In 1993, a number of species of algae were subjected to treatment with various sterol-inhibiting fungicides (SIFs) to determine the effects of these chemicals on growth and sterol synthesis in non-target organisms (Orcutt, 1993). One of several findings of this experiment revealed that *Chlorella kessleri* was more resistant to the SIF, propiconazole, than *C. fusca* var. *vacolata* (henceforth referred to as *C. fusca*). The purpose of the current study was to determine the reason for the observed tolerance of *C. kessleri* and the sensitivity of *C. fusca* to the SIF. The specific composition and associated function of membrane lipids may affect an algal cell's ability to tolerate a xenobiotic compound such as propiconazole. When comparing the free sterol (FS) and steryl ester (SE) composition and total lipid of *C. fusca* and *C. kessleri* over time, it was found that these algae respond differently with increasing cell age. Inherent differences based on age were then considered relative to treatment with propiconazole. Following treatment, FS, SE, and total lipid variations were observed as well as differences in the amount of certain free fatty acids (FFAs) and the occurrence of precursor (14α-methyl) sterols. Both FFAs and precursor sterols have been reportedly involved in the toxicity of SIFs on target cells. The combined differences in the various parameters both as a result of cell age and also chemical treatment are crucial to the explanation of sensitivity/tolerance of the two algae. In addition, more well-known SIF resistance mechanisms may also factor into this explanation. Overall it appears that multiple mechanisms may be involved in the response of the two *Chlorella* species to propiconazole.

Earlier research has shown that *Chlorella* represents one of the most diverse genera of algae with respect to sterol composition and it is quite possible that members of the *Chlorella* species are some of the most diverse organisms among plant species. Sancholle *et al.*, 1984 states that there is a general belief that lower fungi are less sensitive to C-14 demethylation inhibitors than the higher fungi, due to the increased diversity of sterol content in lower fungi. This same relationship may be true for algae. It appears that greater diversity of sterol structures is associated with organisms thought to be phylogenetically primitive, while simplification occurs in phylogenetically advanced organisms. For example, unicellular green algae, specifically in the genus *Chlorella* have a much greater diversity of sterols among species (Patterson, 1971) than more advanced algae which typically have only one or two dominant sterols associated with their tissues. Also, Orcutt and Patterson, (1975) noted that colonial diatoms tend to have a more complex sterol profile than those that exist in a solitary state. Ballantine *et al.*, 1979 substantiated the work of Orcutt and Patterson in the observation that diatoms which exist as solitary cells have less complex sterol compositions than those that form colonies. However, in the case of *C. fusca* and *C. kessleri* it appears that there can be variation in sterol complexity even among organisms of the same genus that typically live as single cells.

In *C. fusca*, for example, 6 different FS types were identified compared to 11 in *C. kessleri*. However, in terms of total sterol, *C. fusca* had a significantly higher amount of sterol as a percent lipid than *C. kessleri*. Free sterols accounted for the majority of total sterol produced within both species of algae, although changes between the organisms were noted relative to culture age. In *C. fusca* and *C. kessleri*, FS increased during the early phases of growth and then
declined or reached a plateau during the stationary phase. The sensitive organism had considerably higher FS levels during the early phases of growth compared to tolerant algae, but later stages of growth revealed similar amounts of FS in both organisms. It has been observed in senescing bean cotyledons that membrane bulk lipid fluidity decreases by two-fold with age (Duxbury et al., 1991). This was largely attributed to an increase in relative sterol concentration and was accompanied by a change in average conformation of membrane proteins. The same response could be induced in young cotyledons by increasing the sterol concentration using cholesteryl hemisuccinate.

Additional differences between the two algae relate to the chemical structure of the sterols. Chlorella fusca contains mostly Δ7 sterols, whereas C. kessleri contains a mix of Δ5 and Δ7 sterols. When considering the number of carbon atoms that comprise these algae, C. fusca has a higher percentage of C-29 sterols, while C. kessleri contains more C-28 sterols. Following propiconazole treatment, FSs remain the predominant form of sterol within both organisms and C. fusca continues to have a significantly higher amount of sterol as a percent lipid across all treatments. However, the number of FS types increases to 9 in C. fusca and 12 in C. kessleri, resulting mainly from the propiconazole induced increase in 14α-methyl sterol precursors. Interestingly, of the 9 FS present in C. fusca following treatment, only three of them are completely new sterol types. In C. kessleri 10 novel sterols appear.

Sterol diversity could be one of the most important factors conveying SIF-tolerance to C. kessleri, especially since sterols are important constituents of cell membranes and function to maintain the integrity and fluidity/permeability of the membrane. The results of the current study, show that C. kessleri produces a much greater diversity of sterol types, both under control conditions and following propiconazole treatment, than C. fusca. In looking at patterns of sterol biosynthetic pathways for taxonomic purposes, Holden and Patterson (1982) found that Chlorella, in particular, was unique in the enormous variety of biosynthetic patterns encountered in members of one genus. This seems to be exemplified in C. fusca and C. kessleri. In vitro studies using synthetic lipid vesicles have demonstrated that sterol concentration and the type of sterol in such vesicles can affect proton efflux induced by the polyene antibiotic amphotericin B (Vertut-Croquin et al., 1983). The sterol ergosterol was more effective than cholesterol in promoting proton efflux suggesting that this may be the reason animal cells are less susceptible to these antibiotics than fungal cells. Other studies found that the partition coefficients for the polyene antibiotic, nystatin, in ergosterol/dimyristoyl-L-alpha-phosphatidylcholine vesicles was more than three-fold greater with minute changes (1 mol %) in the sterol concentration on either side of the critical sterol mol fraction of 25.0 mol % (Wang et al., 1998). Kelly et al., 1994 reported differences in the sterol composition of a mutant strain of Saccharomyces cerevisiae that had a fungistatic response to azole treatment versus one that had a fungicidal response. Based on this information, it could be argued that the efficiency of an organism to adjust membrane fluidity/permeability in response to chemical treatment might be enhanced in an organism that has a larger number of sterols from which to select.

Considering that to produce such a variety of sterol types, the pathway in C. kessleri would most likely be more branched and complex than that of C. fusca, which has fewer end-product sterols. This kind of the sterol synthesis pathway has been reported for Chlorella emersonii (Doyle et al., 1972) in which several branches, including one prior to the C-14-
demethylation step are observed. It is conceivable then, that under chemical stress, C. kessleri would be able to sequester sterol building materials into another branch of the pathway, producing at least the minimum sterols required for growth. C. fusca on the other hand would not have alternative branches to utilize when exposed to chemical stress and would therefore exhibit few options for the production of functional sterols. C. kessleri may also be able to incorporate sterols otherwise thought of as precursor sterols. In marine dinoflagellates, for example, Piretti et al., 1997 found that 4-methyl sterols, usually found in prokaryotes, are principally incorporated within cell membranes suggesting they function as end-product rather than precursor sterols (Loeblick, 1984). Perhaps C. kessleri has retained the capacity to utilize 4-methyl sterols in membranes more efficiently than C. fusca. This ability may not only allow this organism to acclimate to different environments (i.e. air, soil, water) but may also convey SIF-tolerance.

Although little is known of the precise physiological roles that sterols play in the cell, work with mutant strains of Saccharomyces cerevisiae has led to the prediction of at least four growth-dependent functions for sterols including roles in mating efficiency/conjugation, mannosylation/glucosylation, membrane permeability, and maintenance of phospholipid composition (Parks and Casey, 1995). The importance of sterols to the growth of organisms may further help to explain the comparable reduction in growth of C. fusca treated with 2, 4 and 6 ppm and C. kessleri treated with 6, 12, and 24 ppm propiconazole. Other researchers have reasoned that the altered growth patterns commonly seen in SIF treated cells were most likely due to changes in enzyme activity brought about through alterations in the physical properties of the membranes as a result of the blockage of sterol production (Vanden Bossche et al., 1992).

To further understand the role of sterols as they affect the sensitivity or tolerance of an organism, the SE fractions of C. fusca and C. kessleri were analyzed at various growth stages. Table 1.3.1 reflects previous work with various species of algae and indicates the considerable variability regarding the presence or absence of SEs relative to a particular growth stage. Both species tested in the current study contained esterified sterols (5 in C. fusca, 10 in C. kessleri) within their cells although the occurrence relative to growth varied between the algae. Steryl esters were found in C. kessleri at every growth stage, while in C. fusca, SEs were not observed until 144 HAI. On a per cell basis, C. kessleri contained more SE than C. fusca, although the amount of SE in C. kessleri fluctuated over time resulting in a decrease rather than the increase seen in C. fusca. Expressed on a µg/mg lipid basis, C. kessleri still contained more SE, but the amount remained fairly stable over time while the SE in C. fusca increased with time. When compared to changes in total sterol, there appears to be an inverse relationship between FS and SE in both organisms (i.e. when SE is low, total sterol is high and the opposite).

Bailey and Parks, 1975 found that SEs begin accumulating in yeast at the onset of the exponential phase of growth and increased greatly through the stationary phase. Through their work with a sterol-requiring mutant, Taylor and Parks, (1981) suggested that freely interconvertible pools of FSs and SEs were functional within yeast cells. Taylor and Parks, (1981) went on to show preferential esterification of sterols lacking the Δ⁷ or Δ²² bond or the 24β-methyl group within these same cells. It has further been found (Lewis et al., 1987) that while yeast cells must maintain an essential, low level of FS for growth, that which is not needed accumulates into a pool of bulk FS and as the pool grows, the FS are removed through
esterification and deposited into a SE pool. Parks and Casey, (1995) report at least two beneficial physiological ends for esterification in yeast cells: proofing and salvage. Proofing refers to the sequestering of non-ergosterol sterols to prevent participation in ergosterol-dependent functions during growth. Salvage involves the capture of excess sterols that result from over-synthesis. Such interconversions could also affect the sterol/lipid composition of membranes impacting fluidity, permeability and possibly enzyme function. Therefore, the process of esterification, in and of itself, could suggest a potential mechanism of SIF-tolerance in C. kessleri. For example, the cells of C. kessleri may be better able to esterify those sterols unsuited for growth, resulting in the less sensitive response of this algae to SIF treatment. Also, C. kessleri has a greater capacity for esterification and SE increases with increasing SIF concentration.

Following propiconazole treatment, no new SEs were found in C. fusca, while 7 were identified in C. kessleri. Those SEs occurring in C. fusca were consistently the same as those observed in the untreated cultures and also corresponded to the FSs found in the treated cultures. In C. kessleri, the SEs occurring in the treated cultures were the same as those occurring as FSs, but were quite different from those identified in cells not receiving propiconazole. When measured on a per cell basis, C. fusca was found to have less SEs than C. kessleri. This was also true of SE measured in µg/mg lipid, however more significance was noted. Although no significant treatment differences were observed for either parameter there seemed to be a slight trend toward SE increase in C. kessleri compared to C. fusca with increasing treatment concentration (SE µg/mg lipid significant at T1, T3 and T5). The prevalence of new SEs relative to the FSs which occur following treatment of C. kessleri seems to show that this alga is more adept than C. fusca at utilizing the esterification process. Esterification/deesterification plays an important role in the relative removal/incorporation of sterols within cell membranes, ultimately affecting membrane structure. Thus, the ability of C. kessleri to esterify the precursor sterols more so than C. fusca, along with other features of this species of algae, could help to explain its apparent SIF-tolerance.

Very early work showed that SIF treated fungal cells contained an increased amount of 14α-methyl or precursor sterols. It was from this that researchers reasoned that the mechanism of SIF action involved the sterol biosynthetic pathway. Through competitive inhibition, the fungicide molecule replaces the 14α-methyl sterol within the CP450 enzyme binding site and prevents it from becoming demethylated for incorporation into the cell membrane (Hajek et al., 1982; Hajek and Novak, 1982; Gadher et al., 1983; Henry and Sisler, 1984; Vanden Bossche et al., 1984, and Wiggins and Baldwin, 1984). Presumably, these intermediates are unable to be correctly incorporated within the membrane lipid bi-layer, ultimately resulting in alterations in cell membrane fluidity/permeability (Watson, et al., 1989; Baldwin, 1990; Henry, 1990; Schuler et al., 1990; Basarab et al., 1991; Doignon and Rozes, 1992).

There are differing opinions, however, as to the role 14α-methyl sterols play in growth inhibition of fungal cells following SIF treatment. Several researchers (Bean, 1973; Chang et al., 1977; Nes et al., 1978; Dahl et al., 1980; Watson et al., 1989) have reported that 14α-methyl sterols are deleterious to cell membranes resulting either from 1) an increase in the permeability of the plasma membrane, which would 2) impair the regulation of cytosol composition, and 3) alter membrane-associated enzymatic activity. However, others (Marcireau et al., 1990; Kelly et
al., 1994; Joseph-Horne et al., 1996) have found that the fungistatic nature of SIFs was not due to the accumulation of abnormal sterols in treated cells, but rather to a reduction in growth supporting sterols. Hernandez et al., 1997 suggests the intracellular ATP depletion by H⁺-ATPase rather than the accumulation of precursor sterols as a mechanism of action of SIFs. In this study, researchers report that the ATP required for fungal cell growth is depleted following the toxic-stress-activation of the plasma membrane H⁺-ATPase, which increases activity as a result of SIF treatment. Still others suggest a little of both (Rangel et al., 1996). It has been reported (Loeffler and Hayes, 1992) that various fungal species were inhibited when atypical sterols were used to replace normal sterol components. In our research, sterol intermediates accumulated in both algae following propiconazole treatment, although to a much greater degree in C. kessleri.

While we have shown that C. kessleri seems to be able to esterify sterol intermediates more efficiently than C. fusca, multiple mechanisms may be operative in explaining algal sensitivity and tolerance. The CP450 enzymes, for example, have multiple metabolic roles within the cells of both eukaryotic and prokaryotic organisms. While no specific research was conducted to determine the type or amount of CP450 enzymes within the SIF-tolerant and -sensitive species of algae, it is possible that differences could be found. Multiple forms of these enzymes have been identified within animals, plants, and fungi (Niggi et al., 1986; Leslie et al., 1988; Donaldson and Luster, 1991; Vanden Bossche et al., 1992). Various isoforms also occur in yeast from cells harvested at different growth phases (Niggi et al., 1986) and grown on different carbon sources (Sanglard et al., 1986). In addition to these potential variations, the affinity of a given pesticide for the CP450 enzyme target site may differ depending on the organism and greatly affect the toxicity of a particular compound. Therefore, work to further address the SIF-sensitivity or -tolerance issue might focus on isolating these enzymes and characterizing the similarities or differences between them. Other more well-known mechanisms of fungal resistance which may be functioning to varying degrees within the tolerant, C. kessleri include 1) an altered uptake/efflux mechanism (De Waard and van Nistelrooy, 1980; Siegel and Solel, 1981; De Waard and van Nistelrooy, 1987; Parkinson et al., 1995; Sanglard et al., 1995), 2) an altered target affinity or possibly over expression of the CP450 enzyme (Kramer, 1986; Vanden Bossche et al., 1987), 3) differences in the isozymes of the CP450 enzyme (Donaldson and Luster, 1991), 4) mutations in the sterol Δ⁵,₆ desaturase enzyme during ergosterol biosynthesis and 5) differences in sterol/phospholipid ratio within the lipid bilayer (Hitchcock et al., 1993).

In addition to these resistance mechanisms, FFAs were also important to consider given their potential role in cell toxicity. Increases in the FFA content of cells and a shift toward greater unsaturation have been previously documented as responses to SIF treatment (Hancock and Weete, 1985). Ikawa et al., 1997 reported that exogenously applied linolenic acid (18:3) is one of the most inhibitory fatty acids towards green algae. These researchers further found that polyunsaturated fatty acids, in general, could inhibit the growth of Chlorella, though the actual mechanism is unknown. In studying the effects of oleic acid (18:1) on the membrane fatty acid composition of Lemna minor, Zimafulula et al., 1997 showed decreased oxygen evolution and decreased photosynthesis within treated cells. Previous studies by Consantopoulos and Kenyon, (1968) and Ikawa et al., 1984 further support the detrimental affects of fatty acids on photosynthesis. Hatton and Kinderlerer, (1991) have found that medium chain fatty acids inhibit
proton gradients across cell and mitochondrial membranes, thereby reducing the ATP available for growth. In addition to the exogenous effects, Barclay and McKersie (1994) suggest that the presence of FFAs within cells during periods of environmental stress altered membrane fluidity, thereby changing the susceptibility of lipids to free radical reactions. Changes in the ratios of saturated/unsaturated fatty acids could impact membrane fluidity/permeability given that increasing FFA unsaturation causes membranes to be more fluid and thus, more permeable (Goedhart et al., 1989). Furthermore, membrane alterations resulting from SIF treatment may force a conformational change in the desaturases associated with membrane fatty acids.

In general, *C. fusca* and *C. kessleri* are similar with respect to the qualitative composition of FFAs they contain, however, relative amounts of FFAs differed. The most notable differences included the finding that type 18:1 is higher in *C. fusca* than *C. kessleri*, while 18:0, 18:2 and 18:3 are lower. When considering the ratio of 18:2/18:3 FFAs, *C. kessleri* was found to have consistently higher ratios of these two FFAs than *C. fusca*, regardless of treatment. Primarily this ratio is important as it relates to modifications of membrane fluidity/permeability following an environmental stress, such as chemical treatment. For example, a shift in favor of fewer double bonds (i.e. 18:2 FFA) would tend to make cell membranes less fluid, while the occurrence of FFAs with more double bonds (i.e. 18:3 FFA) would increase membrane fluidity. When comparing a naturally triazole-tolerant with a sensitive species of fungi, Weete and Wise (1987) found increases in the amount 18:2 FFA in the sensitive organism as the concentration of propiconazole increased, indicating more structured membranes. While the higher ratio in the tolerant *C. kessleri* may seem to contradict this report, *C. fusca* had a higher amount of 18:1 FFA (fewer double bonds), which is not accounted for in the ratio. No distinctive trends were observed relative to FFA as a percent lipid or dry weight across treatments, however, it did appear that *C. fusca* had a greater amount of total fatty acid than *C. kessleri*.

If FFAs, rather than precursor sterols, were detrimental to cell membranes, *C. kessleri* may have been more able to negate the effects of excess FFAs through esterification, therefore reducing the toxicity of these molecules. In *C. kessleri*, it appeared that precursor sterols were esterified as readily as end-product sterols. This did not appear to be the case in *C. fusca*. Esterification, therefore, could serve a dual purpose, not only could this process help sequester sterol building blocks for future use, but it could also help to reduce the number of potentially toxic FFAs from inhibiting cell growth. Again, factors such as sterol diversity, energy potential of the cell, CP450 monoxygenase enzymes along with the amount of FFAs could all confer the tolerance of *C. kessleri*.

As a whole, the total lipid composition of algal cells has been suggested as having a possible role in the uptake and sequestering of toxic xenobiotics (Kent and Currie, 1995). Therefore, comparing lipid compositions of the two species of algae may shed further light on why *C. fusca* is more sensitive to propiconazole than *C. kessleri*. The amount of total lipid produced in cells of *C. fusca* is higher than in *C. kessleri* during all growth stages. The difference becomes even greater in the stationary phase of growth. It is also interesting to note that the amount of lipid per cell parallels lipid expressed as a percentage of dry weight in *C. fusca* and in both cases increases with age. However, in *C. kessleri*, there is an inverse relationship of these parameters. At about 216 HAI, lipid per cell declines and lipid as percent dry weight increases in *C. kessleri*. This is most likely because individual cell weight declines
sharply in *C. kessleri* compared to *C. fusca*. In *C. fusca*, at the same period of time, lipid per cell is higher than in *C. kessleri*. These types of comparisons suggest that total lipid production in the two algae can vary, individually, with stage of growth.

The expression of lipid concentrations can also factor in to the evaluation of lipid content (i.e. lipid per cell versus lipid as a percent dry weight). Kent and Caux (1995) observed that algal cells treated with the insecticide fenitrothion exhibited higher lipid levels than the control when lipid was expressed on a cellular basis and no difference when expressed on a dry mass basis. Based on the findings of Kent and Caux (1995) and Kent and Currie (1995) the fact that lipid per cell was higher in *C. fusca*, at least during the early part of the stationary growth phase, could also account for greater sensitivity of *C. fusca* to propiconazole than *C. kessleri*. In their study as well as with others, dead algal cells have been shown to accumulate more pesticide than live cells (Chiou *et al.*, 1979; Hancock and Weete, 1985; Kent and Currie, 1995). From this information researchers reasoned that lipid-soluble compounds, such as xenobiotics, are absorbed primarily through passive rather than active diffusion prior to being sequestered within algal cells. Kent and Caux, (1995) further found that algae possess a high capacity for sequestering and concentrating lipophilic compounds due also to their small cell size, limited cell barriers, high surface/volume ratios, and effective adsorptive capacity.

Measurements of cell diameter revealed an increase for both *C. fusca* and *C. kessleri* with increasing propiconazole concentration, although neither species nor treatment differences were generally significantly different. Increased cell size has previously been reported in fungi treated with SIFs (Ragsdale and Sisler, 1972). If chemical was being excluded in the tolerant species one would likely expect little or no change in cell size. Exclusion mechanisms could include the production of organic exudates (carbohydrates and proteins) that act as chelating agents (Marsalek and Rojickova, 1996), an altered uptake or efflux mechanism, complexation of metals by biologically synthesized ligands, or oxidation, reduction, or chemical modification of the xenobiotics (Folsom *et al.*, 1986). Treated cells of *C. kessleri*, rather than the sensitive *C. fusca* tended to be slightly larger in diameter across treatments. Thus, exclusion is probably an unlikely mechanism of tolerance in *C. kessleri*. The generally small cell size of both organisms may be important as it impacts surface area and bioaccumulation of propiconazole within these algae.

Previous studies with insecticides (Kent and Caux, 1995), herbicides (Caux *et al.*, 1996) and commonly occurring pollutants in natural aquatic ecosystems (Megharaj *et al.*, 1992) have been conducted to assess the effects of the various compounds on algal growth and lipid content. The latter two studies in particular, were concerned with the algistic or algicidal properties of herbicides and other pollutants, respectively. Thus, one of the objectives of the current study was to determine if propiconazole was algicidal or algistatic. Our results indicate that treated *Chlorella* cells, placed into fresh medium, showed regrowth potential equivalent to that of control cultures, suggesting an algistatic interaction with propiconazole. Propiconazole in both sensitive and tolerant *Chlorella* species acted in an algistatic manner. The algistatic response of these algae species is consistent with the other reports of a fungistatic reaction of fungi to this group of compounds (Sancholle *et al.*, 1984; Marcireau *et al.*, 1990; Burpee *et al.*, 1996; Guerin *et al.*, 1996).
The importance of understanding the effects of propiconazole on non-target organisms became increasingly more significant following the results of a comprehensive survey of the Central American watershed (Castillo et al., 1997). The survey reported that propiconazole was the most widely distributed pesticide in aquatic ecosystems within this country. Even more evocative was the finding by Levine and Oris, 1999 that preexposure of fathead minnows (*Pimphales promelas*) to propiconazole resulted in the enhanced acute toxicity of the organophosphate (OP) insecticide, parathion. These researchers suggest that the enhancement of parathion could have resulted from the induction of a novel CP450 enzyme that catalyzes the activation of OPs to their oxon products, although additional work will need to be completed.

Within the United States a 1992 USGS National Water Quality Assessment reports that rice producing regions along the Mississippi River flood plain receive, on average, greater than 0.186 lbs. a.i./mi$^2$ of county/year (Figure 4.1.1). This is special concern given the previous study showing that preexposure to propiconazole may increase OP sensitivity in aquatic organisms. While current pesticide legislation (i.e. Food Quality Protection Act or FQPA) calls for the phase out of most OPs, monitoring of the effects of SIFs on non-target organisms should be a continued focus. Other areas receiving higher amounts include the northern portion of Oregon, stretching the length of the border between Washington, most of North Dakota and also northwestern Ohio. According to the USGS study, propiconazole was used primarily on wheat and grains followed by rice, field and grass seed, pecans, barley, sweet corn, peaches, wild rice, celery and rye.

In summary of the current research, the two non-target *Chlorella* species, *C. kessleri* and *C. fusca* appeared to have inherent differences in the amount of FS, SE and total lipid as well as in the way in which these components change through the aging process. The two organisms also varied in their physiological response to propiconazole treatment with regard to their sterol profiles, esterification efficiency, FFA production, lipid content, and cell size. Thus, it is highly likely that multiple mechanisms are operative relative to the sensitivity/tolerance of these algae to chemical treatment. Such mechanisms may include differences in the affinity of CP450 monoxygenase enzymes, sterol membrane composition, sterol esterification efficacy and solubility, the production and amount of potentially toxic FFAs, differences in lipid amounts affecting lipophilic partitioning, and cell size which could impact bioaccumulation of xenobiotics. Inherent species differences in addition to variations in species' response to treatment make it very difficult to predict the effects of propiconazole on these two species of algae.
Figure 4.1.1. Estimated annual agricultural use of propiconazole (1992) as reported by USGS [from data collected through the Pesticide National Synthesis Project]. On the web at http://water.wr.usgs.gov/pnsp/use92/propconzol.html.