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# TOXICOLOGY OF SYNTHETIC PYRETHROIDS IN AQUATIC ORGANISMS: AN OVERVIEW

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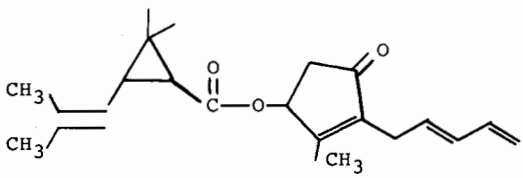
**Abstract**—The aquatic toxicology of the photostable synthetic pyrethroid insecticides as it affects two important groups of susceptible organisms—fish and aquatic insects—is discussed. The sensitivity of these aquatic species to the pyrethroids is dependent on several factors, including toxicokinetics, target site (nervous system), sensitivity and possible secondary mechanisms of action, as well as chemical and physical properties of the aquatic medium that influence toxicity and bioavailability. Uptake rates and routes of fenvalerate greatly affected the toxicity of fenvalerate to mosquito larvae. LD50 values were determined for cuticular and dietary exposure routes by utilizing radiolabeled fenvalerate at the respective LC50 concentrations in the two media (water and food). Fenvalerate was sixfold more toxic to mosquito larvae by the cuticular route. Technical fenvalerate was more toxic to larvae than was the emulsifiable concentrate formulation. Addition of different concentrations of humic acid to the water reduced the toxicity to the larvae. Review and analysis of relevant literature are integrated into a discussion of the principles and details of aquatic toxicology of the pyrethroids.

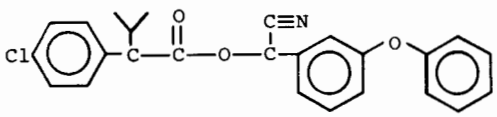
**Keywords**—Synthetic pyrethroids Fish Mosquito larvae Uptake Toxicokinetics

## INTRODUCTION

In recent years, synthetic pyrethroid insecticides have been developed for major uses in agriculture and public health. The current commercial products were evolved from the natural pyrethrins, which possess high insecticidal potency, low mammalian toxicity and very short persistence. The modern synthetic pyrethroids retain some of the attributes of the natural products but have been designed to provide enhanced residual activity through greater photostability. They are also more resistant to chemical and biological degradation by virtue of changes at several sites in the molecule (Fig. 1): (a) the substituted pentenone ring is replaced (typically) by a phenoxybenzyl group, (b) the isobutene group is replaced by a halogenated vi-




pyrethrin I




fenvalerate

Fig. 1. Structures of a natural pyrethrin and fenvalerate, a photostable synthetic pyrethroid.

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nyl group or halogenated phenyl ring and (c) a cyano group is substituted on the benzylic carbon. The first two of these changes increase the photostability of the molecule and reduce its susceptibility to oxidation. The third change stabilizes the ester bond against hydrolysis.

The water solubilities of the photostable synthetic pyrethroids are in the range of 1 to 10 μg/L, and the octanol/water partition coefficients range from $10^4$ to $10^7$.

These halogenated, lipophilic and photostable compounds are exceptionally active against many insects yet still are relatively safe to mammals and birds. However, they are extremely toxic to certain aquatic and marine groups, including fish. This article presents an overview of the aquatic toxicology of the synthetic pyrethroids, including their toxicity and toxicokinetics, summarizing research from this and other laboratories and presenting new data on bioavailability and uptake rates and routes.

## MATERIALS AND METHODS

Technical-grade fenvalerate was provided by the Shell Development Company (Modesto, CA). Purification by silica gel column chromatography resulted in greater than 98% purity. $^3$H-[ring]-Fenvalerate was prepared by Amersham Corporation (Arlington Heights, IL).

The bluegill and fathead minnows used in most studies were obtained from the Kloubec Fish Farm or the U.S. Environmental Protection Agency Environmental Research Laboratory in Duluth, Minnesota. They were acclimated to the laboratory conditions for 10 d before use and were not fed for 24 h before or during exposures. The details of the exposure conditions are provided elsewhere [1,2]. All exposure concentrations were monitored by GC, as were residue levels in fish [3]. LC50 values were calculated using the trimmed Spearman–Karber method [4].

The mosquito larvae were reared from eggs obtained from a laboratory strain of *Culex pipiens pipiens* in distilled water; they were fed ground Tetramin fish food.

The mosquito larval LC50 tests were conducted with distilled water, 50 ml per assay, in 100-ml Pyrex beakers. Twenty second-instar larvae were transferred to each beaker, and the appropriate concentration of fenvalerate insecticide was added in 0.5 ml acetone. Mortality was recorded at 24 h. Insects that did not respond to tapping on the container were judged to be dead.

### *Mosquito larva route-of-entry studies*

Fourth-instar larvae of *C. p. pipiens* were exposed to [$^3$H]fenvalerate by two routes of entry, cuticular and oral. The concentrations used were the LC50 levels: 0.00045 mg/L in the water and 11 mg/kg in the diet. The $^3$H-[ring]-fenvalerate utilized had a specific activity of 3.8 Ci/mmol. For the cuticular exposure, the insecticide was added, in 1 ml acetone solution, to the 100 ml distilled water containing the larvae. Three replicates of 10 larvae each were collected, rinsed and combusted at each of nine time intervals: 0.5, 1, 1.5, 2, 2.5, 3, 16, 24 and 48 h. No mortality occurred in the three control groups. For the oral exposure route, Tetramin fish food was ground by mortar and pestle, treated with [$^3$H]fenvalerate in 1 ml acetone, dried quickly, rinsed with distilled water to remove any freely available fenvalerate and dried again before being fed to the larvae. Three replicates were used for each of the nine time intervals after treatment with the [$^3$H]fenvalerate food. Larvae were starved for 4 h before exposures. The individual samples of 10 larvae were allowed to blot dry on filter paper, were transferred to ashless paper and then were combusted in a Packard Tri-Carb sample oxidizer. The radioactivity was quantified using an LKB Rack-Beta liquid scintillation counter and was expressed as nanograms fenvalerate per larva.

## RESULTS AND DISCUSSION

### *Toxicity*

Synthetic pyrethroids are generally accepted to be relatively safe for mammalian and avian species. Oral LD50s for rats and mice range from 100 to 2,000 mg/kg [5,6]. Acute oral toxicities of greater than 4,000 have been reported for three pyrethroids in three species of birds [3,7,8], indicating that avian species are also highly resistant to pyrethroid intoxication.

Aquatic species seem to be much more sensitive than terrestrial vertebrates to pyrethroids. Permethrin was found to have an i.p. LD50 of 14 mg/kg to rainbow trout [9], and fenvalerate had an i.p. LD50 of 0.7 mg/kg in bluegill [2]. A more environmentally sound comparison would be between the terrestrial oral LD50 and the aquatic LC50 because these are the most likely routes of natural exposure. Most pyrethroids are toxic at extremely low concentrations, with LC50s of generally less than 10 μg/L. Cypermethrin was found to have LC50s of 1.2, 0.9 and 0.5 μg/L in brown trout, carp and

rainbow trout, respectively [10]. In studies using fathead minnows, LC50s were determined to be 0.2 μg/L for flucythrinate [11], 1.1 μg/L for fenvalerate [2] and 16 μg/L for permethrin [12].

Pyrethroids are also very toxic to aquatic insects and crustaceans, with most LC50 values being well below 1 μg/L. When a variety of mosquito and midge larvae and pupae were tested, 24-h LC50 values for deltamethrin, cypermethrin, fenvalerate and permethrin ranged from 0.02 to 13 μg/L [13]. Deltamethrin and cypermethrin have 96-h LC50s of about 0.01 μg/L in lobster (*Homarus americus*) and shrimp (*Crangon septemspinosa*), with fenvalerate being four to five times less toxic [14,15].

In addition to acute toxicity, many pyrethroids may have potentially deleterious effects at sublethal levels. Anderson [16] noted behavioral changes within hours of exposure in several aquatic invertebrates, resulting in a cessation of feeding and insect drift.

Sublethal and chronic studies of pyrethroids have demonstrated that fish are very susceptible to growth effects, and low survivability has been noted in several species, especially affecting the early life stages [11,17,18]. Similarly, no observable effect levels are quite low for chronic and sublethal impact on aquatic invertebrates such as daphnids, copepods and chironomids [19,20]. Many deleterious effects occur at concentrations below 1 μg/L, including effects on reproduction, growth and behavior [21,22].

In contrast to insects and crustaceans, molluscs are relatively tolerant of pyrethroids, with acute effect levels not observed at water solubility [11,16]. The reasons for these differences in species susceptibilities are not clear.

*Toxicity of isomers*

Synthetic pyrethroids generally are a mixture of stereoisomers. Technical permethrin, for example, is a mixture of both the cis and trans isomers. Fenvalerate, which contains chiral centers at the 2C and αC, is a mixture of four stereoisomers. Stereochemical structure affects the lethality of both these compounds to insects and mammals [23,24]. The more potent esters of fenvalerate are those with an S configuration in the acid moiety (i.e., the 2S,RS esters). The isomer with the greatest toxicity to two insects (2.7 to 3.5 times more toxic than the technical material) has an S configuration in the alcohol moiety as well (i.e., the 2S,αS isomer). Esters with an R configuration in the acid moiety have oral LD50s to mice in excess of 5,000 mg/kg and are about 100 times less toxic to insects than the technical material [23].

Stereochemical structure is also an important factor in pyrethroid toxicity to aquatic species. Miyamoto [25] observed the (−)-isomers of permethrin to be much less toxic to killifish than the (+)-isomers, whereas Zitko et al. [14] reported 1*R-cis* permethrin to be more toxic than technical permethrin to salmon. Virtually all the toxicity of fenvalerate to fathead minnows and bluegill was attributed to the 2S,αS isomer, while isomers containing an R configuration in the acid moiety were found to be essentially nontoxic [2]. Differences in stereochemical structure significantly altered the toxicity of fenvalerate, cypermethrin and fenpropanate to mosquito larvae [23].

Although fenvalerate isomers remained resolved throughout an aerobic soil study [26], spontaneous racemization has been found to occur in water, ethanol, methanol, DMF and DMSO [2,27].

Although some optical integrity may be retained, racemization would reduce most pure isomer preparations to a mixture of configurations. For this reason, i.p., i.m. or i.v. exposure routes are preferred because the optical purity of the toxicant delivered can be more closely controlled. Spontaneous racemization may still occur in vivo, however. It was determined that whole-body residues of fenvalerate at mortality were similar in both i.p. and aqueous exposures of fathead minnows and bluegills [2].

*Temperature effects on toxicity*

Pyrethroid insecticides are more toxic at lower temperatures to both insects [28–30] and trout [31]. This phenomenon is known as a negative temperature coefficient and is relatively uncommon. Poikilotherm metabolism is reduced at lower temperatures, so the pyrethroid effect may be a temperature-dependent interaction at the site of action. Nerves may be more sensitive to the effects of pyrethroid-induced toxicity, although toxicokinetic factors such as uptake, distribution and detoxification may contribute to increases in toxicity at lower temperatures. Other environmental factors that can alter the aquatic ecosystem can also stress organisms there, e.g., pH, oxygen concentration, sunlight, nutrient input and turbidity.

*Hardness and salinity effects on toxicity*

Differences in water hardness and salinity have been shown to alter the toxicity of pyrethroids to

aquatic species [32,33]. Because one mode of action is involved with ionic regulation by ATPases, it may be that, in addition to direct nerve toxicity, pyrethroids cause an osmoregulatory imbalance. Ionic homeostasis in fish is largely regulated by the transepithelial potential of the gill and by active transport processes involving enzymes, such as ATPases. Monovalent and divalent ions are actively regulated by Na-K-ATPase [34] and Ca-ATPase [35], respectively. DDT has been shown to inhibit Na-K-ATPase in gill epithelia [36]. Squid axon Ca-ATPase have been inhibited by both DDT and pyrethroids [37]. Ionic characteristics of the water, such as hardness and salinity, have been demonstrated to influence the toxicity of fenvalerate to bluegill [38]. Ion balance in the urine has also been observed to be affected by fenvalerate [39]. Fenvalerate was the least toxic in very soft water. This secondary osmotic stressor may help explain why aquatic species are so extremely susceptible to pyrethroid intoxication.

*Toxicokinetics*

*Uptake.* Efficient uptake of insecticides across the gills and into the bloodstream can result in high toxicity to fish. Water solubility and lipophilicity, parameters generally accepted to influence uptake, have been correlated with the toxicity of insecticides [17], including pyrethroids [18]. Fenvalerate, because of its unusually high lipophilicity (log $P$ of 7.2), is taken up at only a 30% efficiency per pass through the gills of a rainbow trout, compared with twice that rate for many other organic chemicals, and uptake is not dose-dependent [1]. This inverse relation between gill uptake efficiency and lipophilicity noted by McKim et al. [40] tends to limit the amount of highly lipophilic insecticide entering the bloodstream, thereby indicating that efficient uptake is not a factor in the extreme toxicity of fenvalerate to fish.

*Distribution in the body.* The LD50 values for fish exposed to pyrethroids generally are 10 to 1,000 times less than the corresponding values for mammals and birds [41]. Owing to their high lipophilicity, both fenvalerate [1] and permethrin [9] were found to concentrate in the fat of fish. Bradbury et al. [1] also found that more fenvalerate was associated with the packed-cell fraction of the blood than with the plasma. Typically, insecticides are transported in the plasma fraction of the blood.

Synthetic pyrethroid bioconcentration factors (BCFs) determined at the end of chronic studies were generally in the range of several thousand. Mean BCFs for fenvalerate [42], permethrin and flucythrinate [11] of 3,200, 2,800 and 4,000, respectively, were reported for the fathead minnow. BCFs of 480 and 570 have been reported for permethrin and fenvalerate, respectively, in the sheepshead minnow [43]; flucythrinate was not detected in fish that survived exposure. The BCFs for these compounds are substantially lower than would be expected from their high octanol/water partition coefficients (log $P$). The relatively low BCFs noted for pyrethroids may be due to a number of factors, including chemical instability, inability to cross the gill/blood barrier or the ability of the fish to effectively metabolize or eliminate these compounds.

Concentrations of fenvalerate in the liver at death in rainbow trout were 10-fold higher than those measured in the brain and the remaining carcass [39]. This degree of accumulation in the liver was not observed in rainbow trout during sublethal exposures [1] and may reflect changes in kinetics with dose rate. The fenvalerate body burden associated with mortality in rainbow trout was 0.25 mg/kg and was 1.0 mg/kg in fathead minnows [39,44].

A brain residue of approximately 0.15 mg/kg was found to be associated with 100% mortality in rainbow trout for fenvalerate [35], 2 mg/kg for permethrin [45] and 0.2 mg/kg for cypermethrin [46]. These are 3- to 18-fold less than the lethal brain residues in mice for permethrin [45], for cypermethrin in mice and Japanese quail (*Coturnix coturnix*) [46], and for fenvalerate in bobwhite quail (*Colinus virginianus*) [3]. The difference in lethal brain concentrations between species for fenvalerate, permethrin and cypermethrin suggests that the specific mode of action for pyrethroids may be an important factor in aquatic species sensitivity.

*Elimination.* Warm-blooded vertebrates have been shown to be very efficient in eliminating synthetic pyrethroids [3,47]. Aquatic species, however, do not readily eliminate pyrethroids. Half-lives for elimination in trout are well in excess of 24 h, whereas half-lives in mammals and birds are in the range of 6 to 12 h. The carcass and bile of rainbow trout exposed to fenvalerate were found to contain 80 to 90% and 10 to 20% of the gill-absorbed dose, respectively, after 48 h of depuration [1]. No fenvalerate was eliminated via the gills and urine, while feces and blood each contained less than 2% of the dose. These findings are generally similar to those found for permethrin after aqueous and i.p. exposures of rainbow trout [9]. Analysis of biliary metabolites for fenvalerate [1], permethrin [9] and

cypermethrin [46] yielded similar results in that the glucuronide of the 4′-hydroxymetabolite was the only product recovered. This relatively slow rate of pyrethroid elimination may be partly responsible for the sensitivity of this salmonid to these insecticides.

*Biotransformations.* In rats and mice, 80% of the orally administered doses of fenvalerate were eliminated in the excrement as a variety of oxidative and hydrolytic products [48,49]. In trout exposed to fenvalerate and permethrin, however, little or no esterase activity or ester hydrolysis has been observed. The only oxidative step that is noted is at the 4′ position, followed by glucuronidation [1,9]. It was also established that microsomal oxidation of *trans*-permethrin was 35 times slower in trout than in mice [45]. With cypermethrin, minor levels of ester hydrolysis products were recovered from exposed trout along with the glucuronide of 4′-hydroxycypermethrin, but there was still an overall deficiency in enzymatic activity as compared with levels in mouse and Japanese quail [46].

When fenvalerate was studied in a model aquatic ecosystem [50], metabolism of the S-acid isomer in four aquatic species proceeded through hydroxylation at the phenoxy group, hydrolysis of the CN group, and cleavage of the ester linkage. None of these metabolites tended to accumulate to high levels in the organisms, however. In addition to metabolic reactions, it has been suggested that effects on respiratory surface and ion regulation may be associated with the mechanisms of pyrethroid action in fish [39,51]. Although mammals and birds seem to metabolize and excrete synthetic pyrethroids readily via a variety of pathways, aquatic species are less efficient in detoxifying these compounds and are therefore highly susceptible to them.

*Bioavailability.* The sensitivity of aquatic species to pyrethroid toxicity may be altered by a variety of environmental conditions, including temperature and the presence of suspended or dissolved solids. It was demonstrated that fenvalerate 96-h LC50s were 40 times higher in channel catfish when the toxicant was first applied to soil particles than when introduced directly into clean water [52]. A concentration of cypermethrin that caused 100% mortality in rainbow trout in microfiltered water did not cause mortality in trout when pond water containing 14.5 mg/L suspended solids was employed [53]. The bioavailability of insecticides to *Daphnia magna* has been shown to be reduced in the presence of suspended solids [54], and *Chironomus tentans* larvae accumulated significantly more permethrin when allowed to enter sediment than when held in water above the sediment [55]. Pyrethroids, being highly hydrophobic, are adsorbed to particulate matter present in the test system, thereby making them unavailable to aquatic species in the water column. The dynamics of hydrophobic toxicants in sediments, although previously not well understood, now is a rapidly expanding field of study.

In addition to suspended solids, dissolved material can also affect toxicity. One commonly found dissolved organic material, humic acid, is a product of the degradation of organic carbon. When mosquito larvae were exposed to fenvalerate in the presence of different amounts of humic acid, significant differences in toxicity were noted (Table 1). The fenvalerate was six times less toxic to mosquito larvae in water with 50 mg/L humic acid than in clean water.

### *Effect of route of entry on toxicity*

Mosquito larvae are exposed to lipophilic xenobiotics by two routes of entry, cuticular and oral. The pyrethroid fenvalerate can enter the body of the larva by penetration through the cuticle after contact with the chemical in the water or by ingestion after adsorption to food particles in the water. A comparison of the potency of fenvalerate by the two routes of entry was achieved by first determining LC50 values in the water for the cuticular route and in the diet for the oral route. In a second experiment, the larvae were exposed to radiolabeled fenvalerate by the two routes at the respective LC50 concentrations to determine the quantities of the insecticide actually taken up by the organism at those concentrations in the exposure media.

The cuticular-exposure 24-h LC50 to fourth-instar *Culex pipiens pipiens* larvae was 0.00045 mg/L

Table 1. Toxicity of fenvalerate to second-instar mosquito larvae in different concentrations of humic acid

| Humic acid (mg/L) | 24-h LC50[a] (ng/ml) |
|---|---|
| 0.00 | 0.26 ± 0.03 |
| 0.05 | 0.43 ± 0.04 |
| 0.50 | 0.51 ± 0.05 |
| 5.00 | 0.49 ± 0.04 |
| 50.0 | 1.61 ± 0.14 |

[a] ±95% C.I.

in the water. The oral 24-h LC50 for fenvalerate was 11 mg/kg in the diet. The uptake studies using [$^3$H]fenvalerate at the LC50 concentrations in the water and in the food revealed a mean body burden of 0.2 ng/insect for the cuticular-exposure groups through the 48-h trial (Fig. 2) and a higher concentration (up to 1.2 ng/insect) for those feeding on fenvalerate-treated food. The two peak concentration times in Figure 3 reflect the feeding behavior of the mosquito larvae in the dietary-exposure experiment. After feeding for the first hour and attaining a high concentration of fenvalerate in their bodies, the larvae became restless and stopped feeding. The same irritant or feeding-deterrent action of synthetic pyrethroids has been documented for other species of insects [56–59]. By 16 h, the larvae had recovered and resumed feeding, which resulted in the high residue levels in their bodies. If the peak concentration of 1.2 ng/insect is taken as the requisite internal toxic dose for an LD50 response at the dietary LC50 exposure level and the 0.2 ng/insect dose for fenvalerate by a cuticular route of entry, fenvalerate was approximately six times more toxic to mosquito larvae by the cuticular

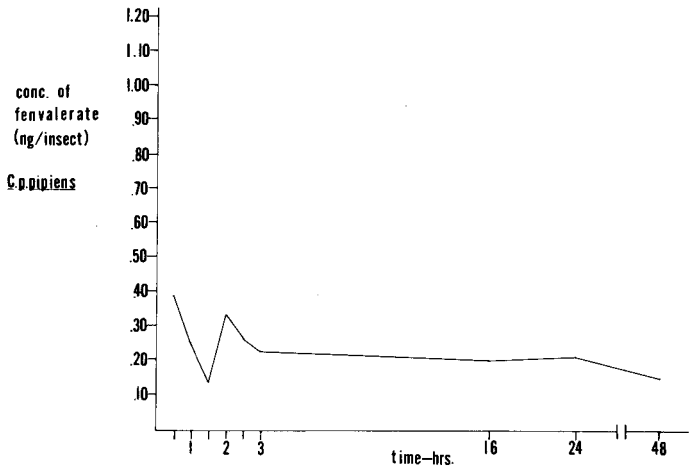


Fig. 2. Uptake of [$^3$H]fenvalerate from water by the mosquito larva *Culex pipiens pipiens* at the LC50 concentration in water.

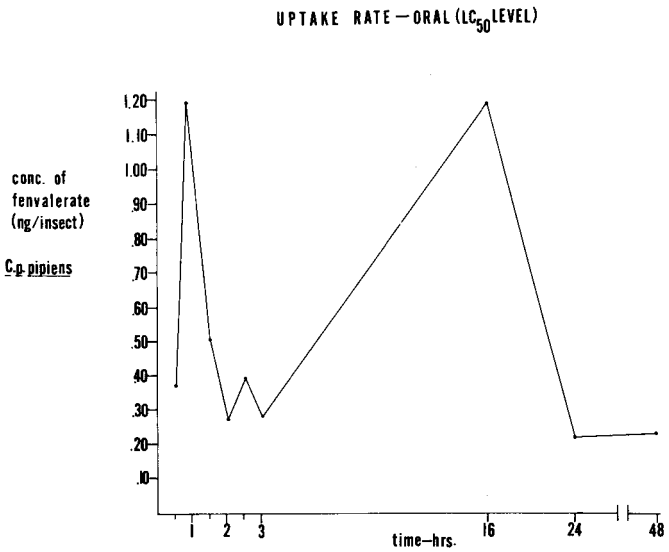


Fig. 3. Uptake of [$^3$H]fenvalerate in food ingested by the mosquito larva *Culex pipiens pipiens* at the LC50 concentration in the diet.

exposure. The lipophilic compound adsorbs quickly to the aquatic dipteran larvae and readily penetrates into the cuticle, while much of the ingested insecticide may remain biologically unavailable in the digestive tract of the immature mosquitoes.

Emulsifiers have been shown to influence the toxicity of pyrethroid insecticides to fish [41], although the magnitude of the effect is variable. Mosquito larvae were 67 times more susceptible to technical fenvalerate (24-h LC50, 0.00045 mg/L; 95% C.I., 0.00040–0.00049) than to the emulsifiable concentrate formulation Pydrin (24-h LC50, 0.030 mg/L; 95% C.I., 0.026–0.033) when corrected for active ingredient. The emulsifier acted to keep more of the toxicant in solution while addition of the pure technical material to the water resulted in rapid adsorption to organic matter (i.e., waxy cuticle of the mosquito larvae) in the clean-water test.

In summary, the photostable synthetic pyrethroid insecticides are innately quite toxic to many species of fish and aquatic arthropods, but many factors influence the degree of hazard that these chemicals present. Toxic mechanisms, isomer constituents, metabolism and bioavailability all affect selectivity and environmental impact. As new, more highly halogenated compounds are developed, there must be concern about environmental fate and effects and an understanding of potential impact, based on the physical, chemical and biological properties of the new products.

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