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Chlorophenols in aquatic environments: Structure-activity correlations

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Chlorophenols are of environmental interest because of their widespread distribution in freshwater habitats. All these compounds are toxic to aquatic species, but to varying degrees depending on the number and the position of the chlorine substituents on the benzene ring. Structurally, chlorophenols fit the requirements for receptor producing-respiratory uncoupling. However, QSAR-based modelling studies indicate that polar narcosis is a mode of toxic action through which many of the chlorophenols elicit their toxicity. Further, the sublethal effects of chlorophenols on respiratory physiology have been linked to their mechanism of toxicity. In this paper, the mechanisms of toxic action for chlorophenols, the structural activity characteristics of chlorophenols, and the sublethal physiological responses are reviewed.

1. Introduction

The chlorinated phenols consist of a group of nineteen different isomers which include mono, di, tri, tetra, and one pentachlorophenol. All these compounds are toxic to aquatic species, but in varying degrees with pentachlorophenol being the most toxic (Buikema et al. 1979). Structurally, chlorophenols fit the requirements for receptor-producing respiratory uncoupling. However, uncoupling oxidative phosphorylation is the most important mode of toxic action for chlorophenols with two or more chlorine substituents, while non-specific polar narcosis is the proposed mechanism in the case of mono substituted phenols (Exon 1984). However, this division is not generally accepted. For example, Schultz (1987) and Bryant and Schultz (1994) classified only the tetrachlorophenols and pentachlorophenol as uncoupling agents, and the mode of toxic action for lower substituted chlorophenols was polar narcotics. On the other hand, *in vitro* studies show that even mono and dichlorophenols have instantaneous uncoupling properties (Ravanel et al. 1985, 1989, Tissut et al. 1987), although pentachlorophenol is a 200-fold more potent uncoupler than mono substituted chlorophenols.

As with other toxic chemicals, understanding the mechanisms, whereby chlorophenols produce their toxicity, forms the basis for the evaluation of the ecological damage that might result from chlorophenol accumulation in biota. Further, knowledge of chemical characteristics that affect environmental partitioning are required to evaluate environmental exposure (Widdows & Donkin

1991, Depledge & Fossi 1994). In this paper, mechanisms of toxic action for chlorophenols, structural activity characteristics of chlorophenols, and the sublethal physiological responses are reviewed.

2. Modes of toxic action

Poisons can only be classified properly when the target organs on which they act and the changes induced in these organs are known (Koeman 1991). Many of the processes affected by a toxicant are fundamental biological processes such as respiration, oxidative phosphorylation, protein synthesis, and osmoregulation. These processes are very similar among organisms that are even taxonomically unrelated. Toxicants that affect fundamental biological (i.e. biochemical) processes act by specific modes of toxic action, unlike compounds acting by a general non-specific narcotic mechanism.

There are several recognized mechanisms of toxic action for aquatic organisms. Recently, McCarty and Mackay (1993) identified eight major modes of toxic action based primarily on effects observed for rainbow trout (*Oncorhyncus mykiss*) (McKim et al. 1987a–c, Bradley et al. 1989a). Modes of toxic action include: 1) narcosis, 2) polar narcosis, 3) respiratory uncoupling, 4) membrane irritation, 5) CNS convulsion, 6) AChE (acetylcholinesterase) inhibition, and 7) respiratory blockade. Further, the effects of dioxin (TCDD) were separated into their own group.

As discussed above, two of these modes — polar narcosis and uncoupling of oxidative phosphorylation — have been proposed for chlorophenols and are described below.

2.1. Polar narcosis

The majority of industrial organic chemicals lack identifiable structural characteristics that result in specific biological activity. Thus, their acute effects on aquatic species is elicited through non-specific narcosis (Bradbury et al. 1989ab, Schultz 1989). The toxicity of these compounds is low, and requires very high critical body residues (2 to 8 mmol/kg on a wet mass basis) compared with compounds (e.g. halogenated organics) with a specific mode of toxic action (McCarty & Mackay 1993). Thus, narcosis represents the baseline toxicity and is currently the most commonly recognized mode of toxic action. Briefly, the expected symptomatology for these compounds includes loss of reaction to external stimuli, loss of equilibrium (in fish), decline in respiratory rate, and subsequent death (Schultz 1989).

Acute potency and/or the physiological and behavioural characteristics of the narcosis response to certain esters, phenols and anilines seems to be unique (Bradbury et al. 1989b and references therein). These agents are termed polar narcotics (corresponds to type II narcosis). QSAR-based studies show that polar narcotics are slightly more toxic by nature than non-ionic narcotics (Bradbury et al. 1989b, Pawlisz & Peters 1993). Increased toxicity of polar narcotics, compared with nonpolar narcotics, was due to greater dipolarity and/or hydrogen-bond donor acidity of the former (Bryant & Schultz 1994). The exact mechanism of narcosis is not known, but they are usually considered to affect nervous system membranes. Leading theories (see Bradbury et al. 1989b, Hermens 1989) propose that narcotic molecules might have multiple sites and mechanisms of action causing the membrane to become inoperative.

2.2. Uncoupling of oxidative phosphorylation

Oxidative phosphorylation, which takes place in the inner mitochondria membrane, is the last step in catabolism and the point at which the major portion of metabolic energy is released. Many of the inner membrane proteins belong to the electron-transport chain, which establish the electrochemical proton gradient across the membrane. Another major component is the large protein complex, enzyme ATP synthetase (ATPase), which catalyses the synthesis of ATP (Stryer 1988, Alberts et al. 1990). ATPase converts one form of energy to another, synthesizing ATP from ADP and Pi in the mitochondrial matrix in a reaction, in which oxidation and phosphorylation are coupled by a proton gradient across the inner mitochondrial membrane. This tight coupling is disrupted by an uncoupler because the protonophoric properties of the H^+-impermeable mitochondrial membrane is disrupted (Terada 1990). Thus, the phosphorylation of ADP is short-circuited from redox-reactions which attempt to maintain the

cross-membrane gradient in a hydrogen ion concentration. Although ATPase and the respiratory chain are not affected, ATP is not formed by the ATPase (Terada 1990). The regulation of ATP production collapses and cell respiration continues to increase until intracellular reserves are exhausted (Okey & Stensel 1993).

Respiratory uncouplers such as chlorophenols (termed weak acid uncouplers by Terada 1990) act on the energy-transducting inner mitochondrial membrane and specifically induce the uncoupling of oxidative phosphorylation. However, they do not have a specific binding receptor site in the membrane, but there is non-specific interaction with the membrane resulting in a specific biological response (Terada 1990). The response is reversible, as for narcotics and polar narcotics (Jaworska & Schultz 1994).

3. Characteristics affecting toxicity of chlorophenols

Chlorophenols possess three characteristics on which their toxicity is based (Terada 1990): 1) an acid-dissociable group (hydroxyl substituent), 2) a strong electron-withdrawing moiety (halogen substituents), and 3) a bulky hydrophobic group (a benzene ring). The hydroxyl group decreases hydrophobicity but increases reactivity (Bryant & Schultz 1994). Replacement of the acid-dissociable group by a non-acid dissociable moiety results in complete loss of uncoupling activity (Terada 1990). The addition of a chloro group increases both hydrophobicity, which dictates exposure, and the acidic strength (reactivity) of phenol. The more acid the chlorophenol is, the more uncoupling the character of the chlorophenol (Saarikoski & Viluksela 1982). Thus, the potency of chlorinated phenols in uncoupling oxidative phosphorylation depends on the degree of chlorine substitution. Compared with 2,4,6-trichlorophenol, 2,4-dichlorophenol and 2-chlorophenol, pentachlorophenol stimulates rat mitochondrial respiration at 50, 200 and 800 times lower a concentration, respectively (Shannon et al. 1991). In the studies of Ravanel et al. (1989) with *Acer* cell suspension and of Tissut et al. (1987) with plant thylakoid, the uncoupling activity exhibited a 200-fold range, from pentachlorophenol which was the most effective compound, to 3- and 2-monochlorophenols which had the lowest activity. Generally, monochlorinated phenols seemed to be poor uncouplers.

Not only do the number of chloro groups affect the toxicity or uncoupling potency of chlorophenols but the position of the chlorine substituents on the benzene ring is extremely important for the toxicity. However, only limited information is available to show how the chlorine position in phenolic compounds affects their toxicity to an organism. The greater the number of chloro substituents in the ortho positions, the lower the observed toxicity (Beltrame et al. 1984, Ravanel et al. 1985, 1989, Shannon et al. 1991, Argese et al. 1995). For instance, 2,6-dichlorophenol was ten-fold less active than 3,5 and 3,4 isomers (Ravanel et al. 1985), or six-fold less active than 2,4-dichlorophenol (Shannon et al. 1991). This ortho effect may be due to the hydrogen bonding and shielding of the phenolic group by the adjacent chloro substituents. The shielding of the phenolic group may in turn reduce the ionization potential of the OH group and therefore decrease the ability of chlorophenol to transport protons through mitochondrial membranes (Shannon et al. 1991).

Due to the polar nature of chlorophenols, toxicity is correlated with the dissociation constant pK_a (Saarikoski & Viluksela 1982, Schultz 1987, Kishino & Kobayshi 1994, 1995). When acidic phenols are evaluated, pK_a is a significant descriptor of toxicity. The pK_a values of the lower chlorinated phenols are sufficiently high that dissociation is not significant over the pH range to which aquatic organisms are usually exposed (Saarikoski et al. 1986). The current understanding of aquatic toxicity suggests that the non-ionized form of chemical is more toxic than the ionized form (Schultz 1987). Further, the neutral and ionized forms of chlorophenols have entirely different modes of action (Saarikoski & Viluksela 1981). However, the main reason for the higher toxicity of the non-ionized form of chemical is the higher potential to bioaccumulate. At the range of pH which exceeds the pK_a value, compound accumulation is greatly reduced because ionization in water decreases the accumulation and therefore the toxicity (Saarikoski et al. 1986, Kishino & Kobayshi 1994, 1995). The phenate ion penetrates the biological membranes more slowly than the neutral molecule, as observed in both the guppy (*Poecilia reticulata*)

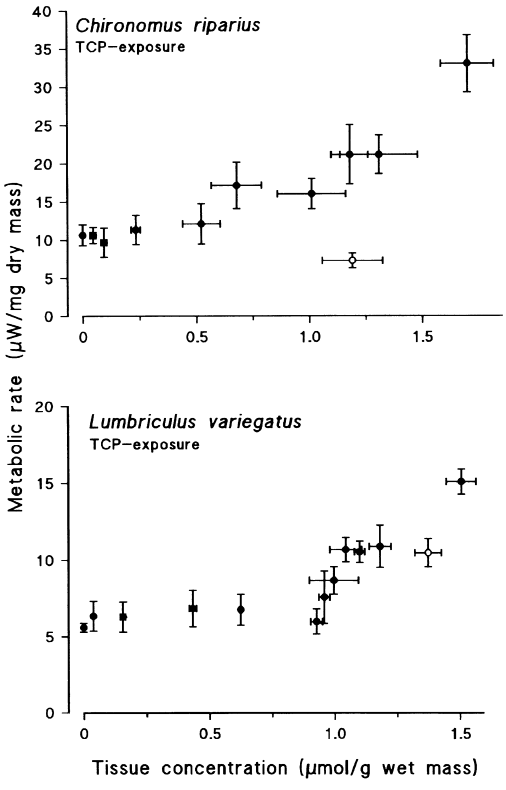

Fig.1. Relationship between concentrations of 2,4,5-trichlorophenol in the tissues of two benthic invertebrates (chironomid larvae, *Chironomus riparius* and the oligochaete worm *Lumbriculus variegatus*) and effects on metabolic rate as measured by direct microcalorimetry (Penttinen & Kukkonen, unpublished data).

(Saarikoski & Viluksela 1982), and in the phospholipid lecithin (Kishino & Kobayshi 1994), one of the major lipid components of the cell membrane. However, when the rate of accumulation is measured at a pH one or more units below the respective pK value, the compound is essentially non-ionized and accumulation from water proceeds at its maximum rate independent of pH (Saarikoski et al. 1986). Although the pK_a value may not have a direct effect on the mode of toxic action of chlorophenols, clearly the exposure conditions must be considered when applying and generalizing the results of in vitro bioenergetic studies to higher levels of organization because potency at the site itself (mitochondria) is not necessarily the same as observed toxicity for experiments with whole, living organisms.

4. Sublethal physiological responses of chlorophenols indicating mode of toxic action

Primary interactions between the toxin and various cell components may induce functional alterations at higher levels of organization, manifested by an impairment of vital functions, such as nerve and muscle functions and respiration (Larsson et al. 1985). Consequently, bioaccumulated chemicals will cause biological responses at whole organism level which should be indicative of the chemical's mode of toxic action. If chlorophenols uncouple oxidative phosphorylation, the sublethal response caused by these toxicants can be detected at the organism level by monitoring the rate of their energy metabolism. The response should reflect an increased metabolic rate in the animal (McKim et al. 1987a, Widdows & Donkin 1991, Wang & Widdows 1993, Penttinen et al. 1996). McKim et al. (1987a) determined the patterns of respiratory-cardiovascular responses for rainbow trout exposed to lethal concentrations of pentachlorophenol. The most evident (and practically only) sublethal toxic response was a rapid increase in ventilation volume and oxygen consumption while the trout survived. This corresponded to a continuously rising metabolic rate, which was not influenced by increased activity, since the exposed animals were immobilized.

For contaminants that increase metabolic rate by uncoupling oxidative metabolism, the maximum rate of sustainable metabolism is measured (Willows 1994). That rate is generally believed to lie within two to three times the basal metabolic rate. This is supported by studies of Widdows and Donkin (1991) for pentachlorophenol, Penttinen et al. (1996) and Penttinen and Kukkonen (unpublished data) for 2,4,5-trichlorophenol. At the maximum, the metabolic rate increased by a factor of three (Fig. 1).

Bradbury et al. (1989a) examined the physiological responses of rainbow trout to polar narcotics including phenol (but no chlorophenols). The most striking characteristics associated with intoxication were the development of tremors and clonic seizures, which were initiated with coughs. This was followed by general depression and cardiovascular collapse. The response to polar narcotics suggests that they affected the central nervous system, or perhaps the peripheral nervous sys-

tem. In general, the changes in the physiological parameters were consistent with increased muscular activity associated with seizures. This was demonstrated by initial rises in metabolic rate, which subsequently declined, reflecting an overall depression of respiratory activity with increasing exposure, consistent with a general narcotic effect. Thus, polar agents had a significant excitatory effect on the nervous system, followed by depression. Although chlorophenols have been commonly cited as polar narcotics, it seems that there is no physiological background for this argument. It is based only on a quantitative structure activity relationship (QSAR).

5. Modelling

Log k_{ow}-dependent QSAR for toxicity is a powerful tool in aquatic systems for predicting the toxicity of chemicals that act especially through narcotic and polar narcotic mechanisms (e.g. Veith et al. 1983, McKim et al. 1987c, Hermens 1989, 1991). For toxins with specific modes of toxic action, effective concentrations are additionally influenced by descriptors other than k_{ow}. Basically, QSARs in chlorophenols are applied either by using log kow as the only descriptor (e.g. Cajina-Quezada & Schultz 1990, Jaworska & Schultz 1994) or the additional descriptors are used including pK_a (Schultz 1987, Bryant & Schultz 1994), ΔpK_a (Saarikoski & Viluksela 1982), ΣD, a steric parameter (Devillers & Chambon 1986, Tissut et al. 1987, Ravanel et al. 1989) and the Hammet constant, the descriptor for electronic effects (Tissut et al. 1987, Ravanel et al. 1989).

In many cases, these equations contain phenols with heterogeneous substituents because, in principle, QSAR makes it possible to predict the accumulation potential and toxicity of related compounds, as shown by Hermens (1989) who has summarised twenty-seven of the derived QSAR equations for substituent phenols with several endpoints. On the other hand, in QSARs given by Devillers and Chambon (1986), Tissut et al. (1987) and Ravanel et al. (1989), chlorophenols were primary compounds in which the structure-activity was studied.

Schultz et al. (1986) observed two separate groups of phenols with different kow relationships. Based on this, Schultz (1987) modelled the biological response to phenol exposure by two log kow-dependent QSARs where structure-activity correlations were based on *in vivo* experiments in the static *Tetrahymena pyriformis* (ciliate protozoans) population growth test. The less toxic chemicals were modelled as a linear 1-octanol/water partition coefficient log k_{ow}-dependent regression,

$$\text{eq. 1. } \log BR = 0.6128 (\log k_{ow}) - 1.1297; n = 14, r^2 = 0.985, s = 0.18.$$

The proposed mechanism was thought to be polar narcosis. The more toxic chemicals were modelled by:

$$\text{eq. 2. } \log BR = 0.4485 (\log k_{ow}) + 0.3007; n = 7, r^2 = 0.925, s = 0.209.$$

Equation 2 models compounds that uncouple oxidative phosphorylation. The criteria for selecting the correct QSAR appears to be the pK_a value. Phenols with a pK_a value > 8.0 are polar narcotics, whereas compounds with the pK_a values < 6.5 are uncoupling agents. Thus, among chlorophenols, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 3,4-dichlorophenol and 3,5-dichlorophenol elicit their toxic action by polar narcosis while only tetrachlorophenols and the pentachlorophenol uncouple oxidative phosphorylation. Chlorophenols which have a pK_a value in the range of 6.5 to 7.8 have been also classified as polar narcotics (Bryant & Schultz 1994).

However, the uncoupling properties of chlorophenols are well established for isolated animal mitochondria (Stockdale & Selwyn 1971, Shannon et al. 1991), plant mitochondria (Ravanel et al. 1985), chloroplasts (Tissut et al. 1987) and for submitochondrial particles (Argese et al. 1995), and QSARs were calculated for the effects of chlorophenols on uncoupling activities (Ravanel et al. 1985, 1989, Tissut et al. 1987). In these studies, *in vitro* respiratory oxygen consumption of cell suspensions was measured and related to the parameters given above. Results indicate that even mono and dichlorophenols have uncoupling properties. Thus, there is a clear discrepancy between results of *in vitro* and *in vivo* experiments, or then the results are open to various interpretations.

How reliable are these QSAR models for polar narcotics when only the chlorophenols are considered? A notable feature of the above models (1 and 2) is the small number of chlorophenols included.

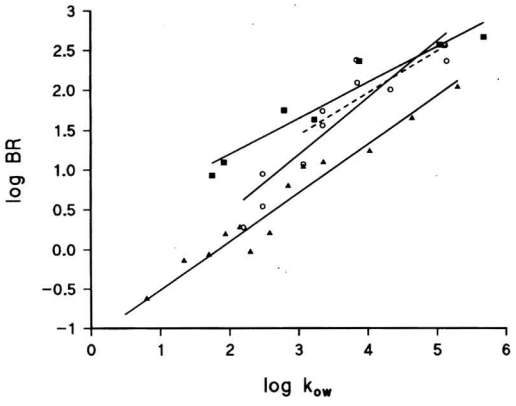

Fig.2. Linear regression of the log BR (biological response) vs. log kow for selected phenols, which act as polar narcotics (triangles) and uncouplers of oxidative phosphorylation (squares) and for chlorophenols (open circles). Dashed line indicates regression line when monochlorophenols are omitted from model of chlorophenols (original data from Schultz 1987, modified by data obtained from Schultz et al. 1986, Cajina-Quazada & Schultz 1990 and Bryant & Schultz 1994).

However, when the results from several studies are combined (Schultz et al. 1986, Schultz 1987, Cajina-Quazada & Schultz 1990, Bryant & Schultz 1994), eleven chlorophenols could be modelled. As a result, chlorophenols (mono to pentachlorophenol) were modelled by:

$$\text{eq. 3. } \log BR = 0.7144\,(\log k_{ow}) - 0.9557;\ n = 10,\ r^2 = 0.896,\ s = 0.324.$$

When the monochlorophenols were omitted, the regression yielded:

$$\text{eq. 4. } \log BR = 0.5269\,(\log k_{ow}) - 0.1442;\ n = 7\ r^2 = 0.689,\ s = 0.304,$$

which models chlorophenols with two or more chlorine substituents that uncouple oxidative phosphorylation (Fig. 2).

5. Conclusions

The toxicity of chlorophenols with two or more chlorine substituents seems clearly related to the uncoupling of the oxidative phosphorylation, and the addition of a chloro group increases the uncoupling character of the chlorophenol. Monochlorinated phenols seem to be poor uncouplers and act as polar narcotics. The uncoupling properties of chlorophenols were demonstrated through *in vitro* respiratory studies or modelling the effects of clorophenols on uncoupling activities. The QSAR equations included descriptors for steric, electronic and acidic properties. Sublethal physiological energetics responses of the uncoupling of oxidative phosphorylation are easily recognizable, at least with higher substituted phenols, and are indicative of the chemical's mode of toxic action. Little evidence was found to support the hypothesis that di and trichlorophenols act as polar narcotics, although polar narcotic QSARs seem to predict the toxicity of lower substituted chlorophenols. Structure-activity correlations based on in vivo experiments are not very useful as such because it is difficult, if not impossible, to find out whether differences between various compounds are due to kinetics factors affecting the concentration at the site of action or real differences in their mode of toxic action. Consequently, mode of toxic action is very difficult to derive reliably from ecotoxicological data.

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