Investigation of natural and synthetic cyanohydrins, insecticidal properties, and their metabolism

by

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ABSTRACT

Many of the commercial fumigants, methyl bromide, chloropicrin, dichlorovos, have environmental problems, and some of them will be phased out. New alternative fumigants need to be developed for safety, biodegradability and selectivity. This research investigated fumigation toxicities of natural and synthetic cyanohydrins against stored-product pests and house fly. Using fumigation toxicity of cyanohydrins to the house fly and the lesser grain borer, quantitative structure-activity relationships (QSAR) were determined. For evaluating what components kill the insects, mode of action of cyanohydrins in insects were also conducted.

Most natural and synthetic cyanohydrins were as effective as or more effective than commercial fumigants against stored-product pests and the house fly. Log P, polarizability and molar refractivity, which are classical parameters for explaining toxicity, were well correlated with the fumigation toxicity of cyanohydrins against the house fly and to a lesser degree for the lesser grain borer. Small quantities of cyanohydrins were detected in the headspace of the experimental chamber and in insects. The total cyanide ion in cyanohydrin-exposed insects was less than in hydrogen cyanide-exposed insects, but some cyanide ion was released *in vivo* to be toxic to the cyanohydrins-exposed insects.

As a result of this research, it seems feasible that natural and synthetic chemicals might be used as alternative fumigants because of their insecticidal activity against stored-product pests and the house fly. Risk assessments, however, should be done to evaluate mammalian toxicity, environmental safety and human health. The QSAR study explained the interactions between the structures of natural and synthetic cyanohydrins and their

biological activities. Finally, this research supports the assumption that naturally occurring cyanohydrins can be degraded to release free cyanide ion *in vivo*, confirming that those cyanogenic compounds kill insects.

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CHAPTER 1. CYANOGENIC GLYCOSIDES: ALTERNATIVE INSECTICIDES?

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Dong-Sik Park and Joel R. Coats

Introduction

Agrochemicals (insecticides, herbicides, and fungicides, etc.) are used for safeguarding the food supply and decreasing pest populations. It is well known that the large-scale use and repeated application of synthetic insecticides, chlorinated hydrocarbon, organophosphorus esters, carbamates, and synthetic pyrethroids, have caused widespread concern regarding environmental problems (air, water and soil), increased resistance, as well as serious acute and chronic toxicity to non-target organisms, sometimes including humans. In addition, some of their metabolites can also be of concern as contaminants in groundwater or as carcinogens or mutagens. One of the types of insecticides used to eliminate pests is the fumigant, which is categorized by its delivery mechanism, i.e., route of exposure. For their fumigation activity, fumigants should be volatile, so they mainly have low molecular weight and are nonpolar, frequently containing chlorine, bromine, or phosphorus. However, several current leading commercial fumigants, methyl bromide, chloropicrin, and dichlorvos, are considered to be contaminants in the environment. For that reason, those commercial fumigants are becoming severely restricted or will be phased out. Those risks and the need for new types of insecticides led us to consider possible reduced-risk insecticides.

Rapid biodegradability in the environment, safety to non-target organisms, and selectivity are considered as good properties of new alternative pesticides. One possible alternative is to use naturally-based pesticides and their close derivatives or analogs. Since the time of the ancient Romans, extracts of plants have been used as insecticides.

Phytochemicals are defined as materials that plants make for growth, reproduction and defense from plant feeding animals. Numerous plants produce metabolites for physiological development, namely, growth and reproduction, called primary metabolites such as carbohydrates, proteins, fats, hormones, *etc.* They also make secondary metabolites which are less abundant or derived from primary metabolites in the plant and have a role in defense against herbivores, pests and pathogens. The secondary plant metabolites can function as feeding attractants, repellents, feeding stimulants, feeding deterrents, oviposition stimulants and deterrents, and toxicants. (Hedin, 1991) Due to their insecticidal characteristics, we have been using plant secondary metabolites as insecticides for many years, for example, pyrethrins extracted from pyrethrum (*Chrysanthemum cinerariaefolium*) flower heads, nicotinoids (*Nicotiana*), rotenonoids isolated from Leguminosae (*Derris*) and terpenoids (mints, pine, and cedar) (Adityachaudhury, 1985; Klocke, 1987; Benner, 1993). Still, many higher plant species have not been surveyed and exploited for insecticidal activity of their secondary metabolites. Discovering and developing plant-based insecticides is a major concern for finding new insecticides in the future.

This overview article will focus on the characteristics and role of cyanogenic glycosides in plants, and their interaction with insects and will cover some aspects of cyanogenic glycosides as alternative insecticides.

Distribution and characteristics of cyanogenic glycosides

Because the cyanogenic glycosides are not ubiquitous in nature, they must be classified as secondary plant products, providing defense mechanisms (allelopathy). Cyanogenic glycosides are polar and water soluble compounds, and are one group of the potentially toxic constituents which is found in some root and seed crops. Those plant

species include bitter almonds (*Prunus amygdalus*), apricot (*Prunus armeniaca*), and cherry (*Prunus avium*), all of which contain amygdalin (a source of HCN); sorghum (*Sorghum bicolor*), which contains dhurrin; cassava (*Manihot esculenta*), lima beans (*Phaseolus lunatus*), and flax (*Linum usitatissimum*), which contain linamarin; and many other vascular plant groups. These plants are grown and used for starch, protein, oil or fiber sources, and as spices or crude drugs. Figure 1 shows the three major cyanogens in plants.

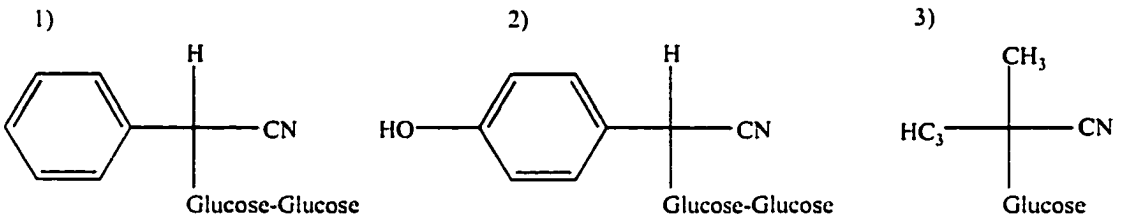

Figure 1. Three major cyanogens in plants (1: amygdalin, 2: dhurrin, 3: linamarin)

The cyanogenic glycosides are biosynthesized by the plants from aromatic or branched-chain amino acids, namely, valine, isoleucine, leucine, phenylalanine and tyrosine. Cyanogenic glycosides can be defined as glycosides of α-hydroxynitriles (cyanohydrins); the cyanohydrins are called aglycones, meaning that the sugar(s) have been hydrolyzed off the glycoside leaving only the cyanohydrin. The glucose with a β-configuration of glycosidic linkage is the sugar which is directly attached to the α-hydroxynitriles (cyanohydrins) of most cyanogenic glycosides (Seigler, 1992; Nahrstedt, 1993). These substances are not themselves toxic; however, the formation of free HCN, a process called cyanogenesis, is associated with a cyanogenic glycoside that is hydrolysed by a β-glycosidase to give a hydroxynitrile, which then decomposes to a carbonyl compound and HCN when tissues of

the plants are crushed or destroyed by animals feeding on them. Their general structure and metabolism is shown in Figure 2.

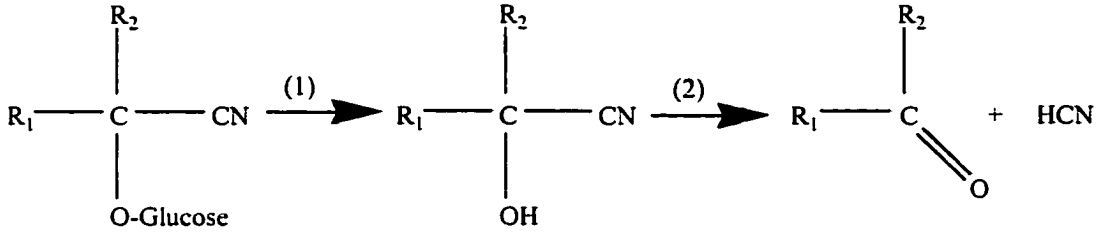

Figure 2. The structure of cyanogenic glycosides, and their metabolism to release hydrogen cyanide (HCN); (1) β-glycosidase, (2) hydroxynitrile lyase.

The pH and temperature influence the reactions that yield carbonyl components and hydrogen cyanide (Eyjolfsson, 1970; Conn, 1981; Nahrstedt, 1993). At an alkaline pH and temperatures in excess of 60 °C, cyanogenesis is faster. The chiral (asymmetric) cyanohydrin carbon in cyanogenic glycosides can be epimerized by dilute base; there are two types of epimers, (R)- and (S)-, in many series of compounds (Eyjolfsson, 1970; Ettlinger et al., 1977; Conn, 1979; Seigler, 1992).

Role of cyanogenic glycosides

Many scientists have already mentioned the role of cyanogenic glycosides in plants (Conn, 1981; Poulton, 1990; Vetter, 2000). In their physiological role, it is clear that cyanogenic glycosides in a plant are related to the production of HCN which is toxic to herbivores; therefore, the plant can be protected from the attack. Several studies have shown that cyanogenic glycosides can act as either feeding deterrents or phagostimulants (Brattsen, et al. 1983; Ellsbury, et al. 1992; Albom, et al. 1992; Belloti, et al. 1993). In fact, cyanogenesis is not confined to plants. For example, millipedes produce cyanoglycosides (Duffey, 1981), and a cyanogenic glycoside is released by larvae of *Zygaena* spp. (Burnet

moth) when they are attacked. This secretion by arthropods is evidence in support of the function of cyanogenic glycosides in plants. Robinson (1930) reviewed the early ideas, which included the cyanogens being nitrogen reserves and precursors for protein synthesis, excretory waste products or protective substances. Consumption of foods containing cyanogenic glycosides by humans has very rarely resulted in cyanide poisoning. However, major livestock losses have occurred due to their consuming cyanogenic glycosides. Cattle and other ruminants are more sensitive to these cyanogenic glycosides because the near-neutral pH in their rumen favors the release of HCN, compared to the acidic stomachs in other mammals. Several research papers and reviews already provide a look at the interaction between cyanogenic glycosides and insects (Jones, 1962, 1966, 1971, 1988; Crawford-Sidebotham, 1972; Ennos, 1981; Compton, 1985; Nahrstedt, 1985; Hruska, 1988). Their research has helped explain the biochemical role of cyanogenic glycosides against herbivores.

Mode of action

Hydrogen cyanide (HCN) is a toxic substance that generates CN^-, and this causes the inhibition of cytochrome c oxidase and other respiratory enzymes. The primary mode of action of cyanide is based on its affinity for the ferric heme form of cytochrome a_3 (cytochrome c oxidase) in the electron transport system (Buck, et al., 1976; Klaassen, 1996). This reaction forms a relatively stable cytochrome c oxidase-CN complex in the mitochondria. When iron is maintained in the trivalent state, electron transport along the cytochrome chain is stopped, and the chain of cellular respiration is brought to a halt. As a result, hemoglobin cannot release its oxygen to the electron transport system.

Alternative insecticides?

As we mentioned above, cyanogenic glycosides can be considered as possible insect control materials by means of their distribution in many plants, their role, and interaction with insects. Previous research (Peterson, 2000a) in our laboratory found that several natural and synthetic cyanohydrins were effective against insects as fumigants. Also, they (Peterson, 2000b) found that topical application of several synthetic cyanohydrin compounds were effective against house flies and mosquitoes. For that reason, we synthesized natural cyanohydrins and some derivatives in the laboratory to test the insecticidal activity against the house fly (*Musca domestica*), and several stored-grain pests (lesser grain borer, *Rhyzopertha dominica;* maize weevil, *Sitophilus zeamais;* sawtoothed grain beetle, *Oryzaephilus surinamensis;* red flour beetle, *Tribolium castaneum*) and to determine which chemical structure is the best one to explain the toxicity, using quantitative structure-activity relationships (QSAR). The structures of 4 representative natural and synthetic cyanohydrins tested in our study are shown in Figure 1. Other compounds and data are available in Park 2002a and Park 2002b. In addition, various concentrations of 1-cyano-1-hydroxy-2-propene acetate (acetoxybutenenitrile) were tested as soil fumigants. Specifically, anti-bacterial and anti-fungal activity, as well as inhibition of weed-seed germination, were measured in treated soils.

As a result, we found that natural and synthetic cyanohydrins are quite effective against stored-product pests (Park, 2002a). Fumigation LC_{50} values of 4 volatile natural and synthetic cyanohydrins against stored-product pest and house fly are shown in Table 1. Three parameters (log P, polarizability, and molar refractivity) are related to fumigation toxicity to the house fly (Park, 2002b). These parameters explain the chemical properties such as lipid solubility, London dispersive forces, and molar volume, respectively. The R^2

values of three parameters and molecular descriptors for cyanohydrins are shown in Table 2 and 3. This result means that the degree of adverse effects on the insects depend on the chemical properties. 1-Cyano-1-hydroxy-2-propene acetate reduced the total soil bacterial and fungal counts significantly, and was effective in inhibiting the germination of weed seeds in soil. Twelve different cyanohydrins or their derivatives were also shown to be nematicidial in laboratory trials and in soil. The possibility of a cyanohydrin (or ester of one) serving as a soil fumigants to replace methyl bromide is intriguing, since some can effectively kill insects, nematodes, weeds, fungi, and bacteria. These results tell us that cyanohydrins might be a useful alternative insecticide.

With laboratory tests and soil tests, we have only limited data for fumigation toxicity and biological activity as a soil fumigant for natural and synthetic cyanohydrins. Mammalian toxicity testing has not been conducted on any of the cyanohydrins discussed here. For a potential commercial fumigant aimed at controlling pests in storage bin, buildings, ships, stored products, soil, on food, or in any closed areas, risk assessments should be done for environmental problems, wildlife effects, and human health. Those include acute and chronic toxicity to mammals, birds, or movement to ground water, and persistence in soil. Also, testing should be done for carcinogenicity, mutagenicity or teratogenecity in mammals.

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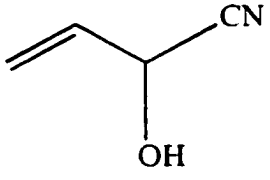
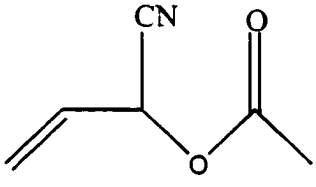
Naturally occurring cyanohydrin

dimethyl ketone cyanohydrin
(DMK)

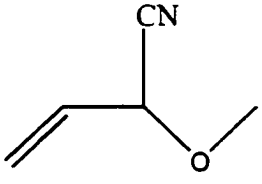
Synthetic cyanohydrins



1-cyano-1-hydroxy-2-propene
(CHP)



CHP-acetate (CHP-ace)



CHP-methyl ether
(CHP-me)

Figure 3. Volatile natural and synthetic cyanohydrins and derivatives.

Table 1. Fumigation LC_{50} values ($\mu g \cdot ml^{-1}$) of volatile natural and synthetic cyanohydrins.

	M. domestica	95% F.L.[a]	*R. dominica*	95% F.L.[a]
Naturally occurring cyanohydrin				
DMK	0.07	0.06, 0.09	0.40	0.35, 0.46
Synthetic cyanohydrin				
CHP	0.056	0.049,0.063	0.37	0.14, 0.42
Cyanohydrin ether				
CHP-me	0.41	0.34, 0.49	0.88	0.75, 1.04
Cyanohydrin ester				
CHP-ace	0.26	0.23, 0.30	0.37	0.32, 0.45

[a] 95% fiducial limits

Table 2. The R^2 of three parameters to house fly and lesser grain borer.

Parameters	R^2	
	House fly	Lesser grain borer
Log P	0.86	0.62
Polarizability	0.79	0.53
Molar refractivity	0.80	0.40

Table 3. Molecular descriptors for cyanohydrins and derivatives.

Compounds	Log P	Polarizability	Molar refractivity
DMK	0.354	7.035	22.5
CHP	0.550	7.796	22.2
CHP-ace	0.679	8.356	31.4
CHP-me	0.828	9.33	27.0

Dissertation objectives

The overall objectives of my research were to investigate the feasibility of using natural and synthetic cyanohydrins as alternative insecticides against the house fly and the stored-product pests, lesser grain borer, red flour beetle, saw-toothed grain beetle, and maize weevil and to evaluate relationships between fumigation toxicity against the house fly and the lesser grain borer and structure of the cyanohydrins, derivatives, and analogues. In order to determine if naturally occurring cyanohydrins kill the insects through their degradation to free cyanide ion, cyanohydrin(s) and cyanide ion in the insect body were determined.

Dissertation organization

This dissertation is composed of a general introduction, three experimental chapters, and a general conclusion. The general introduction was published by the *Korean Journal of Pesticide Science*. In this paper, distribution and characteristics of cyanogenic glycosides were addressed and their role in plants and mode of action were also mentioned. The second chapter is titled "Fumigation toxicity of volatile natural and synthetic cyanohydrins to stored-product pests and activity as a soil fumigant", and it has been accepted for publication in *Pest Management Science*. This paper focused on the insecticidal properties of cyanohydrins against the house fly and several stored-product pests, and on the activity of cyanohydrin soil fumigants. The results indicate that the insecticidal properties of most cyanohydrins were as potent as commercial fumigants. Also, one cyanohydrin ester was effective as a soil fumigant. The third paper evaluates the relationships between fumigation toxicity against the house fly and the lesser grain borer and structure of cyanohydrins, derivatives, and analogues. This paper was published by *Journal of Agricultual and Food Chemistry*. These quantitative structure-activity relationships (QSAR) were developed to predict the toxicity of

cyanohydrins in the house fly and the lesser grain borer, and to help explain the differences in potency of the cyanohydrins. The fourth chapter is focused on which of the components (cyanide ion, or cyanohydrins and their derivatives) kill the insects, and it was prepared for submission to the *Journal of Pesticide Science*. This paper compares the toxicity of hydrogen cyanide to cyanohydrins against house fly and lesser grain borer, and evaluates the quantity of cyanohydrin in the headspace of the experimental chamber and in cyanohydrin-exposed house fly. In addition, cyanide ion within the insects exposed to cyanohydrins, and hydrogen cyanide were measured. The general conclusions follow the fourth chapter.

CHPATER 2. FUMIGATION TOXICITY OF VOLATILE NATURAL AND SYNTHETIC CYANOHYDRINS TO STORED-PRODUCT PESTS AND ACTIVITY AS A SOIL FUMIGANT

A paper accepted in *Pest Management Science*

Dong-Sik Park, Chris Peterson, Shaohan Zhao and Joel R. Coats

Abstract: Insecticidal fumigation toxicity of natural and synthetic cyanohydrins was evaluated with four stored-product pests: the lesser grain borer (*Rhyzopertha dominica*), the red flour beetle (*Tribolium castaneum*), the saw-toothed grain beetle (*Oryzaephilus surinamensis*), the maize weevil (*Sitophilus zeamais*) and the house fly (*Musca domestica*). The fumigation LC_{50} values were calculated by probit analysis. For house flies, all but one of the cyanohydrins tested were more potent than 1,3-dichloropropene (Telone®). Three of them were as efficacious as chloropicrin. For the lesser grain borer, all cyanohydrins tested were more insecticidal than dichloropropene, and all but one of the cyanohydrins tested were more potent than chloropicrin. Four of them were as insecticidal as dichlorvos. The acetate of 1-cyano-1-hydroxy-2-propene (CHP-ace) was also tested in soil for anti-fungal and anti-bacterial activity, and inhibition of weed seed germination. CHP-ace reduced the total soil bacterial and fungal counts significantly, and was effective in inhibiting the germination of weed seeds in soil, as well as nematicidal activity (earlier study), indicating a broad spectrum of activity as a soil fumigant.

Key words: fumigation, cyanohydrin, stored product pests, insecticide, methyl bromide, chloropicrin, dichlorvos, dichloropropene

1 INTRODUCTION

In plants, biosynthetic processes produce some substances such as primary and secondary metabolites. Primary plant metabolites are used for growth and reproduction. However, secondary plant metabolites are useful for defense from herbivores. Many food and feed plants have been shown to synthesize cyanogenic compounds which can decompose to produce hydrocyanic acid (HCN) as a main source of plant defense, which acts as a toxicant or feeding deterrent to herbivores.[1] Cassava, lima beans, peas, almonds, white clover, bamboo, and flax all produce cyanogenic compounds. Some cyanogenic plants are grown and used for starch, protein, oil or fiber sources, and as spices or crude drugs.[2] The cyanogenic glycosides are biosynthesized by the plants from aromatic or branched-chain

amino acids.[3] The biochemical system for the formation of free HCN, cyanogenesis, is associated with a cyanogenic glycoside that is hydrolysed by a β-glycosidase to produce a hydroxynitrile (cyanohydrin), which then decomposes to a carbonyl compound and HCN.[4] As a nonselective respiratory inhibitor, hydrogen cyanide (hydrocyanic acid) has been used for many years as a fumigant for insects, inhibiting cytochrome a_3 in the mitochondrial electron-transport system.[5]

Although there are many commercial fumigants such as methyl bromide, dichlorvos, chloropicrin, and phosphine, regulatory problems exist with several current fumigants. The U. S. Environmental Protection Agency (EPA) has been restricting the use of methyl bromide, an ozone depleter, and dichlorvos a suspected carcinogen.[6] As a result of their toxicity, methyl bromide, phosphine, dichloropropene, chloropicrin, and dichlorvos use is restricted to licensed commercial applicators.[7,8,9]

In our study, we conducted tests on the fumigation toxicity of several natural and synthetic cyanohydrins. This research compares how effective the volatile natural and synthetic cyanohydrins are against five species of insect pests. Their potency is also compared to some commercial fumigants. Various concentrations of 1-cyano-1-hydroxy-2-propene acetate (CHP-ace, acetoxybutenenitrile) were tested as general soil fumigants in this study. Specifically, anti-bacterial and anti-fungal activity, as well as inhibition of weed seed germination, were measured in treated soils. Methyl bromide fumigation has been performed earlier on soil in this laboratory, and the effect on microorganisms was compared. As an additional comparison, the effect of chloropicrin and 1,3-dichloropropene (active ingredient in Telone®) on weeds was also determined.

2 MATERIALS AND METHODS

2.1 Chemicals

The cyanohydrins were synthesized in the laboratory, using two types of reactions: trimethylsilyl cyanide (DMK, MEK, MVK, FDMK, and DDMK) or potassium cyanide (CHP, CPC, CPM) plus a carbonyl starting material, from the methods of Gassman *et al.*[10] Cyanohydrin esters (CHP-piv, and CHP-pro) and one ether (CHP-me) were prepared by the methods of Rice *et al.*[11] and of Ogawa *et al.*,[12] respectively. The cyclopropylmethyl ketone cyanohydrin (CPM) and cyclopropylcarboxaldehyde cyanohydrin (CPC) were synthesized using cyclopropyl methyl ketone and cyclopropanecarboxaldehyde, respectively. All natural and synthetic cyanohydrins' chemical structures were confirmed by proton nuclear magnetic resonance (NMR). CHP-ace, the acetate of 1-cyano-1-hydroxy-2-propene, also known as 2-acetoxybutenenitrile, was purchased from Aldrich (Milwaukee, WI), and dichlorvos and chloropicrin were purchased from Chem Service (West Chester, PA). The active ingredient in Telone®,1,3-dichloropropene, was purchased from TCI America (Portland, OR). The chemical structures of natural and synthetic cyanohydrins that were synthesized and tested in this study are shown in Figure 1.

2.2 Insect Bioassay

2.2.1 Fumigation of Musca domestica

Fifteen adult house flies were placed in a 25-ml jar with dry food and a moist cotton dental wick soaked with distilled water, and the jar was covered with cheesecloth, which was held in place with a rubber band. The jar was then placed in a 2.7-liter amber jar with a folded piece of 9-cm Whatman #4 filter paper. Each test compound was dissolved in corn oil, and 200µl of the appropriate concentration solution was applied to the filter paper. The 2.7-liter glass amber jar was securely capped, and mortalities were recorded after 24 hr. This

test was done in three replications. Nominal concentrations in the air in the jar were calculated using an assumption that all of the compound volatilized off the filter paper.

2.2.2 Fumigation of Stored Product Insects

Ten adult beetles of each species (*Rhyzopertha dominica*, *Tribolium castaneum*, *Oryzaephilus surinamensis*, or *Sitophilus zeamais*), with 1 g of grain for food, were placed into a glass tube (1.5 × 5 cm) fitted with a metal screen, which was secured by paraffin film on each end. This tube was suspended in a 490-ml glass mason jar with a folded piece of 9-cm Whatman #4 filter paper. Each test compound was dissolved in corn oil, and 100µl of the appropriate concentration solution was applied to the filter paper. The jar was securely capped, and mortalities were recorded after 24 hr. This test was done in three replications.

2.3 Soil fumigant activity

2.3.1 Soil bacteria and fungi study

The soil was collected from an agricultural field plot in Ames, IA. The soil type was a loam (44%, 32%, and 24%: sand, silt, and clay). The pH was 7.0, and the percentage of organic matter was 2.7%. The soil was incubated in a Percival environmental growth chamber (Percival Co., Boone, Iowa). The photoperiod was 12:12 L:D and the temperature was 24 °C ± 1 inside the chamber. The soil was incubated on a lab bench for 12 hr before six treatments were applied. The six treatments were: blank control, solvent control (0.5 ml acetone), 10 ppm of CHP-ace (0.5 ml) for a final concentration of 0.5 µg/g of soil; 100 ppm of CHP-ace (0.5 ml): 5 µg/g of soil; 1000 ppm of CHP-ace (0.5 ml): 50 µg/g of soil, and 10,000 ppm of CHP-ace (0.5 ml): 500 µg/g of soil. Each treatment had three replications. Ten grams (dry-weight) soil, at a moisture content of approximately 100% holding capacity, was put into a sterilized 260-ml French square bottle, and the six treatment solutions were applied. After the treatment, the soil was placed in the incubator for an additional 8 hr.

Next, 90 ml of sterilized phosphate buffer was then added to each bottle, and the bottles were shaken for approximately 30 min. Serial dilutions were then carried out, i.e., the solution was progressively diluted by factors of 10. Three or four replicates were used for each concentration of each compound. The active ingredient in Telone® (1,3-dichloropropene) and chloropicrin were used as the commercial standards for comparison. Telone® is known to have fumigation activity against nematodes and weeds, similar to the activity of methyl bromide[13]. A microdrop plate-count technique was then used to count the number of bacteria and fungi. Generally, this method involves applying the solutions to an agar medium in petri dishes. The number of colony-forming units can then be calculated per gram of soil. This method provides a means for determining an estimate of microbial activity in soil.[14] Such results provide a general response of soil microbes to tested compounds in a dose-response assay.

2.3.2 Weed study

In the case of the weed study, three treatments were used initially: A solvent control (0.5 ml acetone); 1,000 ppm of CHP-ace (0.5 ml) for a final concentration of 10 μg/g of soil; and 10,000 ppm of CHP-ace (0.5 ml): 100 μg/g of soil. Later tests also included one-ml treatments of 1000 ppm (10 μg/g of soil) and 10,000 ppm (100 μg/g of soil) of chloropicrin and dichloropropene as noted above. In all cases, 50 g of soil were used in each replication. The soil was put in glass mason jars, and the jars were kept in the greenhouse for three days before the chemical treatments were applied to the surface of the soils. Three replications were done in all instances. For the CHP-ace and 0.5 ml solvent control tests, a count of germinated weeds was made at three and 24 days after treatment. For the chloropicrin and dichloropropene tests, the mason jars were kept in the greenhouse for four days before the

chemical treatments were applied to the surface of the soils. Counts were made 10 days after treatment.

2.3.3 Microbial respiration study

In the case of the microbial respiration study, 20 g of soil (dry weight) were placed in stoppered, 250 ml-glass jars, and soil moisture was adjusted to –33 kPa approximately of field-capacity. The methyl bromide was applied at concentrations of 350 μg/g (i.e., 350 ppm) and 2,733 μg/g as noted above. The soils were incubated in the dark at 25 °C. A complete description of the test set-up and results were described by Rice *et al.*[15] The method used to monitor microbial activity in the soil was the measurement of CO_2 generated per time interval. Specifically, carbon dioxide efflux was measured at 24-hr intervals after an initial 48-hr fumigation period. The sample headspace was purged with moist, CO_2-free air and was analyzed using an infrared gas analyzer. Microbial respiration in the methyl bromide-fumigated and untreated samples was compared. "Microbial activity" refers to normal respiration, metabolism and growth of soil microorganisms. In this case, microbial activity refers to "aerobic" activity, i.e., use of oxygen and carbon sources for energy and growth, producing carbon dioxide and water as waste products. "Normal" microbial activity is determined in any given experiment by the activity in the untreated control soil. "Slightly depressed" activity refers to activity that is about 50% or more of the control activity, such that carbon dioxide production is about 50% or more of the production in the control. "Severely depressed" microbial respiration activity refers to activity that is less than 20% of control activity.

2.3.4 Statistical methods

All of the fumigation LC_{50} values determined in this study were calculated by using Proc Probit on SAS[16] (Tables 1 and 2). Comparisons of compounds' toxicities in the Results section (using Table 1 and 2) are based on the 95% fiducial limits (95% F.L.); LC_{50} values that have overlapping F.L.'s are considered to be comparable; if the F.L.'s for two LC_{50}'s being compared do not overlap, those LC_{50}'s are considered to be different. Standard deviation of Table 3 and 4 was evaluated by Microsoft Excel. For Tables 5 to 9, statistically significant differences were calculated by ANOVA obtaining a p-value.

3 RESULTS AND DISCUSSION

Insect Fumigation. All of the cyanohydrins and derivatives tested were more toxic than dichloropropene except for 1-cyano-1-hydroxy-2-propene pivalate (CHP-piv) in the house fly (*Musca domestica*) fumigation bioassay (Table 1). Dimethyl ketone cyanohydrin (DMK), methylethyl ketone cyanohydrin (MEK) and Fluoro-dimethyl ketone cyanohydrin (FDMK) were as toxic as chloropicrin. In the lesser grain borer (*Rhyzopertha dominica*) bioassay, all the cyanohydrins tested were more toxic than dichloropropene and were more toxic than chloropicrin, with the exception of CHP-piv. FDMK, deutero (D_6)-dimethyl ketone cyanohydrin (DDMK) , 1-cyano-1-hydroxy-2-propene (CHP) and CHP-ace were as potent as dichlorvos.

Fumigation LC_{50} values of several cyanohydrins against three stored product pests are shown in Table 2. CHP-methyl ether (CHP-me) was most toxic compound to the red flour beetle (*Tribolium castaneum*), the least toxic to the saw-toothed grain beetle (*Oryzaephilus surinamensis*), and was nearly the least toxic to the maize weevil (*Sitophilus zeamais*). Comparing species susceptibility, the red flour beetle was the least susceptible to these

fumigants. CPM was the most toxic compound to the maize weevil, and the saw-toothed grain beetle, among the five tested against them.

The synthetic cyanohydrins CHP and DDMK were the most toxic compounds tested against *M. domestica* and *R. dominica*, respectively. Most natural and synthetic cyanohydrins were more toxic than or as toxic as the commercial fumigants. The variation of fumigation toxicity among species of insects is probably due to different metabolism, uptake and detoxification mechanisms, although there may be some differential susceptibility at the site of action. Mammalian toxicity testing has not been conducted in our laboratory on any of the cyanohydrins discussed here.

Antimicrobial activity. Tables 3 and 4 show the total bacterial counts and fungal counts, respectively, after two days, with no treatment of the soil (blank control and solvent control) and with treatments using various concentrations of CHP-ace. The results from Table 3 and 4 show that CHP-ace, particularly at levels of 50 μg/g and 500 μg/g can reduce the total bacterial and fungal counts significantly, i.e., severely depress growth of both fungus and bacteria after two days. As shown in Table 3, the bacterial count actually increased at a dosage of 5 μg/g, indicating that the composition was sufficient to provide a nutritive substrate to the bacteria, i.e., provide them with a carbon source, but was not present in an amount sufficient to be inhibitory (or toxic) to the bacteria.

Inhibition of weed seed germination. Tables 5 and 6 shows the average number of germinated weeds following treatment with 0.5 ml of a solvent control and two different concentrations of CHP-ace for three different replications after three and 24 days, respectively. Tables 5 and 6 show that CHP-ace is effective in inhibiting the germination of weed seeds in soil, and also show the statistically significant difference between control and

each treatment. It was also observed that CHP-ace at both the 10 μg/g and 100 μg/g concentrations can also kill post-emergent weeds. Tables 7 and 8 show the average number of germinated weeds following treatment with one ml of a solvent control and two different concentrations of chloropicrin and dichloropropene, respectively, for three different replications after 10 days, respectively. Tables 7 and 8 show that both chloropicrin and dichloropropene are effective in inhibiting the germination of weed seeds in soil, but less effectively than CHP-ace between control and each treatment. It was also observed during testing that dichloropropene and/or chloropicrin, at both 10 μg/g and 100 μg/g concentrations can also kill post-emergent weeds. Although germinating weeds were not counted on the same days for all tests, and variability was high in the chloropicrin and dichloropropene tests, the compounds in the present study showed effectiveness comparable to chloropicrin and Telone's active ingredient, dichloropropene.

The Table 9 shows the severity and number of days for which microbial activity was depressed by treatment of soil with a fumigant. Soil microbial activity was "slightly depressed" after treatment with 350 μg/g of methyl bromide, i.e., there was a temporary depression in CO_2 efflux, but it returned to normal by Day 4. The soil treated with 2733 μg/g of methyl bromide exhibited "severe depression" of soil microbial activity for the duration of the 24-day experiment. By comparison, CHP-ace at 250 μg/g completely inhibited microbial growth for two days. While methods and measurement in the methyl bromide (Rice *et al.*[15]) studies were different from the ones used in the CHP-ace, application methods and treatment rates were comparable. Methyl bromide and CHP-ace both show anti-microbial activity in soil.

The above results show that CHP-ace is an effective herbicide, bactericide and fungicide in this soil. The results in Table 3-8 further show that the CHP-ace is effective at approximately the same concentrations as methyl bromide. The volatility and cost are very different for CHP-ace and methyl bromide. Further research is needed to determine efficacy in the field and economic feasibility of CHP-ace or other cyanohydrins/derivatives for use as soil fumigants.

These results help us understand the bioactivity of the cyanogenic compounds and may provide valuable leads for future fumigant development including possible utility as a soil fumigant.

ACKNOWLEDGMENT

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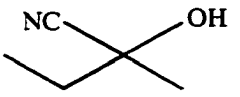
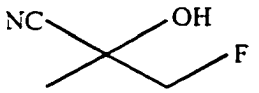
Naturally occurring cyanohydrins

dimethyl ketone cyanohydrin
(DMK)

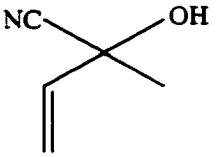


methylethyl ketone cyanohydrin
(MEK)

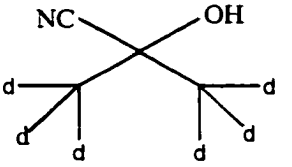
Synthetic cyanohydrins



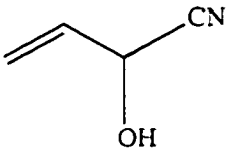
fluoro-DMK (FDMK)



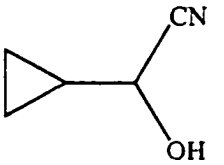
methylvinyl ketone
cyanohydrin (MVK)



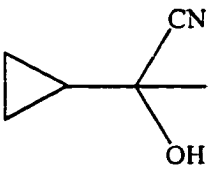
deutero-DMK (DDMK)



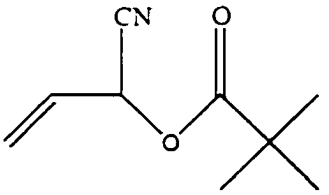
1-cyano-1-hydroxy-2-propene
(CHP)



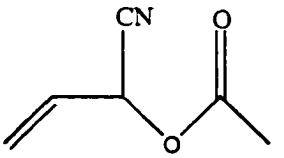
cyclopropylcarboxaldehyde
cyanohydrin (CPC)



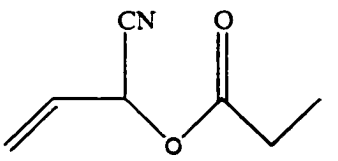
cyclopropylmethylketone
cyanohydrin (CPM)



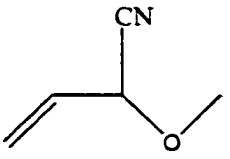
CHP-pivalate
(CHP-piv)



CHP-acetate
(CHP-ace)



CHP-propionate
(CHP-pro)



CHP-methyl ether
(CHP-me)

Figure 1. Structures of cyanohydrins and derivatives tested in this study.

Table 1. Fumigation LC_{50} values ($\mu g \cdot ml^{-1}$) of volatile natural and synthetic cyanohydrins.

	M. domestica	95% F.L.[a]	*R. dominica*	95% F.L.[a]
Naturally occurring cyanohydrins				
DMK	0.07	0.06, 0.09	0.40	0.35, 0.46
MEK	0.09	0.07, 0.10	0.41	0.34, 0.51
Synthetic cyanohydrins				
FDMK	0.09	0.08, 0.10	0.33	0.30, 0.37
MVK	0.22	0.19, 0.24	0.92	0.86, 1.02
DDMK	0.20	0.17, 0.22	0.26	0.23, 0.30
CHP	0.056	0.049,0.063	0.37	0.14, 0.42
CPC	0.15	0.14, 0.17	0.40	0.35, 0.45
CPM	0.15	0.12, 0.19	0.40	0.33, 0.47
Cyanohydrin ether				
CHP-me	0.41	0.34, 0.49	0.88	0.75, 1.04
Cyanohydrin esters				
CHP-piv	1.37	1.17, 1.68	2.41	2.19, 2.68
CHP-ace	0.26	0.23, 0.30	0.37	0.32, 0.45
CHP-pro	0.66	0.58, 0.77	0.70	0.64, 0.76
Commercial fumigants				
Chloropicrin	0.08	0.076, 0.099	1.30	1.20, 1.42
Dichlorvos	0.011	0.009, 0.013	0.29	0.21, 0.41
Dichloropropene	0.90	0.82, 1.15	5.47	4.76, 6.17

[a] 95% fiducial limits

Table 2. Fumigation LC_{50} values ($\mu g \cdot ml^{-1}$) of cyanohydrins and derivatives.

Compounds	*T. castaneum*	95% F.L.[a]	*O.surinamensis*	95% F.L.[a]	*S. zeamais*	95% F.L.[a]
CHP	12.1	10.9, 13.13	0.5	0.4, 0.6	2.5	2.3, 2.8
CHP-ace	22.5	22.2, 27.4	0.7	0.6, 0.8	2.7	2.5, 3.0
CHP-me	4.8	4.2, 5.4	2.1	1.9, 2.3	2.6	2.4, 2.9
CPC-CNOH	36	31.4, 41.6	0.5	0.4, 0.6	1.3	1.1, 1.4
CPM-CNOH	32	23.7, 39.6	0.2	0.2, 0.3	0.7	0.6, 0.8

[a] 95% fiducial limits

Table 3. Total bacterial count in soil two days following application of CHP-ace.

Treatment (CHP-ace)	Average (CFU/g dry wt.)	Std. Deviation
Blank control	4.7×10^6	1.8×10^6
Solvent control	3.2×10^6	0.2×10^6
0.5 μg/g	4.6×10^6	2.6×10^6
5 μg/g	16.2×10^6	3.2×10^6
50 μg/g	0.2×10^6	0.02×10^6
500 μg/g	0.00	0.00

Table 4. Total fungal count in soil two days following application of CHP-ace.

Treatment (CHP-ace)	Average (CFU/g dry wt.)	Std. Deviation
Blank control	1.9×10^4	0.4×10^4
Solvent control	1.4×10^4	0.4×10^4
0.5 μg/g	2.0×10^4	0.5×10^4
5 μg/g	0.5×10^4	0.4×10^4
50 μg/g	0.00	0.00
500 μg/g	0.00	0.00

Table 5. Number of germinated weed seeds three days after application of CHP-ace.

Treatment	Number of Germinated Weeds					
	Replication	1	2	3	ave.	p-value
Solvent Control		6	8	11	8.3	0.0006
10 μg/g		0	0	0	0	
100 μg/g		0	0	0	0	

Table 6. Number of germinated weed seeds 24 days after application of CHP-ace.

Treatment	Number of Germinated Weeds					
	Replication	1	2	3	ave.	p-value
Solvent Control		10	20	27	19	0.0048
10 μg/g		0	0	0	0	
100 μg/g		0	0	0	0	

Table 7. Number of germinated weed seeds 10 days after application of chloropicrin.

Treatment	Number of Germinated Weeds					
	Replication	1	2	3	ave.	p-value
Solvent Control		0	12	3	5	0.2963
10 μg/g		0	3	0	1	
100 μg/g		0	0	0	0	

Table 8. Number of germinated weed seeds 10 days after application of 1,3-dichloropropene.

Treatment	Number of Germinated Weeds					
	Replication	1	2	3	ave.	p-value
Solvent Control		0	12	3	5	0.3313
10 μg/g		4	4	8	5.3	
100 μg/g		0	2	0	0.7	

Table 9. Severity and number of days microbial activity was depressed by treatment of soil with fumigant (data from Rice *et al.*[15]).

Treatment	Severity	Days
Control	Normal activity	0
350 μg/g MeBr	Slightly depressed	4
2,733 μg/g MeBr	Severly depressed	24
250 μg/g of CHP-ace	No bacteria or fungal colonies formed after 2 days	

CHPATER 3. A QSAR EVALUATION OF CYANOHYDRINS' FUMIGATION TOXICITY TO HOUSE FLY (*Musca domestica*) AND LESSER GRAIN BORER (*Rhyzopertha dominica*)

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ABSTRACT

Using fumigation toxicity data of 11 natural and synthetic cyanohydrins to house fly (*Musca domestica*) and a stored-product pest, the lesser grain borer (*Rhyzopertha dominica*), the quantitative structure-activity relationships (QSAR) of cyanohydrins were examined by Oxford Molecular CAChe 3.2™ and Microsoft Excel™. This analysis used nine physicochemical parameters. Correlation between the LC_{50}'s for house fly and lesser grain borers were also evaluated. The results showed that log P, polarizability, and molar refractivity are the best descriptors to explain the relationship between the structure of cyanohydrins and biological effects in house flies, and to a lesser degree in lesser grain borers. A significant relationship was also found between the toxicity to house flies and lesser grain borers.

Key words: *Quantitative Structure-Activity Relationship, QSAR, cyanohydrins, fumigation, toxicity, stored-product pest, insecticide, house fly*

INTRODUCTION

There are several commercial fumigants on the market today, such as methyl bromide, dichlorvos, chloropicrin, and phosphine. However many of these fumigants have negative ecological or human health effects. Methyl bromide is a known ozone depleter, whose use as a fumigant is being phased out by the U. S. Environmental Protection Agency (EPA), dichlorvos is a suspected carcinogen, and phosphine has new restriction on its use.

Recent research in this laboratory demonstrated that volatile cyanohydrins that occur naturally in flax and cassava are very potent insect fumigants (1). Topical toxicity has been tested with several cyanohydrins, but the toxicity values were not as good as for the fumigation toxicity (2). Cyanohydrins' bioactivity is similar to that of several commercial fumigants. Their function in plants is to act as a chemical defense mechanism against herbivores, including insects, and pathogens. These secondary plant metabolites are stored in a stable glucose form until feeding damage occurs; when the glucone is hydrolyzed by activated enzymes, the free cyanohydrin is released and is toxic to insects (3,4,5,6). Nematicidal activity has also been demonstrated for many of the volatile cyanohydrins as part of the current research project. Current research focuses on the development of quantitative structure-activity relationships (QSARs) for prediction of cyanohydrins' insecticidal toxicity.

The descriptors used in the QSARs development were selected to explain one of the major principles of toxicology, which is the dose makes the poison. This principle states that for a chemical to have adverse effects on an organism, the organism must be exposed to and absorb sufficient amounts of the chemical. Fumigation studies support this principle and show that knowing the volatility of compounds is essential in determining the amount of chemical to which an organism is exposed (7). In addition, lipid solubility of chemicals is essential for penetration into the insect cuticle for contact toxicity, although fumigants may enter through spiracles as well. Therefore, volatility and lipid solubility may be critical in determining the toxicity of a compound.

Vapor pressure, which is strongly influenced by intermolecular interactions, is a standard measurement of volatility. These intermolecular interactions can be explained by

London dispersive forces, which include dipole-dipole, dipole-induced dipole, and induced dipole-induced dipole interactions. Hydrogen bonding and electrostatic interaction of certain functional groups also play a major role in affecting vapor pressure. Descriptors in the QSAR models were chosen to explain important features of intermolecular interactions. Polarizability, a descriptor used in this study, is a key component in determining the London dispersive forces. Molar refractivity, which is the representation of molar volume and polarizability was also used in these QSAR models. Log P, which represents octanol-water partition coefficient of a chemical, is one of several determinants in the penetration of a chemical into the insect (8) and is also important descriptor in the QSAR models. We also examined molecular weight, molecular connectivity index (0,1,2), shape index (1,2,3), valance connectivity index (0,1,2), highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) to explain various components of intermolecular interactions to develop insecticidal QSAR relationships for volatile cyanohydrins.

MATERIALS AND METHODS

Chemicals. The structures of natural and synthetic cyanohydrins tested in this study are shown in Figure 1. The cyanohydrins were synthesized in the laboratory following the methods of Gassman and Tally (9). Purification was achieved by silica gel column chromatography. Cyanohydrin esters were prepared from the cyanohydrins by the methods of Rice et al., (10). The chemical structures for all cyanohydrins that were synthesized in this study were confirmed by proton NMR. Commercial fumigants, dichlorvos and chloropicrin, were purchased from Chem Service (West Chester, PA). The active ingredient in Telone®,1,3-dichloropropene, was purchased from TCI America (Portland, OR).

Fumigation toxicity testing on two species. Insecticidal fumigation toxicity of natural and synthetic cyanohydrins was tested with the house fly and lesser grain borer as described by Peterson et al., (11). Adult house flies and borers were placed in a 2.7-liter amber jar and a 490-ml glass mason jar, respectively, and a measured quantity of a test compound was applied to the filter paper in each jar. The jar was securely capped, and mortalities were recorded after 24 hours. The fumigation LC_{50} values were calculated by Proc Probit on SAS (12), and the results are presented in Table 1. All concentrations were nominal, and results were calculated assuming 100% volatilization of the fumigant from the filter papers in the glass chamber (jar). It is not known if the toxicity is mostly due to the cyanohydrins entering the insects and killing them or HCN from decomposition of the cyanohydrin killing the insect. Vapor pressures were not measured in this study.

QSAR Calculations. Descriptors examined were molecular weight, molecular connectivity index (0,1,2), shape index (1,2,3), valance connectivity index (0,1,2), molar refractivity, polarizability, log P, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). These parameters have been used for explaining the size, shape, and volatility of compounds and for predicting the insecticidal activity. All descriptors and structures were calculated using Oxford Molecular CAChe® 3.2 (Beaverton, Oregon). Regression analysis, and cross-validation were calculated using Stat View™. The results are shown in Table 2. Cyanohydrin structures and energies were obtained by using PM3 calculations.

RESULTS AND DISCUSSION

Fumigation toxicity data and nine parameters were chosen in order to explain cyanohydrin toxicity to house flies. Six of the descriptors evaluated, molecular weight,

molecular connectivity index (0,1,2), shape index (1,2,3), valance connectivity index (0,1,2), HOMO and LUMO, showed a lower correlation with toxicity than did log P, polarizability, and molar refractivity. The R^2 values with molecular connectivity indices (0,1,2) were 0.74, 0.73, 0.47 and for shape indices (1,2,3) were 0.73, 0.53, 0.00, respectively. The R^2 values for valance connectivity indices (0,1,2) were 0.75, 0.56 and 0.32. The R^2 values for HOMO and LUMO were 0.32 and 0.36, respectively. Molecular weight had an R^2 of 0.71. Three of the descriptors examined, log P, polarizability and molar refractivity, showed highly significant correlation between certain structural features of the cyanohydrins and their toxicity to house flies (Figure 2). Our results showed that as log P of the cyanohydrins decreased, their toxicity increased. Log P has the highest correlation with toxicity of cyanohydrins than the other parameters. Our results also revealed a linear trend of increasing toxicity with decreasing polarizability. A linear correlation was also obtained for molar refractivity (Figure 2). This descriptor is related to the potency of London dispersive forces, but it also takes into consideration size and shape of the molecules. Log P, polarizability, and molar refractivity are highly cross-correlated, so the results obtained with those three parameters all describe similar relationships. The high cross-correlation among the three parameters is shown in Table 3.

QSARs obtained for lesser grain borer utilized the same three parameters to predict cyanohydrins toxicity as house fly (Figure 3). The types of relationships were similar, but the correlation (R^2-values) were not as high. In addition, the relationship between the house fly and lesser grain borer was not highly correlated ($R^2 = 0.68$) as shown in Figure 4. Table 2 shows the comparison of the R-squared (fit of the regression) and cross validation (predictive power of the regression) of the three parameters for house fly and lesser grain borer.

The results indicate that log P, polarizability, and molar refractivity values can be used to help predict the toxicity of cyanohydrins in house flies, and to a lesser degree for lesser grain borers. Although a small set of analogs was used to develop these QSAR models, the models will provide insight in designing insecticidally active cyanohydrins. These compounds were selected because they were synthesized to be close analogs of the potent natural cyanohydrins generated in the flax plant. As shown by the R^2 values among the three parameters, log P was the best descriptor in predicting toxicity. Polarizability was previously used to calculate the vapor pressures of 479 compounds with good accuracy (7). Since polarizability has been previously used to calculate predicted vapor pressures and is used in calculating log P and molar refractivity (13), the toxicity correlations for cyanohydrins are probably explained by an increase in exposure of the insects to the chemical, through high volatility. Our assumption is that toxicity of the volatile cyanohydrins is caused by the availability of the cyanohydrin in the vapor phase to enter the insect and/or its decomposition *in vivo* to release cyanide ion. Research is in progress to determine if cyanohydrins cause toxicity in the fumigation chamber, or if toxicity stems from spontaneous decomposition of cyanohydrins to hydrogen cyanide in the fumigation chamber.

The QSAR may also explain the reactivities of the various cyanohydrins in the insect body, presumably generating cyanide ion. The fumigation potency of these low-molecular-weight cyanohydrins is probably dependent on their (1) volatility, and/or (2) their reactivity inside the insect. Research is currently in progress to determine concentrations of the cyanohydrins and HCN in the headspace of the chamber and in exposed insects. If the cyanohydrins are decomposing in the chamber to release HCN, then the additional products formed would be volatile carbonyl compounds (acrolein, acetone, fluoroacetone, etc.) which

could also contribute to toxicity. If the cyanohydrins are degraded *in vivo*, the carbonyl compounds may enhance the toxicity to the insect in an additive or synergistic mode.

CONCLUSIONS

The insecticidal potencies of 11 volatile natural and synthetic cyanohydrins against house fly and lesser grain borer are shown in Table 1, as well as three commercial fumigants. Log P, polarizability, and molar refractivity can be used to predict toxicity of volatile cyanohydrins in house flies. Significant relationships were found between those three parameters and the toxicity to the house fly and lesser grain borer. Some of these cyanohydrins were as potent as current commercial fumigants or more potent. The current research does not allow deduction of the moiety that enter/kills the insects. The more toxic cyanohydrins may be more volatile or may decompose more readily to yield HCN (in the chamber or in the insect). Although the results here do not conclusively explain the underlying principle of the QSAR's, those relationships are still of value in predicting toxicity to the two species of insects.

ACKNOWLEDGMENT

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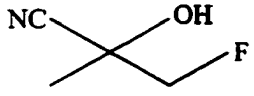
Naturally occurring cyanohydrins

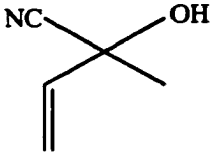
dimethyl ketone cyanohydrin (DMK) **(I)**

methylethyl ketone cyanohydrin (MEK) **(II)**

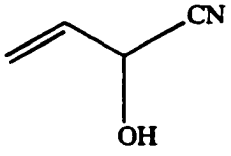
Synthetic cyanohydrins



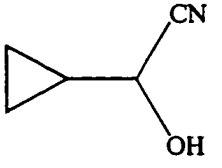
fluoro-DMK cyanohydrin (FDMK) **(III)**



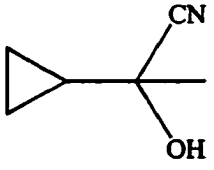
methylvinyl ketone cyanohydrin (MVK) **(IV)**



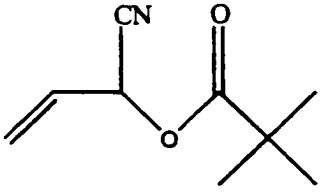
1-cyano-1-hydroxy-2-propene (CHP) **(V)**



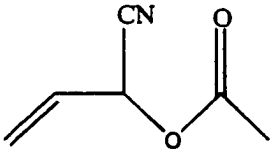
cyclopropylcarboxaldehyde cyanohydrin (CPC) **(VI)**



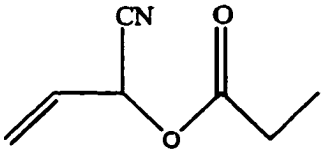
cyclopropylmethylketone cyanohydrin (CPM) **(VII)**



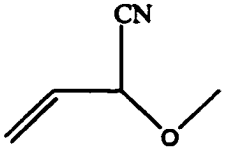
CHP-pivalate (CHP-piv) **(VIII)**



CHP-acetate (CHP-ace) **(IX)**



CHP-propionate (CHP-pro) **(X)**



CHP-methyl ether (CHP-me) **(XI)**

Figure 1. Structures of volatile natural and synthetic cyanohydrins and derivatives tested in insect fumigations.

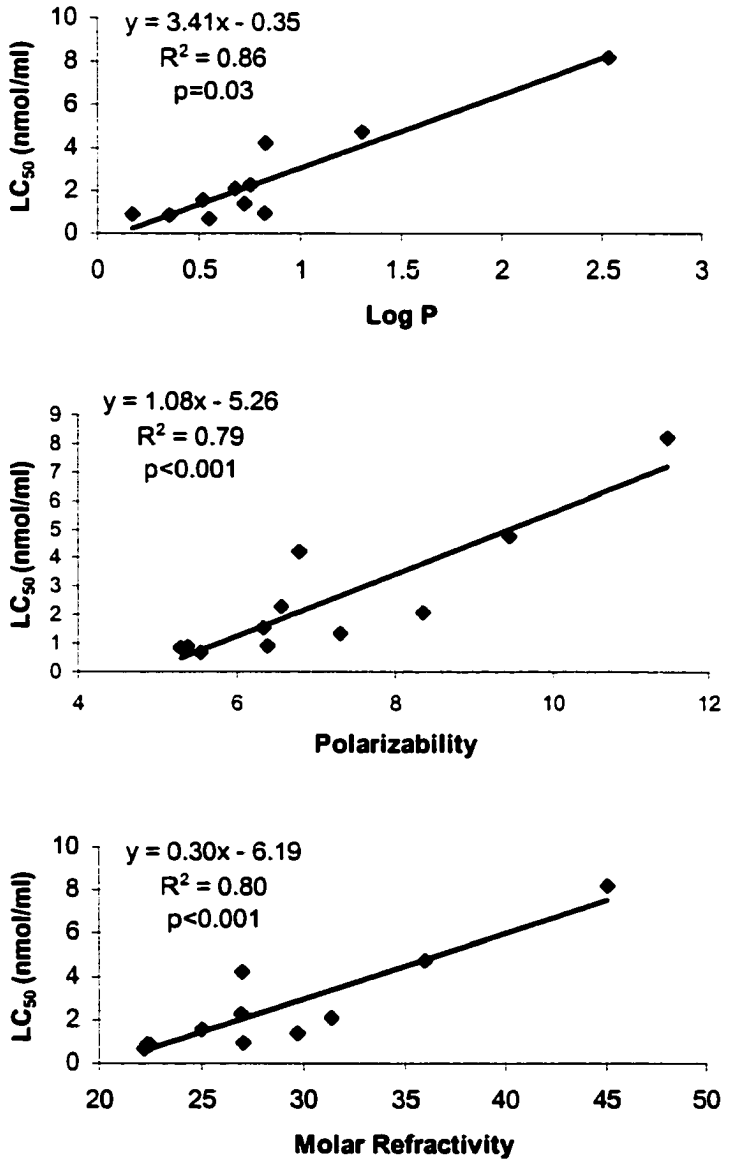


Figure 2. Relationships between three parameters and the toxicity of volatile cyanohydrins to the house fly. For the Molar Refractivity figure, three data points are superimposed.

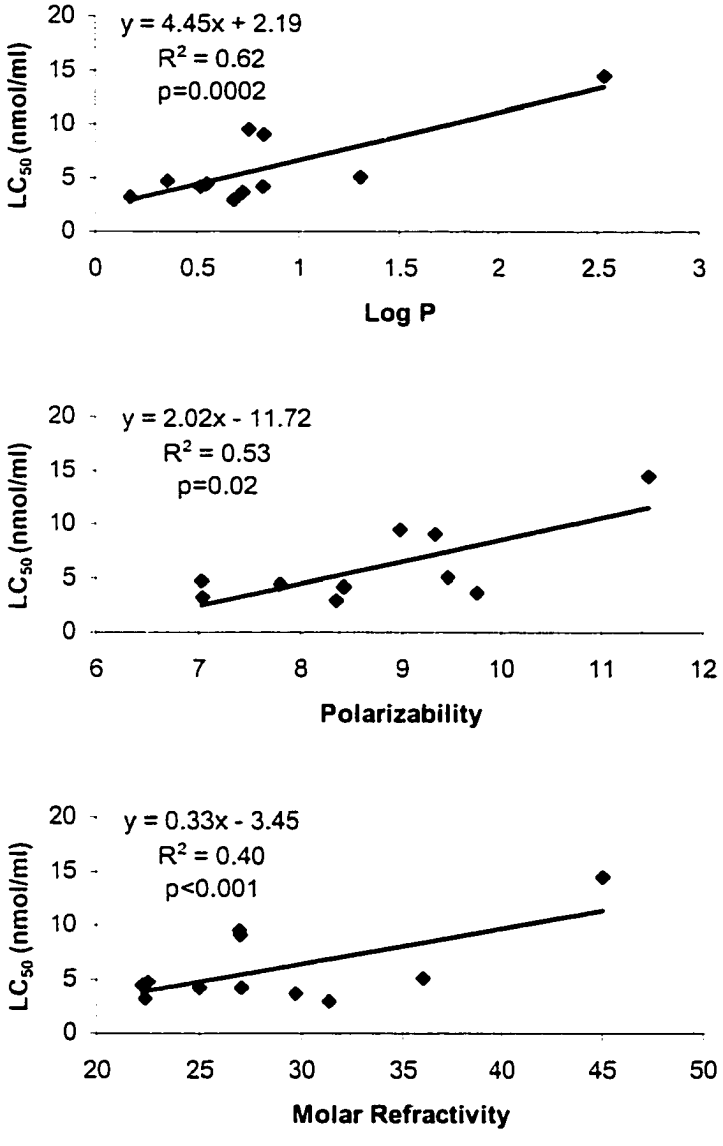


Figure 3. Relationships between three parameters and the toxicity of cyanohydrins to the lesser grain borer. For the Polarizability figure, two data points are superimposed.

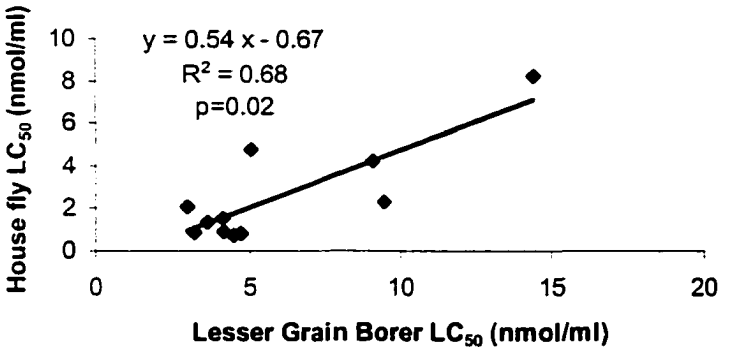


Figure 4. The correlation between the toxicities of volatile cyanohydrins to the house fly and lesser grain borer, using their 24-hr fumigation LC_{50} values (nmol/ml).

Table 1. Toxicity and molecular descriptors for 11 cyanohydrins and derivatives and three commercial fumigants.

Compounds	House fly		Lesser grain borer		Log P	Polarizability	Molar refractivity
	LC_{50}[a]	95% F. L.[b]	LC_{50}[a]	95% F. L.[b]			
I	0.82	0.71, 1.06	4.70	4.11, 5.41	0.354	7.035	22.5
II	0.91	0.71, 1.00	4.14	3.43, 5.14	0.823	8.421	27.1
III	0.87	0.78, 0.97	3.20	2.91, 3.59	0.172	7.04	22.4
IV	2.27	1.96, 2.47	9.47	8.86,10.50	0.752	8.981	26.9
V	0.67	0.59, 0.76	4.45	1.68, 5.05	0.550	7.796	22.2
VI	1.54	1.44, 1.75	4.12	3.60, 4.63	0.519	8.43	25.0
VII	1.35	1.08, 1.71	3.60	2.97, 4.23	0.722	9.757	29.7
VIII	8.19	6.70,10.05	14.41	13.10,16.03	2.534	11.481	45.1
IX	2.08	1.84, 2.40	2.96	2.56, 3.60	0.679	8.356	31.4
X	4.74	4.17, 5.53	5.03	4.60, 5.46	1.307	9.462	36.0
XI	4.22	3.50, 5.05	9.06	7.72,10.71	0.828	9.33	27.0
Chloropicrin	0.49	0.46, 0.60	7.91	7.30, 8.64			
Dichlorvos	0.05	0.04, 0.06	1.31	0.95, 1.86			
Dichloropropene	8.11	7.39,10.36	49.29	42.89,55.60			

[a] nmol/ml

[b] 95% fiducial limits

Table 2. The R^2 (and cross validation) of three parameters to house fly and lesser grain borer.

Parameters	R^2 (and rCV^2)	
	House fly	Lesser grain borer
Log P	0.86, (0.79)	0.62, (0.19)
Polarizability	0.79, (0.62)	0.53, (0.14)
Molar refractivity	0.80, (0.68)	0.40, (0.18)

Table 3. Cross-correlation among the three parameters.

R^2	Log P	Polarizability	Molar Refractivity
Log P	1.0	0.79	0.89
Polarizability	0.79	1.0	0.78
Molar Refractivity	0.89	0.78	1.0

CHAPTER 4. MODE OF ACTION OF CYANOHYDRINS IN INSECTS

A paper prepared for submission to *Journal of Pesticide Science*

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In previous research, fumigation toxicities of natural and synthetic cyanohydrins against house fly and stored-product pests were evaluated. Toxicity of those cyanohydrins to insects was as potent as commercial fumigants such as chloropicrin, dichlorvos and 1,3-dichloropropene. This toxicity, however, was obtained by assuming 100 % evaporation of the natural and synthetic cyanohydrins in the chamber and did not determine which of the components (cyanide ion, the cyanohydrin, or metabolites) kill the insect. Using this assumption, fumigation toxicity of hydrogen cyanide (HCN) was compared to natural and synthetic cyanohydrins against the house fly and the lesser grain borer. Concentrations of the cyanohydrins in the headspace of the chamber and of the CHP-ace (a cyanohydrin derivative)-exposed house flies were investigated by gas chromatography. Quantities of cyanide ion in the insect body exposed by topical application and fumigation to cyanohydrins and only by fumigation with HCN were determined by a colorimetric method. LC_{50} fumigation toxicity of hydrogen cyanide against the house fly and the lesser grain borer was 0.02 μg/ml and 0.16 μg/ml, respectively. The percentage of cyanohydrin in the headspace of the chamber varied from 1 to 7 % of the amount applied. 53% of the CHP-ace was detected in the house fly bodies after fumigation exposure. In the topical exposure experiments, cyanide ion in the house flies treated with CHP-ace, CHP, and DMK was 56, 52, and 54 μg/g, respectively. In fumigation exposure experiments, cyanide ion in the house fly was 48, 38, and 39 μg/g, respectively. Cyanide ion of the CHP-ace, CHP, and DMK treated lesser grain borer was 43, 35, and 33μg/g, respectively. The total amount of cyanide ion *in vivo* after the fumigation test with HCN was 680 μg/g, which is higher than the cyanide ion that resulted from fumigation with cyanohydrins.

INTRODUCTION

Cyanogenesis, liberation of hydrogen cyanide (HCN), is well known in a wide variety of species in the plant kingdom. For protection from herbivores, many plants can synthesize cyanogenic compounds. The distribution, characteristics, and role of these cyanogenic glycosides were reviewed by Park.[1)] In this laboratory, we found that natural and synthetic cyanohydrins were potent fumigants to house flies and stored-product pests and as effective as commercial fumigants.[1,2,3)] Commercial fumigants such as methyl bromide, dichlorvos,

and chloropicrin have been used for many years to control insects and pathogens. Those fumigants are being restricted or phased out due to their adverse effects to humans and the environment. So, alternative fumigants are needed for reasons of safety and biodegradability. The insect LC_{50} values of cyanohydrins were obtained with the assumption that the natural and synthetic cyanohydrins had been totally volatilized in the chamber and available to enter the insect body and to release cyanide ion in the insect body.[2)]

The objective of this study was to determine the component which kills the insects. Fumigation toxicity of hydrogen cyanide (HCN) against the house fly and the lesser grain borer was compared to natural and synthetic cyanohydrins. Concentrations of the cyanohydrins in the headspace of the chamber and in exposed house fly were investigated by gas chromatography. After topical or fumigation exposure of house fly and lesser grain borer to cyanohydrins, the total amount of cyanide ion in the insect body was determined by a spectrophotometric method. The total amount of cyanide ion in the house fly was also measured after a fumigation test with HCN. This total concentration of cyanide was also compared with the concentration of cyanide ion in the house fly body as a result of exposure to cyanohydrins.

MATERIALS AND METHODS

1. Chemicals

The chemical structures of cyanohydrins tested in this study are shown in Figure 1. 1-Cyano-2-hydroxy-propene (CHP) and dimethylketone cyanohydrin (DMK) were synthesized in this laboratory.[4)] CHP-acetate (CHP-ace), 2-acetoxy-3-butenenitrile, was purchased from Aldrich (Milwaukee, WI). DMK is a cyanohydrin that occurs naturally, e.g., in flax.

2. Fumigation toxicity of hydrogen cyanide to house fly and lesser grain borer

2.1 Fumigation of Musca domestica *and* Rhyzopertha dominica

Fumigation toxicity of HCN against the house fly and lesser grain borer was tested by method used previously research in this laboratory.[4] Ten to 15 adult house fly or lesser grain borer were placed in a small jar (35ml) with food (powdered sugar and dried milk for house fly or wheat for lesser grain borer). One ml of 100,000 μg/ml of KCN solution (100,000 μg KCN) was put into a 35-ml glass french square bottle. Both the small jar and french square bottle were put into a 490-ml glass mason jar. The lid was capped immediately after 50 μl of concentrated sulfuric acid was put into the 35-ml french square jar to adjust the pH to 3. The concentration of KCN used was based on an approximate LC_{99} (24-hr). Mortalities were recorded after 24 hr. The fumigation LC_{50} values were calculated by using Proc Probit on SAS.[5] Three replications were conducted in this test.

3. Determination of cyanohydrins in the headspace of the chamber and in the CHP-ace-exposed house fly

3.1 Gas chromatography

A Hewlett-Packard 5890 GC equipped with a flame ionization detector and a carbowax column (25m × 0.25mm) was used for determining cyanohydrins. For CHP-ace, the oven temperature was programmed at 40°C for 4 min, then increased at 10°C/min to 120°C, and then held for 15 min. The injection and detector temperatures were 180°C and 280°C, respectively. For CHP and DMK, the oven temperature was programmed at 80°C for 4 min, then increased at 10°C/min to 220°C, and then held for 15 min. The injection and detector temperatures were 180°C and 280°C, respectively.

3.2 Cyanohydrins in the headspace of the chamber

The highest concentration of CHP-ace and pure CHP and DMK cyanohydrins was used for testing in the headspace of the chamber due to the detection limits. One ml of 100,000 μg/ml of CHP-ace solution (100,000 μg of CHP-acetate) in acetone was applied to filter paper in a glass french square bottle (260 ml). After 24 hr, 25μl of air was taken by a gas-tight syringe through a septum in the lid and was injected onto the GC. For CHP and DMK, pure compound was used with same method as for CHP-ace. After analysis of CHP and DMK with the GC, the chromatogram was compared with a standard curve of CHP-ace to obtain an estimate of their concentrations.

3.3 Cyanohydrin in CHP-ace-exposed adult house fly

Fifty adult house flies were put into a small mason jar with food. Filter paper was treated with 100 μl of 10,000 μg/ml of CHP-ace (1,000 μg CHP-ace). After 24 hr, adult house flies were ground with 100 ml of methanol and centrifuged. 2 μl of the supernant was injected onto the GC.

4. Concentration of cyanide ion in *Musca domestica* and *Rhyzopertha dominica*

The determine cyanide ion concentrations in insects the method of Epstein was followed.[6] One ml of each supernant solution of the water extract was taken in a tube with 0.2 ml of 1% aqueous chloramine-T solution to which 6 ml of a pyridine-pyrazolone reagent (500 ml of saturated water of phenyl methyl pyrazolone with 100 ml of pyridine and 0.1 g of bis-pyrazolone) was added. After 20 minutes, absorbance was measured by a spectrophotometer at 630nm using a Bausch & Lomb Spectronic®21 spectrophotometer.

4.1 Topical

One μl of 10,000 μg/ml of CHP-ace (10 μg CHP-ace) and 100,000 μg/ml of CHP and DMK (100 μg) were dosed onto the dorsal thorax of the house fly. After 24 hr, 50 house flies were ground with 100 ml of distilled water and centrifuged. The concentration of cyanide ion in the extracts was measured by the spectrophotometric method.

4.2 Fumigation

The fumigation exposure test used the same method as described previously in this laboratory. [4] Based on an approximate LD_{99}, filter paper in a mason jar was treated with 100 μl of 10,000 μg/ml of CHP-ace (1,000 μg CHP-ace) and 100,000 μg/ml of CHP and DMK (10,000 μg CHP or DMK). After 24 hr, 50 house flies were ground with 100 ml of distilled water and the extract centrifuged. The concentration of cyanide ion in the extracts was analyzed using the spectrophotometeric method. For the lesser grain borer, 1 g of adult lesser grain borer was used in the same method as used for the house fly. After the fumigation test with 100 μl of 100,000 μg/ml of KCN (10,000 μg KCN) to the house fly, which generated HCN gas at an approximate LD_{99} concentration, the concentration of cyanide ion was also measured in the flies.

RESULTS

1. *Fumigation toxicity of HCN against the house fly and lesser grain borer*

LC_{50} values for the fumigation toxicity of hydrogen cyanide and some natural and synthetic cyanohydrins are shown in Table 1. Two natural cyanohydrins from flax (*Linum usitatissimum*) and the synthetic analog,CHP, were all very effective fumigants with LC_{50}'s similar to chloropicrin and nearly as potent as HCN.

2. *Cyanohydrins in the headspace of the chamber and in the CHP-ace-exposed house fly*

The GC standard curve of CHP-ace is shown in Figure 2. The percentage of cyanohydrins in the headspace of the chamber is shown in Table 2. The percentage of cyanohydrin in the CHP-ace-exposed adult house fly was 53 %.

3. *Concentration of cyanide ion in house fly and lesser grain borer*

Table 3 shows the amount of total cyanide ion in the insect body after topical and fumigation test with cyanohydrins and after a fumigation test with hydrogen cyanide. Figure 3 gives a calibration curve for cyanide ion using the spectrophotometeric method.

DISCUSSION

Comparison of the fumigation toxicity (LC_{50}) of hydrogen cyanide to the cyanohydrins showed that hydrogen cyanide was somewhat more toxic than the cyanohydrins against the house fly and lesser grain borer. One possibility is that cyanohydrins could have been transformed to other forms or could have stayed on the filter paper or the jar wall. It is well known that HCN is a highly toxic fumigant that has been used to control insects. Hansen[7] reported, for example, that HCN fumigation was highly effective against pests of Hawaiian cut flowers and foliage.

As a result of determination of the cyanohydrins in the headspace of the chamber, less than 10 % of the total in the air indicates that the rest of the cyanohydrins could have stayed in the filter paper or on the jar wall or could have degraded. Filter papers treated with cyanohydrins were extracted with acetone, and the cyanohydrins were measured by GC. Residual cyanohydrins were not detected in the filter paper. This may be due to transformation or to reaction with the filter paper. 53 % of CHP-ace was detected in the house fly body after the fumigation exposure.

The release of hydrogen cyanide is not confined to higher plants. Cyanogenic glycosides are released by millipedes[8)] and larvae of *Zygaena* spp. (the Burnet moth). Detecting 53 % of CHP-ace in the house fly body after the fumigation exposure means that some of cyanohydrin stayed on the filter paper or the jar wall, the same assumption as for the headspace experiment, or was transformed or excreted by the house flies.

After the house fly and the lesser grain borer were treated with cyanohydrins or hydrogen cyanide at the approximated LC_{99} concentrations, it was determined that the total amount of cyanide ion in the insect body exposed to hydrogen cyanide was higher than in those that were exposed to a cyanohydrin. This indicates that cyanohydrins entered the insect body but generated less cyanide ion gas than hydrogen cyanide. Comparing fumigation LC_{50} values to total cyanide ion for the CHP- and DMK-exposed house fly and lesser grain borer at the same treatment concentration, the total cyanide ion from the CHP-exposed insects should be higher than DMK exposed insects because the CHP LC_{50} was lower than the DMK LC_{50}. However, it turned out that quantity of cyanide ion in the DMK-exposed insects was higher than for the CHP-exposed insects, except for the topical-application for the house fly. This might be due to different degrees of breakdown of the molecule in the insect body. Cyanide ion generated in the house fly was higher than in the lesser grain borer. This result supports the fact that fumigation toxicity for the house fly was higher than for the lesser grain borer. Responsibility for toxicity of the cyanohydrins depends on the quantity of cyanide ion released in the insect body. Total cyanide ion in the HCN-exposed house fly was higher than in the cyanohydrin-exposed house fly at concentrations that were approximately the LC_{99}.

It is thought that these natural and synthetic cyanohydrins are highly volatile compounds. With this study, these results did not completely support my assumption. However, the natural and synthetic cyanohydrins were almost as potent as hydrogen cyanide against house fly and lesser grain borer. Although cyanohydrins were not totally broken down to cyanide ion in the insect body, the cyanohydrins generated enough cyanide ion to kill the house fly and lesser grain borer.

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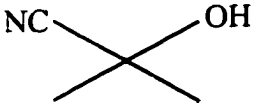
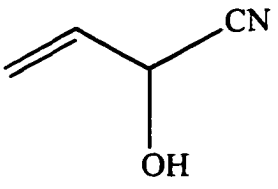
Naturally occurring cyanohydrins

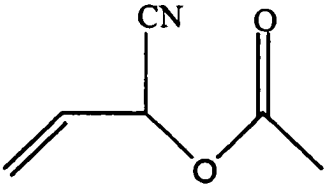


dimethyl ketone cyanohydrin
(DMK)

Synthetic cyanohydrins



1-cyano-1-hydroxy-2-propene
(CHP)



CHP-acetate
(CHP-ace)

Figure 1. Structures of cyanohydrins and derivatives tested in this study.

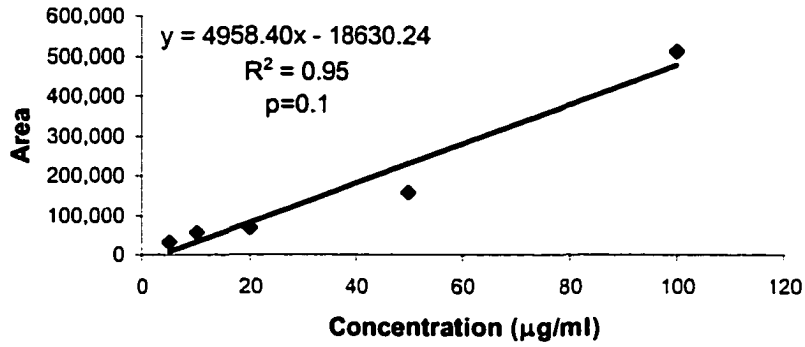


Figure 2. The standard curve for CHP-ace from a gas chromatograph with a nitrogen-phosphorus detector.

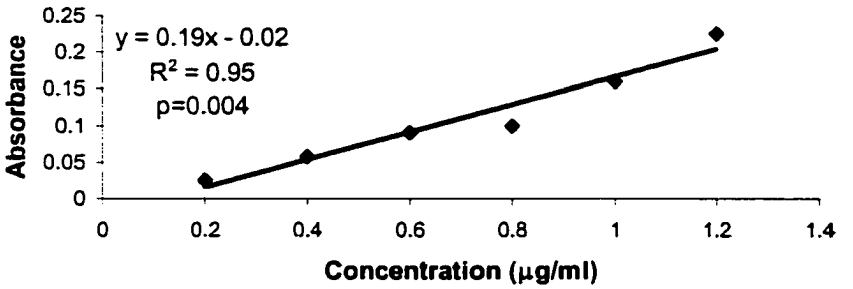


Figure 3. Standard calibration curve for cyanide ion with a spectrophotometer.

Table 1. Fumigation LC_{50} values ($\mu g \cdot ml^{-1}$) of natural and synthetic cyanohydrins, HCN and commercial fumigants.

	M. domestica	95% F.L.[a]	*R. dominica*	95% F.L.[a]
Naturally occurring cyanohydrins				
DMK	0.07	0.06, 0.09	0.40	0.35, 0.46
MEK[b]	0.09	0.07, 0.10	0.41	0.34, 0.51
Synthetic cyanohydrin				
CHP	0.056	0.049,0.063	0.37	0.14, 0.42
Cyanohydrin ester				
CHP-ace	0.26	0.23, 0.30	0.37	0.32, 0.45
Hydrogen cyanide (HCN)	0.02	0.015, 0.033	0.16	0.144, 0.172
Commercial fumigants				
Chloropicrin	0.08	0.076, 0.099	1.30	1.20, 1.42
Dichlorvos	0.011	0.009, 0.013	0.29	0.21, 0.41
Dichloropropene	0.90	0.82, 1.15	5.47	4.76, 6.17

[a] 95% fiducial limits

[b] MEK = methylethyl ketone cyanohydrin

Table 2. Percentage of cyanohydrin or derivative in the headspace of fumigation chamber.

Compound	% of cyanoydrin in the headspace
DMK	1
CHP	1
CHP-ace	7

Table 3. Concentration of cyanide ion in insects as μg CN^-/g insect body (ppm).

Compound	House fly		Lesser grain borer	
	Topical	Fumigation	Topical	Fumigation
DMK	56 ppm	48 ppm	NT	43 ppm
CHP	52	38	NT	35
CHP-ace	54	39	NT	33
HCN	NT	680	NT	NT

NT: not tested

CHAPTER 5. GENERAL CONCLUSIONS

This dissertation has investigated the natural and synthetic cyanohydrins, their insecticidal properties, quantitative structure-activity relationships (QSAR), and their metabolism.

Chapter 1 contained an overview of background information on cyanogenic glycosides. Because cyanogenic glycosides, which are plant secondary metabolites, have been assumed for many years to be defensive compounds against herbivores, natural and synthetic cyanohydrins were synthesized and tested for fumigation toxicity against the house fly and several stored-product pests (Chapter 2). In order to determine the relationships between fumigation toxicity against house fly and lesser grain borer and the structures of cyanohydrins, derivatives, and analogues, quantitative structure-activity relationships (QSAR) were developed (Chapter 3). The quantity of cyanohydrin in the headspace of the experimental fumigation chamber and in cyanohydrin-exposed adult house fly was measured (Chapter 4). Cyanide ion within the insects exposed to cyanohydrins was also measured. The last experimental chapter focused on whether or not the cyanohydrins are degraded to cyanide ion in the insects and kill them by that mode of action.

Distribution and characteristics of cyanogenic glycosides were addressed and their role in plants and mode of action were also discussed in the general introduction. Conclusions of the general introduction were (1) cyanogenic glycosides in plants probably provide protection from insects and (2) some of the cyanohydrins could be alternative insecticides.

Most natural and synthetic cyanohydrins tested in this study were more toxic than or as potent as the commercial fumigants against the house fly and several stored-product pests.

Many of the commercial fumigants such as methyl bromide, chloropicrin, dichlorvos, and phosphine have either toxicology problems or environmental concerns. The significance of the fumigation bioassay is that it proves that alternative fumigants may be feasible from natural sources or synthetic analogues that are equally biodegradable. This result could provide valuable leads for future fumigant development.

Their fumigation toxicity against the house fly was correlated to log P, polarizability and molar refractivity. The relationships between the toxicity to the lesser grain borer and chemical structure were similar to those for the house fly for these three parameters, but the correlations were not as high for the lesser grain borer. In general, as log P, polarizability and molar refractivity increased, fumigation toxicity to house fly decreased. These three parameters can be used to help predict the fumigation toxicity of other cyanohydrins in the house fly and the lesser grain borer.

Fumigation toxicity of hydrogen cyanide was more potent than the cyanohydrins against the house fly and the lesser grain borer, and this indicated that the cyanohydrins probably generated less cyanide ion in the insect body. After determination of cyanohydrins in the headspace and in exposed insects, the results did not support the assumption that natural and synthetic cyanohydrins were highly volatile; significant quantities of the cyanohydrins might have stayed on the filter paper or jar wall. Another possibility was that they could have been degraded or transformed in the chamber and insect body. For testing this assumption, further research needs to be done. Detecting cyanide ion in the cyanohydrin-exposed insects showed that cyanohydrins entered the insect body and generated cyanide ion which was toxic to the insect. This result supported the hypotesis that fumigation toxicity depends on the quantity of the cyanide ion in the insect body.

As a view of the chemical ecology of cyanogenic glycosides, this research proves that natural chemicals in flax or other cyanogenic plants can be quite potent insecticides. Therefore, this research has identified alternative compounds with potential for development into commercial pesticides. These naturally occurring cyanohydrins kill insects through their degradation to free cyanide ion. This has previously been assumed to be the mode of action, but it has not been experimentally demonstrated or quantified prior to this project. Using this QSAR study, the toxicity of other cyanohydrins and their derivatives can be understood and predicted. Mammalian toxicity studies, however, still need to be done to help evaluate any potential hazards to humans, livestock, pets and wildlife.