloro-2-pyridinol. Each point represents the mean of data for two rats. The broken lines indicate the terminal depletion to the minimum limit of detection (x).

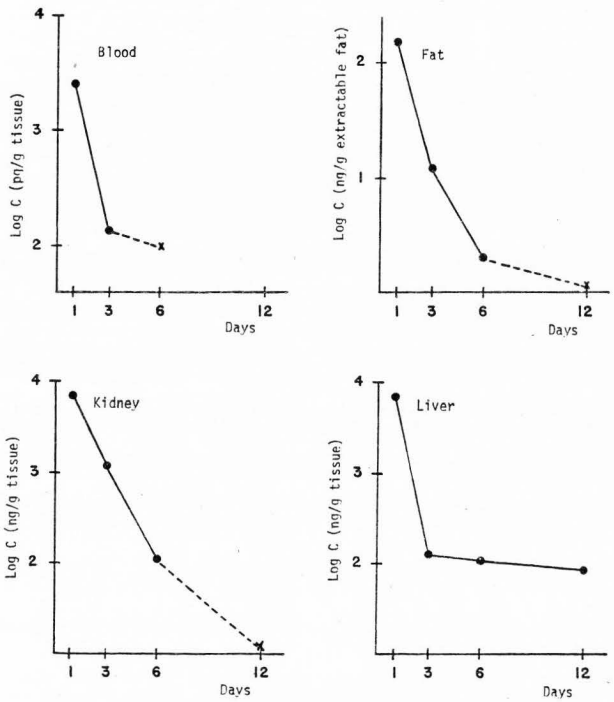


Figure 7. Depletion of 4-iodo-2,5-dichlorophenol in blood, fat, kidney and liver tissues of rats following a single orally administered dose of 0.33 m moles/kg. Each point represents the mean of data for two rats. The broken lines indicate the terminal depletion to the minimum limit of detection (x).

was observed (Figure 4). By the twelfth day depletion reached 96.7%. Also the initial rates of the liver 3,5,6-TC-2-Pyridinol 24 h concentration rapidly declined in the first three days then declined more slowly from that time on (Figure 6).

Table 5 presents the tissue:blood ratios of halogenated phenols and pyridinol at varying time intervals after single oral administration 1.66 m moles/kg. These ratios indicate that the rate of decline of halogenated phenols and pyridinol in blood were greatest when compared to other tissues, therefore, the ratios increased as time increased. With respect to blood, all tissues showed accumulation of halogenated phenols and pyridinol with time. The highest accumulation of administered halogenated phenols was in kidney, followed by fat and liver, which showed the least accumulation; whereas the highest accumulation of halogenated pyridinol was in liver, followed by kidney and fat, respectively. Table 6 presents the tissue:blood ratios of all compounds used in the study at varying time intervals after administration of a single oral dose of 0.33 m moles/kg body weight. The higher tissue:blood ratios of residues following the low dose of these compounds (Table 6) indicates a proportionally more rapid clearance of the blood and distribution of residue to tissues than that occurring with the higher dose level (Table 5).

Distribution in Organs

Data on the distribution of halogenated phenols and pyridinol in organs are shown in Appendix C. Following administration, each of the halogenated phenols and pyridinol was rapidly removed from

Table 5. Tissue:Blood Ratios of Halogenated Phenols and Pyridinol at Various Time Periods After the Single Administration of a 1.66 m moles/kg Dose in Rats[a]

Compounds	Tissues	Time days 1	3	6	12
2,4,5-trichloro-phenol	Fat	40.6 ± 5.7	63.5 ± 47.1	37.4 ± 5.3	*29.8 ± 0.9
	Kidney	30.4 ± 4.1	81.1 ± 11.3	339.5 ± 145.0	*25.7 ± 2.5
	Liver	5.2 ± 0.4	12.7 ± 1.7	7.2 ± 2.9	*6.99 ± 2.9
4-bromo-2,5-dichlorophenol	Fat	21.8 ± 15.1	92.5 ± 24.2	50.9 ± 6.3	92.9 ± 43.8
	Kidney	37.5 ± 3.2	234.1 ± 34.1	102.9 ± 17.2	ND
	Liver	19.7 ± 6.3	113.1 ± 41.5	50.5 ± 19.9	ND
3,5,6-trichloro-2-pyridinol	Fat	15.1 ± 3.1	40.6 ± 32.8	39.0 ± 0.01	ND
	Kidney	13.9 ± 0.1	242.4 ± 107.7	39.5 ± 0.0	*7.5 ± 0.02
	Liver	133.4 ± 20.1	1272.4 ± 108.4	2438.3 ± 102.3	*1989.3 ± 529.5

[a]Mean ± SE of two animals in each case. The values were obtained by dividing mean tissue concentration by mean blood concentration for each animal.

*The values were obtained by dividing mean tissue concentration by lower limits of detectability of blood concentration.

ND = not detected in kidney, liver and fat.

Table 6. Tissue:Blood Ratios of Halogenated Phenols and Pyridinol at Various Time Periods After Administration of a 0.33 m moles/kg Dose in Rats[a]

Compounds	Tissue	Time days 1	3	6	12
2,4,5-trichlorophenol	Fat	122.8 ± 80.6	57.3 ± 5.9	*47.9 ± 21.9	*30.1 ± 0.1
	Kidney	171.1 ± 2.8	75.9 ± 14.7	*326.4 ± 71.6	*17.3 ± 7.2
	Liver	22.9 ± 6.9	21.0 ± 4.2	*40.4 ± 0.1	ND
4-bromo-2,5-dichlorophenol	Fat	84.3 ± 42.8	195.3 ± 149.3	*80.5 ± 36.5	*42.4 ± 0.1
	Kidney	149.9 ± 16.0	567.4 ± 106.3	*525.4 ±19.5	*147.3 ± 37.6
	Liver	51. 2 ± 2.3	21.3 ± 9.5	ND	ND
4-iodo-2,5-dichlorophenol	Fat	61.7 ± 7.1	91.4 ± 15.6	*23.4 ± 10.1	ND
	Kidney	63.6 ± 0.9	169.6 ± 9.3	*20.7 ± 0.1	ND
	Liver	59.0 ± 15.8	25.0 ± 4.8	*13.2 ± 3.6	*16.7 ± 1.7
3,5,6-trichloro-2-pyridinol	Fat	6.9 ± 2.6	31.7 ± 24.4	*39.6 ± 26.8	*20.2 ± 9.0
	Kidney	22.5 ± 0.2	37.6 ± 28.9	92.0 ± 0.6	*55.2 ± 1.5
	Liver	203.7 ± 84.3	683.5 ± 131.7	*313.6 ± 91.2	*194.9 ± 28.1

[a]Mean ± SE of two animals in each case. The values were obtained by dividing concentration by mean blood concentration for each animal.

*The values were obtained in dividing mean tissue concentration by lower limits of detectability of blood concentration.

ND = not detected in kidney, liver and fat.

the blood and accumulated in the fat and liver and followed by the kidney. The highest content of halogenated phenols was in fat, whereas, the highest organ content of the halogenated pyridinol was in liver. The percentage of each chemical in the whole organ relative to the administered dose is given in Appendix C. The content of chemicals in the whole organ at 24 h after administration was depleted, approximately by 96%, or reached undetectable limits on the sixth or twelfth day. All compounds appear to be redistributed from blood and perhaps other tissues, thus fat and liver decline rates become slow relative to blood.

DISCUSSION

Animal Studies

No evidence whatsoever of adverse effects were observed in rats. The decrease in body weight which was observed after one day post-administration of the higher dose (1.66 m moles/kg) of the pyridinol was probably due to feed avoidance. However, the decrease in body weight was regained by the third day after dosing. McCollister et al. (1961) reported that the repeated oral administration of 0.1 g/kg/day of 2,4,5-TCP to rats for 24 days produced no adverse effects; whereas rats maintained at 0.3 to 1.0 g/kg/day for 24 days showed mild reversible pathological changes in the liver and kidneys. However, at 1.0 g/kg, the only effect attributable to 2,4,5-TCP was a slight increase in average weight of the kidneys. Anderson et al. (1949) fed groups of cattle dosages of 0.8, 2.4, and 7.2 g per 100 pounds of animal weight per day of zinc, 2,4,5-trichloro-phenate and 2,4,5-trichlorophenyl acetate in the diet for 78 days. They observed no adverse effect at any of the levels fed. Beck (1976) reported that halogenated phenols and pyridinols, when fed to rats at 100, 200, and 400 ppm in their diet, did not show significant difference in feed efficiency (weight gain/feed consumed) from controls. In light of the above, it would be safe to say that halogenated phenols and pyridinol are low in oral toxicity when the rats are given single oral doses at 0.33 and 1.66 m moles/kg. The lower dose, 0.33 m moles/kg is equivalent to 65.6, 80.4, 95.9, and 65.9 mg/kg for

2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol, 4-iodo-2,5-dichlorophenol, and 3,5,6-trichloro-2-pyridinol, respectively. The higher dose, 1.66 m moles/kg, is equivalent to 328, 401.9, and 329.5 mg/kg for 2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol, and 3,5,6-trichloro-2-pyridinol, respectively.

Gas Chromatographic Analysis of Halogenated Phenols and a Pyridinol in Tissues

The recovery of the study compounds in analysis of these tissues was generally very acceptable except for the pyridinol in fat which was 54.1%. No interference peaks were present in the tissues analyzed; however, some liver and kidney tissues analyzed for 2,4,5-TCP and the pyridinol contained small peaks, just beyond the apex of the pyridinol peak on the trailing edge, and for 2,4,5-TCP coincident with the GC peak. This interference was not identified but it was believed to be not due to pyridinol based upon data presented by Ivey et al. (1978) and Ivey (1979). In previous studies with Dursban in plants, it was found that dehalogenation of the [Cl^{36}] 3,5,6-trichloro-2-pyridinol could occur and [Cl^{36}] chloride could be found in the tissues (Smith et al. 1967). Smith et al. (1967) studied the metabolism of [Cl^{36}] chlorine-labeled chlorpyrifos in rats, chickens, and dogs and showed that [cl^{36}] chlorine-labeled material was present in trace quantities in the tissues which were eliminated from the body at a rate different from that of the major compound chlorpyrifos being investigated. They suggested that this minor compound might be present in the original compound as an impurity, or the compound might be undergoing enzymatic dehalogenation with the formation of radioactive chloride.

Today, one must consider the probability that the "dehalogenated" residue observed by Smith et al. (1967) was actually 2,5,6-TC-2-pyridinol. There are several possible benzene oxides which can be derived from i.p. administration of 300 mg 1,2,4-trichlorobenzene to the male rabbit (Kohli et al. 1976), and one of these 2,4,5-trichlorobenzene oxides is the most likely precursor for the metabolite 2,4,5-trichlorophenol. The 2,4,5-trichlorobenzene oxide could give the corresponding phenol by direct opening of the C_2O) bond or by the NIH shift of chlorine. Also, it has been found that 2,4,5-TCP is metabolized to 3,5-dichloro-catechol in soil and water (Rosenberg and Alexander 1980). These results indicate some possible routes to compounds representing the unknown peaks of halogenated phenols and pyridinol metabolites in animals. Studies of these compounds is needed in the future.

Twenty-Four Hour Concentrations

The halogenated phenols and pyridinol were differentially distributed in every tissue sampled following the administration, even though the total amount of halogenated phenols and pyridinol administered was the same. The difference could possible be due to preferential absorption from the gastrointestinal tract or to preferential elimination of certain compounds. As the concentration of the dose administered increased, the percent concentration of the compounds in tissues tended to be lower and the elimination from the tissues became slower. The highest amounts of halogenated pyridinol residues were observed in liver, then followed by kidney, fat, and blood whereas the highest residues of halogenated phenols

were observed in kidney, followed by liver, fat, and blood. In general, the 24 h concentration in fat and kidney tissues showed 2,4,5-TCP < 4-Br-2,5-DCP < 4-I-2,5-DCP > 3,5,6-TC-2-pyridinol, whereas the concentrations of these compounds in liver tissue gave the inverse series. The concentrations of these compounds in blood were similar and not significantly different, however the halogenated pyridinol concentration was higher than that of the halogenated phenols. The average concentrations of these compounds, based on either lipid or tissue weight, follow the same order and the same pattern as above. The only difference found was that the concentrations of all compounds were higher on a lipid basis than that found on a tissue basis in liver.

These findings are consistent with evidence from other animal experiments which indicated that the 3,5,6-trichloro-2-pyridinol concentrates in liver more than kidney and fat such as when cattle were fed chlorpyrifos daily for 30 days at levels of 3 to 10 ppm (Dishburger et al. 1977) and similar results obtained with cattle bearing chlorpyrifos impregnated plastic ear bands (Ivey et al. 1978). Also, McKellar et al. (1972) found the residues of 3,5,6-trichloro-2-pyridinol occurred predominantly in liver and kidney of swine when the animals were fed chlorpyrifos in their diet for 30 days. Similar results were obtained when chickens were fed chlorpyrifos (Dishburger et al. 1972). This means that there must be a very large turnover of halogenated pyridinol in the rat liver since it has been reported that liver contains the predominant

concentration of halogenated pyridinol compared to the kidney and the fat. The increase of halogenated phenol residues in the kidney is not surprising, however, since the kidney would be one of the pathways for the elimination of halogenated phenols from the body. It is known that rats primarily excrete phenols via the kidneys (Goldstein et al. 1974) and that related halogenated phenols may act similarly. That is in agreement with the higher rate of excretion of halogenated phenols in the urine as found by Bradway et al. (1977) and Shafik et al. (1973) who found that the urinary halogenated phenols and pyridinols excretion as metabolites of rats exposed to Ronnel, Leptophos, Dursban, Bromophos, and C-9491 were complete within 1-4 days. However, when the rate of absorption exceeded the rate of excretion, the toxic compound accumulated to a critical concentration in the body. Accumulation may also occur if the compound is so lipid-soluble that it is reabosrbed by the renal tubules after filtration (Goldstein et al. 1974). The accumulation of the halogenated phenols and pyridinol studied herein appear to be consistent with this concept.

Relationship of Lipophilicity and Concentration of Halogenated Phenols and Pyridinol in Tissues

Generally, the most important physio-chemical factor influencing the ability of a foreign compound to achieve effective concentrations in various biological phases is lipid solubility. The lipid-protein structure of cellular membranes requires that the foreign substance have sufficient lipophilicity to partition into the membrane, largely by diffusion, in order to cross into the cell.

Various methods of evaluating relative solubility parameters appear in the literature. The Hansch approach to regression analysis in structure-activity studies generally employs the relative octanol-water partition coefficient, known as π.

$$\pi = \log\left(\frac{P_X}{P_H}\right)$$

In this study the relative lipophilicity values, as measured by R_m for halogenated phenols and pyridinol, were generally very well correlated with the log residue concentration of compounds in all tissues as shown in Table 7 and Table 8. The data presented in Table 7 and in Table 8 are summarized in Figures 8 and 9 to illustrate the relationship between the lipophilicity, R_m, and the halogenated phenols and pyridinol concentrations in tissues 24 h after dosing. Increased concentration in tissue correlated with increased lipophilicity, the R_m, for the cases of fat and kidney.

Tables 9 and 10 show data for halogenated phenols and pyridinol and the results of regression analyses of compound concentrations as a function of lipophilicity. A strong linear correlation was seen for all compounds in fat, kidney, and liver at both dose levels. The compounds had a poorer correlation in the case of blood (at lower dose only). It is possible that the residue concentration in blood peaked much earlier than 24 h and that the measurements made here at 24 h, were at a time well into the process of redistribution from blood to tissues. Measurements made at shorter time intervals might well have shown a strong correlation of blood residues with R_m.

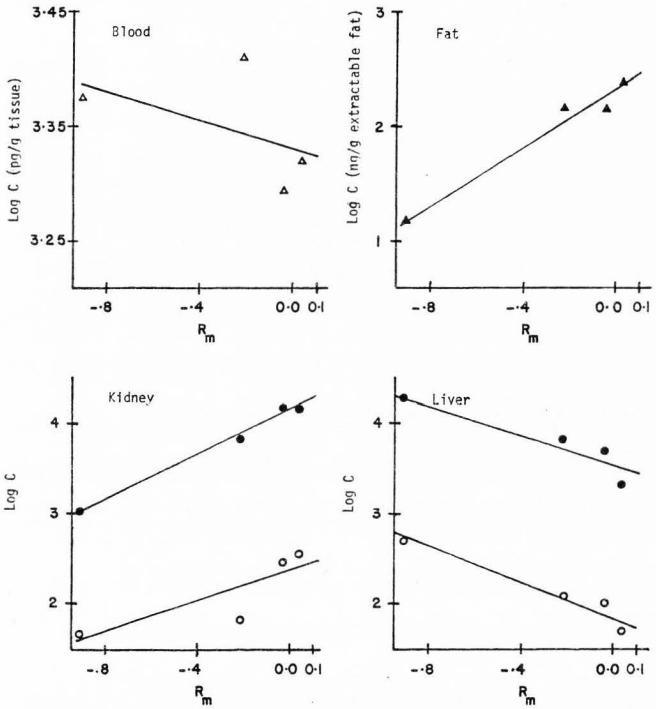


Figure 8. Effect of lipophilicity of halogenated phenols and pyridinol on concentration in tissues 24 h after administration of 0.33 m moles/kg. Solid circles (●) represent ng/g lipid and the open circles (o) represent ng/g tissue.

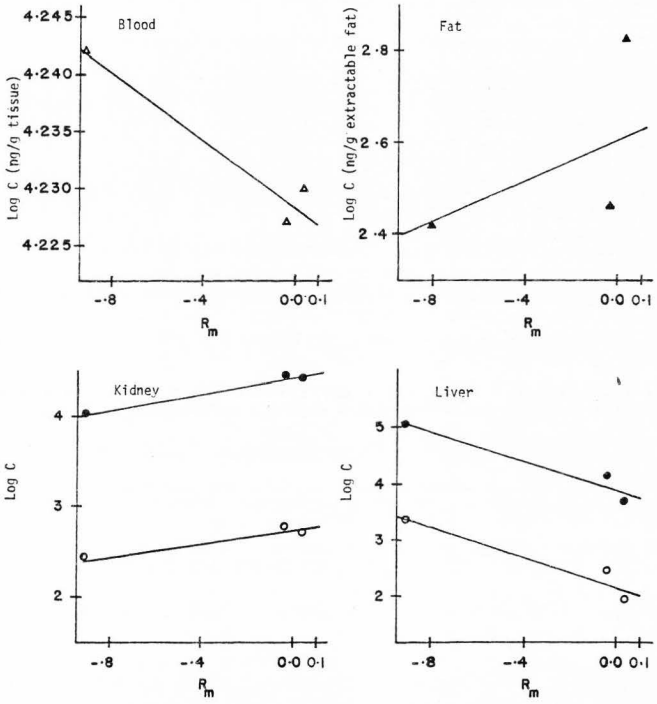


Figure 9. Effect of lipophilicity of halogenated phenols and pyridinol concentration in tissues 24 h after administration of 1.66 m moles/kg. Solid circles (●) represent ng/g lipid and the open circles (o) represent ng/g tissue.

Table 7. Concentrations of Halogenated Phenols and Pyridinol in Tissues 24 h After Administration (0.33 m moles/kg) and their Chromatographic R_m Values

Compound	R_m	Log C					
				Kidney		Liver	
		Blood[a]	Fat[b]	Tissue[c]	Lipid[d]	Tissue[c]	Lipid[d]
2,4,5-trichlorophenol	+0.035	3.32	2.39	2.54	4.16	1.67	3.03
4-bromo-2,5-dichloro-phenol	-0.045	3.29	2.15	2.48	4.17	2.01	3.72
4-Iodo-2,5-dichloro-phenol	-0.231	3.41	2.17	1.76	3.82	2.11	3.81
3,5,6-trichloro-2-pyridinol	-0.908	3.37	1.19	1.67	3.04	2.66	4.26

[a]Log concentration pg/g tissue.

[b]Log concentration ng/g extractable fat.

[c]Log concentration ng/g tissue.

[d]Log concentration ng/g lipids.

Table 8. Concentrations of Halogenated Phenols and Pyridinol in Tissues 24 h After Administration (1.66 m moles/kg) and their Chromatographic R_m Values

Compound	R_m	Log Concentration					
				Kidney		Liver	
		Blood[a]	Fat[b]	Tissue[c]	Lipid[d]	Tissue[c]	Lipid[d]
2,4,5-trichlorophenol	+0.035	4.23	2.84	2.71	4.42	1.95	3.63
4-bromo-2,5-dichloro-phenol	-0.045	4.23	2.47	2.79	4.44	2.48	4.17
3,5,6-trichloro-2-pyridinol	-0.908	4.24	2.40	2.40	4.05	3.36	5.04

[a]Log concentration pg/g tissue.

[b]Log concentration ng/g extractable fat.

[c]Log concentration ng/g tissue.

[d]Log concentration ng/g lipid.

Table 9. Correlation of the Halogenated Phenols and Pyridinol Concentrations in Tissue with R_m 24 h After Administration of 0.33 m moles/kg

Tissues	Regression equation	(n	r	s)[a]
Blood	Log C^b = 3.331 - 0.064 R_m	4	-0.517	.120
Fat	Log C^c = 2.328 + 1.227 R_m	4	0.982	.239
Kidney	Log C^d = 2.363 + 0.879 R_m	4	0.813	.222
	Log C^e = 4.150 + 1.235 R_m	4	0.995	.240
Liver	Log C^d = 1.845 - .924 R_m	4	-0.961	.210
	Log C^e = 3.543 - 0.822 R_m	4	-0.926	.202

[a] n is the number of halogenated phenols and pyridinol in the regression.
r is the correlation coefficient, and
s is the standard error of the estimate.

[b] Log concentration pg/g tissue.

[c] Log concentration ng/g extractable fat.

[d] Log concentration ng/g tissue.

[e] Log concentration ng/g lipid.

Table 10. Correlation of Halogenated Phenols and Pyridinol Concentrations in Tissue with R_m 24 h After Administration of 1.66 m moles/kg

Tissues	Regression Equation	(n	r	s)[a]
Blood	Log C^b = 4.228 - 0.015 R_m	3	-.965	.153
Fat	Log C^c = 2.663 + 3.065 R_m	3	.681	.203
Kidney	Log C^d = 2.751 + .377 R_m	3	.964	.210
	Log C^e = 4.430 + .416 R_m	3	.993	.214
Liver	Log C^d = 2.197 - 1.306 R_m	3	-.955	.357
	Log C^e = 3.883 - 1.299 R_m	3	-.952	.357

[a] n is the number of halogenated phenols and pyridinol in the regression.
r is the correlation coefficient, and
s is the standard error of the estimate

[b] Log concentration pg/g tissue.

[c] Log concentration ng/g extractable fat.

[d] Log concentration ng/g tissue.

[e] Log concentration ng/g lipid.

This was consistent with the concept that compounds must have lipophilicity to cross membranes, but there were some exceptions as indicated in blood and liver tissue concentration which shows the concentration was inverse to the increase of the lipophilicity of the compounds.

This provided one of the most important exceptions to the generalization that increasing lipid solubility enhances concentration. The reason for that exception in the liver is not known at the present time. However, there may be binding of this compound with biological macromolecules in this organ. This would argue from the report that trichlorophenol and the pyridinol reduced thyroxine (T_4) bound to liver mitochondria by 10 to 30% (Beck 1976). Also, it was indicated that the trichlorophenols, the dichlorobromo-phenol and the pyridinol bind at least as well as T_4 (itself) to the T_4-binding site of bovine serum albumin in vitro. The relative binding affinity was found higher for the halogenated pyridinol than that found for 2,4,5-trichlorophenol. So protein and/or organele binding may be a reason for the concentration increase in blood and liver and the delayed clearance from liver.

Often drugs are conjugated in liver first, and then pass into bile as a glucuronide, sulfate, glycinate or gluthione conjugate, and pass into the intestine. If the properties of the drug happen to be favorable for intestinal absorption, a cycle may result (enterohepatic cycle) in which biliary excretion and intestinal reabsorption continue until renal excretion finally eliminates the

drug from the body (Goldstein et al. 1974). It was found that sheep plasma contained the glucuronide of 3,5,6-trichloro-2-pyridinol as well as urine when the sheep were given a single oral dose of chlorpyrifosmethyl or its metabolite 3,5,6-trichloro-2-pyridinol (Bakke and Price 1976). That means the glucuronide pyridinol was reabsorbed from the intestine and recycled causing the little elevation in its blood concentration which was found. The other possibility is that conjugation with glucuronic acid probably takes place in the gastrointestinal tissues prior to the phenol's absorption into the blood (Pekas and Paulson 1970). Bakke and Price (1976) reported that 3,5,6-TC-2-pyridinol was absorbed rapidly into the blood of the sheep and excreted rapidly in urine.

Any other reason for the higher concentration of 3,5,6-TC-2-pyridinol in liver may be related to the electronegativity of nitrogen that makes pyridine unreactive toward electrophilic substitution but makes pyridine highly reactive toward nucleophilic substitution. As is well known, some conjugation reactions occur through nucleophilic attack by the electron rich atom. Thus, 3,5,6-TC-2-pyridinol conjugation with glucuronide occurs very rapidly as reported by Bakke and Price (1976).

Depletion of Halogenated Phenols and Pyridinol in Tissues

The levels of halogenated phenols and the halogenated pyridinol in tissue decline with time, however, the rates of decline were different for different tissues and for different compounds used. This kinetic study of halogenated phenols and pyridinol indicated a persistence of compounds in various tissue after an initial rapid

decline with time, but not always in an apparently log linear fashion. An initial rapid decline phase followed by a slower decline was shown in most tissues. The decline of 3,5,6-TC-2-pyridinol in liver and 2,4,5-TCP in kidney were exceptions which showed very slow decline and persistence of significant residues to the twelfth day.

The reason for persistence of 2,4,5-TCP in kidney and 3,5,6-TC-2-pyridinol in liver may be binding of these compounds with biological macromolecules. Pyridinol association with the liver mitochondrial fraction may be a factor also, in analogy to the specific binding of T_4 and T_3 (Krenning et al. 1980).

The levels of halogenated phenols and pyridinol in blood declined rapidly when compared to most other tissues as suggested by the comparative levels of these compounds at various time periods following administration of a single oral dose of the compound. This may indicate continued partitioning into tissues showing slow kinetics such as kidney and liver, because it is seen that the rats have ability to rapidly detoxify and eliminate phenols from the blood (Goldstein et al. 1974). Although halogenated phenols and pyridinol were not analyzed separately in plasma and blood cells, earlier studies have indicated that most of the pyridinol in blood is found in the plasma (Bakke et al. 1976).

The rates of decline from fat were slower than those found in the blood with time and the persistence of all compounds in fat with time exceeded that found in blood.

The concentrations of compounds in the lipid fraction of each tissue were not identical. These differences may be due to factors

such as the structure of lipid in each tissue, the permeability of tissue vascular systems to compounds and solubility of the compounds which related to the lipophilicity in this study. The compounds may thus display some special affinity for some specific component of lipid such as has been shown for DDT (Tinsely et al. 1971).

The results indicated that there may be possible quantitative differences in the kinetics of halogenated phenols and pyridinol distribution of different dose levels. The relative tissue concentration of compounds as reflected by tissue:blood ratios were consistently increased with time after administration. The accumulation of compounds in tissues appears to be a direct function of the dose administered but both the dose levels used in this study were not in a linear relationship because the lower dose level of compounds produced a significantly greater concentration in tissues than the higher dose level in relation to the percent dose administered; however, it is possibly only at the lower dose levels that a linear relationship may be expected.

If further studies are to be carried out in this area, there are some points that should be implemented. Samples of tissues should be collected between 1 and 24 h in addition to the sample times used in this study to investigate the exact time of peak uptake in tissues. Due to the lipophilicity of these compounds, they are expected to distribute to most tissues, hence other critical tissues should be sampled, especially brain, bile, muscle, and gastrointestinal tract and contents. Bakke et al. (1976) reported

that sheep bile, liver, kidney, and gastrointestinal tract with its contents contained detectable residues of 3,5,6-trichloro-2-pyridinol after a single oral dose of chlorpyrifos or 3,5,6-trichloro-2-pyridinol and that other related halogenated phenols may act similarly. Also, urine and feces should be sampled to know the fate of all compounds in the body of the animal because the rates of halogenated phenols and pyridinols removal from tissues are expected to be reflected in their rates of excretion. It would be instructive to determine the halogenated phenols and pyridinols in tissues in future work following specific organophosphorus pesticides administration to animals for metabolism studies, because halogenated phenols are the major metabolites of these pesticides. Finally, although there were no indications of halogenated phenols and pyridinols metabolites, such as the partial dechlorination or the shift of chlorine which could occur (Smith et al. 1967 and Kohli et al. 1976), studies designed differently might produce evidence of such compounds as metabolites.

SUMMARY

This thesis was initiated to determine the structure-activity relationships among halogenated phenols and a pyridinol in terms of their distribution and accumulation in selected tissues and the kinetics of their decline from those tissues. The compounds used in the studies were 2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol, 4-iodo-2,5-dichlorophenol, and 3,5,6-trichloro-2-pyridinol. There was no evidence whatsoever of adverse effects at the dose levels employed.

The compounds were chromatographed in reversed-phase thin layer chromatography as a measure of their relative lipophilicity values (R_m). The R_m values were found to be generally very well correlated with the log concentration of these compounds in tissues. The correlation coefficients were -.517, .982, .813, and -.961 for the low dose level of these compounds in blood, fat, kidney, and liver based on the tissue weight, respectively. Correlation coefficients of .995 and -.926 were calculated for kidney and liver based on the lipid content. Similar correlation coefficients were -.965, .681, .964, and -.955 for the high dose level of these compounds in blood, fat, kidney, and liver based on the tissue weight, respectively, or .993 and -.952 in kidney and liver based on the lipid content, respectively.

The levels of compounds in tissues declined with time, however, the rates of decline were different for different tissues and for

different compounds. All compounds appear to be redistributed from blood and perhaps other tissues, thus fat and liver decline rates become slow relative to blood.

The persistence of the compounds in tissues after an initial rapid decline with time indicated quantitative differences in the kinetics of compounds distribution at different dose levels.

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APPENDICES

Appendix A

Table 11. Mean Body Weight (g±S.E.[a]) of Rats at Various Time Periods After a Single Oral Dose of Halogenated Phenol or Pyridinol

Treatment	Days				
	0	1	3	6	12
2,4,5-trichlorophenol					
0.33 m moles/kg	182 ± 4 (8)[b]	188 ± 4 (8)	211 ± 5 (6)	230 ± 5 (4)	276 ± 8 (2)
1.66 m moles/kg	186 ± 3 (8)	190 ± 4 (8)	210 ± 5 (6)	241 ± 5 (4)	271 ± 8 (2)
4-bromo-2,5-dichlorophenol					
0.33 m moles/kg	185 ± 3 (8)	190 ± 3 (8)	209 ± 4 (6)	238 ± 7 (4)	248 ± 1 (2)
1.66 m moles/kg	185 ± 3 (8)	190 ± 3 (8)	218 ± 3 (6)	250 ± 4 (4)	288 ± 4 (2)
4-iodo-2,5-dichlorophenol					
0.33 m moles/kg	186 ± 3 (8)	190 ± 3 (8)	210 ± 5 (6)	232 ± 8 (4)	266 ± 2 (2)
3,5,6-trichloro-2-pyridinol					
0.33 m moles/kg	183 ± 3 (8)	186 ± 3 (8)	209 ± 4 (6)	240 ± 6 (4)	280 ± 2 (2)
1.66 m moles/kg	186 ± 2 (8)	185 ± 3 (8)	210 ± 4 (6)	232 ± 12 (4)	274 ± 7 (2)
Control	185 ± 2 (8)	190 ± 2 (8)	207 ± 3 (8)	232 ± 4 (8)	265 ± 5 (8)

[a]Standard error of the mean.

[b]Value in parentheses is the number of animals.

Appendix B

Table 12. Mean Relative Kidney and Liver Weight (g tissue/100 g body weight ± S.E.[a]) of Rats at Various Time Periods After a Single Oral Administration of Halogenated Phenol or Pyridinol

Treatment	Tissue	Days 1	3	6	12
2,4,5-trichlorophenol					
0.33 m moles/kg	Kidney	0.81 ± .01	0.91 ± .04	0.80 ± .02	0.77 ± .05
0.33 m moles/kg	Liver	4.34 ± .02	4.11 ± .08	3.72 ± .05	3.63 ± .17
1.66 m moles/kg	Kidney	0.85 ± .01	0.79 ± .03	0.84 ± .04	0.78 ± .03
1.66 m moles/kg	Liver	4.75 ± .51	4.35 ± .16	4.07 ± .15	3.86 ± .09
4,bromo-2,5-dichlorophenol					
0.33 m moles/kg	Kidney	0.81 ± .03	0.89 ± .02	0.82 ± .01	0.80 ± .04
0.33 m moles/kg	Liver	4.08 ± .25	4.00 ± .14	3.57 ± .44	3.44 ± .19
1.66 m moles/kg	Kidney	0.85 ± .02	0.84 ± .04	0.79 ± .02	0.81 ± .03
1.66 m moles/kg	Liver	4.21 ± .08	4.27 ± .14	3.84 ± .13	3.89 ± .02
4-iodo-2,5-dichlorophenol					
0.33 m moles/kg	Kidney	0.81 ± .09	0.81 ± .04	0.79 ± .04	0.75 ± .03
0.33 m moles/kg	Liver	4.27 ± .18	4.18 ± .01	3.69 ± .08	3.82 ± .18
3,5,6-trichloro-2-pyridinol					
0.33 m moles/kg	Kidney	0.82 ± .03	0.89 ± .02	0.84 ± .02	0.80 ± .03
0.33 m moles/kg	Liver	4.37 ± .00	3.98 ± .01	3.73 ± .29	4.02 ± .01
1.66 m moles/kg	Kidney	0.85 ± .04	0.84 ± .02	0.80 ± .02	0.83 ± .08
1.66 moles/kg	Liver	4.20 ± .18	4.15 ± .06	3.45 ± .00	3.99 ± .17
Control	Kidney	0.81 ± .06	0.78 ± .02	0.83 ± .01	0.78 ± .01
	Liver	4.43 ± .20	3.96 ± .08	3.58 ± .01	3.56 ± .09

[a]Standard error of the mean; each value represents an average of two animals.

Appendix C

Table 13. Distribution of Halogenated Phenols and Pyridinol in Rats Organs Following Oral Administration

Treatment	Organs[a]	Days After Dosing 1	3	6	12
2,4,5-trichlorophenol					
0.33 m moles/kg	Blood[b]	.030 (.0003)	.032 (.0003)	ND[c]	ND
	Fat[b]	3.135 (.030)	2.144 (.019)	.498 (.005)	.384 (.004)
	Kidney	.498 (.005)	.277 (.003)	.247 (.002)	.018 (.0002)
	Liver	.360 (.003)	.344 (.003)	.043 (.004)	ND
1.66 m moles/kg	Blood[b]	.262 (.0004)	.058 (.0001)	.019 (.00003)	.016 (.00003)
	Fat[b]	11.442 (.019)	4.115 (.007)	.695 (.001)	.352 (.001)
	Kidney	.861 (.005)	.457 (.001)	.573 (.001)	.028 (.0001)
	Liver	.826 (.001)	.395 (.001)	.060 (.0001)	.037 (.0001)
4-bromo-2,5-dichlorophenol					
0.33 m moles/kg	Blood[b]	.029 (.0002)	.003 (.00002)	ND	ND
	Fat[b]	1.878 (.015)	.515 (.004)	.187 (.001)	.125 (.001)
	Kidney	.465 (.004)	.035 (.0003)	.010 (.0001)	ND
	Liver	.783 (.006)	.027 (.0002)	ND	ND
1.66 m moles/kg	Blood[b]	.235 (.0004)	.003 (.0004)	.007 (.00001)	ND
	Fat[b]	4.566 (.007)	.613 (.001)	.358 (.0005)	.287 (.0004)
	Kidney	.931 (.001)	.140 (.0002)	.071 (.0001)	ND
	Liver	2.254 (.003)	.320 (.0004)	.162 (.0002)	ND

Table 13. (Continued)

Treatment	Organs[a]	Days After Dosing			
		1	3	6	12
4-iodo-2,5-dichlorophenol					
0.33 m moles/kg	Blood[b]	.038 (.0002)	.002 (.00001)	ND	ND
	Fat[b]	1.903 (.012)	.236 (.002)	.078 (.0005)	ND
	Kidney	.087 (.001)	.051 (.0003)	.005 (.00003)	ND
	Liver	1.026 (.007)	.031 (.0002)	.013 (.0001)	ND
3,5,6-trichloro-2-pyridinol					
0.33 m moles/kg	Blood[b]	.034 (.0003)	.002 (.00002)	ND	ND
	Fat[b]	.266 (.002)	.102 (.001)	.083 (.001)	ND
	Kidney	.069 (.001)	.014 (.0001)	.009 (.0001)	ND
	Liver	3.617 (.033)	.662 (.006)	.333 (.003)	.263 (.0002)
1.66 m moles/kg	Blood[b]	.251 (.0004)	.019 (.00003)	.017 (.00003)	ND
	Fat[b]	5.007 (.009)	.211 (.0004)	.168 (.0003)	ND
	Kidney	.396 (.001)	.121 (.0002)	.013 (.00002)	ND
	Liver	17.839 (.031)	3.146 (.005)	2.157 (.004)	2.609 (.004)

[a]Each value represents the content (μg) of chemical in the whole organ, average of two animals; value in parentheses is percentage of the original dose.

[b]Estimated organ weight was calculated (Lindstrom et al. 1974) as blood $[B_{m(g)} = 77\ (B.W._{kg})^{0.99}]$ and fat $[F_{m(g)} = 180\ (B.W._{kg})^{1.0}]$.

[c]ND, not detected.

Appendix D

Table 14. Concentration of Halogenated Phenols and Pyridinol in Tissue After Administration[a]

Treatment	Days	Tissues Concentration						
		Blood[b]	Fat		Kidney		Liver	
			Tissue[b]	Lipid[c]	Tissue[b]	Lipid[d]	Tissue[b]	Lipid[d]
2,4,5-trichlorophenol								
0.33 m moles/kg	1	2.1	97.3	247.1	345.6	14.5	46.4	2.1
	3	1.9	54.6	111.4	139.0	8.0	38.3	1.7
	6	ND	11.9	24.0	134.5	8.0	5.0	0.2
	12	ND	ND	7.4	8.6	.5	ND	ND
1.66 m moles/kg	1	17.0	328.0	688.0	515.7	26.3	88.8	4.2
	3	3.7	114.4	192.7	289.4	17.1	45.4	1.8
	6	1.0	15.6	34.8	275.5	16.8	5.9	.3
	12	.8	7.3	14.9	13.1	0.8	3.5	.2
4-bromo-2,5-dichlorophenol								
0.33 m moles/kg	1	1.9	55.3	141.9	303.7	14.8	101.9	5.3
	3	0.2	15.1	26.4	19.3	10.1	3.3	0.1
	6	ND	4.3	8.0	5.3	3.1	ND	ND
	12	ND	1.4	2.1	ND	ND	ND	ND
1.66 m moles/kg	1	16.9	142.7	292.6	616.2	27.5	300.9	14.8
	3	0.3	15.5	29.2	77.0	10.3	34.5	1.8
	6	0.4	8.0	19.0	36.3	4.0	17.0	1.0
	12	ND	5.5	9.3	ND	ND	ND	0.3

Table 14. (Continued)

Treatment	Days	Tissues Concentration						
		Blood[b]	Fat		Kidney		Liver	
			Tissue[b]	Lipid[c]	Tissue[b]	Lipid[d]	Tissue[b]	Lipid[d]
4-iodo-2,5-dichlorophenol								
0.33 m moles/kg	1	2.6	56.4	148.1	47.0	6.5	128.2	6.4
	3	0.1	6.0	12.5	29.2	1.3	3.4	0.2
	6	ND	1.6	2.6	2.9	0.1	1.5	0.1
	12	ND	ND	ND	ND	ND	0.8	0.1
3,5,6-trichloro-2-pyridinol								
0.33 m moles/kg	1	2.4	8.2	15.5	46.3	1.1	456.7	18.0
	3	0.1	2.3	6.7	8.0	0.4	82.0	3.9
	6	ND	1.9	4.8	4.6	0.1	37.6	1.5
	12	ND	ND	4.1	ND	ND	23.4	1.0
1.66 m moles/kg	1	17.4	117.3	253.2	253.2	11.2	2313.7	110.7
	3	1.1	5.5	21.4	67.1	4.3	355.1	16.3
	6	1.0	4.4	12.1	7.6	0.2	212.6	13.0
	12	ND	ND	4.6	ND	ND	238.7	11.2

[a]Each value represents an average of two animals.

[b]Concentration ng/g tissue.

[c]Concentration ng/g extractable fat.

[d]Concentration µg/g lipid.

ND = not detected.